Treatment of Chronic Hepatitis C
The Benelux studies

Johannes T. Brouwer

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The publication of this thesis was financially supported by Altana Pharma BV, AstraZeneca BV, Aventis Pharma BV, Gilead/UCB Pharma, F.M.H. Medical BV, Ferring BV, GlaxoSmithKline, Janssen-Cilag BV, Novartis Pharma BV, Roche Nederland BV, Schering-Plough BV, Tramedico Nederland, Zambon Nederland BV

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Proefschrift Rotterdam

ISBN 90-77595-54-6

Printed by Optima Grafische Communicatie Rotterdam

Treatment of Chronic Hepatitis C
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Behandeling van Chronische Hepatitis C
De Benelux studies

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus Prof. dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties
De openbare verdediging zal plaatsvinden op
donderdag 10 juni 2004 om 16.00 uur

door

Johannes Teunis Brouwer

geboren te Vlaardingen

PROMOTIECOMMISSIE

Promotor: Prof. dr. S.W. Schalm

Overige leden: Prof. dr. E.J. Kuipers

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en voor

Johan, Willem Pieter,

Marlies en Hannah

Contents

Chapter 1

General introduction

In the eighties and early nineties of the last century, acute hepatitis occurred in 5-10% of patients receiving blood transfusions in the USA (1), and in more than 90% of cases this could not be attributed to hepatitis A or B ("Non-A, Non-B hepatitis") (2). More than 50% of the hepatitis infections became chronic, and Non-A, Non-B hepatitis led to liver cirrhosis in about 20% of patients, thereby being a serious health burden (3). In 1989 the hepatitis C virus was discovered as the major cause of this post-transfusion hepatitis, but it was also found in many cases of unknown chronic hepatitis without known blood contacts in the past (sporadic HCV). It is estimated that more than 170 million people are infected with hepatitis C worldwide (4–6), leading to more than 280.000 deaths each year due to decompensated liver cirrhosis and liver cancer (7).

Acyclovir and prednisone were not effective for chronic NANB hepatitis (8,9). A pilot study in 10 patients suggested that interferon-alpha could be a potential therapy for chronic hepatitis C (10). Interferon was proven to be effective in controlled trials published in 1989 (11,12). Based on these 2 studies, with 166 and 41 patients, respectively, Interferon (IFN) 3 mega units (MU) thrice a week (tiw) for 6 months was registered as standard therapy in many countries around the world.

Standard IFN treatment of chronic hepatitis C was hampered by two major problems: only 50% of patients had disappearance of liver inflammation during treatment, expressed as normalization of the liver enzyme alanine amino transferase (ALT), and more than 50% of those with normal ALT levels during treatment had a relapse after treatment withdrawal, leading to less than 20-25% sustained ALT response 6 months after the end of treatment (11).

These results led to an initiative in 1990 by physicians and basic scientists in Belgium, the Netherlands and Luxembourg (BeNeLux) to start investigator initiated studies aiming to unravel the mechanisms of non-response and to improve the success rate of treatment. The importance of designing studies based on virological in stead of biochemical assessments was recognized, and basic scientists worked hard to develop reliable HVC RNA assays which could be used in a standardized way in the clinical trials. It was understood that the important issues to study were initial non-response to therapy, breakthrough during treatment after an initial response, and relapse after treatment withdrawal. It was realized that large cohorts were needed to study treatment of these components in a randomized way, and with this aim the Benelux multicenter study group for treatment of chronic hepatitis C was formed. In this group, coordinated from Rotterdam, almost all academic centers from Belgium,

several academic centers from the Netherlands, and many large non-academic centers from the three Benelux countries were involved.

From the start the aim was to closely cooperate with basic scientists, in particular virologist, molecular biologists and immunologists. The evolving techniques for qualitative and quantitative HCV RNA testing and analysis of HCV RNA genotypes were incorporated in the Benelux studies from the early days onward. The associated laboratory investigations and exploratory follow-up studies have been described in separate academic theses (van Doorn, Kleter) (13,14) and publications (15–20). Aims of the basic clinical studies were to make individualization of treatment possible and to reduce the incidence of initial non-response, breakthrough and relapse, by determining predictors and subsequently adapting the dose or duration of treatment. The concepts emerging from those studies were finally tested in a group of patients considered difficult to treat (21-23). This series of investigations led to another academic thesis (Bekkering) (24).

This thesis describes a period of 10 years in which the treatment of chronic hepatitis C developed from occasional sustained response to cure in more than 60% of patients.

REFERENCES

1. Alter HJ. Transfusion-associated non-A, non-B hepatitis: the first decade. In: Zuckerman AJ, editor. Viral hepatitis and liver disease. New York: Alan R. Liss; 1988. p. 537-542.
2. Dienstag JL. Non-A, non-B hepatitis. I. Recognition, epidemiology, and clinical features. Gastroenterology 1983;85(2):439-62.
3. Koretz RL, Stone O, Mousa M, Gitnick GL. Non-A, non-B posttransfusion hepatitis--a decade later. Gastroenterology 1985;88(5 Pt 1):1251-4.
4. WHO. Hepatitis C: global prevalence. Wkly Epidemiol Rec 1997;72(46):341-4.
5. Alter MJ. Epidemiology of hepatitis C. Hepatology 1997;26(3 Suppl 1):62S-65S.
6. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345(1):41-52.
7. Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. Lancet 2003;362(9401):2095-100.
8. Pappas SC, Hoofnagle JH, Young N, Straus SE, Jones EA. Treatment of chronic non-A, non-B hepatitis wih acyclovir: pilot study. J Med Virol 1985;15(1):1-9.
9. Stokes P, Lopez WC, Balart LA. Effects of short-term corticosteroid therapy in patients with chronic non-A, non-B hepatitis (NANB). Gastroenterology 1987;92:1783 (abstract).
10. Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, Peters M, et al. Treatment of chronic non-A,non-B hepatitis with recombinant human alpha interferon. A preliminary report. N Engl J Med 1986;315(25):1575-1578.
11. Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC, Jr, Perrillo RP, et al. Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. N Engl J Med 1989;321(22):1501-1506.
12. Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, et al. Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med 1989;321(22):1506-1510.
13. van Doorn LJ. Detection and characterization of the hepatitis C virus [thesis]. Rotterdam: Erasmus University; 1994.
14. Kleter GEM. Characterization of hepatitis C virus isolates from chronically infected patients [thesis]. Rotterdam: Erasmus University; 1995.
15. Kleter GE, Brouwer JT, Heijtink RA, Schalm SW, Quint WG. Detection of hepatitis C virus RNA in patients with chronic hepatitis C virus infections during and after therapy with alpha interferon. Antimicrob Agents Chemother 1993;37(3):595-7.
16. van Doorn LJ, Kleter B, Stuyver L, Maertens G, Brouwer H, Schalm S, et al. Analysis of hepatitis C virus genotypes by a line probe assay and correlation with antibody profiles. J Hepatol 1994;21(1):122-9.
17. van Doorn LJ, Kleter B, Voermans J, Maertens G, Brouwer H, Heijtink R, et al. Rapid detection of hepatitis C virus RNA by direct capture from blood. J Med Virol 1994;42(1):22-8.
18. Louagie HK, Brouwer JT, Delanghe JR, De Buyzere ML, Leroux-Roels GG. Haptoglobin polymorphism and chronic hepatitis C. J Hepatol 1996;25(1):10-4.
19. Kleter B, Brouwer JT, Nevens F, van Doorn LJ, Elewaut A, Versieck J, et al. Hepatitis C virus genotypes: epidemiological and clinical associations. Benelux Study Group on Treatment of Chronic Hepatitis C. Liver 1998;18(1):32-8.
20. Depraetere S, Van Kerschaever E, Van Vlierberghe H, Elewaut A, Brouwer JT, Niesters HG, et al. Long term response to interferon treatment in chronic hepatitis C patients is associated with a significant reduction in anti-E1 envelope antibody titers. J Med Virol 2000;60(2):126-32.
21. Bekkering FC, Brouwer JT, Schalm SW, Elewaut A. Hepatitis C: viral kinetics. Hepatology 1997;26(6):1691-3.

22. Bekkering FC, Brouwer JT, Leroux-Roels G, Van Vlierberghe H, Elewaut A, Schalm SW. Ultrarapid hepatitis C virus clearance by daily high-dose interferon in non-responders to standard therapy. J Hepatol 1998;28(6):960-4.
23. Bekkering FC, Brouwer JT, Hansen BE, Schalm SW. Hepatitis C viral kinetics in difficult to treat patients receiving high dose interferon and ribavirin. J Hepatol 2001;34(3):435-40.
24. Bekkering FC. Viral kinetics of the hepatitis C virus [thesis]. Rotterdam: Erasmus University; 2001.

Chapter 2

Benelux multicentre trial of alpha interferon treatment for chronic hepatitis C: standard *v* high dose treatment monitored by biochemical and virological markers (interim analysis)

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Benelux Multicentre Trial Study Group

Gut 1993; supplement: S119-S120

ABSTRACT

In an interim analysis of the Benelux trial, no difference in alanine aminotransferase normalisation has been found between the high (6 million units) and standard (3 million units) doses of interferon alfa-2b during the first eight weeks of treatment. The probability of achieving normal alanine aminotransferase activity on at least two successive occasions during treatment is 65% for the standard dose and 73% for the higher dose. Measurement of plasma hepatitis C virus-RNA suggests that hepatitis C virus-RNA negativity after four weeks of treatment is a prerequisite for sustained response to interferon alfa-2b.

INTRODUCTION

A standard six month course of treatment with alpha interferon, 3 million units thrice weekly, leads to alanine aminotransferase normalisation in about 50% of patients with hepatitis C virus infection. Relapses occur in most of the patients, however, after treatment is stopped. So, conceivably, treatment results might he improved if a higher induction dose is used and if the dosage and duration of treatment are attuned to the effects on alanine aminotransferase and hepatitis C virus-RNA.

METHODS

Patients were included in the Benelux multicentre trial if they had: (a) history of alanine aminotransferase ≥2 times the upper limit of normal, for at least six months; (b) history of exposure to blood products or a positive anti-HCV test; (c) no clinical, histological or serological evidence of other viral, alcoholic, drug, or obesity related, hereditary or autoimmune hepatitis. Patients were randomised to receive either: (a) standard treatment; 3 million units interferon alfa-2b (INTRON A) thrice weekly for 24 weeks; (b) experimental treatment: induction phase of 6 million units interferon alfa-2b thrice weekly for eight weeks, followed by a maintenance phase of titrated doses of interferon 6 to 1 million units thrice weekly until biochemical and virological remission (alanine aminotransferase activity normal, plasma hepatitis C virus-RNA undetectable) was achieved while on 1 million units thrice weekly. Interferon was given by subcutaneous injection. Treatment was withdrawn in cases of non-response after 12 weeks, or after 52 weeks in cases of partial response.

RESULTS

To date, 244 patients with hepatitis C virus have been entered into the study. Patient characteristics before treatment are comparable with the standard and experimental treatment group (Table). No statistically significant differences were seen between the groups by either χ square or Wilcoxon's rank sum test.

Data for 12 weeks are available for 188 patients and 149 patients have been followed for at least six months. No difference in alanine aminotransferase normalisation rate has been found between the high (6 million units) and standard (3 million units) induction dose during the first eight weeks, with figures of 53% (95% confidence intervals 46,66) and 54% (95% confidence intervals 44,64), respectively.

Table Patient characteristics before treatment

	Interferon treatment schedule	
	Standard (n=120)	Experimental (n=124)
Men/women	74/46	69/55
Blood exposure	74	79
Anti-HCV positive	116	120
Age*	47.1 (14)	46-7 (14)
Alanine aminotransferase (IU/l)*	168 (98)	150 (91)
Hepatitis C virus-RNA positive†	24/27	37/39
Histological examination		
Chronic persistent hepatitis	20	28
Chronic active hepatitis	65	64
Cirrhosis	21	30

*Mean (SD).
†Tested in 66 patients only.

The probability (actuarial analysis) of reaching normal alanine aminotransferase activity on at least two successive occasions during treatment is 65% (95% confidence intervals 56,74) for the standard scheme and 73% (95% confidence intervals 64,82) for the experimental scheme, with a plateau after 16 weeks (NS).
The plasma hepatitis C virus-RNA has been measured in a subgroup of the first consecutive 24 patients from one centre, and studied sequentially to evaluate whether a long term treatment response could be predicted early. Fifteen patients had detectable hepatitis C virus-RNA at four weeks' treatment despite alanine aminotransferase normalisation in seven. All 15 remained viraemic until the end of treatment (six months), 13 of them with raised transaminase activity. Nine of the 24 patients had undetectable hepatitis C virus-RNA at four weeks while seven remained hepatitis C virus-RNA negative until the end of treatment. These results suggest that, when hepatitis C virus-RNA is detectable after four weeks of interferon treatment, it is predictive for long term non-response. Hepatitis C virus-RNA negativity after four weeks of treatment with interferon seems to be a prerequisite for a sustained treatment response. The hepatitis C virus-RNA value at four weeks is of more importance than alanine aminotransferase activity in predicting the long term treatment response ($p<0.001$, Fisher's exact test).

CONCLUSIONS

High dose induction treatment (6 *v* 3 million units alpha interferon thrice weekly) does not improve the early response rate. Hepatitis C virus-RNA determination at four weeks' treatment seems to be highly predictive for long term treatment outcome.

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Chapter 3

Efficacy of interferon dose and prediction of response in chronic Hepatitis C: Benelux study in 336 patients

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Journal of Hepatology 1998; 28: 951-959

ABSTRACT

Background In an attempt to improve the limited efficacy of treatment of chronic hepatitis C with interferon-alpha 3 MU tiw, we studied the effects of double-dose therapy followed by downward titration, and analyzed the pre- and pertreatment factors associated with response or non-response.

Methods 354 consecutive patients in 19 centres were randomized to interferon-alpha 3 MU tiw for 6 months or 6 MU tiw for 8 weeks followed by down-titration (3,1 MU tiw) till alanine aminotransferase remained normal and plasma HCV RNA was repeatedly undetectable. The primary outcome measure was sustained alanine aminotransferase and HCV RNA response 6 months after treatment.

Results 336 patients received treatment. The sustained response rate for patients receiving 3 MU tiw for 6 months was 14% (9-21%) and for patients receiving double dose tiw for 8 weeks and thereafter titrated therapy 15% (10-21%) (p=0.8). Pretreatment factors associated with a sustained alanine aminotransferase plus HCV RNA response were the absence of cirrhosis, presence of genotype 2 or 3, a low viral load and, in addition, a low alanine aminotransferase/aspartate aminotransferase ratio; a model was developed to allow estimation of the chance of response for the individual patient. The most powerful predictor of sustained response, however, was plasma HCV RNA at week 4; a positive test virtually precluded a sustained response (1.7%, 0.4-5.0%). If week 4 HCV RNA was not detectable, the chance of a sustained response was 21% (12-34%) for genotype 1 versus 40% (28-54%) for the others (p=0.02). Six MU tiw led to a significant higher week 4 HCV RNA response (47% not detectable) than 3 MU (37%) (p=0.02). During down titration this difference in viral on-treatment response was lost.

Conclusions In the treatment of hepatitis C, an early HCV RNA response is a prerequisite for long-term efficacy. Doubling the initial interferon dose increases this early response, but subsequent downward titration negates this effect, especially in genotype 1.

INTRODUCTION

Chronic hepatitis C can be modified by interferon-alpha therapy (1). Most large studies have used the serum alanine aminotransferase (ALT) level as the primary marker to assess the efficacy of treatment. Sustained ALT normalization 6 months after therapy is only found in 15-30% of patients (2,3). The apparent need for enhancing the efficacy of therapy has been addressed in many randomized controlled trials assessing modifications in dose and duration of therapy. Prolongation of therapy increases the ALT sustained response rate (4,5). The role of the interferon dosage beyond 3 million units (MU) thrice weekly (tiw) is still uncertain, and further improvement in treatment efficacy is hampered by the lack of knowledge of the viral mechanisms involved in response. This report of the Benelux Study Group on treatment of chronic HCV with interferon-alpha describes the results of a randomized controlled trial in 336 patients, with special emphasis on evaluation of a double IFN dose initially, treatment monitoring by ALT and HCV RNA, and analysis of pretreatment and early virological on-treatment factors associated with response.

MATERIALS AND METHODS

Patients

Patients between 18 and 70 years of age were eligible for the study if they had a positive blood test for anti-HCV or documented chronic non-A, non-B hepatitis after receiving blood products, serum ALT concentrations at least twice the upper limit of normal for at least 6 months and liver biopsy findings compatible with chronic hepatitis. Patients were excluded in case of additional causes of chronic liver disease: presence of hepatitis B surface antigen, more than 150 grams ethanol per week, ferritin >1000 µg/l, auto-antibodies >1:100. Further exclusion criteria were previous antiviral therapy, immunosuppressives, decompensated liver disease (ascites, bleeding varices, encephalopathy, bilirubin >34 µmol/l, albumin <32 g/l, serum creatinine >140 µmol/l), cytopenia (platelet count $<50x10^9$/l, granulocytes $<1.5x10^9$/l), coinfection with HIV or signs of hepatocellular carcinoma.

The study was approved by the institutional review board of each participating centre; written informed consent was obtained from each patient.

Treatment

Patients were randomized at a ratio of 4:5 to receive either “standard dose” or "titrated dose” treatment. Randomization was performed centrally by opening an envelope (6). Standard dose treatment consisted of 3 MU of recombinant interferon alpha-2b (Intron-A, Schering-Plough, Kenilworth, N.J., USA) injected subcutaneously tiw for 24 weeks. Titrated dose treatment started with 6 MU of interferon alpha-2b tiw for the first eight weeks, followed by dose reduction to 3 and 1 MU tiw at intervals of at least 8 weeks, provided that ALT levels remained normal; treatment was ended when ALT levels remained normal and HCV RNA remained undetectable by PCR for at least 8 weeks of 1 MU tiw; treatment was discontinued because of failure if ALT did not normalize during 6 MU tiw, or after 52 weeks if ALT normalized but HCV RNA remained detectable.

Blood was sampled at 4-week intervals during treatment and 6-week intervals during follow-up. The interferon dosage was reduced to 50 percent in case of leukopenia ($<1.5 \times 10^9$/l) or thrombocytopenia ($<50 \times 10^9$/l) or when severe subjective side-effects persisted. Hematologic and biochemical tests were performed in the certified laboratories of the participating hospitals; results were corrected for local normal values.

Virologic assessments

Blood samples for HCV RNA determinations were taken under strict conditions to avoid cross-contamination or breakdown of RNA. Special tubes and pipettes were provided, and samples were processed to plasma and frozen to -70 ^{o}C within 2 h of collection. All virologic assessments were performed in the Department of Virology in Rotterdam. Plasma HCV RNA was analysed in duplicate by the polymerase chain reaction (PCR) using two sets of primers derived from the 5' noncoding region, followed by a hybridization assay, as described previously (7). The PCR assay tested faultless in the Eurohep proficiency panel (8), with a sensitivity of 0.9 $\times 10^3$ genome equivalents per millilitre (geq/ml) for genotype 1 and 1.2 $\times 10^3$ geq/ml for genotype 3. The amplification products were used in the Line Probe Assay (Inno-LiPA, Innogenetics, Ghent, Belgium) for assessment of the genotype. The genotype was also determined by direct sequence analysis for the first 60 patients and in case of an indeterminate LiPA result. The major HCV types (1–5) were classified correctly by LiPA in all cases; HCV subtype 1a and 1b were misclassified in 8% of cases (9).

Quantification of plasma HCV RNA was performed by branched DNA assay (Quantiplex v 1.0, Chiron, Emeryville, CA, USA). The detection limit of this assay was 0.35 $x10^6$ genome equivalents per millilitre. Results of this first-generation bDNA assay were corrected by a factor 3 for genotype 2 and a factor 2 for genotype 3, according to recent guidelines (10).

Liver Histology

Liver specimens, obtained before the start of therapy and after 6 months of follow-up, were reviewed by two pathologists who were blinded for all clinical information. Histological scoring was based on the original histological activity index by Knodell et al. (11), with a modification according to Desmet et al. (12) in which a distinction between ‘stage’ and ‘grade’ of chronic hepatitis was proposed. Stage (0–4) reflects the degree of fibrosis and architectural changes; grade (0–18) reflects the necroinflammatory activity.

Response Criteria

Response was defined according to the standards outlined at the recent NIH Consensus Development Conference (13,14) as virological and biochemical on-treatment response (OTR), end-of-treatment response (ETR) and sustained response 6 months post therapy cessation (SR). Biochemical and virological responses were calculated both as separate items as well as combined.

SR, which was the main outcome measure of this study, used to estimate the required sample size, was defined according to these guidelines as persistently normal ALT levels and undetectable plasma HCV RNA 6 months after the end of therapy. ETR was defined as the presence of normal ALT levels at the end of treatment and at least 1 month earlier, and no detectable HCV RNA.

The biochemical on-treatment response was assessed at the end of the double dose induction period (week 8) and cumulative during therapy (ALT repeatedly normal on at least two occasions with at least 1 month interval). The virological on-treatment response (HCV RNA not detectable) was assessed at week 4 instead of week 8 because of the earlier observation that the HCV RNA response precedes the ALT response by about 4 weeks (15). As a secondary outcome measure, the change in the histological inflammatory activity and grade of fibrosis was taken, and classified as ‘worsened’ (increase in severity of at least one point), ‘unchanged’ or ‘improved’

(decrease of at least one point).

Statistical Analysis

Categorical variables were compared by the chi-squared test or Fisher exact test, and continuous variables by the Student *t*-test or the Mann-Whitney test, as appropriate. Cumulative response rates were calculated by the Kaplan-Meier method and compared by the log-rank test. Variables with *p*-values ≤ 0.2 in the univariate analysis were included in multivariate analysis. The independent prognostic value of the selected variables was determined by backward stepwise selection procedures by two independent persons, using SPSS software (SPSS Inc, Chicago, IL, USA) and Stata software (Stata Corp, Texas, TX, USA), yielding similar results; predictor variables for biochemical OTR (cumulative over time) were determined by Cox-regression analysis, and predictor variables for virological OTR (week 4) and SR (6 months after treatment cessation) by logistic regression analysis. ALT and HCV RNA concentrations were logarithmically transformed to obtain approximately normal distributions. In the multivariate analysis, continuous parameters were analysed by their actual values and after transformation to categories. The categories for age were predefined, those for biochemical parameters were based on equal group sizes. The two types of analysis led to the same conclusions; only the results for categories are shown because they are easier to interpret. Changes in histology scores for inflammation and fibrosis were compared using the Kruskal-Wallis test for paired data.

All comparisons shown were based on the intention-to-treat method. All tests of significance were two-tailed; *p*-values below 0.05 were considered to be significant.

RESULTS

Patient characteristics

Between 1990 and 1993, 354 patients were recruited from 19 hospitals in the Benelux (Belgium, The Netherlands and Luxembourg); 159 were randomized to receive 'standard dose treatment' and 195 'titrated dose treatment' (ratio 4:5). Eighteen patients were excluded because they did not fit the entry criteria: five had a false-positive anti-HCV test and 13 withdrew before the start of treatment. All remaining 336 patients were included in the efficacy analysis.

Characteristics of the patient groups are given in Table 1; the two groups appeared well balanced, except for the pretreatment ALT and HCV RNA concentrations. To adjust for this imbalance, the study outcome was also calculated after correction for these variables using multivariate analysis.

Treatment

Ten patients withdrew from treatment and follow-up shortly after the start of therapy because of noncompliance; these were classified as non-responders. In addition, in four cases the protocol was violated because titrated instead of standard therapy was given; these patients were classified in the intention-to-treat analysis as if they had received standard therapy. Per-protocol analysis, with exclusion of these 14 patients, led to the same conclusions as the intention-to-treat analysis. Only the results of the latter are shown.

The interferon dose was reduced or stopped because of side-effects in 16 patients (eight in each group). The mean duration of treatment (±SE) was 24 ±1 weeks for the group on standard dose therapy versus 38 ±2 weeks for the group on titrated dose therapy; the cumulative amount of interferon per patient was 219 ±6 million units in the standard dose versus 332 ±12 million units in the titrated dose group ($p=0.0001$).

Table 1

Baseline characteristics of 336 hepatitis C patients treated with Interferon-alpha*

Characteristic	Standard dose* (*n* = 149)	Titrated dose* (*n* = 187)
Age (years)	47 ± 1.2	47 ± 1.0
Male sex (%)	63	57
History of parenteral exposure (%)[a]	62	65
Time since parenteral exposure (years)	14 ± 1.2	12 ± 0.9
Serum Alanine Aminotransferase[b]	5.2 ± 0.3	4.4 ± 0.2
Histological Activity Index		
Grade of inflammation (0–18)	4.9 ± 0.2	4.7 ± 0.2
Stage of fibrosis (0–4)	2.0 ± 0.1	2.0 ± 0.1
Cirrhosis (%)	25	25
Genotype (%)[c]		
1a	6	9
1b	54	60
2	11	8
3	15	12
4	4	5
others	9	5
Plasma HCV RNA concentration[d]	5.2 ± 0.7	4.4 ± 0.5

* Standard dose: 3 MU tiw, 6 months. Titrated dose: 6 MU tiw, 8 weeks, followed by dose reduction to 3 and 1 MU tiw, respectively. Values are means ± SE.
[a] Parenteral exposure: transfusion, iv drug abuse, needle-stick accident.
[b] Expressed as multiples of the upper limit of normal.
[c] Assessed by INNO-LiPA in 322 patients.
[d] Assessed by branched DNA (Quantiplex™ v 1.0) in 308 patients, expressed as multiples of 10^6 genome equivalents per millilitre; a correction was made for genotype 2 and 3.

Table 2

Alanine aminotransferase (ALT) and HCV RNA response during and after standard dose and titrated dose IFN therapy

	% Response to Standard Dose Therapy (C.I.*) *n*=149			% Response to Titrated Dose Therapy (C.I.*) *n*=187		
	Biochemical	Virological	Both	Biochemical	Virological	Both
On-treatment response[a]	51 (43–60)	40 (32–49)	31 (23–40)	51 (43–58)	45 (38–53)	30 (23–38)
End-of-treatment response	48 (40–56)	39 (31–48)	33 (26–42)	38 (31–45)	36 (28–43)	26 (19–33)
Sustained response	16 (11–23)	15 (10–22)	14 (9–21)	19 (13–25)	17 (12–23)	15 (10–21)

* C.I.: 95% Confidence Limits.

[a] HCV RNA response assessed at week 4 and ALT response at week 8

Biochemical and virologic responses

The biochemical on-treatment response was similar for the two groups (Table 2). In contrast, the virological on-treatment response was higher in the 6 MU group: at 4 weeks HCV RNA was not detectable in 45% versus 40% in those with 3 MU. This difference was significant after adjustment for imbalances in pretreatment characteristics by multivariate analysis (Table 3, odds ratio 1.9, p=0.017); the estimated HCV RNA response rate at 4 weeks after adjustment by multivariate analysis was 47% (95% C.I. 44–51%) for titrated therapy versus 37% (34–40%) for standard treatment.

In both groups, most ALT relapses occurred after discontinuation of the 3 MU dose (titrated dose therapy: 20% relapse during 3 MU, 30% during 1 MU, 21% after end of therapy; standard dose therapy 18% during 3 MU, 53% after end of therapy). The initial difference in HCV RNA response between the two groups was lost during the process of downward titration, and six months after the end of treatment the groups had a comparable sustained ALT and HCV RNA response (14% vs 15%, p=0.8).

Virtually all HCV RNA responses occurred within the first 4 weeks: 82% (75–88%) of all HCV RNA responses, including the temporary ones, and 94% (82–99%) of the HCV RNA responses which were followed by a sustained remission. During treatment, plasma HCV RNA remained detectable in about one-third of those with normal ALT levels, and the end point of ALT normalization with undetectable HCV RNA was significantly better for prediction of a sustained response (52%, CI 41–62%) than the classical end point of ALT normalization alone (35%, 27–43%, p=0.01).

Liver Histology

A pretreatment liver biopsy was obtained in 327 of 336 patients. Of the 327 pretreatment biopsies, 287 could be retrieved and scored by two central pathologists. The local and central pathologists were in agreement on the presence or absence of cirrhosis in 87% of cases (kappa concordance 0.66). For analysis, classification by the central pathologists was used unless the biopsy was not available for review.

Paired pre- and post-treatment biopsies could be evaluated in 126 cases. Patients with paired biopsies did not differ from the others with regard to pretreatment characteristics or response to treatment. A sustained response was accompanied by a reduction or disappearance of inflammatory activity in 96% of patients, whereas the inflammation was unchanged or worsened in 47% of those with a relapse or nonresponse ($p<0.001$). In addition, patients with a sustained response showed less progression of fibrosis (16%) compared to patients with a relapse (21%) or nonresponse (31%, $p=0.05$). After 6 months of follow-up, no significant differences were observed between the two treatment groups in changes in inflammatory activity, but the grade of fibrosis had increased more often among those who had received standard dose therapy (33%) than those with titrated dose therapy (16%) ($p=0.04$).

Table 3 Factors associated with an on-treatment and a sustained response to Interferon treatment, respectively

Potential factor	Biochemical On-Treatment Response*		Virological On-Treatment Response[a]		Sustained ALT & HCV-RNA Response[b]	
Categories (*n*)	%Response univariate	Rel. Risk (95% C.I.)[c] multivariate	%Response univariate	Odds Ratio (95% C.I.)[d] multivariate	%Response univariate	Odds Ratio (95% C.I.)[d] multivariate
Treatment						
standard dose (149)	60		40		14	
titrated dose (187)	65	1.2 (0.9–1.6)	45	1.9 (1.0–3.4)0	15	1.1 (0.5–2.5)
Age						
<40 y (119)	76		54		17	
40-60 y (131)	61	1.1 (0.8–1.6)	44	0.8 (0.4–1.8)	18	1.9 (0.7–4.8)
>60 y (86)	42	0.8 (0.5–1.4)	25	0.8 (0.3–2.2)	7	1.2 (0.2–5.8)
AST/ALT ratio						
<0.6 (168)	73		53		20	
≥ 0.6 (167)	51	0.9 (0.6–1.3)	32	0.6 (0.3–1.1)	9	0.3 (0.1–0.7)[00]
Gamma GT						
≤ 1.4 x ULN (165)	76		51		20	
>1.4 x ULN (163)	46	0.6 (0.4–0.9)[00]	35	0.9 (0.4–1.7)	9	0.4 (0.2–1.1)
Serum Ferritin						
<170 µg/l (157)	79		50		17	
≥ 170 µg/l (156)	44	0.5 (0.4–0.7)[000]	36	0.7 (0.4–1.3)	12	1.0 (0.4–2.4)

Table 3 - *continued* -

Potential factor	Biochemical On-Treatment Resp.*		Virological On-Treatment Resp.[a]		Sustained ALT & HCV-RNA Resp.[b]	
Categories (*n*)	%Response univariate	Rel. Risk (95% C.I.)[c] multivariate	%Response univariate	Odds Ratio (95% C.I.)[d] multivariate	%Response univariate	Odds Ratio (95% C.I.)[d] multivariate
Cirrhosis						
absent (246)	68		49		18	
present (80)	41	0.5 (0.3–0.8)^{0}	23	0.2 (0.1–0.5)^{000}	6	0.3 (0.1–1.0)^{0}
Genotype						
1b (194)	57		30		8	
1a (25)	79	1.7 (0.9–2.9)	42	1.5 (0.5–4.5)	0	0
2 (31)	72	2.0 (1.2–3.2)^{00}	77	21 (6.4–73)^{000}	39	20 (5.4–77)^{000}
3 (46)	67	1.3 (0.8–2.0)	66	7.4 (2.8–20)^{000}	24	9.4 (2.7–33)^{000}
4 (16)	69	1.1 (0.5–2.2)	50	3.5 (0.9–13)	19	5.0 (0.9–29)
others (24)[e]	67	1.2 (0.6–2.5)	68	8.0 (1.9–34)^{0}	29	2.5 (0.4–15)
HCV-RNA						
<0.35 (52)	76		61		29	
0.35-2.6 (128)	61	0.6 (0.4–0.9)^{0}	47	0.3 (0.1–0.7)^{00}	15	0.2 (0.0–0.6)^{00}
>2.6 (128) (x106geq/ml)	60	0.6 (0.4–0.9)^{00}	31	0.2 (0.1–0.4)^{000}	7	0.1 (0.0–0.3)^{000}

* Biochemical on-treatment response: ALT repeatedly normal during treatment
[a] Virological on-treatment response: plasma HCV-RNA undetectable by PCR at week 4
[b] Sustained response: ALT normal and HCV-RNA undetectable by PCR 6 months after end of therapy.
[c] Cox regression analysis; o $p<0.05$; oo $p<0.01$; ooo $p<0.001$.
[d] Logistic regression; o $p<0.05$; oo $p<0.01$; ooo $p<0.001$.
[e] Others: genotype 5 (6), multiple types (4), unknown (14)

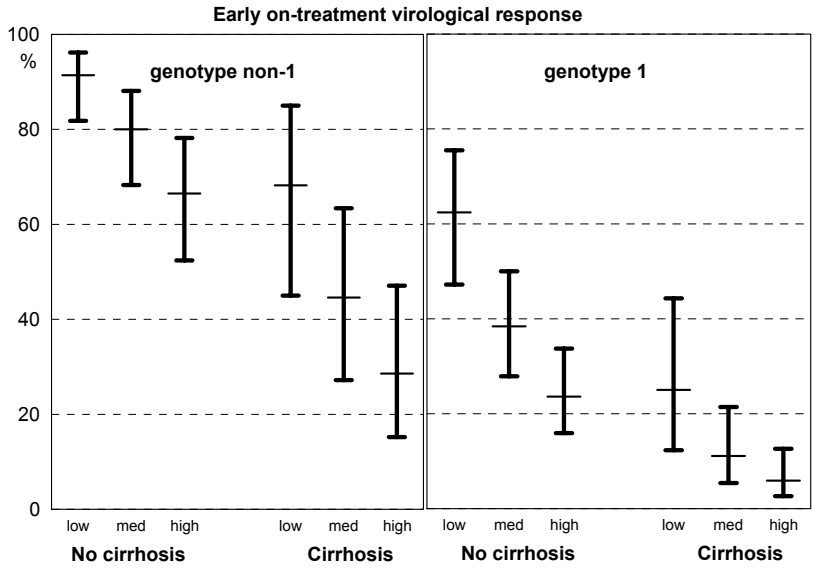
Features predicting response

Responders to therapy were compared to non-responders for pretreatment clinical, biochemical, histological, and virological features. In accordance with other studies, independent predictors of both on-treatment and sustained response were the absence of cirrhosis, the presence of genotype 2 or 3 and a low plasma HCV RNA concentration. The pretreatment gamma glutamyl transferase (γGT) and ferritin levels, often mentioned by others, were independent predictors of a biochemical response (ALT), but had no relation to the effect on the virus (HCV RNA). An additional factor which proved to be an independent predictor of a sustained response was the pretreatment AST/ALT ratio, which predictive value was independent of the presence of cirrhosis. The logistic regression model for virological OTR and for biochemical plus virological SR (Table 3) could be simplified to a model including only the key factors cirrhosis, genotype and viral level without much loss of predictive power (ROC complete model 83%, simplified model 80%). This simplified model, which allows an easy estimate of the chance of response for the individual patient, is shown graphically in Figure 1.

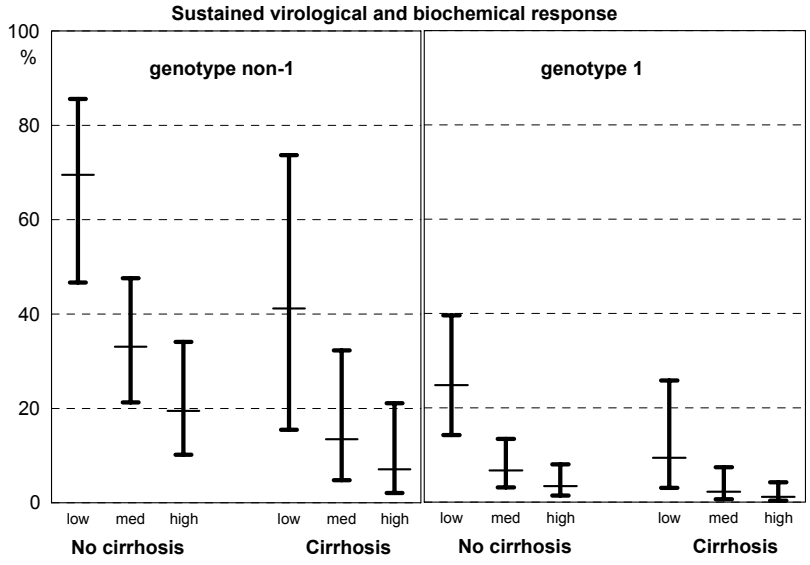
The most powerful predictor of the long-term outcome is the early on-treatment HCV RNA response. A positive HCV RNA 4 weeks after the start of treatment was followed by a nonresponse in 98.3% of cases, even though the ALT levels might be normal. This observation applies within all subcategories of pretreatment factors predictive of response (Table 4). For those in whom HCV RNA became undetectable at week 4, the only significant additional predictor of SR was the genotype 1: genotype 1 (subtype 1a or 1b) was present in 34% of those who remained in remission versus 56% in those who developed a relapse (p=0.02).

Figure 1

a



b



Estimated probability and 95% C.I. of an early on-treatment virological response (a) and a sustained response (b) in relation to genotype, HCV RNA concentration (low: $<0.35 \times 10^6$; medium: $0.35–2.6 \times 10^6$; high: $>2.6 \times 10^6$ geq/ml), and presence or absence of cirrhosis.

Table 4

Value of an early HCV RNA measurement for the prediction of a sustained response.

Pretreatment category (*n*)	% Sustained ALT and HCV RNA response (95%C.I.)	
	if HCV RNA week 4 undetectable*	if HCV RNA week 4 detectable*
Total group (336)	34 (26–43)	1.7 (0.4–5.0)
AST/ALT ratio		
<0.6 (168)	37 (26–47)	2.6 (0.3–9.0)
≥ 0.6 (167)	28 (16–43)	1.0 (0.0–5.6)
Cirrhosis		
absent (246)	35 (26–44)	1.7 (0.2–6.0)
present (80)	25 (7.0–52)	1.8 (0.0–9.0)
Genotype		
1 (219)	21 (12–34)°	1.4 (0.0–5.0)
others (117)	40 (28–54)	2.9 (0.0–15)
HCV RNA (x106geq/ml)		
<0.35 (52)	45 (27–64)	5.0 (0.1–25)
0.35-2.6 (128)	31 (20–46)	3.0 (0.4–11)
>2.6 (128)	25 (12–42)	0.0 (0.0–4.2)

*Plasma HCV RNA 4 weeks after start of IFN treatment, assessed by a validated PCR (detection limit $0.9x10^3$ genome equivalents per milliliter for genotype 1 and $1.2x10^3$ geq/ml for genotype 3).

°Genotype 1a or 1b versus others: p=0.017 (Chi^2).

DISCUSSION

In this large multicenter study, which aimed to compare two different treatment strategies, minimal effect on the final outcome was observed: in effect, the outcome was even more disappointing than usually published. Nevertheless, important new information emerged from the study which may help in the understanding of the factors associated with response and the design of more effective therapy. No effect of doubling the dose from 3 to 6 MU interferon tiw was found on the on-treatment response when assessed by ALT measurements (Table 2), which is in concordance with other reports (4,16–19). In contrast, after 4 weeks of therapy HCV RNA was significantly more often not detectable in those on 6 MU interferon tiw than in those on the standard 3 MU dose (odds ratio=1.9, p=0.017, Table 3), and this condition appeared to be a prerequisite for achieving a sustained response (Table 4). This observation supports further exploration of treatment schedules with higher dosages of interferon.

The treatment end point of normal ALT levels and undetectable plasma HCV RNA was superior for the prediction of a sustained response (52%) compared to the end point of only ALT normalization (35%, p=0.01). In contrast to our initial expectations, the sustained response rate for the group on titrated dose therapy, which had an undetectable HCV RNA as end point, was not enhanced. We observed only a minor increase in the percentage undetectable HCV RNA with increased duration of interferon therapy. In fact 94% of the HCV RNA responses which were sustained had already occurred within the first 4 weeks. Loss of efficacy was observed by downward titration, which was associated with 20% breakthrough when assessed by HCV RNA (Table 2). Most studies suggest less breakthroughs during prolonged treatment with the standard 3 MU dose than with the 1 MU dose (4,5,20–23). Prolonged treatment with 6 MU might even be superior to 3 MU (18,24), which is in line with our observations on the early effect of the interferon dose on the HCV RNA response.

A sustained ALT and HCV RNA response was predicted in the present study by the absence of cirrhosis, the presence of genotype 2 or 3 and a low viral load (Table 3), which is in agreement with other observations (25-32). A new finding was the independent predictive value of the AST/ALT ratio, a clinical parameter thought to be associated with the presence of cirrhosis (33), but now apparently representing extra information in addition to that on cirrhosis in the prediction of a sustained response. An additional finding of multivariate analysis was the significant predictive value of serum ferritin for a primary ALT response but not for the primary HCV RNA response or the sustained ALT and HCV RNA response, suggesting that the iron content affects the inflammatory process but does not influence elimination of the hepatitis C virus. This might also explain the seemingly beneficial effect of iron depletion, when only the ALT levels are assessed (29).

The large size of the study allowed us to construct a predictive model which has to be validated in a prospective way (Fig. 1). In this model the very low chance of sustained response for genotype 1 becomes clear, in particular in combination with cirrhosis, and also the very rewarding response for types 2 and 3 without cirrhosis.

The observation that the HCV RNA response occurs soon after the start of therapy led us to analyse the value of HCV RNA assessment at 4 weeks of treatment as a time-dependent predictor of response. If HCV RNA is detectable, the chance of a sustained response is less than 5%; if HCV RNA is undetectable, the sustained response rate is on the average 34% (Table 4). Furthermore, determination of HCV RNA one month after start of treatment was superior in comparison with evaluation of baseline factors; only the genotype appeared to have additional prognostic value when the HCV RNA result at week 4 was known. If HCV RNA was undetectable at week 4, the sustained response rate was 21% for genotype 1a/b versus 40% for the others (p=0.017).

It is obvious that in view of the costs and adverse effects, interferon monotherapy should be restricted to those whose chances of a sustained response are appreciable; a response of 1 in 3 appears to represent a minimum. Continuation of therapy is then only suitable for those with undetectable plasma HCV RNA at week 4. For patients with an *a priori* low chance of a sustained response, for instance patients with cirrhosis who are clearly in need of antiviral therapy, interferon can be started and continued if HCV RNA becomes undetectable after 4 weeks. Once an early HCV RNA response has occurred, strategies need to be developed to diminish breakthrough and relapse. Avoidance of downward titration below 3MU tiw and maintenance therapy for 12 months are the first elements; whether the addition of other antiviral agents (ribavirin) is helpful is tested in ongoing investigations (34,35).
Our study, however, clearly suggests that major advancements in treatment efficacy can only be made when the early HCV RNA response is enhanced, which might be achieved by increasing the initial interferon dose or dosing frequency (36).

ACKNOWLEDGEMENTS

The Benelux Study Group is grateful to B. de Becker, W. Beukman, J. Boot, E. Fries, G. de Mutsert, H.G.M. Niesters, M. Rensink, L. vd Velden, C.P. van Vliet and J. Voermans for their excellent technical assistance; J.K. Albrecht, Schering Plough, Kenilworth, NJ, USA for logistic support; F.J.W. ten Kate, Academic Medical Center, Amsterdam, The Netherlands, for reviewing the liver biopsies; W.C.J. Hop, Erasmus University Rotterdam, The Netherlands, for statistical advice; J.P. Bonn, M. Urdea and J. Wilber, Chiron, Emeryville, CA, USA for supplying the Quantiplex assay; H.T.M. Cuypers and P.N. Lelie, Central Laboratory for Blood Banks, Amsterdam, the Netherlands, for their expert help in developing a high-quality HCV RNA assay; L.J. van Doorn and W.G.V. Quint, SSDZ, Delft, the Netherlands, for their help in genotype determinations and G. Maertens and L. Stuyver, Innogenetics, Gent, Belgium, for supplying the Inno-LiPA HCV typing assays.

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REFERENCES

1. Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, Peters M, et al. Treatment of chronic non-A,non-B hepatitis with recombinant human alpha interferon. A preliminary report. N Engl J Med 1986; 315:1575-1578.
2. Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC, Jr., Perrillo RP, et al. Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. N Engl J Med 1989; 321:1501-1506.
3. Di Bisceglie AM, Martin P, Kassianides C, Lisker Melman M, Murray L, Waggoner J, et al. Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med 1989; 321:1506-1510.
4. Lin R, Roach E, Zimmerman M, Strasser S, Farrell GC. Interferon alfa-2b for chronic hepatitis C: effects of dose increment and duration of treatment on response rates. Results of the first multicentre Australian trial. Australia Hepatitis C Study Group. J Hepatol 1995; 23:487-496.
5. Poynard T, Bedossa P, Chevallier M, Mathurin P, Lemonnier C, Trepo C, et al. A comparison of three interferon alfa-2b regimens for the long-term treatment of chronic non-A, non-B hepatitis. N Engl J Med 1995; 332:1457-1462.
6. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 1977; 35:1-39.
7. Kleter GE, van Doorn LJ, Brouwer JT, Schalm SW, Heijtink RA, Quint WG. Sequence analysis of the 5' untranslated region in isolates of at least four genotypes of hepatitis C virus in The Netherlands. J Clin Microbiol 1994; 32:306-310.
8. Zaaijer HL, Cuypers HT, Reesink HW, Winkel IN, Gerken G, Lelie PN. Reliability of polymerase chain reaction for detection of hepatitis C virus. Lancet 1993; 341:722-724.
9. Kleter GE, van Doorn LJ, Stuyver L, Maertens G, Brouwer JT, Schalm SW, et al. Rapid genotyping of hepatitis C virus RNA-isolates obtained from patients residing in western Europe. J Med Virol 1995;47:35-42.
10. Hawkins A, Davidson F, Simmonds P. Comparison of plasma virus loads among individuals infected with hepatitis C virus (HCV) genotypes 1,2 and 3 by quantiplex HCV RNA assay versions 1 and 2, Roche Monitor assay, and an in-house limiting dilution method. J Clin Microbiol 1997;35:187-92.
11. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981; 1:431-435.
12. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994; 19:1513-1520.
13. Anonymous. National Institutes of Health Consensus Development conference Panel statement: management of hepatitis C. Hepatology 1997;26:2S-10S.
14. Lindsay KL. Therapy of hepatitis C: overview. Hepatology 1997;26:71S-7S.
15. Kleter GE, Brouwer JT, Heijtink RA, Schalm SW, Quint WG. Detection of hepatitis C virus RNA in patients with chronic hepatitis C virus infections during and after therapy with alpha interferon. Antimicrob Agents Chemother 1993; 37:595-597.
16. Lindsay KL, Davis GL, Schiff ER, Bodenheimer HC, Balart LA, Dienstag JL, et al. Response to higher doses of interferon alfa-2b in patients with chronic hepatitis C: a randomized multicenter trial. Hepatitis Interventional Therapy Group. Hepatology 1996; 24:1034-1040.
17. Bellobuono A, Mondazzi L, Tempini S, Bellati G, Ideo G, for the Italian Multicentre Study Group on Treatment of Chronic Hepatitis C. Comparison between fixed and escalating doses of alpha interferon treatment in chronic hepatitis C. Hepatology 1995; 22:152A.

18. Chemello L, Bonetti P, Cavalletto L, Talato F, Donadon V, Casarin P, et al. Randomized trial comparing three different regimens of alpha-2a-interferon in chronic hepatitis C. The TriVeneto Viral Hepatitis Group. Hepatology 1995; 22:700-706.
19. Marcellin P, Pouteau M, Martinot-Peignoux M, Degos F, Duchatelle V, Boyer N, et al. Lack of benefit of escalating dosage of interferon alfa in patients with chronic hepatitis C. Gastroenterology 1995; 109:156-165.
20. Jouet P, Roudot-Thoraval F, Dhumeaux D, Metreau JM. Comparative efficacy of interferon alfa in cirrhotic and noncirrhotic patients with non-A, non-B, C hepatitis. Le Groupe Francais pour l'Etude du Traitement des Hepatites Chroniques NANB/C. Gastroenterology 1994; 106:686-690.
21. Craxi A, Dimarco V, Loiacono O, Almasio P, Bruno R, Camma C, et al. Transfusion-associated chronic hepatitis c: alpha-n(1) interferon for 6 vs 12 months. J Hepatol 1996; 24:539-546: 99.
22. Shiffman ML, Hofmann CM, Luketic VA, Sanyal AJ, Contos MJ, Mills AS. Improved sustained response following treatment of chronic hepatitis C by gradual reduction in the interferon dose. Hepatology 1996; 24:21-26.
23. Kasahara A, Hayashi N, Hiramatsu N, Oshita M, Hagiwara H, Katayama K, et al. Ability of prolonged interferon treatment to suppress relapse after cessation of therapy in patients with chronic hepatitis C: a multicenter randomized controlled trial. Hepatology 1995; 21:291-297.
24. Poynard T, Leroy V, Cohard M, Thevenot T, Mathurin P, Opolon P, et al. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effects of dose and duration. Hepatology 1996; 24:(4)778-789.
25. Lau JY, Davis GL, Kniffen J, Qian KP, Urdea MS, Chan CS, et al. Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. Lancet 1993; 341:1501-1504.
26. Davis GL. Prediction of response to interferon treatment of chronic hepatitis C . J Hepatol 1994; 21:1-3.
27. Davis GL, Lindsay K, Albrecht J, Bodenheimer HC, Jr., Balart LA, Perrillo RP, et al. Clinical predictors of response to recombinant interferon-alpha treatment in patients with chronic non-A, non-B hepatitis (hepatitis C). Journal of Viral Hepatitis 1994; 1:55-63.
28. Pagliaro L, Craxi A, Cammaa C, Tine F, Di Marco V, Lo Iacono O, et al. Interferon-alpha for chronic hepatitis C: an analysis of pretreatment clinical predictors of response. Hepatology 1994; 19:820-828.
29. Van Thiel DH, Friedlander L, Fagiuoli S, Wright HI, Irish W, Gavaler JS. Response to interferon alpha therapy is influenced by the iron content in the liver. Journal of Hepatology 1994; 20:410-415.
30. Chemello L, Cavalletto L, Noventa F, Bonetti P, Casarin C, Bernardinello E, et al. Predictors of sustained response, relapse and no response in patients with chronic hepatitis C treated with interferon-alpha. J Viral Hepat 1995; 2:91-96.
31. Martinot-Peignoux M, Marcellin P, Pouteau M, Castelnau C, Boyer N, Poliquin M, et al. Pretreatment serum hepatitis C virus RNA levels and hepatitis C virus genotype are the main and independent prognostic factors of sustained response to interferon alfa therapy in chronic hepatitis C. Hepatology 1995; 22:1050-6.
32. Pawlotsky JM, Roudot-Thoraval F, Bastie A, Darthuy F, Remire J, Metreau JM, et al. Factors affecting treatment responses to interferon-alpha in chronic hepatitis C. J Infect Dis 1996; 174:1-7.
33. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. Gastroenterology 1988; 95:734-739.
34. Brouwer JT, Nevens F, Michielsen P, Hautekeete ML, Chamuleau RAFM, Adler M, et al. What options are left when hepatitis C does not respond to interferon? Placebo controlled Benelux multicentre retreatment trial on Ribavirin monotherapy versus combination with interferon. J Hepatol 1994; 21 (suppl 1):S17.
35. Schalm SW, Hansen BE, Chemello L, Bellobuono A, Brouwer JT, Weiland O, et al.

Ribavirin enhances the efficacy but not the toxicity of interferon in chronic hepatitis C. Meta-analysis of individual European patients. J Hepatol 1997; 26: 961-966.
36. Iino S, Hino K, Kuroki T, Suzuki H, Yamamoto S. Treatment of chronic hepatitis C with high-dose interferon alpha-2b. A multicenter study. Dig Dis Sci 1993; 38:612-618.

Chapter 4

High-dose daily Interferon treatment in chronic hepatitis C
A pilot study in 22 patients non-responsive to previous Interferon standard therapy

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SUMMARY

Background/Aims In the early nineties, treatment of chronic hepatitis C with Interferon monotherapy led to a sustained response rate of about 15%. In this pilot study, we aimed to investigate the factors involved in non-response and to evaluate whether daily high dose Interferon could overcome part of the resistance to standard Interferon mono therapy.

Methods Twenty-two patients with chronic hepatitis C who did not have an ALT normalization during previous treatment with Interferon 3–6 MU tiw were retreated with 10 MU daily for 5 days and 10 MU tiw until week 4, followed by stepwise dose reduction in case of initial HCV RNA response or dose withdrawal in case of non-response.

Results In retrospective, out of the 22 ALT non-responders, 13 were absolute HCV RNA nonresponders during previous treatment, 5 patients had a breaktrough and 4 a response-relapse. Sixty-eight percent had genotype 1, 59% a high viral load and 65% had a cirrhosis. Fifteen patients (68%) did not respond to daily high dose retreatment, 3 (14%) had a response-relapse and 4 (22%, 95% C.I. 5–40%) had a sustained response. Sustained responders were characterised by absence of cirrhosis, virological breakthrough or response-relapse on previous therapy and undetectable HCV RNA at week 4. Although HCV RNA became undetectable during treatment in only 32% of patients, all patients had a drop in the level of viraemia during the first 2 weeks with a mean decrease of 2.6 log (99.7%).

Conclusions Daily high dose retreatment of non-responders to interferon leads to additional sustained response. Dose-response observations suggest a variable sensitivity of HCV to IFN, and also other mechanisms involved in non-response.

INTRODUCTION

In the early nineties, sustained virologic response after therapy of chronic hepatitis C was limited to around 15%, and in those groups who needed treatment most – like cirrhosis – a sustained response was only reached in about 5% of patients (1–3). Standard therapy consisted of interferon-alpha 3 MU tiw for 6 to 12 months. For those who did not respond to this treatment, two options were explored in the Benelux studies: daily high dose therapy (4) and combination therapy with Ribavirin (5). Because of the supposedly better efficacy and tolerability of combination therapy, the international research community focused in general on the latter, and reports on daily high dose therapy were neglected outside Japan.

In this manuscript we report on the outcome of the clinical study with daily high dose interferon. Materials from this clinical study have been the basis for pioneer research on viral kinetics in chronic hepatitis C (6, 7). In addition, the marked drop in viral load observed both in genotype 1 and genotype non-1 patients, point to other mechanisms than drug resistance as cause of non-response.

MATERIALS AND METHODS

Patients

Twenty-two patients with chronic hepatitis C who did not respond to previous therapy were included in this pilot study. Non-response was originally defined as lack of 2 sequential normal ALT's at the end of treatment; HCV RNA was tested retrospectively. All subjects had been treated in a Benelux multicenter trial comparing standard IFN monotherapy (Interferon-alpha-2b 3 MU tiw for 24 weeks) with 6 MU tiw for 8 weeks followed by dose reduction based on ALT and HCV RNA response (3).
Candidate for the high dose interferon treatment were all patients not responding to primary interferon treatment and in whom the diagnosis of hepatitis C was confirmed by detection of plasma HCV-RNA and exclusion of other causes of chronic hepatitis. Patients were eligible for high dose interferon treatment after withdrawal of interferon for at least 6 months. Overall entry and exclusion criteria were the same as described in the original protocol for the Benelux study (3). Patients with a high risk for serious or severe side effects were excluded: active cirrhosis with severe cytopenia (platelets $<50 \times 10^9$/l, granulocytes $<1.5 \times 10^9$/l); decompensated liver disease (ascites, bleeding varices, spontaneous hepatic encephalopathy, bilirubin >34 µmol/l, albumin <32 g/l, quicktest/normotest^R less than 35%, serum creatinine >140 µmol/l); autoimmune diseases such as thyreoiditis and glomerulonephritis, or positive antinuclear, antimitochondrial, or smooth muscle antibody $\geq$1:100 dilution of serum; clinically important psychiatric illness; history of seizures; severe adverse events during the primary interferon treatment.

Design of the study

The study was designed in such a way that it made possible later assessment (when adequate techniques became available) of factors thought to be possibly involved in resistance to treatment, e.g. mutations of viral nucleotide sequences and changes in interferon induced pathways like induction of 2'5'-OAS and protein kinases, expression of HLA and activation of NK cells, alterations in interferon kinetics and viral kinetics. In three centers with appropriate laboratory facilities for direct preparation of blood samples 22 patients were included, and approximately 10 of them agreed to frequent blood sampling. The study was approved by the ethical committees of the university hospitals of Rotterdam and Gent, and all patients gave their written informed consent.

When sensitive and reliable techniques for assessment of quantitative HCV RNA became available, out of all possibilities mentioned above we focussed on assessment of viral kinetics. The results of these assessments led to several publications on viral kinetics in interferon mono therapy (6, 7). In this manuscript we report on the actual outcome of the study and address the question whether initial non-response to interferon can be overcome by daily high dose therapy.

Treatment and assessments

Patients were retreated with daily high dosage IFN therapy, consisting of daily 10 MU IFN-a2b sc. for 5 consecutive days, followed by 10 MU thrice weekly until week 4. Patients were allowed to withdraw for non-response in case HCV RNA remained detectable at week 4; in case of HCV RNA response IFN was tapered stepwise to 3 and 1 MU tiw and ended when ALT remained repeatedly normal and HCV RNA undetectable during the lowest IFN dose.

Patients were allowed to take up to 4 grams of paracetamol daily to reduce side effects.

Blood was sampled for liver and haematological tests pre-treatment and every 4 weeks thereafter. HCV RNA was assessed before starting treatment, at week 4, at the end of therapy and 26 weeks thereafter; in addition, frequent sampling for HCV RNA and IFN kinetics was performed in-hospital during the first 4 days and between day 28–30.

The genotype was assessed by a Line Probe Assay (Inno-LiPA, Innogenetics, Gent, Belgium). Quantification of plasma HCV RNA was performed at a single central laboratory by a rt-PCR assay with a sensitivity of 100 copies per milliliter (National Genetics Institute, Los Angeles, USA) (8). Liver biopsies were performed within 6 months before retreatment and were reviewed by a central pathologist for presence or absence of cirrhosis.

Non-response (NR) was defined as detectable HCV RNA at the end of therapy, response-relapse (RR) as HCV RNA undetectable at the end of therapy with re-appearance during follow-up, and sustained response as undetectable HCV RNA at the end of therapy and after at least 24 weeks follow-up.

RESULTS

The patient characteristics are summarized in table 1. In contrast to usual populations, a high percentage of cirrhosis was present in our study. As all patients were originally selected based on absence of ALT normalisation during therapy, retrospective HCV RNA analysis showed that about 60% were virological non-responders, about 20% had a breakthrough and about 20% a virologic relapse during previous standard IFN monotherapy.

Table 1 Baseline characteristics

Characteristic	Number/Total (%)
Gender, male	11/22 (50%)
Age, mean, years	46
Genotype 1	15/22 (68%)
Viral load > 2x 10^6 geq/ml	13/22 (59%)
Cirrhosis	13/20 (65%)
Virologic response to previous IFN therapy	
NR (non-response)*	13/22 (59%)
BT (breaktrough)[a]	5/22 (23%)
RR (response-relapse)[b]	4/22 (18%)

* NR defined as persistently detectable HCV RNA at treatment weeks 4, 12 and 24, at the end of therapy and after 24 weeks of follow-up.
[a] BT defined as temporary disappearance of detectable HCV RNA during treatment followed by re-appearance before the end of therapy and during follow-up.
[b] RR defined as HCV RNA undetectable at the end of therapy and re-appearance during follow-up.

Table 2 Virologic response to treatment

HCV RNA response to treatment	**Non-response***	**Response-relapse[a]**	**Sustained response[b]**
Total population	15/22 (68%)	3/22 (14%)	4/22 (18%)
By RNA response wk4[c]			
RNA negative	-	1/5 (20%)	4/5 (80%)
RNA positive	14/16 (88%)	2/16 (12%)	-
By grade of fibrosis			
Cirrhosis	7/7 (100%)	-	-
No cirrhosis	6/13 (46%)	3/13 (23%)	4/13 (31%)
By genotype			
Genotype 1	12/15 (80%)	1/15 (7%)	2/15 (13%)
Genotype non-1	3/7 (43%)	2/7 (29%)	2/7 (29%)
By viral load (geq/ml)			
High (>$2x10^6$)	4/9 (44%)	3/9 (33%)	2/9 (22%)
Low (<$2x10^6$)	11/13 (85%)	-	2/13 (15%)
By gender			
Male	8/11 (73%)	1/11 (9%)	2/11 (18%)
Female	7/11 (64%)	2/11 (18%)	2/11 (18%)
By RNA response to previous IFN therapy			
NR	12/13 (92%)	-	1/13 (8%)
BT	1/5 (20%)	2/5 (40%)	2/5 (40%)
RR	2/4 (50%)	1/4 (25%)	1/4 (25%)

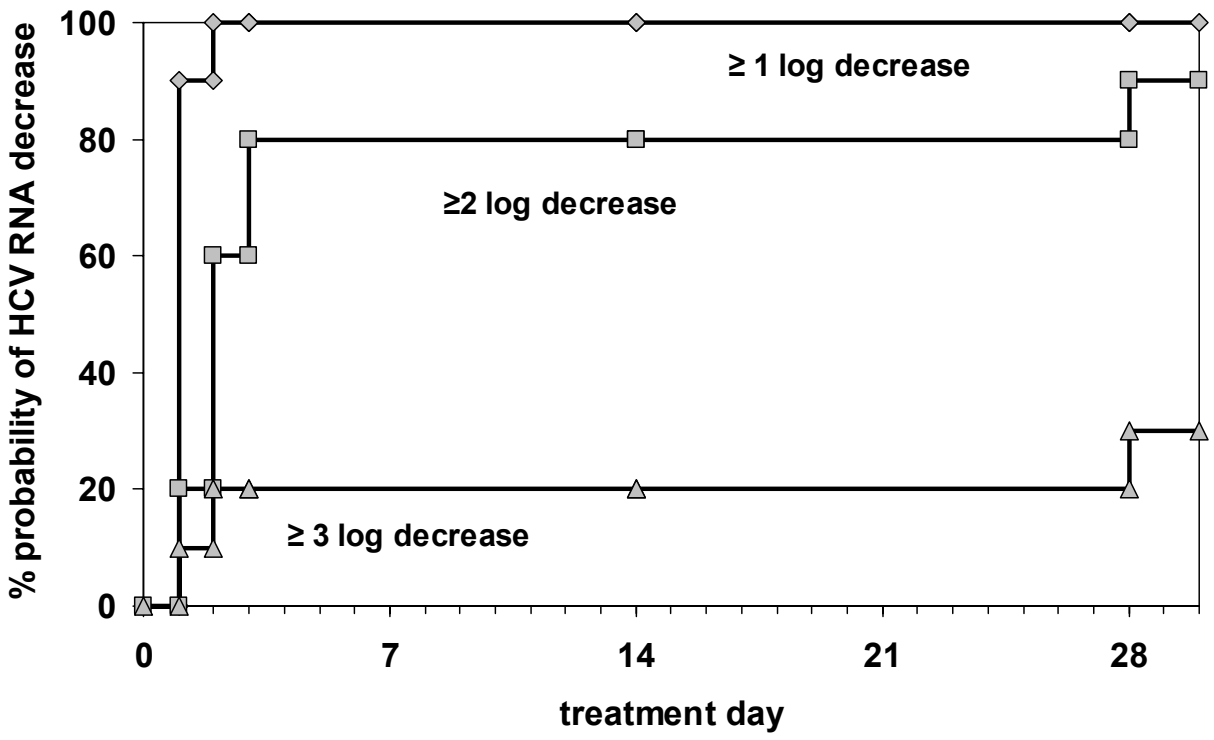
* NR defined as detectable HCV RNA at the end of therapy and after 24 weeks of follow-up.
[a]RR defined as HCV RNA undetectable at the end of therapy and re-appearance during follow-up.
[b]SR defined as undetectable HCV RNA at the end of at least 24 weeks follow-up.
[c] Response at week 4 unknown in 1 patient who eventually had a non-response.

The results are summarized in table 2. Eighteen percent had a sustained response, 14% response-relapse and 68% non-response. Sustained responders were characterised by absence of cirrhosis, virological breakthrough or response-relapse on previous therapy and undetectable HCV RNA at week 4. Eight patients decided to withdraw from treatment because of lack of response at treatment week 4, and 8 patients without a week 4 response continued; of the latter group, none reached a sustained response.

Quantitative HCV RNA analysis of plasma samples showed that the mean drop in viral load was 2.5 log at day 3, and 2.6 log (99.7%) at week 2; at week 4 the average drop in viral load was 2.7 log. At week 4, all patients tested had at least a 1 log drop in viral load, 90% had a 2 log drop and 30% had a decrease of at least 3 log (figure 1).

Common side effects were fatigue and depression, leading to treatment withdrawal in two patients (week 12 and 14). One patient developed hypothyroidism. One patient with compensated liver cirrhosis developed thrombocytopenia complicated by severe epistaxis; stopping treatment resulted in a prompt rise of the platelets. Leukopenia was observed in almost all patients, but did not lead to dose adjustments and was not associated with clinical significant infectious problems. No deaths were reported.

Figure 1



Cumulative percentage of patients with 1, 2 and 3 log decrease in viral load.

DISCUSSION

Retreatment of non-responders with standard doses IFN monotherapy did not lead to additional responses (9–11). For this reason, two treatment modalities for non-responders were explored: combination of standard doses IFN with Ribavirin, and treatment with daily high doses IFN monotherapy. In our study, 18% of non-sustained responders to standard IFN monotherapy became sustained responder by daily high dose IFN. In patients with no HCV RNA response at all during previous treatment, sustained response was 8%; in those with a temporary response followed by a breakthrough or relapse, sustained response was 33%. In a meta-analysis of non-responders to interferon, defined as lack of ALT normalisation during therapy, sustained response to retreatment with standard dose interferon was 2% (95% C.I. 0–4%) versus 18% (14–23%) in retreatment with interferon ribavirin combination therapy (12). In our study in ALT non-responders, the sustained response rate was 18% (5–40%), suggesting that daily high dose interferon retreatment might overcome part of the non-response to standard interferon therapy and approaches the efficacy of interferon ribavirin combination therapy in this difficult to treat patient group. In a meta-analysis of individual patient data of non-responders, defined as lack of RNA response to interferon therapy, retreatment with standard dose combination therapy led to a sustained response in 10% versus 23% in retreatment with high dose combination therapy (13), suggesting that dose increment of interferon might be useful in combination therapy as well.

The RNA response patterns observed during high-dose treatment confirmed observations from the earlier Benelux study (14,15): 80% of patients with undetectable HCV RNA at week 4 (HCV $<10^3$ geq/ml) became sustained responder, in contrast to none of those with detectable RNA at week 4. Other predictors of response were the genotype, in accordance to the literature; another important pre-treatment predictor seemed to be the presence or absence of cirrhosis. Again, this is in accordance with our earlier observations (3), whereas this factor is only of minor importance in combination therapy (16–18).

This pilot study confirms the concepts from Japanese studies, which document an important viral suppression by daily dosing of IFN (4). Measurement of viral load might distinguish between those with a drop in viral level and a chance of sustained response and those with a too low drop in viral levels to achieve a viral response by 6-12 months. When validated techniques for measurement of quantitative HCV RNA became available, it was shown that, although HCV RNA became undetectable during treatment in only 32% of patients, all patients had a drop in the level of viraemia during the first 2 weeks with a mean decrease of 2.6 log (99.7%) (7).

Thus, non-response does not appear to be equivalent to resistance to interferon; it may be the result of decreased sensitivity of HCV to interferon, of a low immune response, of altered pharmacokinetics, or of non-compliance. The resulting insights in viral behaviour during daily high dose interferon mono therapy formed the basis for a series of studies of these concepts in daily high dose combination therapy (19-22), aiming to improve the chances of cure for difficult to treat chronic hepatitis C.

ACKNOWLEDGEMENTS

The authors are grateful to the staff and nurses of the Clinical Research Unit of the Erasmus University of Rotterdam and the personnel of the University of Gent for their support in logistics and patient treatment. We also thank the virology departments of the Erasmus University (head Prof ADME Osterhaus), especially RA Heijtink and HGM Niesters, and of the University of Gent (head prof G Leroux-Roels) for handling and analysis of the multitude of samples collected in this study.

REFERENCES

1. Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC, Jr, Perrillo RP, et al. Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. N Engl J Med 1989;321(22):1501-1506.
2. Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, et al. Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med 1989;321(22):1506-1510.
3. Brouwer JT, Nevens F, Kleter B, Elewaut A, Adler M, Brenard R, et al. Efficacy of interferon dose and prediction of response in chronic hepatitis C: Benelux study in 336 patients. J Hepatol 1998;28(6):951-9.
4. Iino S, Hino K, Kuroki T, Suzuki H, Yamamoto S. Treatment of chronic hepatitis C with high-dose interferon alpha-2b. A multicenter study. Dig Dis Sci 1993;38(4):612-618.
5. Brillanti S, Garson J, Foli M, Whitby K, Deaville R, Masci C, et al. A pilot study of combination therapy with ribavirin plus interferon alfa for interferon alfa-resistant chronic hepatitis C. Gastroenterology 1994;107(3):812-7.
6. Bekkering FC, Brouwer JT, Schalm SW, Elewaut A. Hepatitis C: viral kinetics. Hepatology 1997;26(6):1691-3.
7. Bekkering FC, Brouwer JT, Leroux-Roels G, Van Vlierberghe H, Elewaut A, Schalm SW. Ultrarapid hepatitis C virus clearance by daily high-dose interferon in non-responders to standard therapy. J Hepatol 1998;28(6):960-4.
8. Tong MJ, Hwang SJ, Lefkowitz M, Lee SD, Co RL, Conrad A, et al. Correlation of serum HCV RNA and alanine aminotransferase levels in chronic hepatitis C patients during treatment with ribavirin. J Gastroenterol Hepatol 1994;9(6):587-91.
9. Marriott E, Quiroga JA, Carreno V. Retreatment of chronic hepatitis C with interferon-alpha. J Infect Dis 1992;166(5):1200-1.
10. Marcellin P, Pouteau M, Boyer N, Castelnau C, Erlinger S, Benhamou JP. Retreatment with recombinant interferon-alpha in patients with chronic hepatitis C. J Infect Dis 1993;167(3):780-1.
11. Toyoda H, Nakano S, Takeda I, Kumada T, Sugiyama K, Osada T, et al. Retreatment of chronic hepatitis C with interferon. Am J Gastroenterol 1994;89(9):1453-7.
12. Cummings KJ, Lee SM, West ES, Cid-Ruzafa J, Fein SG, Aoki Y, et al. Interferon and ribavirin vs interferon alone in the re-treatment of chronic hepatitis C previously nonresponsive to interferon: A meta-analysis of randomized trials. Jama 2001;285(2):193-9.
13. Camma C, Bruno S, Schepis F, Lo Iacono O, Andreone P, Gramenzi AG, et al. Retreatment with interferon plus ribavirin of chronic hepatitis C non-responders to interferon monotherapy: a meta-analysis of individual patient data. Gut 2002;51(6):864-9.
14. Kleter GE, Brouwer JT, Heijtink RA, Schalm SW, Quint WG. Detection of hepatitis C virus RNA in patients with chronic hepatitis C virus infections during and after therapy with alpha interferon. Antimicrob Agents Chemother 1993;37(3):595-7.
15. Brouwer JT, Hansen BE, Niesters HG, Schalm SW. Early prediction of response in interferon monotherapy and in interferon-ribavirin combination therapy for chronic hepatitis C: HCV RNA at 4 weeks versus ALT. J Hepatol 1999;30(2):192-8.
16. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998;352(9138):1426-32.

17. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 1998;339(21):1485-92.
18. Brouwer JT, Nevens F, Bekkering FC, Bourgeois N, Van Vlierberghe H, Weegink CJ, et al. Reduction of relapse rates by 18-month treatment in chronic hepatitis C. A Benelux randomized trial in 300 patients. J Hepatol 2004;40:689-695.
19. Bekkering FC, Brouwer JT, Hansen BE, Schalm SW. Hepatitis C viral kinetics in difficult to treat patients receiving high dose interferon and ribavirin. J Hepatol 2001;34(3):435-40.
20. Bekkering FC, Stalgis C, McHutchison JG, Brouwer JT, Perelson AS. Estimation of early hepatitis C viral clearance in patients receiving daily interferon and ribavirin therapy using a mathematical model. Hepatology 2001;33(2):419-23.
21. Bekkering FC, Neumann AU, Brouwer JT, Levi-Drummer RS, Schalm SW. Changes in anti-viral effectiveness of interferon after dose reduction in chronic hepatitis c patients: a case control study. BMC Gastroenterol 2001;1(1):14.
22. Vrolijk JM, Bekkering FC, Brouwer JT, Hansen BE, Schalm SW. High sustained virological response in chronic hepatitis C by combining induction and prolonged maintenance therapy. J Viral Hepat 2003;10(3):205-9.

Chapter 5

Retreatment of hepatitis C non-responsive to Interferon. A placebo controlled randomized trial of Ribavirin monotherapy versus combination therapy with Ribavirin and Interferon in 121 patients in the Benelux

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BMC Gastroenterol 2003;3(1):24

ABSTRACT

Background : Evidence based medicine depends on unbiased selection of completed randomized controlled trials. For completeness it is important to publish all trials. This report describes the first large randomised controlled trial where combination therapy was compared to placebo therapy and to ribavirin monotherapy, which has not been published untill now.

Methods: One hundred and twenty one patients with chronic hepatitis C and elevated transaminases who did not respond to previous treatment with standard interferon monotherapy, were included from 16 centers in Belgium, the Netherlands and Luxembourg between 1992 and 1996. Patient poor-response characteristics were: genotype 1 (69%), HCV RNA above $2x10^6$ copies/ml (55%) and cirrhosis (38%). Patients were randomized to 6 months combination therapy with interferon alpha-2b (3 MU tiw) and ribavirin (1000-1200 mg / day), 6 months ribavirin monotherapy (1000-1200 mg / day) or 6 months ribavirin placebo. The study was double blinded for the ribavririn / placebo component. One patient did not fit the entry criteria, and 3 did not start. All 117 patients who received at least one dose of treatment were included in the intention to treat analysis.

Results: At the end of treatment, HCV RNA was undetectable in 35% of patients on combination therapy and in none of the patients treated with ribavirin monotherapy or placebo. The sustained virological response rate at 6 months after therapy was 15% for patients treated with interferon and ribavirin.
During the 6 months treatment period 13% of patients on interferon ribavirin combination therapy, 13% of patients on ribavirin monotherapy and 11% of patients on placebo withdrew due to side effects or noncompliance. At 24 weeks of treatment the mean Hb level was 85% of the baseline value, which means a mean decrease from 9.1 mmol/l to 7.8 mmol/l. The end of treatment Hb levels were not significantly different from patients treated with ribavirin monotherapy ($p=0.76$). End of treatment WBC was significantly lower in patients treated with combination therapy, compared to ribavirin ($p<0.01$) as well as for patients treated with ribavirin monotherapy compared to placebo ($p<0.01$).

Discussion: This belated report on the only placebo controlled study of interferon ribavirin combination therapy in non responders to standard doses of interferon monotherapy documents the effectiveness, be it limited, of this approach as well as the dynamics of the effects on blood counts.

BACKGROUND

Until a decade ago, interferon was the only drug available for the treatment of hepatitis C. Only a minority of patients had a sustained response to standard doses of interferon monotherapy. However, non-responders might be still amendable to therapy by either high-dose daily interferon (1) or by standard interferon in combination with ribavirin (2). This is a belated full report on the first and probably only RCT where combination therapy was assessed in comparison to ribavirin monotherapy and to placebo therapy.

METHODS

Participants

Patients between 18 and 70 years of age with chronic hepatitis C, no ALT normalisation during and after treatment and positive HCV RNA after treatment with interferon monotherapy, were elegible for the study. The previous treatment course consisted of three mega units interferon thrice weekly for 24 weeks or at least eight weeks of treatment with 6 mega units interferon thrice weekly or at least 4 weeks of 10 mega units interferon thrice weekly.

Patients with hepatitis of other viral origin, patients with inherited metabolic liver disease, patients with acquired metabolic liver disease related to alcohol, hepatotoxic drugs or obesity and patients with autoimmune hepatitis were excluded from the study. Other exclusion criteria were significant medical illness within the past five years, pregnancy or likelihood of becoming pregnant, immune modulating therapy (corticosteroids) within the past 6 months, inadequate levels of hemoglobin (<6 mmol/l), platelet count (<50x10e9/l), white blood cells (<3x10e9/l), signs of hepatocellular carcinoma or decompensated liver disease, recent drug or alcohol addiction and unlikeliness to attend regularly for treatment and follow up.

Data were collected in 16 centers in Belgium and in the Netherlands; central data collection was done in the Erasmus Medical Center in Rotterdam, the Netherlands. The study received ethics committee approval in all centers involved and all patients gave signed informed consent.

Interventions

Patients were randomised and assigned to receive either ribavirin (ICN pharmaceuticals, Bucks, United Kingdom) monotherapy or a matched placebo orally twice a day in a total dose of 1200 mg a day (body weight ≥75 kg) or 1000 mg a day (body weight <75 kg) for 24 weeks, or interferon (Intron A, Schering Plough, Kenilworth, NJ, USA) 3 mega units thrice a week combined with ribavirin 1000 or 1200 mg according to body weight during 24 weeks.

All patients were assessed in an outpatient setting for safety, tolerance and efficacy at the end of weeks 2, 4, 8, 12, 16, 20 and 24 and every 4 weeks during follow up until week 48. Initial HCV-RNA was measured by bDNA with a lower detection limit of 2000 copies/ml, HCV-RNA negativity at week 24 and week 48 was measured by PCR. HCV-RNA and hematologic- and biochemical parameters were measured in the certified laboraties of the participating hospitals; results were corrected for local normal values. Liver biopsies, taken withion 12 months before treatment were reviewed by the local pathologist for the presence or absence of cirrhosis.

Objectives

The objective of this study was to evaluate if interferon ribavirin combination therapy or ribavirin monotherapy would be a therapeutic option for non-responders to previous interferon therapy.

Outcomes

The primary endpoint for this study was defined as loss of detectable serum HCV RNA 24 weeks after the end of treatment. The secondary endpoint was normalisation of alanine aminotransferase 24 weeks after treatment.

Sample size

It was calculated that 120 patients (40 per treatment arm) were needed in order to have 90% chance of detecting a difference of 30% at the significance level of p=0.05 (single sided testing) in disappearance of HCV RNA or normalization of ALT levels, assuming the frequency of these endpoints would be ten percent or less in the placebo group.

Criteria for 50% dose reduction of ribavirin were anemia with Hb <5.4 mmol/l or excessive subjective symptoms such as myalgia, fatigue or dyspepsia interfering severely with daily activities. Criteria for a 50% dose reduction of the total weekly interferon dose included leucopenia <1.5 x10e9/l, thrombocytopenia <40 x10e9/l or excessive subjective symptoms interfering with daily activities.

Randomization

Randomization was performed centrally at the coordination center in Rotterdam, the Netherlands after receiving all entry data, by opening an envelope (3). Patients were randomized to either combination therapy or monotherapy at a ratio of 1:2 after stratification for the presence of cirrhosis. The ribavirin and placebo arms were double blinded. Patients allocated to monotherapy received blinded study medication containing either ribavirin or placebo.

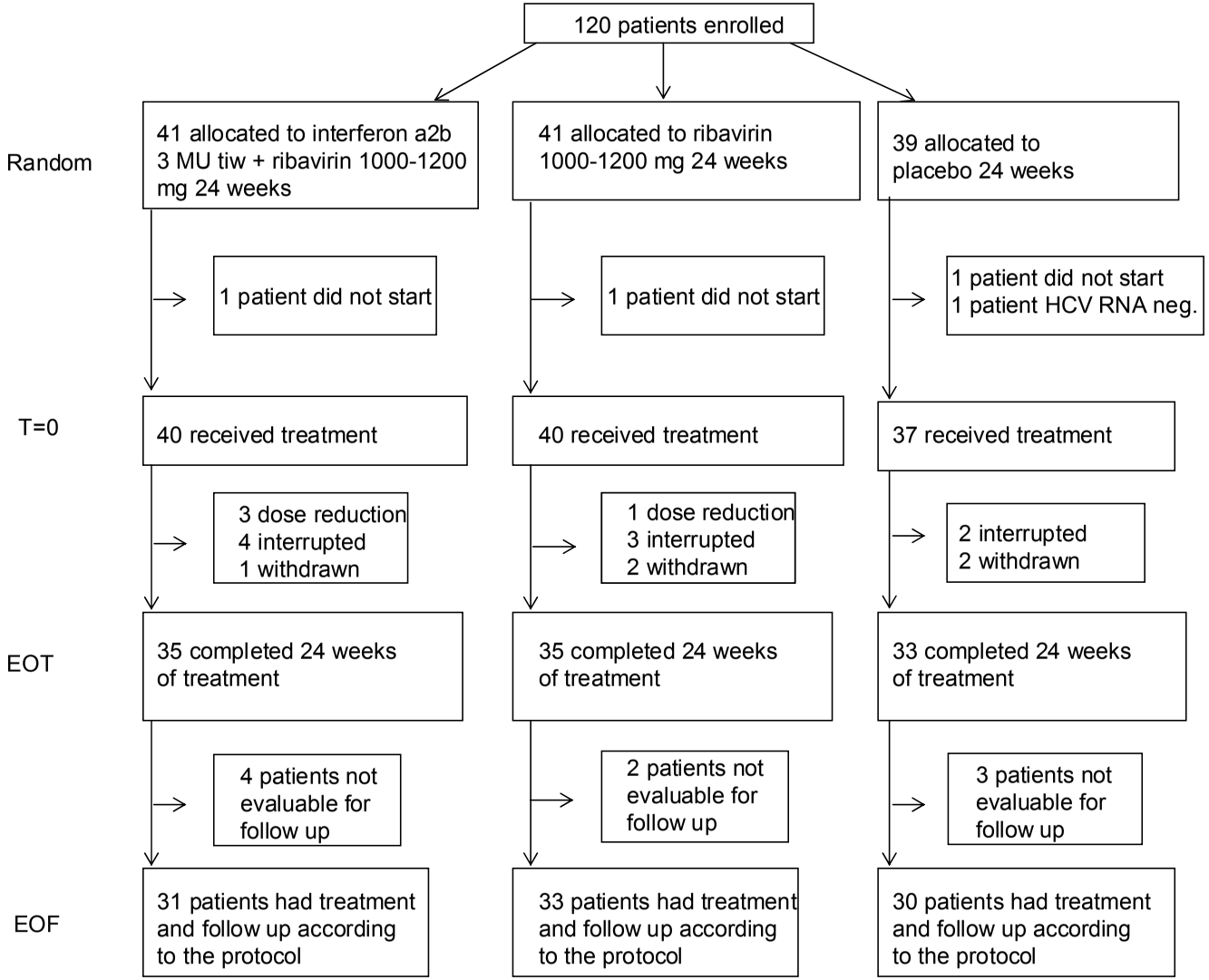
Allocation concealment

The randomization code of the monotherapy arms of the study remained concealed until all patients had completed their follow up and the database had been verified and closed.

Statistical analysis

All statistical analyses were performed using SPSS for Windows (Version 10 SPSS Inc, Chicago, IL, USA). The Kaplan Meier method was used to evaluate the time of ALT normalisation according to treatment using the log rank test to assess statistical significance. The Pearson Chi-square test was used to compare the differences in mean end of treatment and end of follow up response between the different groups. The Mann Whitney test was used to compare the differences in mean end of treatment blood counts.

Figure 1 Trial profile. Patients who had a dose reduction following the protocol were included in all analyses.



RESULTS

Participant flow

121 patients were recruited from 16 university and affiliated hospitals in Belgium, the Netherlands and Luxembourg ('Benelux'). Seventy seven patients had been treated in a previous Benelux study on interferon mono therapy (4), the other 44 had been treated with a comparable treatment schedule of 6 MU interferon thrice a week for at least 12 weeks or 3 MU for at least 24 weeks.

One patient was excluded in view of a negative HCV RNA at baseline and three never started therapy. All remaining 117 patients were included in the analysis (fig 1).

Recruitment

Patients were recruited between 1992 and 1996.

Baseline data

There were no differences in patient characteristics between the 3 treatment groups (table 1). Patient poor-response characteristics were: genotype 1 (69%), HCV RNA above $2x10^6$ copies/ml (55%) and cirrhosis (38%).

Numbers analyzed

The results are presented on the basis of patients who received at least one dosis of study medication, the intention to treat population. 40 patients received at least one dose of combination therapy, another 40 patients received at least one dose of ribavirin and 37 patients received at least one dose of placebo.

Outcomes and estimation

Patients who received combination therapy had significantly better responses than patients treated with ribavirin monotherapy or placebo, both virologically (HCV-RNA negativity) as well as biochemically (ALT normalisation) (fig 2). At the end of treatment, HCV RNA was undetectable in 35% of patients on combination therapy and in none of the patients treated with ribavirin monotherapy or placebo.

The sustained virological response rate at 6 months after therapy was 15% for patients treated with interferon and ribavirin (table 2).

Eighteen percent of patients receiving combination therapy, 2.7% of patients receiving ribavirin monotherapy and 2.5% of patients receiving placebo treatment had persistently normal ALT levels during 24 weeks follow up (table 3).

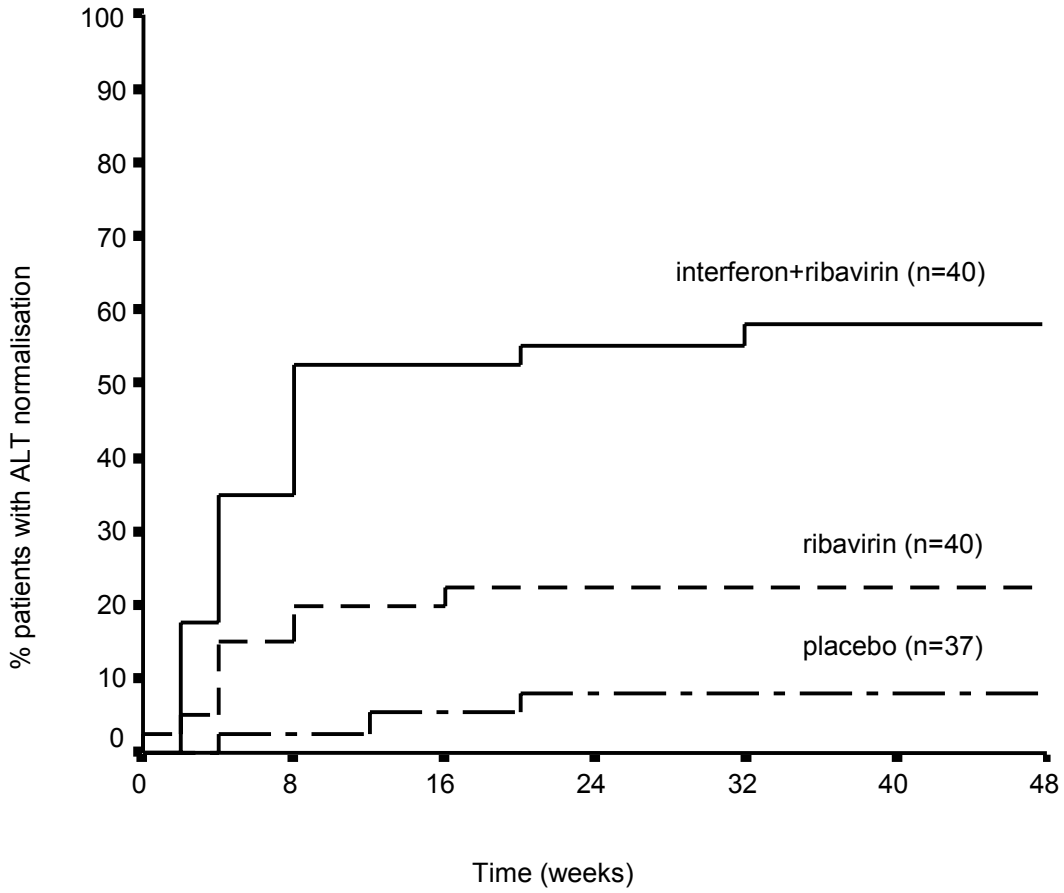
Table 1 Base-line characteristics of the patients (intention-to-treat population).

Characteristic*	Interferon and Ribavirin 6 mo (n=40)	Ribavirin 6 mo (n=40)	Placebo 6 mo (n=37)
Age – yr	47 ± 12	48 ± 12	46 ± 12
Sex – M/F	28/12	28/12	31/6
Serum ALT – times ULN	3.8 ± 2.0	3.8 ± 2.0	3.7 ± 1.6
Serum HCV RNA†			
Copies/ml – log10	6.2 ± 0.8	6.3 ± 0.8	6.3 ± 0.7
≥2 x 10^6 copies/ml – %	54	55	55
Genotype – n (%)			
1	29 (79)	24 (62)	28 (76)
2 or 3	6 (16)	11 (22)	8 (23)
other	2 (5)	4 (6)	1 (3)
Cirrhosis – n (%)	18 (45)	13 (33)	14 (38)

*means ± SD.

†Assessed by bDNA in 68 patients (24 interferon+ribavirin, 22 ribavirin, 22 placebo).

Figure 2 Cumulative probability of ALT response during treatment.



Intention to treat population, per treatment group. Response was defined as normal ALT on at least two sequential occasions, the time of response was taken at the first normal ALT.

P=0,00 interferon + ribavirin vs placebo

P=0,02 ribavirin vs placebo

P=0,02 interferon + ribavirin vs ribavirin (Log rank test)

Table 2 HCV RNA response at end of therapy and end of follow up, by therapy.

HCV RNA negativity – n (%)	Interferon and Ribavirin 6 mo (n=40)	Ribavirin 6 mo (n=40)	Placebo 6 mo (n=37)
end of therapy	14 (35) [1]	0	0
end of follow-up	6 (15) [2]	0	0

[1] p<0.01 (Pearson Chi-square)

[2] p<0.01 (Pearson Chi-square)

Table 3 ALT response, by therapy group.

ALT response – n (%)	Interferon and Ribavirin (n=40)	Ribavirin (n=40)	Placebo (n=37)
On therapy[1]	23 (58%)	9 (23%)	3 (8.1%)
End of treatment[2]	19 (48%)	4 (10%)	2 (5.4%)
End of follow up[3]	7 (18%)	1 (2.7%)	1 (2.5%)

[1]normal ALT on at least two occasions with at least 1 month interval, p<0.01 (Pearson Chi-square).

[2]normal ALT at week 24 and at least one month earlier, p<0.01 (Pearson Chi-square).

[3]persistently normal ALT levels during 24 weeks follow up, p=0.02 (Pearson Chi-square).

Adverse events

Hemoglobin levels decreased in patients treated with combination therapy and in patients treated with ribavirin monotherapy (fig 3A). After week four a plateau-phase was reached. There was a significant difference between the end of treatment Hb change of the patients receiving combination therapy or ribavirin monotherapy, compared to patients treated with placebo ($p<0.01$). The difference in mean end of treatment Hb change between combination therapy and ribavirin monotherapy was not statistically significant ($p=0.76$).

Hemoglobin levels returned to baseline values for both groups within 8 weeks after cessation of treatment. Placebo treatment did not significantly affect hemoglobin levels.

White blood counts decreased in patients treated with combination therapy with interferon and ribavirin (fig 3 B). At week 12 the mean WBC for this group was 64% from the baseline value.After week 12 a plateau-phase was reached. Ribavirin monotherapy also caused a progressive decrease in mean WBC.Placebo treatment did not affect WBC levels.

The mean change in WBC at the end of treatment was greater for the patients treated with combination therapy than for patients treated with ribavirin monotherapy ($p<0.01$). There was also a significant difference between the end of treatment WBC change in patients receiving ribavirin monotherapy compared to patients treated with placebo ($p<0.01$).

Figure 3A Changes in hemoglobin over time, by treatment.

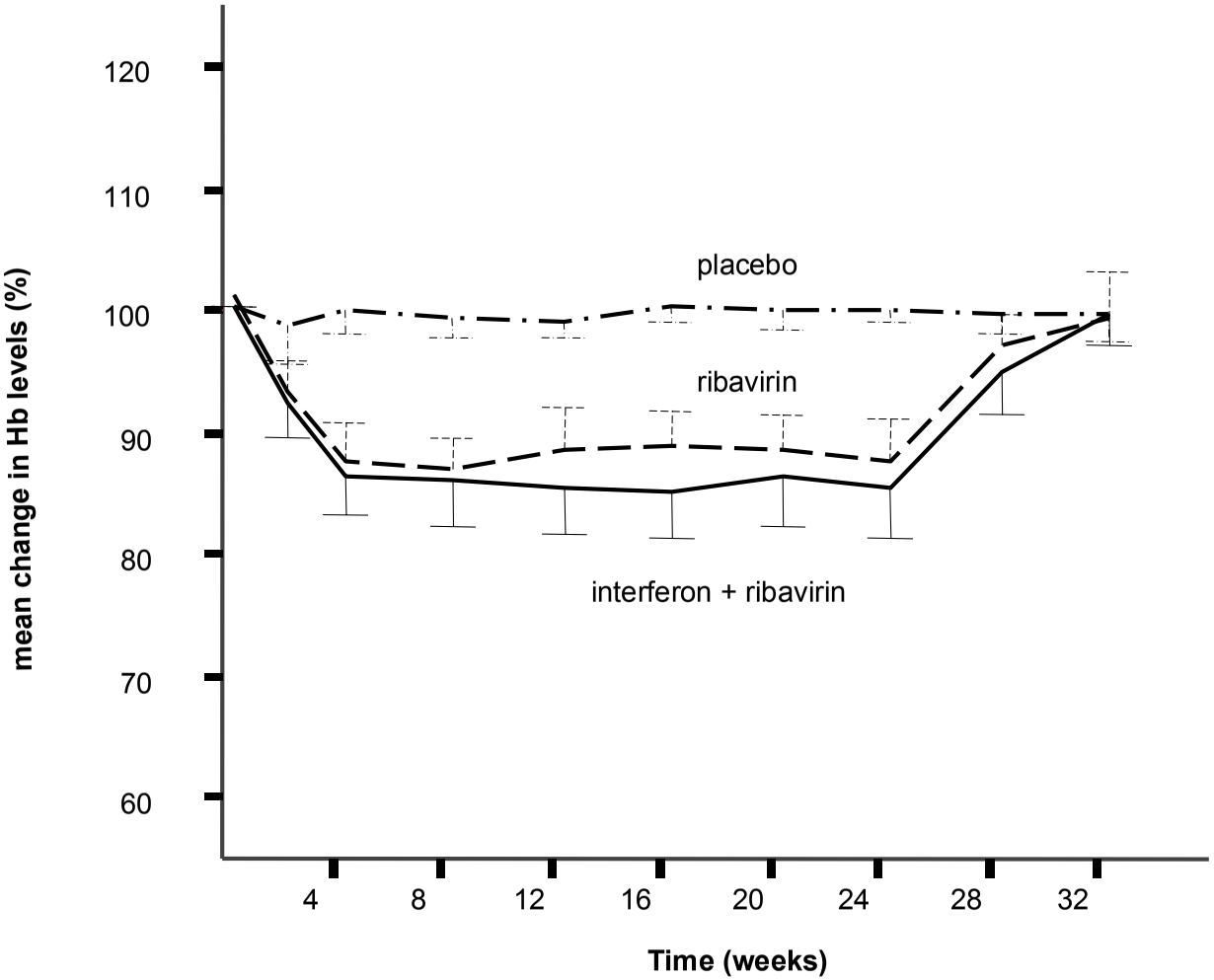
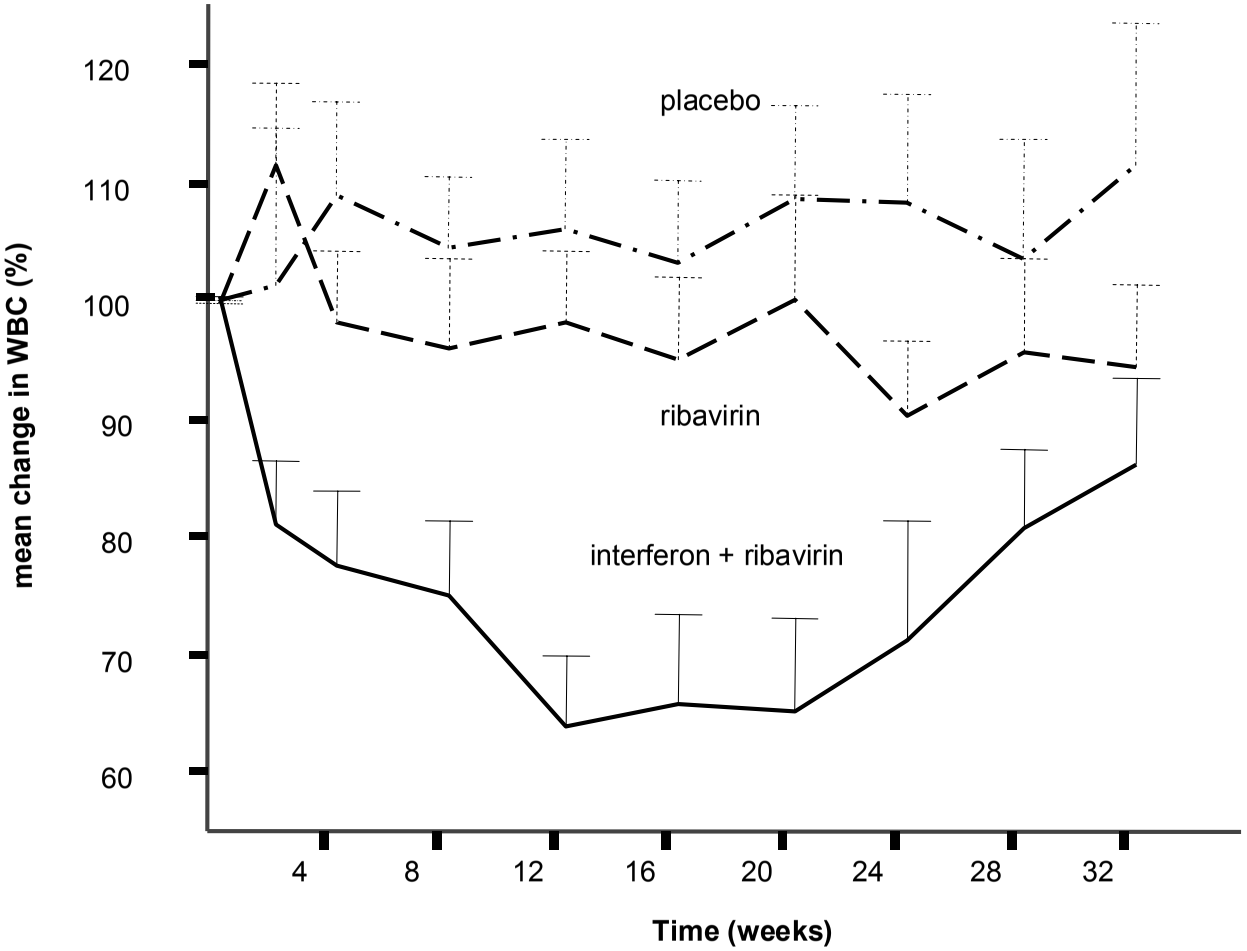


Figure 3B Changes in total white blood cells over time, by treatment.



Side effects

During the 6 months treatment period 12.5% of patients on interferon ribavirin combination therapy, 12.5% of patients on ribavrin monotherapy and 10.8% of patients on placebo withdrew due to side effects or noncompliance (figure 1).

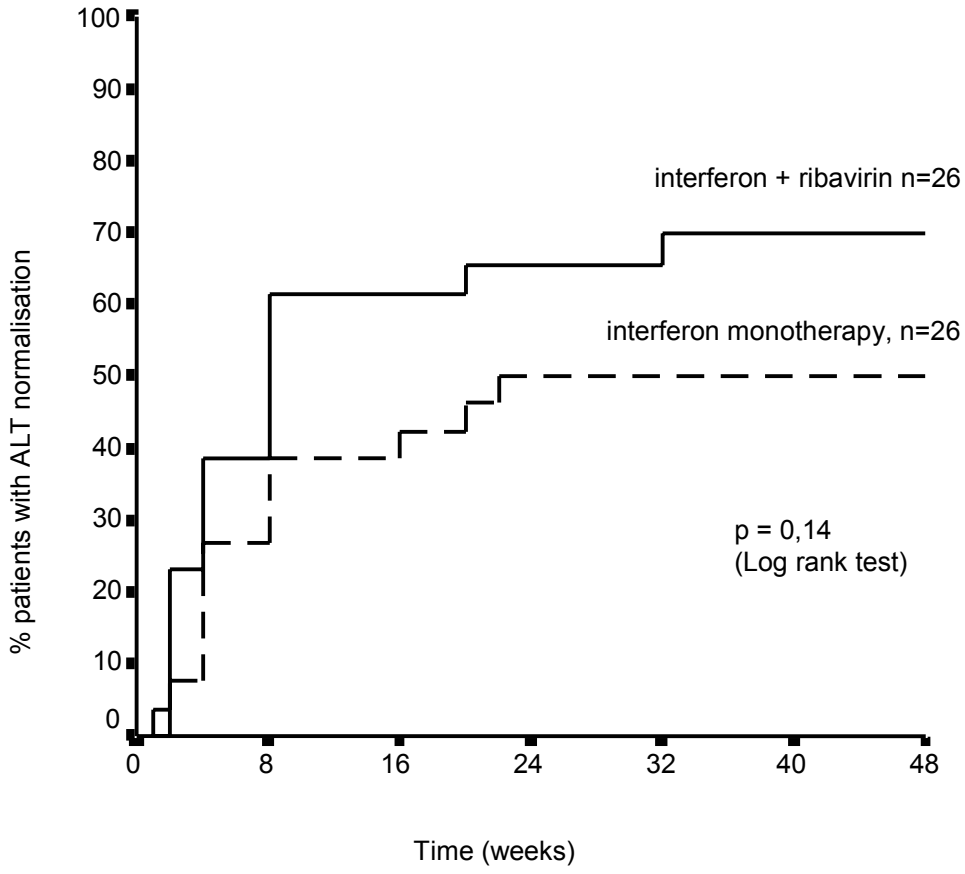
In the group receiving combination treatment 3 patients needed dose reduction of interferon because of dyspnoea, depression and pyelonephritis One patient stopped interferon and had a dose reduction of ribavirin because of subjective side effects and anemia.

Among patients treated with ribavirin, one had a dose reduction because of anemia which gave rise to cardiovascular complaints. One interrupted therapy because of vasculitis.

Ancillary analyses

Figure 4 shows the rates of ALT normalisation of 26 patients who were treated in a previous trial with interferon monotherapy (4) compared to the rates of ALT normalisation the same patients showed in this trial on combination therapy. There is a tendency towards increased ALT normalisation rate with interferon and ribavirin combination therapy.

Figure 4 Cumulative probability of ALT response during treatment.



Patients treated with combination therapy in this trial vs results of the same patients in a previous trial with interferon monotherapy (4). There is a trend towards increased normalisation rate with interferon and Ribavirin combination therapy.

DISCUSSION

In 1992 there were two therapeutic options for patients not responding to standard doses of interferon monotherapy: treatment with high daily doses of interferon (1) or combination therapy with interferon and ribavirin.

To test this second option we started this randomised controlled trial, in which we compared combination therapy with interferon and ribavirin to ribavirin monotherapy and to placebo. This trial is scientifically sound, with high enough numbers treated to draw reliable conclusions. The feasibility of such a trial was, however, low because of the placebo arm included; consequently it took several years for inclusion of all patients.

Part of the results of this study were published in meta-analyses (5). This full report can still be of value for meta-analysis by the Cochrane approach and can serve as a reference for ribavirin monotherapy in non-responders and for placebo therapy in non-responders.

This study documents a significant, though small effect of adding ribavirin to the treatment of non-responders. Since then various RCT have been published comparing combination therapy with interferon monotherapy (6–9). Saracco et al. reported sustained response rates comparable to the 15% we found in this study in patients assigned to a comparable treatment schedule (7). In meta-analyses that have been published on this subject, also response rates of 14–16% have been reported (10–13). One way to increase response rates in patients retreated with combination therapy, is to make a stricter selection and to treat only subgroups of patients who are more likely to have a sustained response. Camma et al state that sustained response rates can be increased to 30% by selecting patients less than 45 years old with normal gammaglutamyltransferase levels and by treating them with high dose long course combination therapy (12). Indeed Saracco et al have shown that non-responders benefit the most from prolonged treatment with high dose interferon, achieving response rates up to 23 % in these patients.

In naive patients good results are obtained by treatment with pegylated interferon combined with ribavirin (14,15). New studies are on their way evaluating the effect of pegylated interferon and ribavirin in non-responders to interferon.

This trial where combination treatment is compared to ribavirin monotherapy and placebo therapy, gives us the opportunity to determine which adverse effects can be expected from which medication. The decrease in WBC in combination therapy is mainly due to interferon, but also ribavirin causes a progressive and significant decrease in WBC.

The decrease in Hb levels seen during combination therapy is almost entirely caused by ribavirin, with only a marginal additional effect of interferon.

Conclusion:

This belated report on the only placebo controlled study of interferon ribavirin combination therapy in non responders to standard doses of interferon monotherapy documents the effectiveness, be it limited, of this approach as well as the dynamics of the effects on blood counts.

ACKNOWLEDGEMENTS

Participants in this study of the Benelux Study Group on treatment of chronic hepatitis C are: [1]M. Adler, [2]G.P. van Berge Henegouwe, [1]N. Bourgeois, [3]J.T. Brouwer, [4]P. Buydens, [5]R.A.F.M. Chamuleau, [6]J. Delwaide, [7]Th.J.M. van Ditzhuysen, [8]A. Elewaut, [9]J. Fevery, [10]H. Fierens, [11]C. de Galocsy, [3]B. Hansen, [2]J. van Hattum, [5]M.L. Hautekeete†, [12]B. van Hoek, [10]P. Michielsen, [13]C.J. Mulder, [9]F. Nevens, [14]J.W. den Ouden-Muller, [5]H.W. Reesink, [3]S.W. Schalm, [15]H. Tuynman, [8]J. Versieck, [8]H. van Vlierberghe, [13]R.A. de Vries, [5]C.J. Weegink, [16]P.J. Wismans.

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REFERENCES:

1. Iino S, Hino K, Kuroki T, Suzuki H, Yamamoto S. Treatment of chronic hepatitis C with high-dose interferon alpha-2b. A multicenter study. Dig Dis Sci 1993;38(4):612-8.
2. Brillanti S, Garson J, Foli M, Whitby K, Deaville R, Masci C, et al. A pilot study of combination therapy with ribavirin plus interferon alfa for interferon alfa-resistant chronic hepatitis C. Gastroenterology 1994;107(3):812-7.
3. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. analysis and examples. Br J Cancer 1977;35(1):1-39.
4. Brouwer JT, Nevens F, Kleter B, Elewaut A, Adler M, Brenard R, et al. Efficacy of interferon dose and prediction of response in chronic hepatitis C: Benelux study in 336 patients. J Hepatol 1998;28(6):951-9.
5. Schalm SW, Hansen BE, Chemello L, Bellobuono A, Brouwer JT, Weiland O, et al. Ribavirin enhances the efficacy but not the adverse effects of interferon in chronic hepatitis C. Meta-analysis of individual patient data from European centers. J Hepatol 1997;26(5):961-6.
6. Bellobuono A, Mondazzi L, Tempini S, Silini E, Vicari F, Ideo G. Ribavirin and interferon-alpha combination therapy vs interferon-alpha alone in the retreatment of chronic hepatitis C: a randomized clinical trial. J Viral Hepat 1997;4(3):185-91.
7. Saracco G, Ciancio A, Olivero A, Smedile A, Roffi L, Croce G, et al. A randomized 4-arm multicenter study of interferon alfa-2b plus ribavirin in the treatment of patients with chronic hepatitis C not responding to interferon alone. Hepatology 2001;34(1):133-8.
8. Pol S, Couzigou P, Bourliere M, Abergel A, Combis JM, Larrey D, et al. A randomized trial of ribavirin and interferon-alpha vs. interferon-alpha alone in patients with chronic hepatitis C who were non-responders to a previous treatment. Multicenter Study Group under the coordination of the Necker Hospital, Paris, France. J Hepatol 1999;31(1):1-7.
9. Barbaro G, Di Lorenzo G, Soldini M, Giancaspro G, Bellomo G, Belloni G, et al. Interferon-alpha-2B and ribavirin in combination for chronic hepatitis C patients not responding to interferon-alpha alone: an Italian multicenter, randomized, controlled, clinical study. Am J Gastroenterol 1998;93(12):2445-51.
10. Cummings KJ, Lee SM, West ES, Cid-Ruzafa J, Fein SG, Aoki Y, et al. Interferon and ribavirin vs interferon alone in the re-treatment of chronic hepatitis C previously nonresponsive to interferon: A meta- analysis of randomized trials. Jama 2001;285(2):193-9.
11. Cheng SJ, Bonis PA, Lau J, Pham NQ, Wong JB. Interferon and ribavirin for patients with chronic hepatitis C who did not respond to previous interferon therapy: a meta-analysis of controlled and uncontrolled trials. Hepatology 2001;33(1):231-40.
12. Camma C, Bruno S, Schepis F, Lo Iacono O, Andreone P, Gramenzi AG, et al. Retreatment with interferon plus ribavirin of chronic hepatitis C non- responders to interferon monotherapy: a meta-analysis of individual patient data. Gut 2002;51(6):864-9.
13. Camma C, Giunta M, Chemello L, Alberti A, Toyoda H, Trepo C, et al. Chronic hepatitis C: interferon retreatment of relapsers. A meta- analysis of individual patient data. European Concerted Action on Viral Hepatitis (EUROHEP). Hepatology 1999;30(3):801-7.
14. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358(9286):958-65.

15. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347(13):975-82.

Chapter 6

Early prediction of response in interferon monotherapy and in interferon-ribavirin combination therapy for chronic hepatitis C: HCV RNA at 4 weeks versus ALT

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Journal of Hepatology 1999; 30: 192-198

ABSTRACT

Background/Aims: There is consensus to stop interferon for hepatitis C if ALT remains elevated after 12 weeks; however, this may lead to unjust treatment withdrawal in ca 20% of potential sustained responders. No consensus exists for interferon-ribavirin combination therapy. The aim of this study was to assess the predictive value of an HCV RNA test at 4 weeks in comparison with ALT, both in interferon monotherapy and in interferon-ribavirin combination therapy.

Methods: Plasma HCV RNA was tested at 4 weeks in 149 naive patients with 6 months and 187 with up-to 12 months interferon monotherapy, and in 40 non-responders treated with 6 months interferon-ribavirin combination.

Results: For 6 and up to 12 months interferon monotherapy, the predictive value for non-response was 99% resp. 97% for a positive HCV RNA at week 4, versus 97% resp. 91% for an elevated ALT at week 12. Using a positive HCV RNA at week 4 as a stopping rule would lead to missing 5% resp. 12% of potential sustained responders, versus 10% resp. 28% for an elevated ALT at week 12. In interferon-ribavirin combination therapy, the predictive value for non-response was 100% for week 4 HCV RNA versus 95% for week 12 ALT, and the potential sustained responders missed by a test was 0% for week 4 HCV RNA versus 20% for week 12 ALT. The overall sensitivity and specificity of a week 4 HCV RNA test was significantly better (area under ROC 0.85) as compared to testing ALT at either week 4 (0.78, $p<0.001$), week 8 (0.76, $p<0.001$) or week 12 (0.78, $p<0.001$).

Conclusion: a positive HCV RNA test ($\geq 10^3$ copies/ml) at 4 weeks is highly predictive for non-response and leads to significantly less misidentification of potential sustained responders than ALT at week 4, 8 or 12, both in 6 or up to 12 months interferon monotherapy and in 6 months interferon-ribavirin combination therapy of chronic hepatitis C.

INTRODUCTION

For chronic hepatitis C the accepted duration of treatment with alpha interferon is - anno 1998 - 12 months (1,2), but treatment efficacy is still limited (3–5). To reduce the costs and prevent unnecessary treatment, it was recommended at consensus meetings in 1997 (1,2) to stop treatment if ALT levels did not normalize within 12 weeks; the additional value of testing for HCV RNA was questioned (1,6).

Recently, Tong et al. reported that measurement of HCV RNA at 12 weeks was more accurate than ALT in early identification of non-(sustained) responders to interferon therapy (7). In their study with 6 months interferon treatment (3 MU t.i.w.), an elevated ALT level at week 12 was 92% predictive for non-sustained response, but application of an elevated ALT at week 12 as a stopping rule would have led to missing 33% of the sustained responders. In contrast, a positive HCV RNA at week 12 was 98% predictive for non-sustained response, and misidentified only 7% of the sustained responders. The question was raised whether testing for HCV RNA before week 12 might lead to even greater benefits and whether the results are also applicable to treatment for longer than 6 months and for the emerging new standard therapy of hepatitis C with interferon-ribavirin combination.

In our centre we observed that plasma HCV RNA became undetectable already at 4 weeks in sustained responders, and suggested a high predictive value of this time point (8). In an earlier paper on a large interferon monotherapy study in the Benelux, we already briefly mentioned the superior predictive value of this assessment compared to pretreatment factors like genotype, viral load and grade of fibrosis (9). In this paper, we extend this observation and report on the predictive value of HCV RNA testing at week 4 versus ALT at either week 4, 8 or 12, both for the large cohort of patients treated with interferon monotherapy (9) as well as for a cohort of patients treated within the Benelux with interferon-ribavirin combination therapy (10).

MATERIALS AND METHODS

Patients

For this study, data from patients, treated in two large randomized controlled trials performed in 19 hospitals in the Benelux were used. The first cohort was a group of 336 naive chronic hepatitis C patients, with ALT levels at least twice the upper limit of normal, who were recruited between 1990 and 1993. One hundred and forty-nine patients were randomized to receive interferon monotherapy, 3 MU thrice a week (t.i.w.) for 6 months, and 187 patients received 6 MU interferon t.i.w. for 8 weeks, followed by dose reduction with a variable duration (dependent on response) with a maximum of 12 months (9).

The second cohort was a group of 120 non-responders, recruited between 1992 and 1996, characterized by presence of elevated ALT levels at the end of interferon monotherapy and elevated ALT levels and detectable plasma HCV RNA, 6 months after the end of treatment (10). These patients were randomized to receive placebo, 6 months ribavirin monotherapy, or 6 months combination therapy of interferon 3 MU t.i.w. and ribavirin 1–1.2 g per day; only the latter group was included in the analysis (*n*=40).

Assessment of ALT and HCV RNA

ALT assessments were routinely performed every 4 weeks during treatment and every 4–6 weeks during the 6-month follow-up. ALT levels were determined at the participating trial centres and corrected for local normal values. Plasma samples for HCV RNA determination were routinely collected at start, week 4, end of treatment and end of follow-up. Samples for HCV RNA determination were processed within 2 hours and stored at -70^{o} C; all HCV RNA determinations were performed centrally at the coordinating centre in Rotterdam, using a validated in-house qualitative PCR assay with a sensitivity of ±10^3 copies per ml according to the Eurohep standard (11).

Response criteria

Sustained response (SR) was defined as undetectable HCV RNA at the end of the 6-month follow-up, together with persistently normal ALT levels during the 6-month

follow-up (measured on at least two occasions)[1]; all other patients were classified as non-sustained responders. The early biochemical and virological on-treatment responses were evaluated for their value to predict SR and non-SR. Biochemical on-treatment response was assessed at weeks 4, 8 and 12 respectively and was defined as drop of the serum ALT level below the upper limit of normal. The virological on-treatment response was assessed at week 4 and was defined as plasma HCV RNA not detectable by the qualitative validated in-house assay.

Statistical analysis

For each test (ALT at week 4, 8 or 12, HCV RNA at week 4) we calculated its positive predictive value (chance of sustained response if the test is normal), its negative predictive value (chance of non-sustained response if the test is abnormal), its sensitivity (fraction of all sustained responders identified by a normal test) and its specificity (fraction of all non-responders identified by an abnormal test). Because of its clinical relevance, we also calculated the reverse forms of the sensitivity and specificity, i.e. the fraction of all (non-)sustained responders not identified by the test. For all tests the areas under the ROC curves were calculated and compared according to the method described by DeLong et al. (13). All calculations were made using SPSS software (SPSS Inc, Chicago, IL, USA) and Stata software (Stata Corp, Texas, TX, USA).

[1] In contrast to the definition proposed by Lindsay at the recent NIH consensus meeting (12), we did not include the criterion of repeatedly normal ALT's at the end of treatment in this definition because of the observation that in 10–15% of proven long-term (>5 years) sustained responders, ALT levels remained slightly elevated until the end of treatment.

RESULTS

Interferon monotherapy

Three hundred thirty-six naive HCV patients participated in a Benelux study on interferon monotherapy. The SR rate was 15% (95% confidence interval (CI) 11–18%).

In 280 patients a complete set of HCV RNA results at week 4 as well as ALT values at week 4, 8 and 12 was available. Table 1 displays the results of the analysis of this cohort according to treatment duration (6 months versus up-to 12 months). Using the outcomes of the ALT or HCV RNA tests as a stopping criterion early during treatment, the clinical most relevant goals are a high predictive value for non-response and no exclusion of potential sustained responders. If HCV RNA was still detectable at week 4, the chance of non-SR was 99% and 97%, respectively, whereas it was 97% and 91%, respectively, in case of ALT normalization at week 12. If ALT normalization at week 12 had been used as stopping criterion, two out of 20 (10%) sustained responders undergoing 6 months of treatment and seven out of 25 (28%) sustained responders undergoing treatment for up to 12 months would have been missed, compared to only 5% and 12%, respectively, using HCV RNA assessment at week 4. Taking various combinations of ALT and HCV RNA criteria, e.g. HCV RNA response at week 4 with ALT normalization at week 12, did not lead to improvement of these results when compared to HCV RNA at week 4 alone (data not shown). In six out of the 45 (13%) proven long-term sustained responders, the ALT level even remained slightly elevated until the end of treatment.

Out of the 336 naive patients treated with interferon monotherapy, 65% had genotype 1a or 1b infection. SR rates were 7% for genotype 1 versus 28% for the others. For genotype 1, the most relevant test characteristics were comparable for PCR at week 4 and ALT at week 12 (99% versus 98% prediction of non-response, 14% versus 13% misidentification of response), while the overall test performance was better for HCV RNA at week 4 due to a better prediction of sustained response (20% versus 13%) and lower misidentification of non-response (27% versus 45%). For the other genotypes, HCV RNA at week 4 was superior over ALT assessment in all respects (Table 2).

Table 1 Predictive value, sensitivity and specificity of an HCV RNA test at 4 weeks versus ALT at week 4, 8 or 12. Results for 6 months and for 12 months interferon monotherapy

Treatment:		%SR if test is normal*	%non-SR if test is abnormal**	%SR not identified by test†	%non-SR not identified by test‡	Odds Ratio§
Interferon mono, 6 mo						
ALT	week 4	31	96	15	33	11.5^{000}
	week 8	25	98	5	51	18.3^{000}
	week 12	26	97	10	47	10.4^{000}
HCV RNA	week 4	36	99	5	31	46.3^{000}
Interferon mono, ≤12 mo						
ALT	week 4	25	91	32	39	3.4^{00}
	week 8	21	91	24	52	2.9^{0}
	week 12	21	91	28	49	2.7^{0}
HCV RNA	week 4	31	97	12	37	13.6^{000}

* Predictive value of a negative/normal test for a sustained response (SR).
** Predictive value of a positive/abnormal test for a non-sustained response (treatment failure).
† 100% minus sensitivity (=% SR identified by test).
‡ 100% minus specifity (=% non-SR identified by test).
§ Ods Ratio: ^{0} $p<0.05$, ^{00} $p<0.001$, ^{000} $p<0.0001$

Table 2 Predictive value, sensitivity and specificity of an HCV RNA test at 4 weeks versus ALT at week 4, 8 or 12. Results for interferon monotherapy in HCV genotype 1 and in other (non-1) genotypes

Treatment:		%SR if test is normal*	%non-SR if test is abnormal**	%SR not identified by test†	%non-SR not identified by test‡	Odds Ratio§
Interferon mono, type 1						
ALT	week 4	12	95	40	35	2.8^{0}
	week 8	10	95	33	50	2
	week 12	13	98	13	45	7.8^{00}
HCV RNA	week 4	20	99	14	27	16.5^{000}
Interferon mono, non-1						
ALT	week 4	50	89	17	39	16.1^{000}
	week 8	44	93	7	56	10.9^{000}
	week 12	40	81	23	55	2.7^{0}
HCV RNA	week 4	48	93	7	52	12.6^{000}

* Predictive value of a negative/normal test for a sustained response
** Predictive value of a positive/abnormal test for a non-sustained response (treatment failure)
† 100% minus sensitivity (=% SR identified by test)
‡ 100% minus specifity (=% non-SR identified by test)
§ Ods Ratio: ^{0} $p<0.05$, ^{00} $p<0.001$, ^{000} $p<0.0001$

Interferon-ribavirin combination therapy

Forty non-responders to interferon monotherapy received 6 months treatment with combination of interferon and ribavirin; 80% had a genotype 1a or 1b. Five patients (12.5%, 95% C.I. 4–27%) had a sustained response. A complete set of ALT data at week 4, 8 and 12, together with HCV RNA results at week 4, was available for 31 patients (including all five sustained responders).

If week 4 HCV RNA remained detectable, the chance of non-SR was 100%, versus 95% in case of ALT remained elevated at week 12 (Table 3); furthermore, one out of five (20%) SR would have been missed if ALT normalization at week 4, 8 or 12 had been used as criterion; in this patient, ALT remained elevated until the end of therapy and only normalized after treatment withdrawal. All five sustained responders were correctly identified by an undetectable HCV RNA at week 4. The odds ratios for ALT normalization as criterion did not reach statistical significance, whereas the odds for week 4 HCV RNA were infinite (95% CI 4.7–∞, p=0.0002).

ROC curves

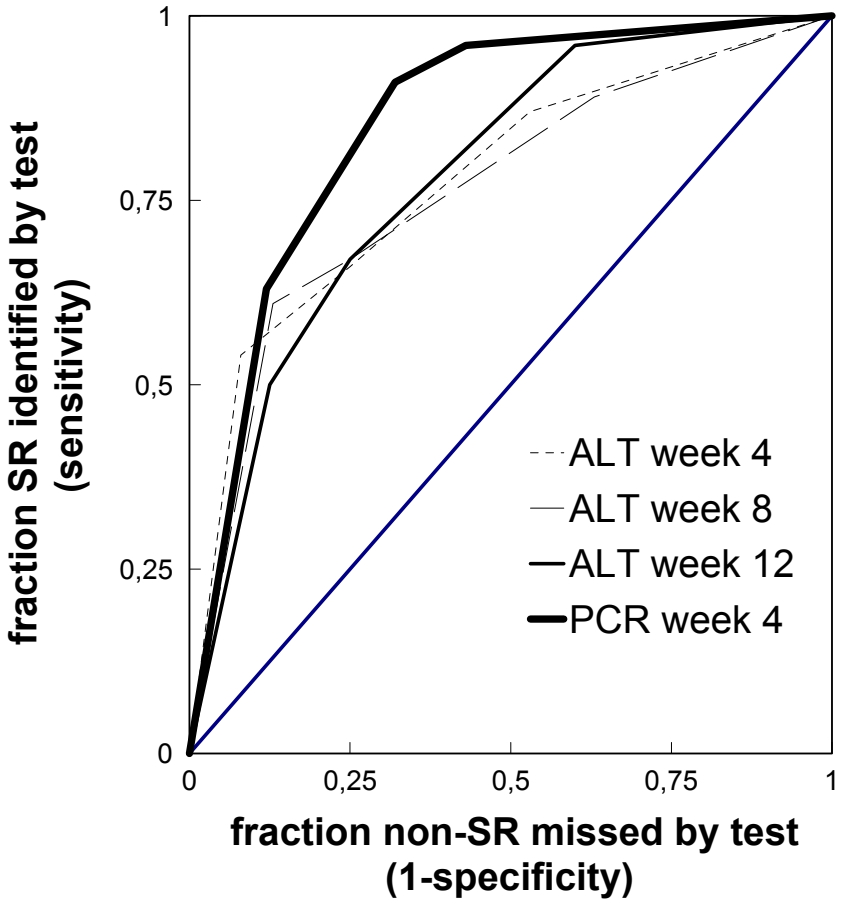
Figure 1 shows the receiver operating characteristic (ROC) curves for HCV RNA testing at week 4 and ALT testing at week 4, 8 and 12 in the total group (125 patients with monotherapy for 6 months, 155 with monotherapy for up to 1 year, and 31 with interferon-ribavirin combination therapy). The area under the ROC curve was significantly higher for HCV RNA testing at week 4 (0.85) as compared to ALT testing at either week 4 (0.78, p<0.001), week 8 (0.76, p<0.001) or week 12 (0.78, p<0.001). The areas under the ROC curves were not significantly different between the ALT tests.

Table 3 Predictive value, sensitivity and specificity of an HCV RNA test at 4 weeks versus ALT at week 4, 8 or 12. Results for interferon-ribavirin combination therapy.

Treatment:		%SR if test is normal*	%non-SR if test is abnormal**	%SR not identified by test†	%non-SR not identified by test‡	Odds Ratio§
Interferon-ribavirin 6 mo						
ALT	week 4	23	96	25	30	6.9
	week 8	18	93	20	55	3.3
	week 12	22	95	20	42	5.4
HCV RNA	week 4	46	100	0	19	∞^{000}

* Predictive value of a negative/normal test for a sustained response
** Predictive value of a positive/abnormal test for a non-sustained response (treatment failure)
† 100% minus sensitivity (=% SR identified by test)
‡ 100% minus specifity (=% non-SR identified by test)
§ Ods Ratio: ^{0} $p<0.05$, ^{00} $p<0.001$, ^{000} $p<0.0001$

Figure 1 Value of testing ALT at week 4, 8 or 12 or HCV RNA at week 4 for early discrimination between eventual sustained responders and non-sustained responders.



The ROC curves show the relation per test between the chance of correctly identifying an eventual sustained responder versus the chance of giving a false positive result. An optimal test would approach 100% sensitivity at 0% false positivity, while a test without discriminative value would only reach 100% sensitivity at 100% false positivity. Differences between curves are evaluated by comparing the area under the ROC curves. The area under the ROC curve was significantly higher for testing HCV RNA at week 4 (0.85) as compared to ALT at either week 4 (0.78, $p<0.001$), week 8 (0.76, $p<0.001$) or week 12 (0.78. $p<0.001$), whereas no significant differences were observed between the ALT tests at week 4, 8 or 12.

DISCUSSION

Treatment of chronic hepatitis C has been improved by prolonging treatment duration from 6 to at least 12 months (14) and by combining interferon with Ribavirin (15,16), but its efficacy is still limited. To reduce unnecessary exposure to treatment and its potential side-effects, and to reduce costs, guidelines have been sought to decide in an early stage whether continuation of treatment leads to a reasonable chance of success. Although pretreatment factors like viral load, genotype and grade of fibrosis can be used to predict the mean treatment outcome for study cohorts, they are of limited value in the individual patient (6,9). Based on observations in interferon monotherapy studies, it was proposed at the consensus conferences that – in addition to delineating a group with potential benefit of therapy – interferon treatment should be stopped if ALT values did not normalize within the first twelve weeks.

This study shows that an HCV RNA test performed at 4 weeks of antiviral therapy has a high predictive value in identifying patients that have virtually no chance of reaching a sustained response with currently accepted treatment regimens; in addition, use of this test criterion affects the total number of sustained responders less than criteria based on ALT at 4, 8 or 12 weeks.

Tong et al. (9) showed in his study with interferon monotherapy for 6 months that HCV RNA testing at week 12 had a high predictive value for non-response (98%) and only missed 1 out of 15 (7%) of the sustained responders. In our cohort with 6 months interferon monotherapy, we found a similar high predictive value for non-(sustained) response (99%, Table 1), with 5% misidentification of SR, when HCV RNA was assessed as early as 4 weeks after the start of therapy. Furthermore, a comparable efficacy of HCV RNA testing was observed in the cohort with variable treatment duration up-to one year. The latter is in accordance with the report of Gavier et al. on 181 patients treated with interferon monotherapy for 12 months (17), where it was observed that persistence of serum HCV RNA at 4 weeks of treatment predicted non-SR in 95% and missed only 8% of sustained responders versus 39% when ALT at week 12 had been used.

The predictive value of an early ALT and HCV RNA assessment has not yet been reported for interferon-ribavirin combination therapy. Although limited in study size (*n*=40), our observation suggests that HCV RNA is at least as predictive as in interferon monotherapy, and its value in comparison with ALT is probably even larger

because ribavirin is known to lead to temporary ALT normalisations which are not accompanied by virus suppression or elimination. In addition, we made the same observation as in interferon monotherapy that in some of the proven long-term sustained responders ALT levels remain elevated until the end of therapy whereas HCV RNA was already undetectable at week 4 in all of them. This stresses the superiority of the HCV RNA detection over the ALT, which should not be used as a surrogate assay.

Recently, Zeuzem et al. (18) argued that a quantitative evaluation of the initial response might be preferred over a qualitative test at week 4. Based on an assumed half-life of the virus, he calculated that all sustained responders should have at least 3 log drop in viral load between start and week 4. Based on this assumption, he expected that sustained responders with a high pretreatment viral load ($>2.10^6$ copies/ml) might still be HCV RNA positive at week 4 by qualitative PCR. However, 15 of the 49 sustained responders in our group with interferon monotherapy had pretreatment viremia levels greater than 2.10^6 genome Eq/ml by bDNA, and all were HCV RNA negative at week 4 of therapy by qualitative PCR (sensitivity 10^3 copies/ml). A so-called late response, i.e. PCR positive at week 4 and negative at end of treatment, followed by a sustained response, was observed in only 3 out of the 336 patients in our cohort treated with interferon monotherapy, and in none of the 40 patients on interferon-ribavirin combination therapy. These observations are supported by the finding of Gavier et al. that the predictive value of the test is not improved when HCV RNA is assessed at week 12 in stead of week 4 (17).

An objection to introducing our findings into clinical practice might be the variable cut-off of the HCV RNA-PCR test (19). If the test is more sensitive than 10^3 copies per ml, as evolving technology is now producing, a qualitative test might lose its current predictive value; replacement with a quantitative test with a sensitivity below 10^3 copies might then become the final answer. However, quantitative HCV RNA assays are at this moment poorly standardized, and either have limited sensitivity or lack linearity in the higher ranges (19,20). Thus, until the standardization issue is solved and performance of quantitative tests is established, a single qualitative HCV RNA assay at week 4 with a cut-off at 10^3 geneq/ml may be at least as predictive, easier in use, and more cost-effective than two quantitative assays. Another objective to implementation of a positive HCV RNA at 4 weeks as a stopping criterion might be that, although chances are very limited, a few potential responders might still be

missed. However, for these patients with chances of less than 5% of success if treatment is continued, it might be better to change to a more intense treatment modality like high-dose induction therapy which offers better chances of success in these specific cases (21,22). We suggest therefore that an HCV RNA test at 4 weeks is not used as a stopping rule, but as a management tool in deciding in whom to continue standard treatment and in whom to adjust it. Future research should explore whether this concept also holds for daily high-dose interferon therapy and for prolonged (≥1 year) interferon-ribavirin combination therapy.

ACKNOWLEDGMENTS

The authors are grateful to the participants in the Benelux Study Group on Treatment of Chronic Hepatitis C who contributed to the database which was used for this report: M. Adler[1], V. Arendt[2], N. Bourgeois[1], J. Belaiche[3], R. Brénard[4], C.M. Bronkhorst[5], J.T. Brouwer[6], P. Buydens[7], R.A.F.M. Chamuleau[5], J. Delwaide[3], Th.J.M. van Ditzhuysen[8], L.J. van Doorn[9], A. Elewaut[10], A.E. Elewaut[10], H. Fierens[11], J. Fevery[12], C. de Galocsy[13], A.P. Geubel[4], J de Groote[12], B.E. Hansen[6], J. van Hattum[14], M.L. Hautekeete[7], R.A. Heijtink[6], J. Henrion[15], B. van Hoek[16], W.C.J. Hop[6], P.L.M. Janssen5, F.J.W. ten Kate5, G.E.M. Kleter6, G. Leroux-Roels10, P. Michielsen[11], J.P. van Munster[17], F. Nevens[12], H.G.M. Niesters[6], J.W. den Ouden[18], D. Overbosch[19], J. Pirotte[3], M. Rasch[14], H.A.R.E. Tuynman[20], W.G.V. Quint[9], H.W. Reesink[5], S.W. Schalm[6], van Steenbergen[12], J. Versieck[10], R.A. de Vries[21], J. Weber[2], P.J. Wismans[22], S.H. Yap[12].

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The authors thank G.R. Jhinkoe Rai for assistance in the data collection and S.N. de Vlaming for assistance in preparation of the manuscript.

REFERENCES

1. Anonymous. National Institutes of Health Consensus Development conference Panel statement: management of hepatitis C. Hepatology 1997;26:2S-10S.
2. Dhumeaux D, Doffoel M, Galmiche JP. A French consensus conference on hepatitis C: screening and treatment. J Hepatol 1997;27:941-4.
3. Chemello L, Bonetti P, Cavalletto L, Talato F, Donadon V, Casarin P, et al. Randomized trial comparing three different regimens of alpha-2a-interferon in chronic hepatitis C. The TriVeneto Viral Hepatitis Group. Hepatology 1995;22:700-6.
4. Lin R, Roach E, Zimmerman M, Strasser S, Farrell GC. Interferon alfa-2b for chronic hepatitis C: effects of dose increment and duration of treatment on response rates. Results of the first multicentre Australian trial. Australia Hepatitis C Study Group. J Hepatol 1995;23:487-96.
5. Poynard T, Bedossa P, Chevallier M, Mathurin P, Lemonnier C, Trepo C, et al. A comparison of three interferon alfa-2b regimens for the long- term treatment of chronic non-A, non-B hepatitis. Multicenter Study Group. N Engl J Med 1995;332:1457-62.
6. Davis GL, Lau JY. Factors predictive of a beneficial response to therapy of hepatitis C. Hepatology 1997;26:122S-7S.
7. Tong MJ, Blatt LM, McHutchison JG, Co RL, Conrad A. Prediction of response during interferon alfa 2b therapy in chronic hepatitis C patients using viral and biochemical characteristics: a comparison. Hepatology 1997;26:1640-5.
8. Kleter GE, Brouwer JT, Heijtink RA, Schalm SW, Quint WG. Detection of hepatitis C virus RNA in patients with chronic hepatitis C virus infections during and after therapy with alpha interferon. Antimicrob Agents Chemother 1993;37:595-7.
9. Brouwer JT, Nevens F, Kleter B, Elewaut A, Adler M, Brenard R, et al. Efficacy of interferon dose and prediction of response in chronic hepatitis C: Benelux study in 336 patients. J Hepatol 1998;28:951-9.
10. Brouwer JT, Nevens F, Michielsen P, Hautekeete ML, Chamuleau RAFM, Adler M, et al. What options are left when hepatitis C does not respond to interferon? Placebo controlled Benelux multicentre retreatment trial on ribavirin monotherapy versus combination with interferon. J Hepatol 1994;21 (suppl 1):S17 Abstract.
11. Kleter GE, van Doorn LJ, Brouwer JT, Schalm SW, Heijtink RA, Quint WG. Sequence analysis of the 5' untranslated region in isolates of at least four genotypes of hepatitis C virus in The Netherlands. J Clin Microbiol 1994;32:306-10.
12. Lindsay KL. Therapy of hepatitis C: overview. Hepatology 1997;26:71S-7S.
13. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837-45.
14. Poynard T, Leroy V, Cohard M, Thevenot T, Mathurin P, Opolon P, et al. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effects of dose and duration. Hepatology 1996;24:778-89.
15. Schalm SW, Hansen BE, Chemello L, Bellobuono A, Brouwer JT, Weiland O, et al. Ribavirin enhances the efficacy but not the adverse effects of interferon in chronic hepatitis C. Meta-analysis of individual patient data from European centres. J Hepatol 1997;26:961-6.
16. Reichard O, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Randomised, double-blind, placebo-controlled trial of interferon alpha- 2b with and without ribavirin for chronic hepatitis C. The Swedish Study Group. Lancet 1998;351:83-7.
17. Gavier B, Martinez-Gonzalez MA, Riezu-Boj JI, Lasarte JJ, Garcia N, Civeira MP, et al. Viremia after one month of interferon therapy predicts treatment outcome in patients with chronic hepatitis C. Gastroenterology 1997;113:1647-53.
18. Zeuzem S, Lee JH, Franke A, Ruster B, Prummer O, Herrmann G, et al.

Quantification of the initial decline of serum hepatitis C virus RNA and response to interferon alfa. Hepatology 1998;27:1149-56.
19. Pawlotsky JM. Measuring hepatitis C viremia in clinical samples: can we trust the assays? Hepatology 1997;26:1-4.
20. Damen M, Cuypers HT, Zaaijer HL, et al. International collaborative study on the second EUROHEP HCV-RNA reference panel. J Virol Methods 1996;58:175-85.
21. Iino S, Hino K, Kuroki T, Suzuki H, Yamamoto S. Treatment of chronic hepatitis C with high-dose interferon alpha-2b. A multicenter study. Dig Dis Sci 1993;38:612-8.
22. Bekkering FC, Brouwer JT, Leroux-Roels G, Vlierberghe H van, Elewaut A, Schalm SW. Ultrarapid hepatitis C virus clearance by daily high-dose interferon in non-responders to standard therapy. J Hepatol 1998;28:960-4

Chapter 7

Reduction of relapse rates by 18-month treatment in chronic hepatitis C. A Benelux randomized trial in 300 patients

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Journal of Hepatology 2004; 40: 689–695

ABSTRACT

Background /Aims Treatment of chronic hepatitis C with Interferon can be ineffective due to relapse. We aimed to reduce the 40% relapse rate of 6 months interferon–ribavirin combination therapy by prolonging treatment to 18 months.

Methods Three hundred patients with treatment-naive hepatitis C, were randomized to 18 months combination therapy with Interferon (3 MU tiw) and Ribavirin (1000–1200 mg/day), 18 months Interferon combined with placebo, or 6 months combination therapy with Interferon and Ribavirin, in a double blinded manner. All 295 patients who received at least one dose of treatment were included in the intention to treat analysis.

Results At the end of treatment, HCV RNA was undetectable in 55 and 49% of those on 6 and 18 months combination therapy, respectively, versus 26% of those on monotherapy (p<0.001). The relapse rate was 38% for 6 months combination therapy, 38% for 18 months monotherapy, and only 13% for 18 months combination treatment (p=0.002). The sustained response rates were 34% for 6 months combination therapy, 16% for 18 months monotherapy and 43% for 18 months combination therapy (p<0.05).

Interpretation Reduction of relapse rates to 15 percent or less is feasible by prolongation of interferon–ribavirin treatment to 18 months.

INTRODUCTION

Sustained virological response to treatment of chronic viral hepatitis C (HCV) is hampered predominantly by two biological phenomena: incomplete efficacy of interferon (IFN) in blocking viral replication, resulting in an initial virological non-response, and incomplete immune-mediated removal leading to relapse after treatment withdrawal (1–3). The introduction of Pegylated-interferon (PEG-IFN) has reduced the initial virological non-response significantly, but did not affect relapse rates (4–7). Ribavirin leads to both an enhanced initial response and a reduction of relapse, especially in genotype 2 and 3 patients (8–10). However, relapse rates remain 30 to 40% in genotype 1, even with optimal dosed PEG-IFN and Ribavirin combination therapy (11–13). Relapse is also significantly related to duration of treatment (14). The first indications that relapse rates could be reduced by prolonging therapy beyond 12 months came from IFN mono-therapy studies (15,16). The Benelux study presented here aimed at reducing the relapse rate in IFN-Ribavirin combination therapy by prolonging the treatment duration from 6 months (the expected standard at the time of the design) to 18 months. To assess whether a comparable reduction of relapse could be achieved by prolonged IFN monotherapy, the 6 months combination therapy was compared not only to 18 months combination therapy, but also to 18 months of IFN mono-therapy.

MATERIALS AND METHODS

Patients

Patients aged 18 years or older were eligible if they had biopsy documented chronic hepatitis C (anti-HCV antibodies, detectable HCV RNA, compatible liver biopsy in the past 12 months) and elevated serum ALT levels on at least 2 occasions within the 2 months prior to study entry. Patients were excluded if they had had previous antiviral treatment, decompensated liver disease, co-infection with HBV or HIV, other causes of liver disease, cytopenia (Hb below lower limit of normal, platelet count $<100x10^9$/l, granulocyte count $<1.5x10^9$/l), substance abuse or any other significant medical illness that might interfere with the trial.

The study was initiated in 25 centers in Belgium, the Netherlands and Luxembourg. The study was approved by the institutional review board of each participating center and written informed consent was obtained from each patient before entry.

Study design

The sample size was based on an estimated sustained response rate of 65% for long-term combination therapy, 40% for short-term combination therapy and 25% for long-term monotherapy. It was calculated that 83 patients per group were needed in order to have a power of 80 percent to detect a difference of 25 percent in sustained response rate between long-term combination therapy and the other two groups at a 2.5 percent level of significance (two two-sided tests with Bonferroni correction for multiple testing). To account for a potential dropout rate of about 10%, the study size was set to 300 patients.

Patients who met the inclusion criteria were randomly assigned to one of the three treatment arms in a ratio of 1:1:1 with stratification for center and presence or absence of cirrhosis. Treatment arms consisted of Interferon alpha-$_{2b}$ (Intron-A, Schering-Plough, Kenilworth, NJ, USA) 3 million units thrice weekly combined with ribavirin (Rebetol, Schering-Plough) 1200 mg/day (1000 mg if bodyweight < 75 kg) for either 6 months (short-term combination therapy) or 18 months (long-term combination therapy), or in combination with placebo for 18 months (long-term monotherapy). The study was performed double-blinded. At randomization the patient received a trial-number, which corresponded to blinded study medication containing either Ribavirin or placebo and to a closed envelop at the coordination

center with information on allocated treatment duration. At 6 months the envelop was opened and the treating physician was informed whether the patient had to stop. Patients were allowed to discontinue therapy at month 6 if HCV RNA remained detectable with ALT levels at least 1.5 times the upper limit of normal. Criteria for 50% dose reduction were Hb <10 g/dl or <6.2 mmol/l (ribavirin), WBC <1.5x10^9/l (IFN), platelets <50x10^9/l (IFN) or intolerable side effects.

The randomization code of the study remained concealed until all patients had completed their follow-up and the database had been verified and closed.

Assessments and study end points

Patients were seen at treatment week 2 and 4, every month till month 6 and every 3 months thereafter; follow-up visits were at 3 and 6 months after the end of therapy. The efficacy end-points were virological response, defined as undetectable HCV RNA at the end of treatment and the end of 6 months follow-up. HCV RNA was measured at a single central laboratory by rt-PCR assay with a sensitivity of 100 copies per milliliter (National Genetics Institute, Los Angeles, USA) (17). The HCV genotype was determined with the INNO-LiPA HCV second generation assay (Innogenetics, Zwijnaarde, Belgium). Hematologic and biochemical tests were performed in the certified laboratories of the participating hospitals; results were corrected for local normal values. Liver biopsies, taken within 12 months before treatment, were reviewed by the local pathologists for the presence or absence of cirrhosis.

All patients who were randomized and who received at least one dose of study medication were included in the intention to treat analysis. Patients with missing follow-up data were classified as remaining HCV RNA positive. In addition, a per protocol analysis was performed including all evaluable patients who completed their treatment and follow-up or who had dose reduction or discontinued prematurely according to the criteria of the protocol.

Statistical analysis

The baseline characteristics of the treatment groups were compared with use of the chi-square test or the Wilcoxon rank-sum test. The treatment responses were compared with use of the chi-square test or the Fisher's exact test where appropriate. The relapse rate was calculated by the fraction of individuals with undetectable HCV RNA at the end of treatment who had detectable HCV RNA after 6 months follow-up. The relation between pretreatment variables and response or relapse was examined by backwards stepwise logistic regression. The analysis was performed using SPSS v. 10 software (SPSS Inc, Chicago, IL, USA).

RESULTS

Between March 1996 and April 1997, 300 patients were enrolled in the study. Of those, 295 patients received at least one dose of study medication and were included in the intention to treat analysis. The groups were well balanced (Table 1). With regard to poor-response characteristics, 71% had a genotype 1 infection, 61% had a viral load $\geq 2x10^6$ copies/ml and 14% had liver cirrhosis.

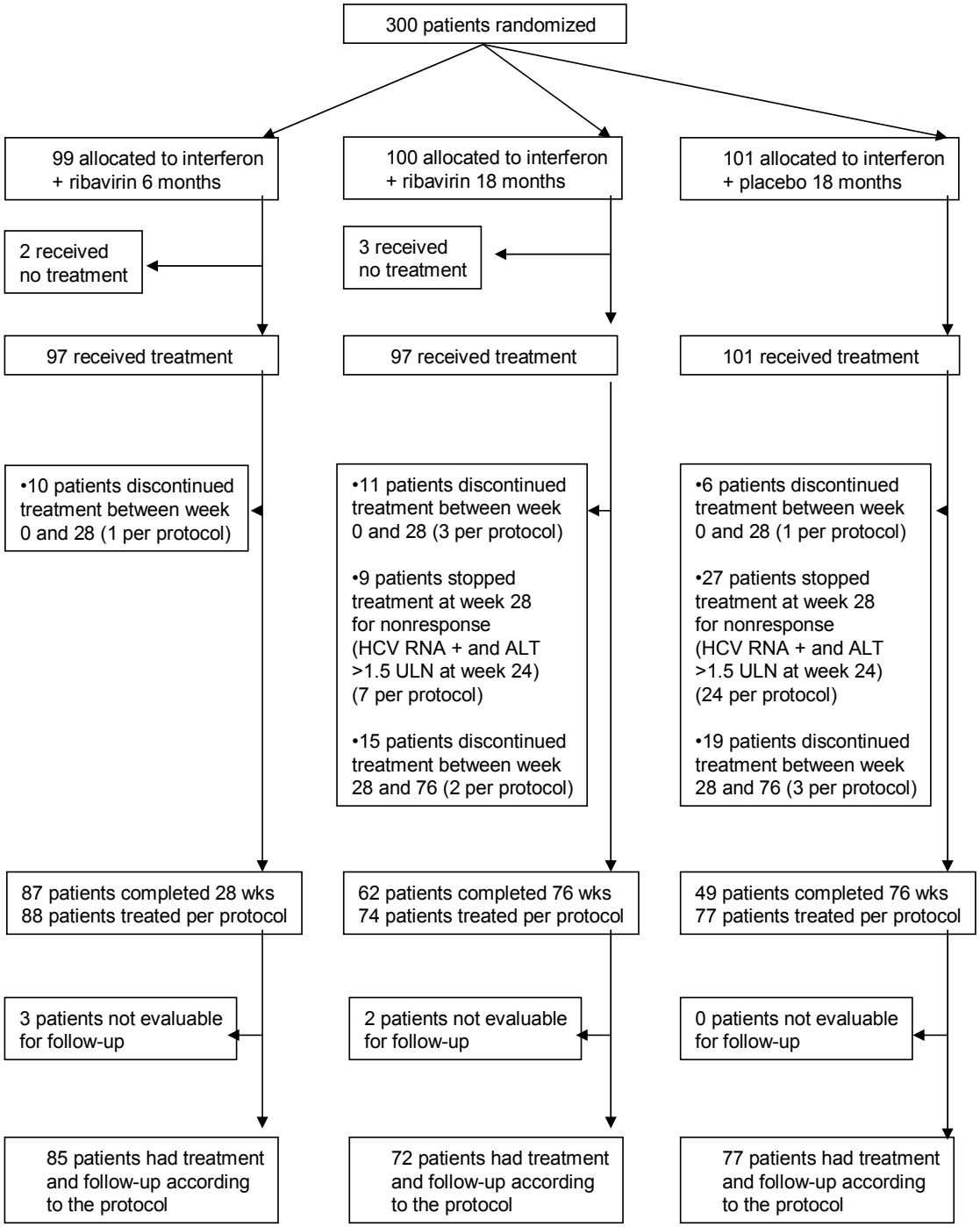
During the first 6 months, 11% of patients on combination therapy and 6% of those on interferon monotherapy discontinued treatment because of adverse events or noncompliance (Figure 1) (n.s.). Seventy-two out of 97 patients on 18 months combination therapy (74%), 77 patients out of 101 on 18 months monotherapy (76%) and 85 patients out of 97 on 6 months combination therapy (88%) completed treatment and follow-up, had dose reduction or withdrew from treatment in accordance with the protocol and were included in the per protocol analysis (*n*=234).

Table 1 Base-line characteristics of the patients (intention-to-treat cohort)

Characteristic[a]	Interferon and Ribavirin 6 months (*n*=97)	Interferon and Ribavirin 18 months (*n*=97)	Interferon and placebo 18 months (*n*=101)
Age (yr)	43 ± 12	47 ± 14	45 ± 14
Sex (M/F)	43/54	61/36	64/37
Weight (kg)	73.0 ± 16	74.2 ± 14	74.5 ± 13
Serum ALT – times ULN	2.9 ± 2.1	3.1 ± 1.9	3.6 ± 2.7
Serum HCV RNA			
copies/ml – log10	6.3 ± 0.7	6.4 ± 0.7	6.3 ± 0.8
$\geq 2 \times 10^6$ copies/ml – %	54	68	61
Genotype – *n* (%)			
1	73 (75)	68 (70)	68 (67)
2 or 3	18 (19)	22 (23)	23 (23)
other	6 (6)	7 (7)	10 (10)
Cirrhosis – *n* (%)	12 (12)	15 (16)	14 (14)

[a] Plus–minus values are means ± SD.

Figure 1 Trial profile

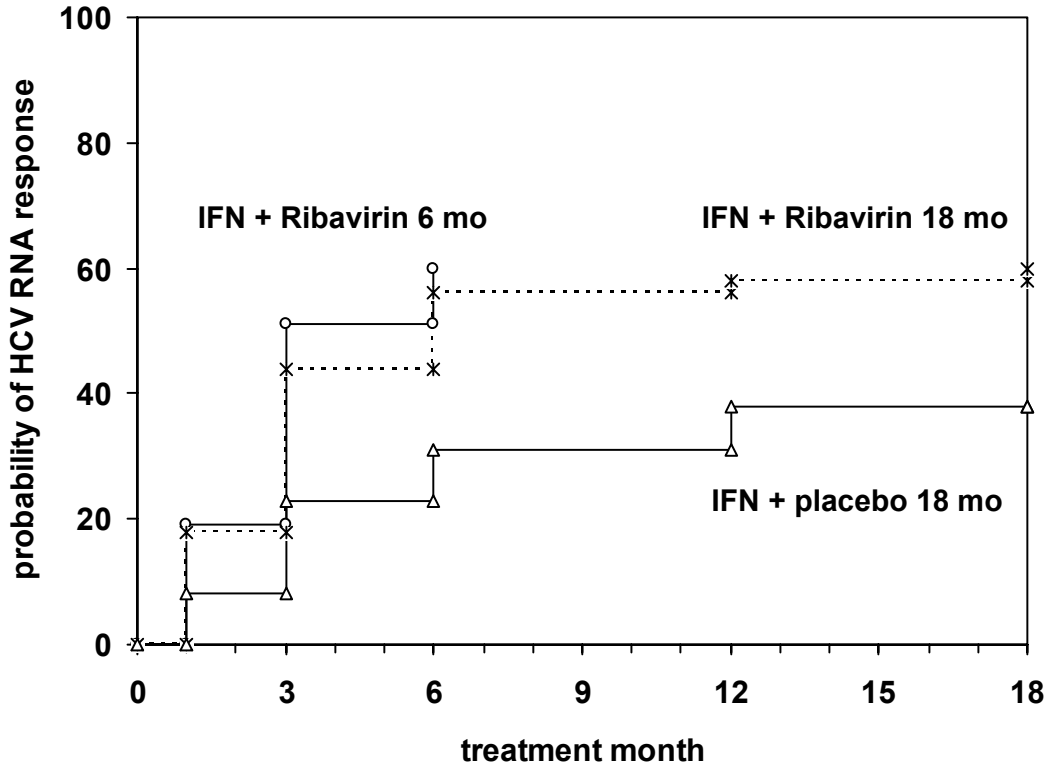


Virologic responses

More than 80% of the virologic responses on combination therapy and 70% of those on monotherapy occurred within the first 3 months of treatment (Fig. 2). Almost no increase in HCV RNA response was observed in prolonged combination therapy after month 6.

A breakthrough with recurrence of detectable HCV RNA before the end of treatment occurred in 8% of patients on combination therapy and 24% of those on IFN monotherapy (p=0.015). At the end of treatment, HCV RNA was undetectable in 49% (95% C.I. 39–60%) of patients on long-term combination therapy, 55% (45–65%) of those on short-term combination therapy and 26% (18–35%) of those on long-term monotherapy (p<0.001).

Figure 2



Cumulative probability of HCV RNA response during treatment. Total study population, per treatment group

The HCV RNA relapse rate after 18 months combination therapy was 13% (5–25%), compared to 38% (20–59%) with 6 months combination therapy (p=0.006) and 38% (25–52%) with 18 months monotherapy (p=0.017) (Table 2).

Table 2 Relation of baseline characteristics to HCV RNA relapse during follow-up in the 127 patients who had a HCV RNA response at the end of treatment

Characteristic[a]	Interferon and ribavirin 6 months (n=53)	Interferon and ribavirin 18 months (n=48)	Interferon and placebo 18 months (n=26)
Total HCV RNA relapse (%)			
Intention to treat	20/53 (38)	6/48 (13)[b,c]	10/26 (38)
Per protocol[d]	20/50 (40)	3/39 (8)[b,c]	6/19 (32)
Cirrhosis			
N	17/49 (35)	6/40 (15)[b,c]	8/22 (36)
Y	3/4	0/8[b,c]	2/4
Serum HCV RNA			
<2 x 10^6 copies/ml	7/28 (25)	2/17 (12)	1/10 (10)
≥2 x 10^6 copies/ml	13/23 (57)	4/29 (14)[b,c]	8/14 (57)
Genotype			
1 Intention to treat	16/34 (47)	4/29 (14)[b,c]	6/15 (40)
Per protocol[d]	16/32 (50)	1/25 (4)[b]	2/10 (20)
2 or 3	3/16 (19)	2/16 (13)	4/10 (40)
other	1/3	0/3	0/1

[a] Intention to treat analysis was used in all cases unless stated otherwise
[b] Significant difference between 18 months IFN + ribavirin and 6 months IFN + ribavirin (p<0.05)
[c] Significant difference between 18 months IFN + ribavirin and 18 months IFN + placebo (p<0.05)
[d] Treated, had dose reduction or stopped earlier, according to the criteria of the protocol (included dose reduction or stopping earlier because of nonresponse or persisting side-effects despite dose reduction; excluded non-compliance, protocol violations, non-classifiable because of incomplete follow-up)

A sustained HCV RNA response was achieved in 43% (33-53%) of patients on long-term combination therapy, 34% (24-44%) of those on short-term combination therapy and only 16% (9-23%) of those on long-term monotherapy (p<0.001) (Table 3).

Table 3 Relation of baseline characteristics to sustained HCV RNA response

Characteristic[a]	Interferon and ribavirin 6 months (n=97)	Interferon and ribavirin 18 months (n=97)	Interferon and placebo 18 months (n=101)
Total sustained response (%)			
Intention to treat	33/97 (34)	42/97 (43)[b]	16/101 (16)
Per protocol[c]	30/85 (35)	36/72 (50)[b,d]	13/77 (17)
Cirrhosis			
N	32/85 (38)	34/82 (41)[b]	14/86 (16)
Y	1/12 (8)	8/15 (53)[b,d]	2/14 (14)
Serum HCV RNA			
<2 x 10^6 copies/ml	21/42 (50)	15/30 (50)[b]	9/37 (24)
≥2 x 10^6 copies/ml	10/50 (20)	25/63 (40)[b,d]	6/58 (10)
Genotype			
1 Intention to treat	18/73 (25)	25/68 (37)[b]	9/68 (13)
Per protocol[c]	16/63 (25)	24/55 (44)[b,d]	8/50 (16)
2 or 3	13/18 (72)	14/22 (64)[b]	6/23 (26)
other	2/6	3/7	1/10 (10)

[a] Intention to treat analysis was used in all cases unless stated otherwise
[b] Significant difference between 18 mo IFN + Ribavirin and 18 mo IFN + placebo (p<0.05)
[c] Treated, had dose reduction or stopped earlier, according to the criteria of the protocol
[d] Significant difference between 18 mo IFN + Ribavirin and 6 mo IFN + Ribavirin (p<0.05)

Factors associated with relapse and sustained response

Table 2 shows the relation of baseline characteristics to HCV RNA relapse in the 127 patients who had a response at the end of treatment. Eighteen months combination therapy led to a significant reduction of relapse in all poor response categories (cirrhosis, high viral load, genotype 1). In fact, the relapse rates after 18 months combination therapy approached those of the favorable categories (no cirrhosis, low viral load, genotype 2 or 3). This effect was visible both in early responders, who got undetectable HCV RNA within the first 3 months of treatment, and late responders. Relapse rates in early responders were 8% (2–21%) with 18 months combination therapy versus 30% (17–46%) in 6 months combination treatment and 28% (10–53%) in 18 months monotherapy *(p*=0.03). In late responders, relapse rates were 30% (7–65%) with 18 months combination therapy versus 70% (35–93%) with 6 months combination therapy and 63% (24–91%) with 18 months IFN monotherapy (*p*=0.2). Factors associated with sustained response (SR) in the univariate analysis are shown in Table 3.

In the multivariate analysis, the only significant factor which influenced the response for genotypes 2 and 3 was the additional use of ribavirin: IFN monotherapy led to a significant lower on-treatment response (odds ratio 0.1, 95% C.I. 0.02–0.5, *p*=0.007) and sustained response (OR 0.1, C.I. 0.02–0.5, *p*=0.004) compared to combination therapy. Neither any of the pretreatment factors (gender, cirrhosis, viral load) nor the prolongation of combination treatment from 6 to 18 months significantly affected the response and relapse rate in genotype 2 or 3.

For genotype 1, factors which had an independent effect on the response and relapse rates were the type of treatment and the pre-treatment viral load. Prolongation of treatment from 6 to 18 months significantly reduced the chance of relapse, both after IFN monotherapy (OR 0.03, C.I. 0.002–0.6, *p*=0.02) and after IFN-Ribavirin combination therapy (OR 0.01, 95% C.I. 0.001–0.1, *p*<0.001). The resulting sustained response rate for 18 months IFN monotherapy did not differ significantly from that of 6 months combination therapy. In contrast, 18 months combination therapy in genotype 1 patients led to a significantly increased chance of sustained response, both in comparison to 18 months IFN monotherapy (OR 6, C.I. 2–19, *p*=0.001) and in comparison to 6 months combination treatment (OR 4, C.I. 2–10, *p*=0.004).

Patients with genotype 1 and a high viral load (≥2 x10^6 copies/ml) had, compared to those with a low viral load, a reduced chance of response during treatment (OR 0.4, C.I. 0.2–0.9, *p*=0.02), a markedly increased risk for relapse (OR 18, C.I. 3–121, *p*=0.03) and a significantly reduced chance for sustained response (OR 0.2, C.I. 0.09–0.5, *p*<0.01).

Adverse events

Ten percent of patients in the group with 6 months combination therapy, 25% of those with 18 months combination therapy and 25% of those with 18 months IFN monotherapy discontinued their treatment prematurely because of adverse events or because of noncompliance (Figure 1). The profile of adverse events for monotherapy and combination therapy was comparable to that of previous reports (9,10) (data not shown). Adverse events leading to treatment withdrawal and/or hospitalisation occurred in six cases with 6 months combination therapy, in 15 cases with 18 months combination therapy (eight before month 6, four between month 6 and 12, two after month 12 and one in the follow-up period) and in six cases with 18 months monotherapy (four before month 6, one between month 6 and 12, and one thereafter).

DISCUSSION

Treatment results in chronic hepatitis C have considerably improved over the last decennium, but the efficacy of therapy is still limited for the most common genotype 1 due to a low response rate during therapy and a high relapse rate after therapy.

Efforts have been made to improve the initial response rate by giving higher and daily IFN dosages as induction treatment (18,19). Although induction leads to a higher initial response, this effect is often lost after dose reduction (20–23). Prolonged induction treatment is still under consideration (24,25).

The documentation that relapse could be reduced by prolongation of treatment came from the early HCV studies, which were assessed by the ALT response (26). The studies of Poynard et al. (15) and Lin et al. (16) showed a reduction of ALT relapse from around 70% with 6 months IFN monotherapy to 50% with 18 months treatment, respectively, 46% with 24 months therapy. The marked effect of combining interferon with ribavirin with reduction of relapse rates by 50% or more (8–10,27) appeared to negate the need for therapy beyond 12 months.

Recent experiences with PegInterferon (PEG-IFN) and ribavirin have shown (11–13) that such treatment of patients with genotype 2 or 3 is associated with cure rates over 80 percent. However, even with 12 months of PEG-IFN plus ribavirin, sustained response rates in genotype 1 remain less than 50% which in part is due to the fact that the relapse rates remain 30 percent or higher (11). PEG-IFN leads to a higher initial response but not to reduction of relapse rates (4–7). The Benelux study presented here shows that by prolonging the duration of IFN–ribavirin combination therapy to 18 months the relapse rate in genotype 1 patients could be reduced to less than 14%, which is comparable to current treatment results in genotype 2/3 patients.

How valid are these results? The patient population included is large enough to prevent bias by small numbers. The patients were recruited from 25 major hospitals in 3 countries, and no special selection process was used. In fact, poor response criteria like cirrhosis and genotype 1 were more prevalent than in several recently performed studies. The study was designed and performed at the same time as the IFN–ribavirin pivotal studies by Poynard et al. (9) and McHutchison et al. (10) and made use of the same brand of interferon and the same central laboratory for HCV RNA determination (17). Although our study did not include an arm with 12 months

IFN plus ribavirin, results for the 6 months combination treatment are comparable with those of 6 months in the above-mentioned studies, whereas results for 18 months combination therapy in genotype 1 patients appear to be better than those reported for 12 months. When we compare the results of the studies with 6 months combination treatment, the results of the Benelux study are with 25% sustained response for genotype 1 and 72% for genotype 2/3 well within the range of 16%–23% for genotype 1 and 50–69% for genotype 2/3 found in the pivotal studies. More specific, the relapse rate of 47% in genotype 1 and 19% in genotype 2/3 is comparable with the relapse rates of 44%, respectively, 19% in the only 6 months combination study from which details on relapse per genotype could be retrieved (8).

Response and relapse rates for genotypes 2 and 3 do not change substantially by prolongation of treatment. However, for genotype 1 they do. Sustained response rates associated with IFN–ribavirin combination treatment in genotype 1 increase from 16–25% with 6 months therapy to 28–33% with 12 months therapy (8–10). The relative contribution of increased end-of-therapy response versus reduction of relapse is poorly defined, but in one study the relapse rate in genotype 1 patients after 12 months IFN–ribavirin combination therapy was still about 40% (25). In this Benelux study-arm with 18 months combination therapy, the relapse rate is reduced to 14%. This is not merely an effect of prolongation of the interferon part of the treatment, as prolongation of IFN monotherapy to 18 months reduces relapse in genotype 1 to only 40%.

Do the benefits of prolonged treatment outweigh the additional costs and exposure of patients to potential side effects? Studies on combination therapy suggest that prolongation of treatment is especially cost-effective in patients with a poor response profile, like genotype 1 and/or a high viral load (28–30). In our study, 25% of patients withdrew from 18 months combination therapy prematurely because of side effects or noncompliance, which is comparable to the 21-27% reported by the pivotal studies (9, 10). Interestingly, most of the serious adverse events occurred in our study during the first 6 months of treatment, and only 2 cases were reported between month 12 and 18.

Are these observations still of value in the PEG-IFN era? Interestingly enough, combination treatment with PEG-IFN and ribavirin increases the on-treatment response and thereby the sustained response rates in genotype 1, but does not affect the relapse rates in a major way. Hadziyannis et.al (11) reported relapse rates

of 48% for 6 months therapy and 27% for 12 months, which is comparable to relapse rates with regular combination therapy. The highly significant reduction in relapse rate observed in our study led to a borderline significant 9–15% increase of sustained virological response. The effect of relapse reduction on the sustained response rate will increase as the percentage of end-of-treatment response increase, as it is now the case with pegylated–IFN–ribavirin combination. Therefore, prolongation of treatment might be a worthwhile option especially for patients with a poor response profile including the presence of cirrhosis and/or a high pretreatment viral load with genotype 1. These observations regarding the potential benefit of prolonged antiviral therapy in the PEG-IFN era are yet to be proven and should be explored in new prospective trials on prolonged therapy with pegylated interferons and ribavirin.

ACKNOWLEDGEMENTS

Participants in the Benelux Study Group on Treatment of Chronic Hepatitis C were:

JT Brouwer[1-2], F Nevens[3], FC Bekkering[2], J Henrion[4], V Lefebvre[5], C Weegink[6], J Delwaide[7], A Elewaut[8], MC Rasch[9], N Bourgeois[10], R Brénard[11], H Van Vlierberghe[8], J Weber[12], M Adler[10], A Hayani[13], ML Hautekeete[†8], B Servais[14], C de Galoscy[15], CMJ van Nieuwkerk[16], JW den Ouden-Muller[17], M Delhaye[10], M Van Gossum[18], P Michielsen[19], J Pirotte[7], RA de Vries[20], P Druez[11], Ph Golstein[10], JW Jongebreur[21], SH Yap[3], C Brixko[22], J Fevery[3], Y Horsmans[11], DPF van Houte[23], MJ Kerbert-Dreteler[24], CJ Mulder[20], De Reuck[18], PJ Wismans[25], BJM Witteman[26], AP Geubel[11], J van Hattum[9], G Leroux-Roels[8], RAFM Chamuleau[6], HW Reesink[6], CM Bronkhorst[27], FJW ten Kate[6], ADME Osterhaus[2], WCJ Hop[2], HGM Niesters[2], BE Hansen[2], SW Schalm[2].

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The authors are grateful to Schering-Plough Research International, Kenilworth, USA & Schering Plough Benelux, Brussels, Belgium, for financial support and Mrs. AM Otte-Denys for excellent support in quality assurance and logistics.

REFERENCES

1. Zeuzem S, Schmidt JM, Lee JH, Ruster B, Roth WK. Effect of interferon alfa on the dynamics of hepatitis C virus turnover in vivo. Hepatology 1996;23(2):366-371.
2. Bekkering FC, Brouwer JT, Schalm SW, Elewaut A. Hepatitis C: viral kinetics. Hepatology 1997;26(6):1691-3.
3. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science 1998;282(5386):103-7.
4. Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000;343(23):1666-72.
5. Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, Balart L, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000;343(23):1673-80.
6. Reddy KR, Wright TL, Pockros PJ, Shiffman M, Everson G, Reindollar R, et al. Efficacy and safety of pegylated (40-kd) interferon alpha-2a compared with interferon alpha-2a in noncirrhotic patients with chronic hepatitis C. Hepatology 2001;33(2):433-8.
7. Lindsay KL, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, et al. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology 2001;34(2):395-403.
8. Reichard O, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Randomised, double-blind, placebo-controlled trial of interferon alpha- 2b with and without ribavirin for chronic hepatitis C. The Swedish Study Group. Lancet 1998;351(9096):83-87.
9. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998;352(9138):1426-32.
10. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 1998;339(21):1485-92.
11. Hadziyannis SJ, Cheinquer T, Morgan T, Diago M, Jensen DM, Sette Jr. H, et al. Peginterferon alfa-2a (40kD) (Pegasys) in combination with Ribavirin (RBV): efficacy and safety results from a phase III, randomized, double-blind, multicentre study examing effect of duration of treatment and RBV dose. J Hepatol 2002;36(S1):3 (abstract).
12. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347(13):975-82.
13. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358(9286):958-65.
14. Poynard T, Leroy V, Cohard M, Thevenot T, Mathurin P, Opolon P, et al. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effects of dose and duration. Hepatology 1996;24(4):778-789.
15. Poynard T, Bedossa P, Chevallier M, Mathurin P, Lemonnier C, Trepo C, et al. A comparison of three interferon alfa-2b regimens for the long-term treatment of chronic non-A, non-B hepatitis. Multicenter Study Group. N Engl J Med 1995;332(22):1457-62.

16. Lin R, Roach E, Zimmerman M, Strasser S, Farrell GC. Interferon alfa-2b for chronic hepatitis C: effects of dose increment and duration of treatment on response rates. Results of the first multicentre Australian trial. Australia Hepatitis C Study Group. J Hepatol 1995;23(5):487-496.
17. Tong MJ, Hwang SJ, Lefkowitz M, Lee SD, Co RL, Conrad A, et al. Correlation of serum HCV RNA and alanine aminotransferase levels in chronic hepatitis C patients during treatment with ribavirin. J Gastroenterol Hepatol 1994;9(6):587-91.
18. Iino S, Hino K, Kuroki T, Suzuki H, Yamamoto S. Treatment of chronic hepatitis C with high-dose interferon alpha-2b. A multicenter study. Dig Dis Sci 1993;38(4):612-618.
19. Bekkering FC, Brouwer JT, Hansen BE, Schalm SW. Hepatitis C viral kinetics in difficult to treat patients receiving high dose interferon and ribavirin. J Hepatol 2001;34(3):435-40.
20. Brouwer JT, Nevens F, Kleter B, Elewaut A, Adler M, Brenard R, et al. Efficacy of interferon dose and prediction of response in chronic hepatitis C: Benelux study in 336 patients. J Hepatol 1998;28(6):951-9.
21. Bekkering FC, Neumann AU, Brouwer JT, Levi-Drummer RS, Schalm SW. Changes in anti-viral effectiveness of interferon after dose reduction in chronic hepatitis c patients: a case control study. BMC Gastroenterol 2001;1(1):14.
22. Pol S, Nalpas B, Bourliere M, Couzigou P, Tran A, Abergel A, et al. Combination of ribavirin and interferon-alfa surpasses high doses of interferon-alfa alone in patients with genotype-1b-related chronic hepatitis C. Hepatology 2000;31(6):1338-44.
23. de Ledinghen V, Trimoulet P, Winnock M, Foucher J, Bourliere M, Desmorat H, et al. Daily or three times a week interferon alfa-2b in combination with ribavirin or interferon alone for the treatment of patients with chronic hepatitis C. J Hepatol 2002;36(5):672-80.
24. Ferenci P, Brunner H, Nachbaur K, Datz C, Gschwantler M, Hofer H, et al. Combination of interferon induction therapy and ribavirin in chronic hepatitis C. Hepatology 2001;34(5):1006-11.
25. Mangia A, Santoro R, Piattelli M, Leandro G, Minerva N, Annese M, et al. High doses of interferon in combination with ribavirin are more effective than the standard regimen in patients with HCV genotype 1 chronic hepatitis. J Hepatol 2002;37(1):109-16.
26. Chemello L, Bonetti P, Cavalletto L, Talato F, Donadon V, Casarin P, et al. Randomized trial comparing three different regimens of alpha-2a-interferon in chronic hepatitis C. The TriVeneto Viral Hepatitis Group. Hepatology 1995;22(3):700-706.
27. Schalm SW, Weiland O, Hansen BE, Milella M, Lai MY, Hollander A, et al. Interferon-ribavirin for chronic hepatitis C with and without cirrhosis: analysis of individual patient data of six controlled trials. Eurohep Study Group for Viral Hepatitis. Gastroenterology 1999;117(2):408-13.
28. Younossi ZM, Singer ME, McHutchison JG, Shermock KM. Cost effectiveness of interferon alpha2b combined with ribavirin for the treatment of chronic hepatitis C. Hepatology 1999;30(5):1318-24.
29. Shepherd J, Waugh N, Hewitson P. Combination therapy (interferon alfa and ribavirin) in the treatment of chronic hepatitis C: a rapid and systematic review. Health Technol Assess 2000;4(33):1-67.
30. Wong JB, Poynard T, Ling MH, Albrecht JK, Pauker SG. Cost-effectiveness of 24 or 48 weeks of interferon alpha-2b alone or with ribavirin as initial treatment of chronic hepatitis C. International Hepatitis Interventional Therapy Group. Am J Gastroenterol 2000;95(6):1524-30.

Chapter 8

High dose induction and prolonged daily interferon plus ribavirin therapy improves effectiveness in HCV patients with unfavorable baseline characteristics

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ABSTRACT

Background and aims: Results of interferon-based treatments of chronic hepatitis C have markedly improved except for patients with unfavorable characteristics. To assess whether such patients can respond to modified interferon regimens we performed a meta-analysis on data of individual patients treated in our center with high-dose induction and prolonged daily interferon plus ribavirin.

Methods: Fifty-four patients selected for poor-response characteristics such as genotype 1, cirrhosis or non-response to previous therapy were enrolled sequentially to one of three therapy schedules. Treatment consisted of 10 MU interferon-a2b (IFN) daily for 2–4 weeks followed by daily 3–5 MU IFN until week 52 and 3 MU daily or thrice weekly until week 76 in combination with ribavirin.

Results: Sustained response rates varied between 75–83% for patients with 1 unfavorable characteristic (n=23). In patients with combinations of such characteristics, sustained response rates were 60%, but only 21% for genotype 1 and cirrhosis (n=14). Six patients (11%) stopped therapy because of adverse effects; clinically serious adverse events occurred in 2/7 patients with advanced cirrhosis.

Conclusion: High-dose induction and prolonged daily interferon–ribavirin therapy is an acceptable and potentially highly effective treatment modality for selected patients with unfavorable characteristics such as genotype 1, cirrhosis or previous non-response.

INTRODUCTION

The interferon-based treatment of chronic hepatitis C has made remarkable progress over the past ten years. Since the introduction of pegylated interferon (PEG-IFN) ribavirin combination therapy, results of large international trials indicate cure-rates of 77-82% in genotype 2 and 3 infection. However, the predominant genotype in many parts of the world is genotype 1 and the sustained response rate in this type of infection is still only 42-46% (1,2).

Morbidity and mortality of chronic hepatitis C is predominantly in patients with cirrhosis (3). Responses in patients with cirrhosis are generally less than in those without cirrhosis although treatment with pegylated interferon diminishes the difference (4). It is therefore of note that the large trials of interferon–ribavirin combination as well as those assessing PEG-IFN with or without ribavirin comprised only a small number of patients with cirrhosis (1,2,5,6).

For patients with unfavorable baseline characteristics, we started in 1998 a special treatment program of high induction dose (10 MU daily) of interferon (IFN) (7,8) followed by long-term daily IFN for 12-18 months in combination with ribavirin. To assess whether such treatment is still of clinical value for certain patient types in the era of PEG-IFN we performed a meta-analysis on individual treatment data of 54 patients with unfavorable baseline characteristics such as genotype 1 or 4, cirrhosis, non-response to IFN or combinations of these criteria treated in our center with high-dose induction and prolonged daily IFN plus ribavirin.

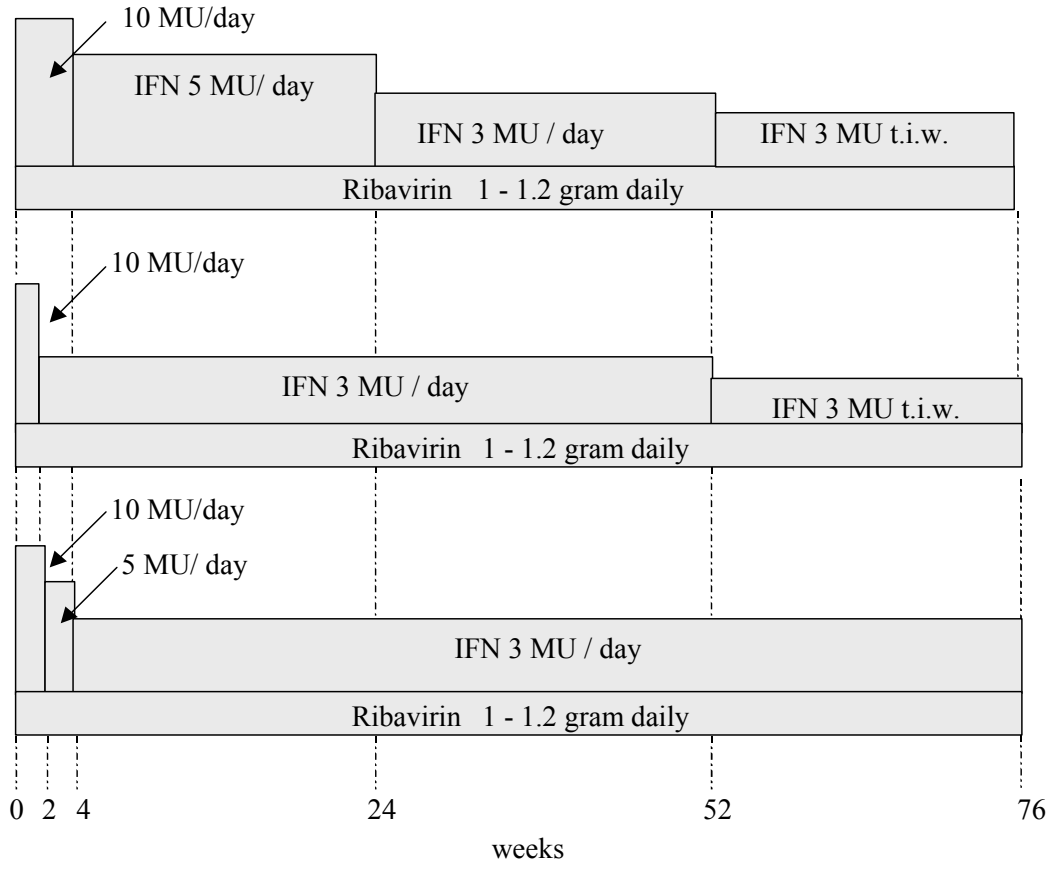
Three treatment schedules of high dose induction and prolonged daily IFN in combination with ribavirin were used. The initial study (9) was designed to explore by viral kinetics the IFN induction dose leading to a maximal initial response. The patients were treated with a relative intensive and long induction regimen of IFN. Subsequently, two other regimens were designed to preserve the favorable results in terms of viral kinetics but to reduce the intensity in terms of side effects and costs. In all three protocols IFN was given daily during at least 12 months in order to maintain a constant drug level and thereby maximize viral inhibition and to minimize virological breakthrough. Thereafter, treatment was continued up to 76 weeks in an attempt to reduce viral relapse (10,11).

PATIENTS AND METHODS

Study population

Between February 1998 and March 2001, 55 consecutive patients with documented chronic hepatitis C were enrolled in a program of high dose induction and prolonged daily IFN in combination with ribavirin therapy. The ethical committee approved the study and patients gave written informed consent for inclusion. Patients were eligible if their maximal chance of reaching a sustained response with standard therapy was considered less than 30% according to the 1998 EASL consensus statement (12). Such patients had genotype 1 or 4, cirrhosis, non-sustained response to prior treatment or combinations of these criteria. In this study non-sustained response was defined as a positive HCV-RNA serum test after at least 6 months of IFN monotherapy (3 MU tiw) or a relapse after 12 months of IFN–ribavirin combination treatment. Patients with clinical relevant concomitant diseases were excluded according to criteria previously described (11,13).

Figure 1 Study design



Study design

Patients were allocated successively to receive one of the following treatment regimens (Figure 1). The first 24 consecutive patients received 10 million units (MU) IFN-a2b (Intron A, Schering-Plough, Kenilworth, NJ, USA) daily for 4 weeks followed by 5 MU daily until week 24, 3 MU daily until week 52 and 3 MU thrice weekly until week 76 (group A). The next fifteen patients were assigned to receive 10 MU IFN-a2b daily for 10 days followed by 3 MU daily until week 52 and 3 MU thrice weekly until week 76 (group B). The following 16 patients were assigned to receive 10 MU IFN-a2b daily for 2 weeks followed by 5 MU daily until week 4 and 3 MU per day until week 76 (group C). Ribavirin was supplied to all patients as 200-mg capsules given orally in 2 divided daily doses of 1,000 mg (weight <75 kg) or 1,200 mg (weight ≥75 kg) throughout the entire treatment period.

All patients were admitted to the ward for the first 7 days of treatment and thereafter intensively monitored on an outpatient basis. Clinical and biochemical assessments were conducted every 4 to 6 weeks during therapy and during a 6-month follow-up period.

Dose adjustment was based on clinical intolerance, granulocytopenia below 0.5 x $10^6/mm^3$ (IFN) or hemoglobin below 6 mmol/l (ribavirin). In such cases interferon was reduced to the next level of the treatment schedule, ribavirin was reduced by steps of 200 mg/day. Combination therapy was discontinued in case of virological non-response, defined as detectable HCV RNA at week 12.

Histology

All patients underwent liver biopsy before the start of therapy. An experienced pathologist who was unaware of the clinical outcome evaluated each liver biopsy for the presence of cirrhosis, comparable to Metavir F4.

Virology

Blood was drawn at baseline and at week 4, 12, 16, 24, 48, 76, 88 and 100. Blood samples were collected in plasma preparation tubes (Becton-Dickenson, Plymouth, UK), which were spun directly after collection in order to avoid RNA breakdown.

A qualitative HCV RNA assay was performed in a single local laboratory to assess viremia (Cobas Amplicor HCV test, Roche Diagnostics, Almere, The Netherlands). Genotypes were identified before the start of treatment by in-house sequence analysis.

Descriptive analysis

The primary end point of this analysis was the rate of sustained response after a 24-week treatment free follow-up period. Results were calculated for the total population (intention-to-treat analysis). The breakthrough rate was calculated at the end of treatment for those reaching HCV RNA negativity during treatment and the relapse rate at 24 weeks of follow-up for those who were HCV RNA negative at the end of treatment.

Statistics

Data were analyzed using SPSS for Windows (Version 10.1 SPSS Inc, Chicago, IL, USA). Percentages are given with 95% confidence intervals (CI).

RESULTS

Patient characteristics

In table 1, baseline characteristics of the study population are shown per treatment schedule and for the whole group. One patient, who was assigned to treatment regimen C and who had a sustained response, did on retrospect not comply with the inclusion criteria associated with poor response and was excluded from the analysis. The intention-to-treat population (ITT) consisted of 54 patients of whom 44 completed the study (81%) according to the protocol.

The proportion of cases with cirrhosis was 46%. Genotype 1 was the predominant genotype (69%). More than half of the patients (54%) had a failure to prior IFN treatment. Fourteen patients (26%) had both a genotype 1 and cirrhosis. The majority of patients (81%) had a high viral load ($\geq 2 \times 10^6$ copies/ml).

Table 1 Baseline characteristics of the patients (intention-to-treat cohort)

Characteristic[a]	Treatment regimen			
	Group A (*n*=24)	Group B (*n*=15)	Group C (*n*=15)	Total (*n*=54)
Age (yr)	46 ± 10	45 ± 7	45 ± 10	45 ± 9
Sex (M/F)	17/7	11/4	12/3	40/14
ALT (IU/l)	103 ± 83	125 ± 78	117 ± 51	113 ± 73
Cirrhosis–*n* (%)	12 (50%)	1 (7%)	12 (80%)	25 (46%)
Serum HCV-RNA				
copies/ml	$1.2x10^7$	$3.2x10^7$	$7.9x10^6$	$1.6x10^7$
≥ 2 x 10^6 –*n* (%)	17 (71%)	14 (93%)	13 (87%)	44 (81%)
Genotype				
1 & 4[b] (%)	12 (50%)	15 (100%)	10 (67%)	37 (69%)
2 & 3 (%)	12 (50%)	0 (0%)	5 (33%)	17 (32%)
Prior treatment failure	15 (63%)	11 (73%)	3 (20%)	29 (54%)

[a] Plus–minus values are means ± SD.
[b] Only one patient had genotype 4.

Outcome

In table 2, intention-to-treat results are shown per treatment arm and for the total study population. The ITT sustained response rates were 67% (95% CI: 45-84%), 53% (95% CI: 27-79%), 47% (95% CI: 21-73%) and 57%(95% CI: 43-71%) for respectively treatment regimen A, B, C and the total study population. The outcomes for the three treatment regimens were not significantly different according to multivariate logistic regression analysis performed to correct for differences in baseline characteristics and study groups.

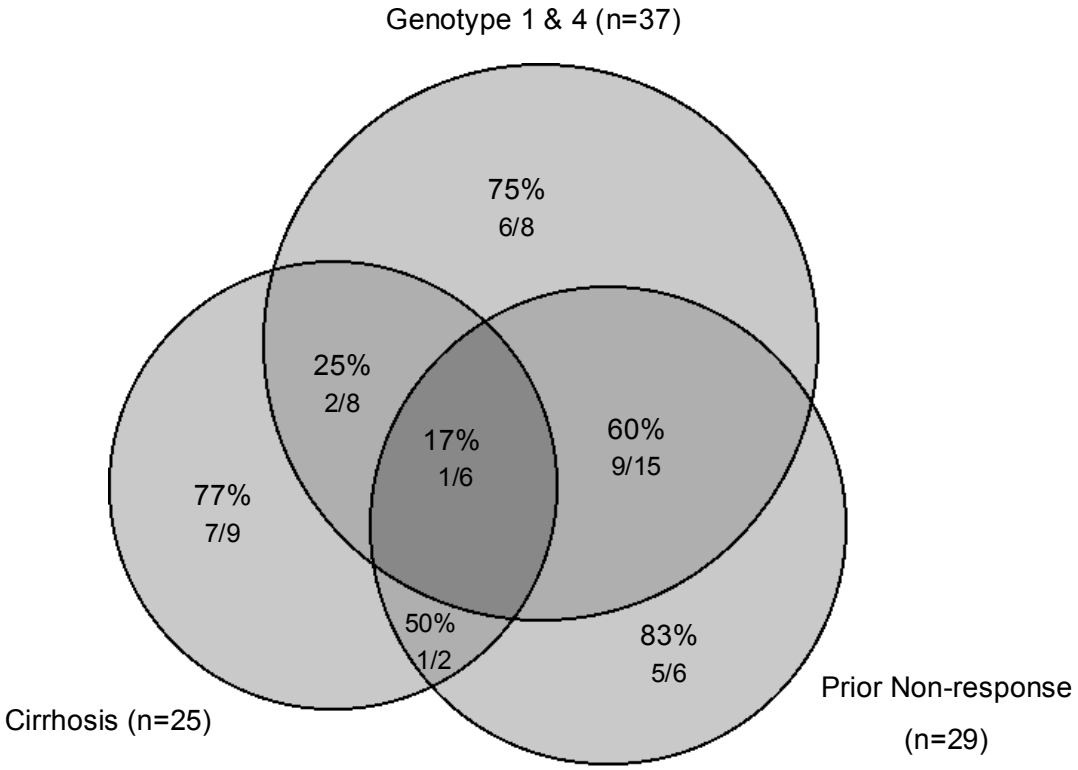
Table 2 Treatment outcome by treatment regimen and for the total study population (Intention to treat analysis)

Treatment regimen[a]	Group A *n*=24	Group B *n*=15	Group C *n*=15	Total[a] *n*=54
Non compliance (0 – 4 weeks)	1	1	2	4/54 (7%) *(2-18%)*
Non-response (at 12 weeks)	2	5	3	10/54 (19%) *(9-31%)*
Stop due adverse event (12 - 76 weeks)	4	0	2	6/54 (11%) *(4-23%)*
Breakthrough during treatment	0	1	1	2/54 (4%) *(0-13%)*
Relapse after treatment [b]	1	0	0	1/32 (3%) *(0-16%)*
Sustained response [a]	16 (67%) *(45-84%)*	8 (53%) *(27-79%)*	7 (47%) *(21-73%)*	31/54 (57%) *(43-71%)*

[a] numbers–*n*, (%), *(95% CI)*.
[b] The relapse rate was calculated at 24 weeks of follow-up for those who were HCV RNA negative at the end of treatment.

Patients with either cirrhosis, genotype 1/4 or non-response to prior therapy had sustained responses in 44% (95% CI: 24-65%), 49% (95% CI: 32-66%) and 55% (95% CI interval: 36-74%) respectively. When only 1 unfavorable characteristic was present, sustained response rates varied between 75-83%. In patients with combination of such criteria, sustained viral response rates were 60% (95% CI: 32-84%) for genotype 1 and previous non-response (n=15), but only 21% (95% CI: 5-51%) for genotype 1 and cirrhosis (n=14) irrespective of the presence or absence of previous non-response (fig. 2).

Figure 2 Sustained response rates per baseline characteristic(s) associated with unfavorable response



The sustained response for 1 unfavorable characteristic varied between 75-83%, and for 2 characteristics between 25-60%; the sustained response was 17% if 3 unfavorable characteristics were present.

Virological non-response (detectable HCV RNA at week 12 or later) occurred in only 10/54 (19%; 95% CI: 9-31%). Four patients were not compliant: they stopped in the first four weeks after discharge from the hospital. In six patients (11%; 95% CI: 4-23%), therapy was stopped because of adverse effects (hepatic decompensation (n=2), depression (n=2), cardiac complaints (n=1) and Staphylococcus sepsis (n=1)). Both patients that developed decompensated liver disease, had advanced cirrhosis: according to the Child-Pugh classification their baseline scores were 6, but both had elevated bilirubin and low platelets (3). All other adverse effects occurred within the first 8 weeks of treatment.

DISCUSSION

This study documents the efficacy rates of high dose induction and prolonged daily interferon plus ribavirin treatment in patients with baseline characteristics associated with poor response. Sustained response rates of 49%, 44% and 55% were observed for patients with either genotype 1, cirrhosis or non-response to previous therapy. Results with modern schedules of PEG-IFN-α and ribavirin yield similar percentages, but the baseline characteristics in our study population were vastly more unfavorable. In patients with combinations of these unfavorable criteria, sustained virological response rates were considerably decreased: 25% in patients with cirrhosis and genotype 1 and 17% in those who met all three criteria.

The studies were originally designed to explore the optimal IFN-α induction dose by viral kinetics (9,14,15). The first group of patients was treated with an intensive schedule resulting in a sustained response rate of 67% (15). The next two strategies were attempts to decrease morbidity and cost without loosing efficacy. However, by shortening the induction period adverse effects were somewhat decreased but the effectiveness was also reduced in some patients (14). No clear advantage of either treatment schedules B or C was found over treatment schedule A.

How can these favorable results be explained? First, in all three treatment groups, therapy was started with a daily high-dose interferon “induction” period in order to reach early HCV-RNA negativity. Early viral response is a strong predictor for sustained response (1,15,16). In this study, 17 patients were HCV-RNA negative after 4 weeks of treatment. Fourteen of them had a sustained response (82%) as compared to 17 out of 37 patients (46%) who had their HCV RNA response after week 4. Second, given the short half-life of IFN, daily IFN injections were given to obtain a more constant drug level in order to optimize viral inhibition and minimize viral breakthrough. The importance of constant antiviral pressure has become more clear since the introduction of PEG-IFN with its prolonged half-life (1,2). Third, we prolonged the treatment duration in order to prevent relapse (10,11). As a result, the relapse rate could be reduced to less than 5% in this study.

The intensive treatment schedules were expected to be associated with an increased rate of intolerance and side effects. Due to intensive monitoring and supportive care the rate of non-compliance could be kept low. Clinically serious adverse effects occurred in 2 patients with impaired liver function due to advanced cirrhosis. Since the sustained response rate in patients with advanced cirrhosis was limited to 1 out of 7, we conclude that risks outweigh benefits in this category of patients.

How should these results be implemented into future treatment schedules? The results of this study show that patients with either genotype 1, cirrhosis or non-response to previous therapy can benefit from high daily dosing and prolonged therapy and this treatment still remains an excellent option. In cirrhotic patients with genotype 1 the indication to treat should be made on an individual basis in view of the response rate of about 20%. Patients with advanced cirrhosis, in our view, should not be treated with this intensive regiment.

ACKNOWLEDGEMENT

This investigator-initiated study was supported by the Foundation of Liver Research as well as by a research grant from Schering-Plough, Kenilworth, NJ, USA.

REFERENCES

1. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., Haussinger D, et al. Peginterferon Alfa-2a plus Ribavirin for Chronic Hepatitis C Virus Infection. N Engl J Med 2002;347:975-982.
2. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958-965.
3. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, Nevens F, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterolgy 1997;112:463-472.
4. Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, Balart L, Reindollar R, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 2000;343:1673-1680.
5. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998;352:1426-1432.
6. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 1998;339:1485-1492.
7. Iino S, Hino K, Kuroki T, Suzuki H, Yamamoto S. Treatment of chronic hepatitis C with high-dose interferon alpha-2b. A multicenter study. Dig Dis Sci 1993;38:612-618.
8. Bekkering FC, Brouwer JT, Leroux-Roels G, Van Vlierberghe H, Elewaut A, Schalm SW. Ultrarapid hepatitis C virus clearance by daily high-dose interferon in non-responders to standard therapy. J Hepatol 1998;28:960-964.
9. Bekkering FC, Brouwer JT, Hansen BE, Schalm SW. Hepatitis C viral kinetics in difficult to treat patients receiving high dose interferon and ribavirin. J Hepatol 2001;34:435-440.
10. Lin R, Roach E, Zimmerman M, Strasser S, Farrell GC. Interferon alfa-2b for chronic hepatitis C: effects of dose increment and duration of treatment on response rates. Results of the first multicentre Australian trial. Australia Hepatitis C Study Group. J Hepatol 1995;23:487-496.
11. Brouwer JT, Nevens F, Bekkering FC, Bourgeois N, Van Vlierberghe H, Weegink J, Lefebvre V, et al. Reduction of relapse rates by 18 month treatment in chronic Hepatitis C. A Benelux randomized trial in 300 patients. J Hepatol 2004;40:689-695.
12. EASL International Consensus Conference on Hepatitis C. Paris, 26-28, February 1999, Consensus Statement. European Association for the Study of the Liver. J Hepatol 1999;Press release no.30:956-961.
13. Brouwer JT, Nevens F, Kleter B, Elewaut A, Adler M, Brenard R, Chamuleau RA, et al. Efficacy of interferon dose and prediction of response in chronic hepatitis C: Benelux study in 336 patients. J Hepatol 1998;28:951-959.
14. Bekkering FC, Neumann AU, Brouwer JT, Levi-Drummer RS, Schalm SW. Changes in anti-viral effectiveness of interferon after dose reduction in chronic hepatitis c patients: a case control study. BMC Gastroenterol 2001;1:14.
15. Vrolijk JM, Bekkering FC, Brouwer JT, Hansen BE, Schalm SW. High sustained virological response in chronic hepatitis C by combining induction and prolonged maintenance therapy. J Viral Hepat 2003;10:205-209.

16. Kleter GE, Brouwer JT, Heijtink RA, Schalm SW, Quint WG. Detection of hepatitis C virus RNA in patients with chronic hepatitis C virus infections during and after therapy with alpha interferon. Antimicrob Agents Chemother 1993;37:595-597.
17. Hadziyannis SJ, Cheinquer H, Morgan T, Diago M, Jensen DM, Sette J, Ramadori HC, et al. Peginterferon alfa-2a (40KD) (PEGASYS®) in combination with ribavirin (RBV): efficacy and safety results from a phase III, randomized, double-blind, multicentre study examining effect of duration of treatment and RBV dose. J Hepatol 2002;37:A536.

Chapter 9

General discussion and Summary

In this thesis a decade of clinical research on chronic hepatitis C is described. At the beginning, around 1990, the disease entity was clearly established but the hepatitis C virus was only identified the year before and serologic tests were just making their entrance (1,2). Interferon had been tested in non-A, non-B chronic viral hepatitis and found to induce normalization of serum transaminases in 50% of patients with persistence of serum ALT normality after stopping interferon therapy in 25% (3,4). The interferon trials were conducted with a fixed dose (2-3 Mega Units, three times a week) and fixed duration (6 months).

The hypothesis was formulated that the initial response might be higher with a two- to three-fold increase of the dose of interferon, and that relapse could be reduced by prolonging the duration of treatment according to the individual need.

Academic centers were eager to conduct clinical research parallel to pharmaceutical industry-driven trials; pharmaceutical companies were willing to provide financial support for consortia of academic units. Physicians in hepatology became aware of the necessity to enter a large number of patients into a trial in order to show differences in response of 15% or less. The need for multicenter studies became apparent. In that setting the Benelux studies on the treatment of chronic viral hepatitis C started with study 90-01.

Benelux study HCV 90-01

Precise aim
- enhance efficacy of standard interferon therapy by double IFN dose initially
- individualized treatment guided by ALT and HCV RNA response

Number of centers, number of patients
- 19 centers, 354 patients randomized, 336 treated

Two arms
- standard: IFN-a2b, 3 MU tiw, 6 months
- experimental: 6 MU tiw, 8 weeks, followed by downward titration to 3 and 1 MU tiw if ALT remained normal and treatment withdrawal if ALT remained normal and HCV RNA undetectable on at least 2 occasions during at least 8 weeks

Outcome measure
- primary: sustained ALT and HCV RNA response 6 months after treatment
- secondary: on-treatment HCV RNA and ALT response; predictors of response; change in histological activity index pre- versus post-treatment

Results
- SR (HCV RNA + ALT): 14% (9-21) standard vs 15% (10-21) experimental treatment
- HCV RNA response week 4: 37% standard vs 47% experimental (p=0.02)
- independent predictors of response: 1) pre-treatment: absence of cirrhosis, genotype 2/3, low viral load, 2) per-treatment: HCV RNA week 4 (negative predictive value 98.3%)
- paired pre- and post treatment biopsies could be retrieved for central scoring in 126 cases: SR response patients showed less (16%) progression of fibrosis than relapse (21%) or non-response (31%) patients; experimental therapy showed less (16%) progression of fibrosis than standard therapy (33%)

Implications
- an early HCV RNA response is a prerequisite for long-term efficacy
- doubling the interferon dose increases the initial response
- subsequent downward titration negates this effect, especially in genotype 1
- IFN treatment inhibits the progression of fibrosis, even in patients without a sustained response

While the Benelux study 90-01 explored a higher initial dose of interferon to increase the early biochemical and virological response, and adjusted the duration of therapy to the individual need to reduce relapse, new developments took place in other areas of the world.

In Japan elaborate dose response studies are the initial step in clinical evaluation and initial virological response up to 90% were reported with daily doses of 10 mega-unit of interferon (5). Since the total dose of interferon was fixed, the duration of therapy was short; the relapse rate was high.

In Sweden and the USA ribavirin, a nucleoside analogue licensed for suppressing another RNA virus: RSV (respiratory syncitial virus), was in evaluation in view of its efficacy in reducing serum ALT (6–8). Neither histological improvement nor reduction in plasma HCVRNA could be demonstrated and the interest in the drug was fading

In Italy standard interferon therapy (3 x week 3 Mega Units) was combined with ribavirin; a very important synergistic effect was reported in a single center uncontrolled study in 7 patients (9). The need to study combination therapy in a controlled manner was evident, as well as exploring whether daily high dose interferon could induce an early virological response in those not responding to standard 3x week 3 mega-units. Both concepts were tested in two subsequent Benelux studies (91-04, 91-07) in patients that came out the 90-01 study because of non-response.

Benelux study HCV 91-04

Precise aim
- can daily high dose IFN induce HCV RNA response in patients who are resistant to standard IFN treatment?

Number of centers, number of patients
- 3 centers, 22 HCV patients without ALT response during previous therapy
- subset of 10 virologic non-responders studied with frequent plasma sampling at 1 site

One arm
- 10 MU IFN daily for 5 days, 10 MU t.i.w. until week 4, followed by stepwise dose reduction in case of response or withdrawal in case of non-response

Outcome measure
- primary: sustained HCV RNA response, 6 months after the end of treatment
- secondary: early viral kinetics; predictors of response

Results
- sustained HCV RNA response in 4/22 patients (18%)
- 9/10 patients studied with viral kinetics had a ≥2 log drop in viral load at week 4
- predictors of SR were absence of cirrhosis, temporary viral response at previous therapy, and undetectable HCV RNA at week 4 (positive predictive value 80%, negative predictive value 100%)

Implications
- an early (week 4) HCV RNA response is a prerequisite for long-term efficacy
- true resistance to interferon is rare, other factors are involved in non-response to therapy which should be explored

Benelux study HCV 91-07

Precise aim
- can ribavirin as monotherapy or in combination to interferon enhance the disappearance-rate of plasma HCV-RNA and/or ALT normalization rate in patients who did not respond to Interferon therapy

Number of centers, number of patients
- 16 centers, 121 patients randomized, 117 treated

Three arms
- 6 months combination therapy with IFN 3MU tiw and ribavirin 1000-1200 mg/d
- 6 months mono therapy with ribavirin 1000-1200 mg/d
- 6 months mono therapy with ribavirin placebo

Outcome measure
- primary: sustained HCV RNA response, 6 months after the end of treatment
- secondary: HCV RNA and ALT response; acceptability and safety

Results
- end of therapy response 35% and sustained HCV RNA response 15% in combination therapy vs no response in ribavirin mono therapy or placebo
- on-treatment ALT response 58% in combination therapy, 23% in ribavirin, and 8% in placebo; sustained in 18%, 3%, and 3%, respectively
- withdrawal for side effects or non-compliance 13% of patients on combination therapy, 13% of those on ribavirin, and 11% of patients on placebo; mean decrease of Hb in ribavirin mono and combi therapy 15%

Implications
- interferon and ribavirin combination therapy has additional effectiveness in non-responders to IFN mono therapy
- a large part (almost 60%) of the response on combination therapy is lost when treatment is withdrawn at 6 months; longer treatment duration might be needed

Treatment results improved markedly with the introduction of ribavirin in 1993, and many small studies were started worldwide to evaluate the efficacy of combination therapy in non-responders and relapsers to prior IFN mono therapy and in naïve patients. A meta-analysis of individual patient data comprising more than 90% of the published data till 1997 showed that the efficacy of combination therapy was enhanced 2-3 fold over mono therapy, leading to a sustained response in about 50% of naïve patients and relapsers and in about 15% of non-responders to prior IFN monotherapy (10).

Although these results meant a major step forward for patients with chronic hepatitis C, still 50% of them had no long-term benefit from therapy. Awaiting further potential treatment improvements by modifications of dose and duration, it was important to identify in an early stage of treatment those patients who would eventually prove to be treatment failures, so that costs and unnecessary exposure to treatment and its potential side effects could be reduced and alternative therapies could be offered in an early stage. In 1997, both the European and the US consensus statements recommended to use a lack of ALT normalisation at 12 weeks

as a stopping rule (11,12). Although this criterium identified over 90% of eventual non-responders in an early stage, it also could lead to premature treatment withdrawal in almost 20% of potential sustained responders. Early Benelux studies (13,14) pointed to the importance of an early week 4 HCV RNA response. This criterium was evaluated in a large group of patients treated in Benelux studies 90-01 and 91-07.

Early prediction of response (Benelux studies HCV 90-01 and 91-07)

Precise aim
- to compare the predictive value for non-response of an early HCV RNA determination at week 4 with the standard week 12 ALT determination (recommended by the European and US consensus in 1997) in interferon monotherapy and in IFN ribavirin combination therapy

Number of centers, number of patients
- IFN monotherapy: 19 centers, 336 patients, 280 with a complete set of HCV RNA data at week 4 and ALT data at week 4, 8 and 12
- IFN ribavirin combination therapy: 16 centers, 40 patients, 31 with a complete data set of HCV RNA at week 4 and ALT at week 4, 8 and 12

Three arms
- IFN monotherapy: standard 3 MU IFN tiw for 6 months or experimental 6 MU tiw for 8 weeks followed by downward titration (study HCV 90-01)
- IFN ribavirin combination therapy: 3 MU IFN tiw in combination with 1000-1200 mg ribavirin daily, for 6 months (study HCV 91-07)

Outcome measure
- predictive value for non-response by week 4 HCV RNA (detection limit 10^3 copies/ml) and week 12 ALT
- fraction of potential responders lost if the test is used as a stopping rule

Results
- predictive value for non-response by week 4 HCV RNA: 99% (std IFN), 97% (exp IFN) and 100% (IFN ribavirin combi); by week 12 ALT: 97%, 91% and 95%, respectively
- fraction of potential responders lost if week 4 HCV RNA is used as stopping rule: 5%, 12% and 0%; if week 12 ALT is used: 10%, 28% and 20%, respectively

Implications
- use of a week 4 HCV RNA leads to a better prediction of non-response and a lower rate of missing potential responders than a week 12 ALT
- an early (week 4) HCV RNA response is a prerequisite for long-term efficacy

After the publication of two large pivotal trials in 1998 (15, 16), combination of IFN 3 MU tiw and ribavirin 1000-1200 mg/day became the standard treatment of chronic hepatitis C. Twenty-four weeks of combination therapy proved to be sufficient for genotypes 2 and 3, leading to sustained responses in 70-80% of patients, with no additional improvement after prolongation of therapy. However, for genotype 1 sustained response rates after 24 weeks of combination reached only up to 20%, due to limited on-treatment response and high relapse

rates up to 50% after treatment withdrawal (17). On-treatment responses did not improve by prolonging treatment to 48 weeks, but relapse rates were reduced, leading to sustained response rates around 30% in genotype 1. In the era of IFN monotherapy, further reduction of relapse rates were shown to be accomplished by prolonging treatment to 18 or even 24 months (18,19). The next logical step for the Benelux group was therefore to explore the possibility of further increasing combination treatment efficacy by prolonging treatment duration beyond 12 months.

Benelux study HCV 96-01

Precise aim
- can we reduce the high relapse rate after 6 months IFN ribavirin combination therapy by prolonging the treatment to 18 months

Number of centers, number of patients
- 25 centers, 300 patients randomized, 295 started treatment

Three arms
- IFN 3 MU tiw + ribavirin 1000-1200 mg/day, 6 months
- IFN 3 MU tiw + ribavirin 1000-1200 mg/day, 18 months
- IFN 3 MU tiw + ribavirin placebo, 18 months

Outcome measure
- undetectable HCV RNA at the end of treatment and after 6 months follow-up
- HCV RNA response during treatment

Results
- HCV RNA is undetectable at the end of treatment in 55% and 49% after 6 and 18 months combination therapy, and in 26% after 18 months monotherapy ($p<0.001$)
- relapse rates are 38% after 6 months combination therapy, 38% after 18 months mono therapy and only 13% after 18 months combination therapy ($p=0.002$)
- sustained rate 34% for 6 months combination therapy, 16% for 18 months mono therapy, and 43% for 18 months combination therapy ($p<0.05$)
- more than 80% of the virologic responses (HCV RNA $\leq 10^2$ copies/ml) on combination therapy and 70% of those on monotherapy occurred within the first 3 months of treatment
- relapse rates in the poor response categories (genotype 1, high viral load, cirrhosis) were significantly higher than the relapse rates of the favourable categories for 18 months monotherapy and 6 months combination therapy but approached the results of the favourable groups for 18 months combination therapy
- prolongation of combination therapy from 6 to 18 months does not significantly influence the relapse (19 v 13%) and sustained response rates (72 v 64%) in genotype 2 and 3

Implications
- relapse rates of 15% or less are feasible by prolongation of IFN ribavirin treatment to 18 months
- prolongation of treatment is especially worthwhile in patients with a poor response profile, like genotype 1, high viral load, and/or cirrhosis

Treatment of patients with genotypes 2 or 3 appears no longer a major issue in view of the high efficacy of 24 weeks combination therapy. However, even with prolonged treatment, sustained response rates were still limited in patient categories that need therapy the most, like genotype 1, high viral load, and cirrhosis. In addition, re-treatment with combination therapy of prior non-responders to IFN mono therapy led to a limited success of only about 15%. For these patients with a poor response profile, especially genotype 1, cirrhosis or prior treatment failure, a series of exploratory studies were performed, combining the concepts of induction therapy to increase the initial / early response, daily dosing to reduce breakthrough, prolonged treatment to reduce relapse, in combination with ribavirin. These series also formed the source for our studies on viral kinetics in combination therapy (20).

HIRO studies

Precise aim
- assess whether combination therapy with high dose induction and prolonged daily IFN and ribavirin leads to an increased sustained response in difficult to treat patients, characterized by genotype 1, cirrhosis and/or non-response or relapse to previous therapy

Number of centers, number of patients
- single center, 55 consecutive patients with a poor response profile enrolled and 54 treated in three sequential cohorts; meta-analysis of individual patient data

Three arms
- first cohort (n=24): IFN 10, 5 and 3 MU daily until week 4, week 24 and week 52, respectively, followed by 3 MU tiw until week 76, in combination with ribavirin 1000-1200 mg/day
- second cohort (n=15): IFN 10 and 3 MU daily until day 10 and week 52, respectively, followed by 3 MU tiw until week 76, in combination with ribavirin 1000-1200 mg/day
- third cohort (n=15): IFN 10, 5 and 3 MU daily until week 2, week 4 and week 76, respectively, in combination with ribavirin 1000-1200 mg/day

Outcome measure
- primary: sustained HCV RNA response 6 months after treatment withdrawal
- secondary: breakthrough and relapse rates; tolerability; factors associated with response

Results
- sustained response total group 57%; ca. 80% if only 1 unfavorable factor, ca. 20% for the combination of (advanced) cirrhosis and genotype 1 or 4
- low breakthrough (4%) and relapse (3%) rates
- low drop-out rate for non-compliance (7%) and adverse events (11%); clinically serious adverse events occurred in 2 patients with advanced cirrhosis (characterized by low platelets and elevated bilirubin)

Implications
- patients with genotype 1, cirrhosis and/or prior treatment failure can benefit from prolonged treatment with high dose daily interferon and ribavirin
- the additional value of an induction phase followed by dose reduction has not (yet) been established and should be further explored; however, low breakthrough rates on dose reduction were observed in this exploratory study
- patients with advanced cirrhosis should not be treated with this intensive regiment

IFN has a short half-life of only 7 hours (21), and dosing thrice weekly leads to large fluctuations in antiviral pressure, reducing the chances of an early viral response and increasing the chances of a breakthrough during therapy (22). The beneficial effect of reducing fluctuations in IFN levels, acquired in our studies by daily dosing, was also observed with the introduction of the long-acting pegylated interferons with prolonged absorption half-lives up to 50 hours and elimination half-lives between 60 and 70 hours(23, 24). With PEG-IFN ribavirin combination therapy, sustained response rates in genotype 1 have increased to almost 50%, mainly as a result of an increase of the on-treatment response. The relapse rate after 12 months treatment is, however, still high – up to 30% (table 1) – and new studies should explore the possibility of reducing relapse by prolonged treatment, shown to be of value in the Benelux studies with standard IFN and ribavirin.

Table 1 Reported relapse and sustained response rates in genotype 1 and genotypes 2/3 for (PEG) IFN monotherapy and combination with ribavirin, according to treatment duration

		Relapse‡		Sustained response	
Author	**Treatment schedule†**	**g1**	**g2/3**	**g1**	**g2/3**
	IFN monotherapy				
Reichard 1998[17]	3x3 IFN-a2b, 6 mo	78%	63%	9%	23%
McHutchison 1998[16]	3x3 IFN-a2b, 6 mo			2%	16%
Poynard 1998[15]	3x3 IFN-a2b, 12 mo			11%	33%
McHutchison 1998[16]	3x3 IFN-a2b, 12 mo			7%	29%
Benelux study[25]	3x3 IFN-a2b, 18 mo	40%	40%	13%	26%
	PEG-IFN monotherapy				
Lindsay 2001[26]	1.5 ug/kg PEG-a2b, 12 mo	66%	36%	14%	49%
Fried 2001[27]	180 PEG-a2a 12 mo			21%	45%
	IFN + Ribavirin combi therapy				
Reichard 1998[17]	3x3 IFN-a2b + Rbv 1000-1200, 6 mo	44%	19%	23%	50%
Poynard 1998[15]	3x3 IFN-a2b + Rbv 1000-1200, 6 mo			18%	64%
McHutchison 1998[16]	3x3 IFN-a2b + Rbv 1000-1200, 6 mo			16%	69%
Benelux study[25]	3x3 IFN-a2b + Rbv 1000-1200, 6 mo	47%	19%	25%	72%
Poynard 1998[15]	3x3 IFN-a2b + Rbv 1000-1200, 12 mo			31%	64%
McHutchison 1998[16]	3x3 IFN-a2b + Rbv 1000-1200, 12 mo			28%	66%
Manns 2001[28]	3x3 IFN-a2b + Rbv 1000-1200, 12 mo			33%	79%
Mangia 2002[29]	3x3 IFN-a2b + Rbv 1000-1200, 12 mo	42%	11%	19%	61%
Fried 2001[27]	3x3 IFN-a2b + Rbv 1000-1200, 12 mo			37%	61%
Benelux study[25]	3x3 IFN-a2b + Rbv 1000-1200, 18 mo	14%¶	13%	37%	64%
	PEG-IFN + Ribavirin combi therapy				
Hadziyannis 2002[30]	180 PEG-a2a + Rbv 1000-1200, 6 mo	48%	12%	41%	78%
Manns 2001[28]	1.5 ug/kg PEG-a2b + Rbv 800, 12 mo			42%	82%
Fried 2001[27]	180 PEG-a2a + Rbv 1000-1200, 12 mo			46%	76%
Hadziyannis 2002[30]	180 PEG-a2a + Rbv 1000-1200, 12 mo	27%	9%	51%	77%

‡ Virological relapse could only be calculated for those studies which gave detailed information on both end-of-treatment response and sustained response rates in genotype 1 and genotype 2/3 patients; g1: HCV genotype 1; g2/3: HCV genotype 2 or 3

† Treatment schedule: IFN dose in MU per week; PEG-IFN dose in ug per week; Ribavirin dose in mg per day

¶ Intention to treat 14% (95% C.I. 3.9%-31.7%), per protocol 4% (95% C.I. 0.0%-20.3%)

What is the biological background of these observations? Viral kinetics during treatment with IFN show a fast initial decline in viral load followed by a slower second phase (31–33); similar observations were made with PEG-IFN (34). Biomathemathical analysis of these patterns shows that the initial viral decline is related to the efficacy by which interferon blocks the intracellular viral replication of HCV. This efficacy (ε) is dependent on the dose of interferon used (22,32,35,36) and on the genotype present (37). The second phase of viral decline is also dependent on the blocking efficacy (ε) of IFN (38), but its major determinant is the immune mediated removal of virus producing cells (δ). A significant influence of Ribavirin on early viral kinetics has not been established (36,39). The infected cell loss rate (δ) is the best predictor of sustained response (40), and its major determinants are the genotype (34,37) and the pretreatment viral load (37,38). Patients with a genotype 2 or 3 have, besides a higher blocking efficacy (ε) of IFN, a higher infected cell loss rate (δ) than patients with genotype 1 (37), which explains the low relapse rates and the lack of benefit by prolongation of combination therapy beyond 6 months. In contrast, the infected cell loss rate (δ) is significantly lower in genotype 1 and here a major benefit in reducing relapse rates can be obtained by prolonging treatment. The observation that the infected cell loss rate (δ) is also determined by the pretreatment viral load (37,38) is in accordance with our findings in the Benelux study 96-01 that the pretreatment viral load is the major predictor of relapse for genotype 1 patients. Genotype 1 patients with a high viral load will therefore benefit the most from prolonging treatment duration.

Further improvement of treatment results might be acquired by individualization of therapy based on the early viral response. The importance of an early viral response is still valid, but the definitions have to be modified in view of the improved detection limits of HCV RNA assays. With a detection limit of 10^3 copies / ml, used in the 90-01 and 91-07 studies, a detectable HCV RNA at week 4 almost precluded the chance of reaching a sustained response. With the more sensitive test of 10^2 copies / ml, used in the 96-01 study, a substantial number of eventual sustained responders lost detectable virus between week 4 and 12. It is therefore logical to use quantitative in stead of qualitative criteria, and a stopping rule of less than 2 log decrease in the first 12 weeks is advocated nowadays (41).

Even earlier and maybe better individualization might be derived by assessing viral kinetics during the first weeks (42) or even after a single dose of IFN (43).

However, it would not be prudent to recommend a strategy based on virological characteristics alone. Besides other independent factors of response, like age, sex, fibrosis and steatosis (44), one of the most important determinants of success is patient compliance (45). Large discrepancies exist between the results of therapy of the registration trials and the overall results in general practice (46). Side effects like flue-like symptoms, depression and anemia can now effectively be managed by appropriate drugs, but the major determinant of success is motivation and continuous education, not only of the patient and his relatives but also of the people involved in giving the treatment. And, in the end, teamwork was essential for the success of the Benelux studies and will remain essential for the treatment of individual patients.

REFERENCES

1. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989;244(4902):359-62.
2. Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989;244(4902):362-4.
3. Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC, Jr, Perrillo RP, et al. Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. N Engl J Med 1989;321(22):1501-1506.
4. Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, et al. Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med 1989;321(22):1506-1510.
5. Iino S, Hino K, Kuroki T, Suzuki H, Yamamoto S. Treatment of chronic hepatitis C with high-dose interferon alpha-2b. A multicenter study. Dig Dis Sci 1993;38(4):612-618.
6. Reichard O, Andersson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. Lancet 1991;337(8749):1058-61.
7. Di Bisceglie AM, Shindo M, Fong TL, Fried MW, Swain MG, Bergasa NV, et al. A pilot study of ribavirin therapy for chronic hepatitis C. Hepatology 1992;16(3):649-54.
8. Reichard O, Yun ZB, Sonnerborg A, Weiland O. Hepatitis C viral RNA titers in serum prior to, during, and after oral treatment with ribavirin for chronic hepatitis C. J Med Virol 1993;41(2):99-102.
9. Brillanti S, Garson J, Foli M, Whitby K, Deaville R, Masci C, et al. A pilot study of combination therapy with ribavirin plus interferon alfa for interferon alfa-resistant chronic hepatitis C. Gastroenterology 1994;107(3):812-7.
10. Schalm SW, Hansen BE, Chemello L, Bellobuono A, Brouwer JT, Weiland O, et al. Ribavirin enhances the efficacy but not the adverse effects of interferon in chronic hepatitis C. Meta-analysis of individual patient data from European centers. J Hepatol 1997;26(5):961-6.
11. Dhumeaux D, Doffoel M, Galmiche JP. A French consensus conference on hepatitis C: screening and treatment. J Hepatol 1997;27(5):941-944.
12. Anonymous. National Institutes of Health Consensus Development Conference Panel statement: management of hepatitis C. Hepatology 1997;26(3 Suppl 1):2S-10S.
13. Brouwer JT, (Anonymous). Benelux multicentre trial of alpha interferon treatment for chronic hepatitis C: standard v high dose treatment monitored by biochemical and virological markers (interim analysis). Benelux Multicentre Trial Study Group. Gut 1993;34(2 Suppl):S119-S120.
14. Kleter GE, Brouwer JT, Heijtink RA, Schalm SW, Quint WG. Detection of hepatitis C virus RNA in patients with chronic hepatitis C virus infections during and after therapy with alpha interferon. Antimicrob Agents Chemother 1993;37(3):595-7.
15. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998;352(9138):1426-32.
16. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for

chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 1998;339(21):1485-92.
17. Reichard O, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Randomised, double-blind, placebo-controlled trial of interferon alpha- 2b with and without ribavirin for chronic hepatitis C. The Swedish Study Group. Lancet 1998;351(9096):83-87.
18. Poynard T, Bedossa P, Chevallier M, Mathurin P, Lemonnier C, Trepo C, et al. A comparison of three interferon alfa-2b regimens for the long-term treatment of chronic non-A, non-B hepatitis. Multicenter Study Group. N Engl J Med 1995;332(22):1457-62.
19. Lin R, Roach E, Zimmerman M, Strasser S, Farrell GC. Interferon alfa-2b for chronic hepatitis C: effects of dose increment and duration of treatment on response rates. Results of the first multicentre Australian trial. Australia Hepatitis C Study Group. J Hepatol 1995;23(5):487-496.
20. Bekkering FC, Brouwer JT, Hansen BE, Schalm SW. Hepatitis C viral kinetics in difficult to treat patients receiving high dose interferon and ribavirin. J Hepatol 2001;34(3):435-40.
21. Wills RJ. Clinical pharmacokinetics of interferons. Clin Pharmacokinet 1990;19(5):390-9.
22. Bekkering FC, Stalgis C, McHutchison JG, Brouwer JT, Perelson AS. Estimation of early hepatitis C viral clearance in patients receiving daily interferon and ribavirin therapy using a mathematical model. Hepatology 2001;33(2):419-23.
23. Glue P, Rouzier-Panis R, Raffanel C, Sabo R, Gupta SK, Salfi M, et al. A dose-ranging study of pegylated interferon alfa-2b and ribavirin in chronic hepatitis C. The Hepatitis C Intervention Therapy Group. Hepatology 2000;32(3):647-53.
24. Zeuzem S, Welsch C, Herrmann E. Pharmacokinetics of peginterferons. Semin Liver Dis 2003;23 Suppl 1:23-8.
25. Brouwer JT, Nevens F, Bekkering FC, Bourgeois N, Van Vlierberghe H, Weegink CJ, et al. Reduction of relapse rates by 18-month treatment in chronic hepatitis C. A Benelux randomized trial in 300 patients. J Hepatol 2004;40:689-695.
26. Lindsay KL, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, et al. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology 2001;34(2):395-403.
27. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347(13):975-82.
28. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358(9286):958-65.
29. Mangia A, Santoro R, Piattelli M, Leandro G, Minerva N, Annese M, et al. High doses of interferon in combination with ribavirin are more effective than the standard regimen in patients with HCV genotype 1 chronic hepatitis. J Hepatol 2002;37(1):109-16.
30. Hadziyannis SJ, Cheinquer T, Morgan T, Diago M, Jensen DM, Sette Jr. H, et al. Peginterferon alfa-2a (40kD) (Pegasys) in combination with Ribavirin (RBV): efficacy and safety results from a phase III, randomized, double-blind, multicentre study examing effect of duration of treatment and RBV dose. J Hepatol 2002;36(S1):3 (abstract).
31. Zeuzem S, Schmidt JM, Lee JH, Ruster B, Roth WK. Effect of interferon alfa on the dynamics of hepatitis C virus turnover in vivo. Hepatology 1996;23(2):366-371.
32. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science 1998;282(5386):103-7.

33. Bekkering FC, Brouwer JT, Leroux-Roels G, Van Vlierberghe H, Elewaut A, Schalm SW. Ultrarapid hepatitis C virus clearance by daily high-dose interferon in non-responders to standard therapy. J Hepatol 1998;28(6):960-4.
34. Zeuzem S, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, et al. Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon alpha2a. Gastroenterology 2001;120(6):1438-47.
35. Lam NP, Neumann AU, Gretch DR, Wiley TE, Perelson AS, Layden TJ. Dose-dependent acute clearance of hepatitis C genotype 1 virus with interferon alfa. Hepatology 1997;26(1):226-31.
36. Zeuzem S, Schmidt JM, Lee JH, von Wagner M, Teuber G, Roth WK. Hepatitis C virus dynamics in vivo: effect of ribavirin and interferon alfa on viral turnover. Hepatology 1998;28(1):245-52.
37. Neumann AU, Lam NP, Dahari H, Davidian M, Wiley TE, Mika BP, et al. Differences in viral dynamics between genotypes 1 and 2 of hepatitis C virus. J Infect Dis 2000;182(1):28-35.
38. Bekkering FC, Neumann AU, Brouwer JT, Levi-Drummer RS, Schalm SW. Changes in anti-viral effectiveness of interferon after dose reduction in chronic hepatitis c patients: a case control study. BMC Gastroenterol 2001;1(1):14.
39. Pawlotsky JM, Neumann AU, Dahari H, Conrad A, Hezode C, Schmid P, et al. Hepatitis C vrius (HCV) dynamics during induction therapy with interferon (IFN) alpha and/or ribavirin. Hepatology 2000;32:223A (abstract).
40. Rosen HR, Ribeiro RR, Weinberger L, Wolf S, Chung M, Gretch DR, et al. Early hepatitis C viral kinetics correlate with long-term outcome in patients receiving high dose induction followed by combination interferon and ribavirin therapy. J Hepatol 2002;37(1):124-30.
41. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38(3):645-52.
42. Neumann AU, Hagai E, Schalm SW, von Wagner M, Germanidis G, Lurie Y, et al. Early viral kinetics prediction of sustained virological response after 1 or 4 weeks of PEG-Interferon and Ribavirin therapy (DITTO-HCV project). Hepatology 2003;38(4, Suppl. 1):68A.
43. Jessner W, Gschwantler M, Steindl-Munda P, Hofer H, Watkins-Riedel T, Wrba F, et al. Primary interferon resistance and treatment response in chronic hepatitis C infection: a pilot study. Lancet 2001;358(9289):1241-2.
44. Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. Lancet 2003;362(9401):2095-100.
45. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002;123(4):1061-9.
46. Bräu N, Bini EJ, Currie S, Shen H, Anand BS, Hu K-Q, et al. Treatment of chronic hepatitis C with interferon alfa-2b and ribavirin in the community-based practice. A prospective study of 813 U.S. veterans. Hepatology 2003;38(4, Suppl. 1):643A.

Chapter 10

Associated research in the context of the Benelux studies

I. Theses with publications based on materials derived from the Benelux studies:

L.J. van Doorn – Detection and characterization of the hepatitis C virus. Rotterdam, 1994.

- van Doorn LJ, Kleter B, Stuyver L, Maertens G, Brouwer H, Schalm S, Heijtink R, Quint W. Analysis of hepatitis C virus genotypes by a line probe assay and correlation with antibody profiles. J Hepatol 1994;21(1):122-9

- van Doorn LJ, Kleter B, Voermans J, Maertens G, Brouwer H, Heijtink R, Quint W. Rapid detection of hepatitis C virus RNA by direct capture from blood. J Med Virol 1994;42(1):22-8

B. Kleter - Characterization of hepatitis C virus isolates from chronically infected patients. Rotterdam, 1995.

- Kleter GEM, Brouwer JT, Heijtink RA, Schalm SW, Quint WGV. Detection of hepatitis C virus RNA in patients with chronic hepatitis C infections during and after therapy with alpha interferon. Antimicrob Agents Chemother 1993;37:595-7.

- Kleter B, Brouwer JT, Nevens F, Doorn LJ van , Elewaut A, Versieck J, Michielsen PP, Hautekeete ML, Chamuleau RAFM, Brénard R, Bourgeois N, Adler M, Quint WGV, Bronkhorst CM, Heijtink RA, Hop WJC, Fevery J, Schalm SW. Hepatitis C virus genotypes: epidemiological and clinical associations. Liver 1998;18:32-8

F.C. Bekkering – Viral kinetics of the hepatitis C virus. Rotterdam, 2001

- Bekkering FC, Brouwer JT, Elewaut A, Schalm SW. Hepatitis C: viral kinetics. Hepatology 1997;26:1691-2.

- Bekkering FC, Brouwer JT, Leroux-Roels G, Vlierberghe H van, Elewaut A, Schalm SW. Ultrarapid hepatis C virus clearance by daily high dose interferon in non-responders to standard therapy. J Hepatol 1998;28:960-4.

- Bekkering FC, Brouwer JT, Hansen BE, Schalm SW. Hepatitis C viral kinetics in difficult to treat patients receiving high dose interferon and ribavirin. J Hepatol 2001;34:435-40.

II. Other international publications based on materials derived from the Benelux studies:

- Louagie HK, Brouwer JT, Delanghe JR, Heijtink RA, De Buyzere ML, Leroux-Roels GG. Haptoglobin polymorphism and chronic hepatitis C. J Hepatol 1996;25:10-4.

- Depraetere S, Kerschaever E van, Vlierberghe H van, Elewaut A, Brouwer JT, Niesters HGM, Schalm SW, Maertens G, Leroux-Roels G. Long term response to interferon treatment in chronic hepatitis C patients is associated with a significant reduction in anti-E1 envelope antibody titers. J Med Virol 2000;60:126-32.

III. Other international publications

- Van Vlierberghe H, Leroux-Roels G, Adler M, Bourgeois N, Nevens F, Horsmans Y, Brouwer J, Colle I, Delwaide J, Brenard R, Bastens B, Henrion J, de Vries RA, de Galocsy C, Michielsen P, Robaeys G, Bruckers L. Daily induction combination treatment with alpha 2b interferon and ribavirin or standard combination treatment in naive chronic hepatitis C patients. A multicentre randomized controlled trial. J Viral Hepat 2003;10(6):460-6

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Dankwoord

DANKWOORD

Het doen van multicenter studies betekent samenwerken. Ik realiseer me dat samenwerken met mij niet altijd eenvoudig was. Mijn hang naar perfectie heeft sommigen wellicht soms tot wanhoop gedreven. Vaak moest het (in mijn optiek) net nog een beetje beter en dan meestal op het laatste moment. Desondanks is de periode van samenwerking binnen de Benelux studies zeer stimulerend geweest en zijn er vele mooie dingen uit voortgekomen.

Allereerst wil ik natuurlijk Solko Schalm bedanken, niet alleen omdat hij me begeleid heeft om uiteindelijk toch te promoveren, maar met name ook voor de vele jaren van interactie die voor mij zeer leerzaam zijn geweest. Solko, ik heb bewondering voor je gedrevenheid en organisatietalent. Je neiging om alles nog net een tandje beter te krijgen, kostte soms de nodige energie maar leidde altijd wel tot fraaie resultaten. Je rol als mede-initiator van de Benelux groep is essentieel geweest. Er is veel werk verzet in de afgelopen jaren en ik heb goede herinneringen aan de samenwerking met je binnen de vele projecten die er waren op locaal, nationaal en internationaal niveau. Ten slotte koester ik goede herinneringen aan de vele donderdagavonden bij jou thuis waar het harde werken steevast werd beloond met een mooi glas rode wijn. (staat de vereniging OWIOZ nog open voor nieuwe leden?). Daarbij wil ik ook Atie bedanken voor de vele momenten van gastvrijheid.

Ik wil alle patiënten bedanken die bereid waren te participeren in de studies. Natuurlijk was het doel steeds om voor hen de meest optimale therapie te vinden, maar het kost soms toch ook vertrouwen (en moed) als jouw arts na het falen van een vorige zware kuur voorstelt om het nog eens te proberen, maar nu nog wat intensiever.

Met plezier kijk ik terug op de samenwerking met de vele collega's en vrienden uit België, Nederland en Luxemburg. De meetings in de E10 Hoeve, net over de grens in België, waren altijd inspirerend. Velen heb ik leren waarderen, niet alleen als arts of wetenschapper, maar ook als mens. Johan Fevery en Andre Elewaut, ik beschouw jullie naast Solko als mede-grondleggers van de studiegroep, waarvoor mijn dank.

Frederik Nevens, het samenwerken met jou was altijd van hoog niveau. Ik waardeer je zeer om jouw intelligentie, organisatietalent en warme menselijkheid. Peter Michielsen, Richard de Vries, Pieter Wismans, Carin van Nieuwkerk, Bart van Hoek en Jannie den Ouden, dank dat we altijd op jullie konden rekenen. Marc Hautekeete: je was helaas veel te kort bij ons, op sommige momenten meen ik nog steeds je gulle lach te horen.

Dat de taalgrens geen barrière hoeft te zijn voor samenwerking, bewezen de vele Franstalige collega's, met wie ik dank zij mijn beperkte talenknobbel voornamelijk in het Engels communiceerde. Michael Adler, Nadine Bourgeois, Reginald Brenard, Jean Pirotte, Jean Delwaide, Jean Henrion, C de Galocsy en vele anderen hebben een belangrijke bijdrage geleverd en waren trouw in het bezoeken van de meetings. Vanwege de reisafstand verliep het contact met de collega's Weber en Arendt in Luxemburg meer elektronisch dan fysiek, maar de bijdrage was er niet minder om en de spaarzame momenten dat we elkaar zagen waren nuttig.

De samenwerking met de verschillende centra in de Benelux is altijd een boeiend proces geweest. Als Benelux groep vonden we het belangrijk om ernaar te streven steeds maar één klinische studie tegelijkertijd te doen, zodat deze snel aan zijn beoogde recruitment kwam waarna een nieuwe studie kon worden geïnitieerd.

Terwijl de bereidheid tot samenwerking vanuit België en Luxemburg steeds overweldigend was, evenals die vanuit vele Nederlandse 'perifere' centra, was de samenwerking tussen de Nederlandse universiteiten in de loop der jaren sterk wisselend. Desondanks heb ik goede herinneringen aan de contacten met de andere Nederlandse universitaire centra, met name aan de groepen van Rob Chamuleau en Henk Reesink, en aan die van Jan van Hattum, zowel tijdens perioden van hechte samenwerking als tijdens perioden waar ieder organisatorisch zijns weegs ging.

Dank ook aan de vele jonge onderzoekers die vaak het meeste werk achter de schermen verrichten, zoals Frank Bekkering, Hans van Vlierberghe en Christine Weegink, en de mensen die sterk betrokken waren bij de vervolgstudies, zoals Jan Maarten Vrolijk en Bart Veldt.

Alle basale wetenschappers van het eerste uur, dank voor jullie fantastische inzet. Ik realiseer me dat we de eerste Benelux trial hebben opgezet in een tijd dat de PCR techniek nog volledig moest worden ontwikkeld. Met een patiënten aanbod van meer dan 350 en een PCR capaciteit in de beginperiode van slechts enkele tientallen per week hebben jullie een enorme klus geklaard. Daarbij lag onze nadruk soms teveel op kwantiteit maar die van jullie steeds op kwaliteit, op een verdere verdieping en betering. De mensen van het eerste uur, zoals Bernhard Kleter, Leen Jan van Doorn, Ruud Heijtink, Bert Niesters, Wim Quint, Nico Lelie en Theo Kuipers, hebben hierbij heel veel belangrijk werk verzet.

Ook de wetenschappers op andere vlakken hebben een onschatbare bijdrage geleverd, zoals Fibo ten Kate en Carolien Bronkhorst die niet alleen vele uren hebben besteed aan het classificeren van de biopten maar daarnaast ook gewaardeerde gesprekspartners waren op de meetings. Geert Leroux-Roels die een aantal fraaie ancillary studies heeft opgezet en deze ook succesvol heeft afgerond, Wim Hop en Bettina Hansen die altijd bereid waren hun hersenen op de meest onmogelijke tijden te doneren aan de wetenschap.

De mensen van het trialbureau en het secretariaat zorgden achter de schermen dat alles goed liep. Dat ging niet altijd automatisch, er is heel wat gezwoegd en gesteund. Ik heb bijzondere waardering voor jullie inspanning, jullie waren de motor die het project nodig had. Vele mensen zijn in de loop van de jaren langsgetrokken, mogelijk dat ik er enkele vergeet. Willeke Beukman, je hebt jarenlang de studies op een geweldige manier ondersteund en je was nooit te beroerd om op het laatste moment over te werken om alles toch nog op tijd klaar te krijgen. Jan Boot, er was altijd wel nood, dank voor al je programmeerwerk. Elke Verheij, dank zij jou heeft het trialbureau aan professionaliteit gewonnen. De mensen van de clinical research unit, de verpleging, de trial nurses, het poli personeel en in het bijzonder ook Heleen van Santen, dank voor al jullie ondersteuning, niet alleen van de projecten maar met name ook van de patiënten; patiëntenzorg is teamwork, en jullie vormen daarin een essentiële schakel. Ook dank aan de vele anderen, zoals Sylvia de Vlaming, Mieke Pruijsten, Margriet Leeuwesteijn, Marion Hoogendoorn, die stuk voor stuk altijd klaar stonden om te helpen.

Ik wil ook alle collega's van de hepatologie groep in Rotterdam bedanken waarmee ik vele jaren met plezier heb samengewerkt. Rob de Man, Herold Metselaar, Sjoerd de Rave, Harry Janssen, het waren interessante tijden waarin de afdeling hepatologie en levertransplantatie in 10 jaar tijd een enorme groei heeft doorgemaakt. Henk van Buuren, paranimf en gewaardeerd collega, ondanks alle drukte had je altijd tijd voor iedereen; de plezierige momenten op je kamer, als besluit van een drukke week, onder het genot van wat wijn uit een plastic bekertje, ze zullen me nog lang heugen.

Ook de collega's van de 'holle buizen' sectie wil ik bedanken voor de leerzame momenten, in het bijzonder Jan Dees en Jelle Haringsma die mij voorafgaand aan mijn overstap naar Delft hebben geholpen mijn endoscopische vaardigheden weer helemaal op peil te brengen. Voor Ernst Kuipers heb ik grote waardering zoals hij in relatief korte tijd Rotterdam weer helemaal op de kaart heeft gezet binnen gastroenterologisch Nederland. Beste Ernst, ook dank voor de manier waarop je mij gestimuleerd hebt tot reflectie, waardoor ik keuzes heb gemaakt in mijn carrière waar ik tot nu toe geen spijt van heb. Ik hoop ook in de toekomst op een goede band tussen Rotterdam en Delft op gebied van patiëntenzorg, onderwijs en onderzoek.

Mijn collega's in Delft wil ik bedanken voor de ruimte die ik kreeg, binnen het overvolle programma, om het proefschrift af te ronden. Ik heb me vanaf het begin bij jullie thuis gevoeld en waardeer jullie combinatie van professionaliteit en menselijkheid. In het bijzonder wil ik mijn directe MDL collega's bedanken, Joost Scherpenisse, Clemens Bolwerk, tevens paranimf, en Willemien Erkelens, en de MDL collega's in internisten-vermomming, Herman Walinga en Iwan Meynaar, voor de altijd plezierige samenwerking en de hoge mate van collegialiteit.

Daarnaast zijn er nog vele andere collega's, teveel om op te noemen, die allemaal hun specifieke en gewaardeerde bijdrage hebben geleverd, leidend tot het succes van de diverse studies. Het feit dat jullie niet bij name zijn genoemd maakt de dank er niet minder om.

Mijn vrienden en familie wil ik bedanken voor alle geduld die ze met me hebben gehad. Hoewel de intenties goed waren, waren de contacten in de praktijk vaak spaarzaam door de grote veelheid aan werk. Ik hoop dat daar nu enige verandering in komt. Peter die mij eens terzijde stond in de mist, Rick die mij altijd wist te stimuleren met de wijze woorden van Goethe, Marijke die mij zo nu en dan vriendelijke woorden zond, mijn tennisvrienden die altijd zorgden voor welkome onderbrekingen, ik waardeer jullie.

Mijn vader, die mij veel te vroeg ontviel, en mijn moeder wil ik danken voor alle stimulans, mogelijkheden en liefde. Ze hebben me laten inzien dat we soms veel te druk zijn met kleine dingen en dat er veel belangrijker dingen in het leven zijn om je mee bezig te houden.

En ten slotte mijn geliefden, Annemarie en de kinderen, Johan, Willem Pieter, Marlies en Hannah. Ik waardeer jullie enorm, en ik hoop……Wat ik hier op papier wilde zetten, kan ik veel beter in de praktijk waarmaken……..

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Curriculum Vitae

CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 2 april 1958 te Vlaardingen. In 1976 behaalde hij het VWO diploma aan het CSG Westland-Zuid te Vlaardingen. Tijdens zijn studie geneeskunde aan de Erasmus Universiteit te Rotterdam deed hij gedurende een half jaar onderzoek aan het Albert Einstein College of Medicine in New York. In 1983 behaalde hij zijn artsexamen (Cum Laude). Voor het vervullen van zijn militaire dienstplicht was hij gedetacheerd in het Militair Hospitaal 'Dr. A. Mathijsen' te Utrecht, waar hij werkte op de afdelingen cardiologie en intensive care. In 1985 begon hij zijn opleiding tot internist in het St Clara Ziekenhuis te Rotterdam (opleider dr. J. Bruins Slot) waarbij hij vanaf 1990 een stage gastro-enterologie/ hepatologie volgde op de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Rotterdam/Dijkzigt (hoofd prof. J.H.P. Wilson). In 1991 werd hij ingeschreven in het specialistenregister als internist. Van 1991 tot 2002 was hij werkzaam als staflid in het Academisch Ziekenhuis Rotterdam, eerst op de eerdergenoemde afdeling Inwendige Geneeskunde II en vanaf 1998 op de afdeling Maag-, Darm- en Leverziekten (hoofd ad interim prof. dr. S.W. Schalm, vanaf augustus 2000 hoofd prof. dr. E.J. Kuipers). Hij werd in 2001 ingeschreven in het specialistenregister als maag-darm-leverarts. Vanaf 2002 tot heden is hij werkzaam als maag-darm-leverarts in de Reinier de Graaf Groep te Delft.

Vanaf 1990 werden studies op gebied van virale hepatitis C verricht onder supervisie van en in nauwe samenwerking met prof. dr. S.W. Schalm. Hij was daarbij intensief betrokken bij het opzetten en uitvoeren van diverse studies in internationaal verband, hetgeen de basis vormde voor dit proefschrift. Hij was mede-initiator en coördinator van de Benelux studie groep (1990-2002); daarnaast was hij sterk betrokken bij twee Europese onderzoeksprojecten, EUROHEP (1991-1999) en DITTO HCV (2000-2002).

Hij is gehuwd met Annemarie en heeft vier kinderen, Johan, Willem Pieter, Marlies en Hannah.

Johannes T. Brouwer