

Optimizing Interferon Alfa Based Therapy for Chronic Hepatitis C

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Optimizing Interferon Alfa Based Therapy for Chronic Hepatitis C

Optimalisering van op interferon alfa gebaseerde behandeling van chronische hepatitis C

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GENERAL INTRODUCTION



INTRODUCTION

The hepatitis C virus was first discovered in 1989 as the major cause of chronic non-A non-B hepatitis (1). The hepatitis C virus is a single stranded RNA virus that belongs to the family of flaviviruses (2). The primary target of the hepatitis C virus are hepatocytes where viral particles replicate extremely fast with the production of 10^{12} viral particles per day. Worldwide 6 major HCV genotypes occur whose prevalence varies geographically. The predominant risk factor for infection is injection drug use. Other risk factors include blood transfusions before 1992, high lifetime number of sexual partners and iatrogenic transmission (3). Infection with the hepatitis C virus leads to a chronic infection in 55-80% of patients. A recently discovered single nucleotide polymorphism near the IL-28b gene is associated with spontaneous clearance of the hepatitis C virus (4).

Worldwide 180 million people suffer from chronic hepatitis C virus (HCV) infection with 3-4 million people newly infected people and more than 350000 deaths each year (5). Although the natural history of chronic hepatitis C is highly variable, approximately 15-30% of patients infected with HCV will develop liver cirrhosis over the ensuing 3 decades (6). Cirrhosis can lead to the development of hepatocellular carcinoma and end stage liver disease. Consequently, chronic HCV infection is the leading indication for liver transplantation in developed countries (7).

Treatment

The primary goal of antiviral treatment for chronic hepatitis C is to prevent complications and death from HCV infection. This goal can be achieved by eradicating the virus permanently. Great strides have been made in the treatment of chronic HCV infection. In 1986 the first patients with non-A non-B chronic hepatitis were treated with interferon alfa for 24 weeks resulting in normalization of ALT in 8 out of 10 patients (8). To date, more than two decades later, the combination of peginterferon alfa and ribavirin has become the standard of care for all HCV genotypes on the basis of results of multiple randomized controlled trials (9-13). Optimization of treatment by altering treatment doses and duration has led to increased SVR rates in the past decade. The current treatment regimen consists of a weekly subcutaneous injection of peginterferon alfa (2a or 2b) combined with oral daily ribavirin for 24 or 48 weeks depending on on-treatment virological response and HCV genotype (14). This treatment regimen leads to sustained virological response rates (SVR) of approximately 80% of patients infected with HCV genotype 2 and 3. However, for patients infected with genotype 1, the most common genotype in the western world, a sustained virological response is only achieved in about 40 to 50% of patients (9-10, 13). Besides genotype several pre-treatment host factors have been identified as predictors of response. These factors include race, fibrosis stage, body weight, age, gender, baseline viral load, baseline ALT and IL-28b genotype. Recent data from genome wide association studies have shown that single nucleotide polymorphisms

near or in the interleukin-28b gene are highly predictive of sustained virological response in genotype 1 patients (15). Patients with IL28b SNP rs 12979860 CC genotype had a more than two-fold higher chance of achieving SVR compared to patients with rs12979860 TT genotype (78% for the CC vs. 38% for the CT and 26% SVR for the TT genotype). The IL28b genotype has become the most important pretreatment predictor of virological response in HCV genotype 1 patients.

Treatment predictors

Besides pretreatment predictors, chances of a sustained virological response can also be determined during treatment. A rapid virological response defined as HCV RNA negativity at week 4 of treatment is associated with high chances (80%-90%) of virological response regardless of HCV genotype. Patients without an early virological response defined as a more than 2 log drop in HCV RNA at week 12 or patients with detectable HCV RNA (HCV RNA >50 IU/ml) at week 24 of treatment have very little chance of achieving an SVR (16). Another on treatment factor determining the probability of SVR is treatment adherence. Patients receiving more than 80% of the prescribed peginterferon alfa and ribavirin dose for more than 80% of the expected duration of therapy have higher chances of SVR (16-17).

Direct Acting Antiviral Agents

In the past years, the growing insight in the HCV life cycle and the structural features of HCV proteins has enabled the development of new direct acting antiviral agents (DAAs) (18-19). Most DAAs inhibit nonstructural proteins which are necessary for viral replication. The most evaluated group of DAAs are the protease inhibitors which inhibit viral replication by inhibiting the NS3/4A protease which is responsible for cleavage of the other nonstructural proteins and unwinding of viral RNA (20-21). Another type of DAAs is the (non)nucleoside NS5B polymerase inhibitor. Nucleoside analogue inhibitors are incorporated into the growing RNA chain which leads to termination of nucleic acid synthesis. Nonnucleoside inhibitors inactivate the NS5B by binding to 1 out of 4 allosteric sites of the polymerase enzyme (22).

Currently, the most advanced DAAs are telaprevir and boceprevir which are both inhibitors of the HCV NS3 protease. Results of phase 2 and 3 trials investigating both drugs show promising results with significant increases of SVR rates in HCV genotype 1 patients (23-26). Due to pre-existing resistant HCV quasiespecies and the development of resistant mutations DAA's cannot be given as monotherapy and are only effective when combined with peginterferon alfa and ribavirin (27). Unfortunately DAA's are not without adverse effects and adding them to the combination of peginterferon alfa and ribavirin makes antiviral therapy for chronic HCV even more difficult to endure.

Side effects

Treatment with peginterferon alfa and ribavirin is associated with many side effects. The major types of side effects include fatigue, influenza-like illness, gastrointestinal, dermatological and neuropsychiatric side effects and hematologic abnormalities. These side effects impair adherence to therapy, lead to dose reductions and/or discontinuation of therapy. Sometimes side effects even lead to hospital admissions or death (28-29). Management of adverse effects is thus crucial to prevent serious adverse events and to minimize treatment failure (17).

There is some evidence from animal studies that treatment with peginterferon alfa and ribavirin therapy is teratogenic (30-33). For this reason strict guidelines exist for the exclusion of pregnant patients or patients with a pregnancy wish. It is strongly recommended to use double contraception till 4 months after treatment for female and till 7 months for male patients (34-35). Furthermore male patients with pregnant partners are excluded from antiviral therapy and female partners are strictly advised to use double contraception. It is however unknown whether treatment in male patients can lead to teratogenicity.

Dose reductions

Most dose reductions are performed because of cytopenias such as anemia, neutropenia and thrombocytopenia. Neutropenia is the most common reason for dose reductions (9). However, studies investigating the correlation between neutropenia and infections could not find any relation between absolute neutrophil counts (36-37). To prevent severe bleedings, patients with baseline thrombocytopenia, who are often cirrhotic and need therapy the most, are excluded from antiviral therapy. Furthermore dose reductions are performed during treatment in the case of platelet counts $<50000/\mu\text{L}$. It is however unknown of these patients are at risk to develop severe bleedings. Dose reductions reduce treatment efficacy and guidelines for dose reductions might be overly cautious. For this reason it is important to investigate the occurrence of side effects associated with cytopenias during antiviral treatment.

The focus of this thesis is the improvement of current standard therapy for hepatitis C by investigating risk factors for side effects, necessity for dose reductions or exclusion of treatment, alternative forms of administration of interferon alfa and the use of the most optimal HCV RNA assay for on-treatment response monitoring.

Goals and aims of this thesis

To investigate which factors are associated with an increased risk of infection and bleeding and whether absolute neutrophil counts and platelet counts are associated with an increased incidence of infection and bleeding respectively.

To investigate whether the use of a very sensitive HCV RNA TMA assay would change the negative predictive value in the prediction of response and whether the current stopping rules are still applicable using more sensitive assays.

To investigate the safety, feasibility and efficacy of the continuous delivery of interferon alfa-2b in combination with ribavirin in patients who previously failed antiviral therapy.

To investigate the effect of chronic hepatitis C and peginterferon alfa/ribavirin treatment on spermatogenesis, sperm DNA integrity and endocrinological parameters.

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;244:359-362.
2. De Francesco R. Molecular virology of the hepatitis C virus. *J Hepatol* 1999;31 Suppl 1:47-53.
3. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998;282:103-107.
4. Tillmann HL, Thompson AJ, Patel K, Wiese M, Tenckhoff H, Nischalke HD, Lokhnygina Y, et al. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. *Gastroenterology* 2010;139:1586-1592, 1592 e1581.
5. www.who.int/immunization/topics/hepatitis_c/en/.
6. Thein HH, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. *Hepatology* 2008;48:418-431.
7. Davis GL, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. *Liver Transpl* 2003;9:331-338.
8. Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, Peters M, Waggoner JG, 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.
9. 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.
10. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-355.
11. Jacobson IM, Brown RS, Jr., Freilich B, Afdhal N, Kwo PY, Santoro J, Becker S, et al. Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. *Hepatology* 2007;46:971-981.
12. Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, et al. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005;352:2609-2617.
13. 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.
14. Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver D. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009;49:1335-1374.
15. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;461:399-401.
16. Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Goncales FL, Jr., Haussinger D, et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. *J Hepatol* 2005;43:425-433.
17. Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, Lok AS, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007;132:103-112.
18. Lange CM, Sarrazin C, Zeuzem S. Review article: specifically targeted anti-viral therapy for hepatitis C - a new era in therapy. *Aliment Pharmacol Ther* 2010;32:14-28.

19. Lindenbach BD, Evans MJ, Syder AJ, Wolk B, Tellinghuisen TL, Liu CC, Maruyama T, et al. Complete replication of hepatitis C virus in cell culture. *Science* 2005;309:623-626.
20. Kim JL, Morgenstern KA, Lin C, Fox T, Dwyer MD, Landro JA, Chambers SP, et al. Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. *Cell* 1996;87:343-355.
21. Kim JL, Morgenstern KA, Griffith JP, Dwyer MD, Thomson JA, Murcko MA, Lin C, et al. Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding. *Structure* 1998;6:89-100.
22. Deore RR, Chern JW. NS5B RNA dependent RNA polymerase inhibitors: the promising approach to treat hepatitis C virus infections. *Curr Med Chem* 2010;17:3806-3826.
23. Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009;360:1839-1850.
24. Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010;376:705-716.
25. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-1838.
26. McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, et al. Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010;362:1292-1303.
27. Kieffer TL, Kwong AD, Picchio GR. Viral resistance to specifically targeted antiviral therapies for hepatitis C (STAT-Cs). *J Antimicrob Chemother* 2010;65:202-212.
28. Fried MW. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002;36:S237-244.
29. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology* 2003;124:1711-1719.
30. Hibi H, Yokoi K, Yamamoto M. Effects of alpha-interferon on sperm production, concentration, and motility in the rat. *Int J Urol* 1997;4:603-607.
31. Narayana K, D'Souza UJ, Rao KP. Effect of ribavirin on epididymal sperm count in rat. *Indian J Physiol Pharmacol* 2002;46:97-101.
32. Narayana K, D'Souza UJ, Seetharama Rao KP. Ribavirin-induced sperm shape abnormalities in Wistar rat. *Mutat Res* 2002;513:193-196.
33. Ulusoy E, Cayan S, Yilmaz N, Aktas S, Acar D, Doruk E. Interferon alpha-2b may impair testicular histology including spermatogenesis in a rat model. *Arch Androl* 2004;50:379-385.
34. <http://www.roche.nl/fmfiles/re7123011/PRODUCTEN/Copegus/Copegus-200mg-VII-62-101101.pdf>.
35. http://www.schering-plough.nl/binaries/Pati_ntenbijsluiting_Rebetol_tcm_169-189839.pdf.
36. Roomer R, Hansen BE, Janssen HL, de Knegt RJ. Risk factors for infection during treatment with peginterferon alfa and ribavirin for chronic hepatitis C. *Hepatology* 2010;52:1225-1231.
37. Soza A, Everhart JE, Ghany MG, Doo E, Heller T, Promrat K, Park Y, et al. Neutropenia during combination therapy of interferon alfa and ribavirin for chronic hepatitis C. *Hepatology* 2002;36:1273-1279.

ADVERSE EFFECTS AND THEIR MANAGEMENT IN THE CURRENT ERA OF HCV TREATMENT

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ABSTRACT

Chronic hepatitis C virus infection affects about 180 million people worldwide and is a leading cause of end stage liver disease and hepatocellular carcinoma. The current treatment regimen consists of a combination with peginterferon alfa and ribavirin. Between 40-90% of patients achieve a sustained virological response depending on viral genotype and several host factors. Recently developed direct acting antiviral agents will increase these percentages even more but only in combination with peginterferon alfa and ribavirin. Therefore peginterferon alfa and ribavirin will remain the backbone of antiviral therapy for the coming years. Unfortunately this treatment regimen is associated with many side effects which compromise treatment adherence and lead to dose reductions or discontinuations of therapy. Side effects include fatigue, fever, myalgia, headache, skin rashes, infections, cytopenias and psychiatric symptoms. Adding a direct acting viral agent to the current regimen will increase the number of side effects and their management will therefore be crucial to maintain effective doses and to optimize treatment adherence. This review focuses on side effects of the current and future treatment regimens for chronic hepatitis C and their management.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection affects about 180 million people worldwide. Approximately 20% of infected patients will develop cirrhosis which can lead to the development of hepatocellular carcinoma and end stage liver disease (1). Consequently, chronic HCV infection is the leading indication for liver transplantation in developed countries (2-3). The current treatment regimen consists of a weekly subcutaneous injection of peginterferon alfa (2a or 2b) combined with oral daily ribavirin for 24 or 48 weeks depending on virological response and HCV genotype. This treatment regimen leads to sustained virological response rates (SVR) of approximately 80% of patients infected with HCV genotype 2 and 3. For patients infected with genotype 1, the most common genotype in the western world, and genotype 4, a sustained virological response is only achieved in about 40 to 50% of patients (4-6).

Furthermore peginterferon alfa and ribavirin treatment is associated with many side effects. The major types of side effects include fatigue, influenza-like illness, gastrointestinal, dermatological and neuropsychiatric side effects and hematologic abnormalities. These side effects impair adherence to therapy, lead to dose reductions and/or discontinuation of therapy and sometimes they even lead to hospital admissions and death (7-8). Management of adverse effects is thus crucial to prevent serious adverse events and to minimize treatment failure (9).

In the past years, the growing insight in the HCV life cycle and the structural features of HCV proteins has enabled the development of new direct acting antiviral agents (DAAs) (10-11). Most DAAs inhibit nonstructural proteins which are necessary for viral replication. The most evaluated group of DAAs are the protease inhibitors which inhibit viral replication by inhibiting the NS3/4A protease which is responsible for cleavage of the other nonstructural proteins and unwinding of viral RNA (12-13). Another type of DAAs is the (non)nucleoside NS5B polymerase inhibitor. Nucleoside analogue inhibitors are incorporated into the growing RNA chain which leads to termination of nucleic acid synthesis. Nonnucleoside inhibitors inactivate the NS5B by binding to 1 out of 4 allosteric sites of the polymerase enzyme (14).

Currently, the most advanced DAAs are telaprevir and boceprevir which are both inhibitors of the HCV NS3 protease. Results of phase 2 and 3 trials investigating both drugs show promising results with significant increases of SVR rates in HCV genotype 1 patients (15-18). Due to pre-existing resistant HCV quasiespecies and the development of resistant mutations DAA's cannot be given as monotherapy and are only effective when combined with peginterferon alfa and ribavirin (19). Unfortunately DAA's are not without adverse effects and adding them to the combination of peginterferon alfa and ribavirin makes antiviral therapy for chronic HCV even more difficult to endure.

This review focuses on adverse effects of treatment with peginterferon alfa and ribavirin including new or more severe adverse effects which come into prominence due to the addition of DAA's to peginterferon alfa and ribavirin.

CURRENT TREATMENT REGIMENS

Peginterferon alfa-2a or 2b in combination with ribavirin

The current standard treatment for chronic hepatitis C is a 24 to 48 week regimen with weekly peginterferon alfa 2a or 2b injections in combination with daily weight based ribavirin (20). The most common adverse events are listed in table 1. Nearly all patients developed at least one adverse event. Serious adverse events occurred in 8-12% of patients. Adverse events lead to dose reductions in 33-43% of patients treated and to discontinuation of therapy in 7-13% of patients (4-6, 21). In the IDEAL study peginterferon alfa-2b was compared with peginterferon alfa-2a. More patients treated with standard-dose peginterferon alfa-2b developed headache, nausea, pyrexia, myalgia and depression than patients treated with peginterferon alfa-2a whereas rash and neutropenia occurred more often in patients treated with peginterferon alfa-2a (21).

Table 1. Most common adverse events in pivotal peginterferon alfa and ribavirin studies

Adverse events	Manns	Fried	Hadziyannis	IDEAL	IDEAL
	PEGIFN alfa-2b 1.5 µg/kg	PEGIFN alfa-2a 180µg	All arms combined	PEGIFN alfa-2a 180µg	PEGIFN alfa-2b Standard dose
Fatigue	64	54	49	63	66
Headache	62	47	52	42*	50*
Pyrexia	46	43	42	23*	35*
Myalgia	56	42	41	23*	27*
Arthralgia	34	27	26		
Rigours	48	24	30		
Nausea	43	29	32	36*	43*
Diarrhoea	22		20		
Decreased appetite	32	21	18		
Weight loss	29				
Concentration loss	17				
Insomnia	40	37	36		39
Depression	31	22	21	21*	26*
Irritability	35	24	27	25	25
Dyspnoea	26				
Alopecia	36	28	25		
Rash	24	21	19	28*	22*
Pruritus	29	22	24		
Injection site reactions	58				
Serious adverse events			8	12	9
Hematologic abnormalities					
Anemia				34	34
Neutropenia				26*	32*
Dose reductions:	42	32		43	43
Discontinuations**	14	10	12	13	13

* P<0.05

**due to adverse events

FUTURE REGIMENS

Peginterferon alfa-2a in combination with ribavirin and telaprevir

Telaprevir, a HCV NS3/4a protease inhibitor, has been studied extensively in combination with peginterferon alfa and ribavirin (15, 17-18, 21-23). These studies have shown significantly improved SVR rates in patients treated with triple therapy compared to standard of care with peginterferon alfa and ribavirin. The highest SVR rates in treatment naïve HCV genotype 1 patients were achieved in the ADVANCE trial: 75% of patients treated with 12 weeks of triple therapy followed by 12 or 36 weeks of peginterferon alfa and ribavirin achieved SVR (24). SVR rates in HCV genotype 1 patients who previously failed treatment with peginterferon alfa SVR rates ranged from 38% in previous nonresponders to 76% in prior relapsers. A future treatment regimen with peginterferon alfa, ribavirin and telaprevir will most likely exist of 12 weeks of triple therapy followed by 12 or 36 weeks of peginterferon alfa and ribavirin depending on

Table 2. Most common adverse events in peginterferon alfa, ribavirin and telaprevir trials

Adverse events	PROVE 1		PROVE 2		PROVE 3		ADVANCE	
	PR	PRT*	PR	PRT*	PR	PRT*	PR	PRT*
Fatigue	76	75	37	27	56	64**		
Asthenia			32	49				
Influenza like illness			52	39	32	29		
Headache	23	37	45	42	36	39		
Pyrexia	29	29	23	18	12	19**		
Pruritis	23	37	35	57	15	39**		
Generalized pruritis	0	15						
Rash	41	58	35	47	20	55**	37	54
Myalgia	24	16	21	14	18	18		
Arthralgia	21	21	17	10	18	16		
Dyspnea	15	15	16	24				
Cough	19	20	26	18	18	16		
Nausea	29	52	40	48	34	42**		
Vomiting	12	21						
Diarrhea	28	38	28	28	19	38**		
Concentration loss	9	10						
Insomnia	39	39	39	31	17	28**		
Depression	17	18	23	21	17	13		
Irritability	29	13			22	21		
Hemorrhoids	1	18			3	15**		
Injection site reaction	24	32						
Alopecia			21	11	11	18**		
Hematologic abnormalities:								
Anemia	27	34	17	23	8	26**	14	38**
Neutropenia	24	13						
Discontinuations**	11	21	7	12	4	17	7	10

* includes all arms containing peginterferon alfa, ribavirin and telaprevir

** due to adverse events

virological response during treatment (25). Unfortunately telaprevir does not come without side effects (table 2). In the largest telaprevir trial the proportion of patients with rash, pruritus, nausea, diarrhoea and anaemia was at least 10% higher in the telaprevir containing arms compared to the control arm (24). Other adverse events which occurred more often in the telaprevir based arms were vomiting, haemorrhoids and insomnia. Especially rash and anemia were common side effects. Also the proportion of patients who discontinued treatment due to an adverse event was higher in the telaprevir arms. Rash and anemia were the most common reasons for treatment discontinuation. Rashes were mostly maculopapular of origin, appeared after 7 to 28 days after treatment onset. Treatment included topical anti-allergic agents and topical and systemic antipruritic agents. Up to 7% of the rashes were defined as severe (grade 3), and corticosteroid treatment was necessary in some patients. After treatment discontinuation all rashes resolved.

Anemia was more common in the telaprevir arms as well. Median decrease in hemoglobin from baseline was 0.5 to 1.0 g/dL larger compared to the control arms. However, very few patients had discontinuation of treatment due to anemia. Use of erythropoietin was prohibited during the telaprevir phase or during the complete treatment duration in all of these studies . (15, 17-18, 21-23) (26)

Peginterferon alfa-2b in combination with ribavirin and Boceprevir

Boceprevir is a peptidomimetic NS3 protease inhibitor with strong antiviral activity against HCV. Boceprevir in combination with peginterferon alfa-2b and ribavirin has been investigated in the SPRINT-1, SPRINT-2 and the RESPOND-2 studies. In all studies adding boceprevir to standard of care with peginterferon alfa-2b and ribavirin significantly improved SVR rates (16, 27-28). Different from the telaprevir based studies; these studies also included arms with a 4 week peginterferon alfa and ribavirin lead-in period prior to adding boceprevir triple therapy. The rationale of the lead-in period was to eliminate pre-existing viral mutations and to prevent viral mutations by decreasing the viral load prior to treatment with boceprevir.

Side effects of boceprevir in combination with peginterferon alfa-2b and ribavirin are listed in table 3. Similar to triple therapy with telaprevir, triple therapy with boceprevir led to higher rates of treatment discontinuation and dose reductions due to adverse events. Adverse events which occurred more often in the boceprevir containing arms were anemia, neutropenia and dysgeusia (loss of taste). A hemoglobin concentration <100 g/L occurred in 204 out of 416 patients (49%) receiving triple therapy with boceprevir compared to 25 out of 104 patients (25%) receiving standard of care and more patients developed neutropenia (32%) in the boceprevir containing arms compared to standard of care (17%). Unlike telaprevir, boceprevir did not lead to increased rates of rash and pruritus. These results were comparable in all boceprevir trials (16, 27-28).

Table 3. Most common adverse events in peginterferon alfa, ribavirin and boceprevir trials

	SPRINT-1*		SPRINT-2**		RESPOND-2	
	PR	PRB	PR	PRB	PR	PRB
Fatigue	55	62			50	55
Anemia	34	55	29	49	20	44
Headache	43	46			48	40
Nausea	43	45			38	42
Insomnia	38	35			20	30
Pyrexia	34	31				
Alopecia	26	31				
Diarrhoea	22	26				
Dysgeusia	9	27	18	40	11	44
Neutropenia	12	24				
Influenza like illness	24	19				
Arthralgia	20	18				
Vomiting	5	19				
Decreased appetite	12	12				
Injection site reactions	10	8				
Dose reductions due to adverse events	29	57	26	37	14	31
Discontinuation due to adverse events	8	13	16	14	3	10

*percentage in the PRB column is based on all boceprevir containing arms in part 1 of the study

**percentage in the PRB column based on boceprevir containing arms of the SPRINT-2 study

Other protease inhibitors in combination with peginterferon alfa and ribavirin

Besides telaprevir and boceprevir several other protease inhibitors are currently being developed. danoprevir, vaniprevir, narlaprevir and TMC-435 are currently investigated in phase 2 studies. Like boceprevir and telaprevir, danoprevir is associated with an increased incidence of adverse events. Most common adverse events were fatigue, nausea, headache, diarrhoea, myalgia, insomnia, chills and vomiting. Nausea and diarrhoea did occur more often in the danoprevir containing arms. Anemia and neutropenia did not occur more often in the danoprevir containing arms. Furthermore, the arm with the highest dose of danoprevir (900mg BID) was terminated early due to grade 4 ALT elevations (10xULN). ALT elevations occurred between week 6 and 12 and were asymptomatic. In 1 patient bilirubin was elevated as well. All ALT elevations were reversible after discontinuation of danoprevir (29).

Vaniprevir, another NS3/4A protease inhibitor, has been investigated in combination with peginterferon alfa-2a and ribavirin in a small trial. Most common adverse events were headache, nausea, vomiting, fatigue and flu-like symptoms. In the highest dose arms (600mg BID vaniprevir) vomiting occurred more often compared to the control arm. Adverse events were mild to moderate in severity and did not lead to vaniprevir discontinuation. The incidence of rash was similar between vaniprevir-containing arms and the control arm (30).

Narlaprevir is another novel potent oral direct-acting antiviral agent which has been studied in two phases, in the first phase as monotherapy or in combination with ritonavir and in a second phase in combination with peginterferon alfa-2b and ritonavir. Most common adverse events during the first phase were gastrointestinal symptoms (diarrhea, anorectal discomfort, abdominal discomfort and abdominal distension) (31).

TMC-435 is a promising macrocyclic protease inhibitor with strong antiviral activity against hepatitis C. In a phase 1 trial with 49 healthy volunteers and 6 HCV patients occurring adverse events were headache, abdominal pain, abdominal distension and photosensitivity reactions (32). In a phase 2 trial TMC-435 has been investigated in combination with peginterferon alfa-2a and ribavirin. SVR rates of approximately 90% were achieved in all treatment arms receiving triple therapy with TMC-435. Most common adverse events were headache, nausea, pruritis, fatigue and flu-like symptoms with no significant differences between the control arm and the treatment arms containing triple therapy with TMC-435. In the TMC-435 containing arms mild increases in bilirubin were observed the first 2 weeks followed by gradual decreases to baseline levels.

(Non)nucleoside polymerase inhibitors

Several nucleoside NS5b inhibitor have been developed. The clinical development of some nucleoside polymerase inhibitors, e.g. valopicitabine and R1626 have been terminated have been stopped due to side effects and lack of efficacy (33-35).

RG7128, a nucleoside inhibitor and prodrug of PSI-6130 has been investigated in combination with the protease inhibitor danoprevir and is currently being investigated in combination with peginterferon alfa-2a/ribavirin combination therapy. Most common adverse events were headache, lethargy, gastrointestinal symptoms and nausea. Almost all adverse were graded as mild to moderate. There were no grade 3 or 4 laboratory abnormalities observed in this study. Neutropenia was the most common laboratory abnormality occurring in 13 to 23 percent of patients (36).

Nonnucleoside inhibitors are currently being investigated in phase 1 and 2 trials. The most advanced nonnucleoside inhibitors at the moment are tegobuvir (GS-9190) and ANA598. In a study investigating tegobuvir with or without GS-9256, a novel NS3 protease inhibitor and/or peginterferon alfa/ribavirine for 4 weeks followed by 44 weeks of peginterferon alfa-2a and ribavirin the most frequent adverse events were headache, diarrhoea and nausea. Some patients developed hyperbilirubinemia including grade 3 hyperbilirubinemia in 2 patients (37).

ANA598 is another nonnucleoside inhibitor which is being investigated in combination with peginterferon alfa-2a and ribavirin for 12 weeks followed by 36 weeks of peginterferon alfa-2a and ribavirin. ANA598 400mg BID was (the highest dose) associated with an increased rash incidence. In 1 patient treatment was discontinued due to grade 3 rash. In all patients a mild reversible increase in bilirubin levels was observed. Other adverse events rates were comparable with standard of care.

MANAGEMENT OF ADVERSE EVENTS

Managing adverse events during treatment with peginterferon alfa and ribavirin is crucial to optimize adherence to antiviral treatment. Sustained virological response rates are higher in patients with full adherence to the prescribed regimen compared to patients with less than 80% of the prescribed treatment (9, 38). For this reason preventing and minimizing dose reductions and treatment discontinuations because of adverse events is important.

General symptoms

Fatigue, headache, fever, myalgia, arthralgia and loss of appetite are the most common adverse events during treatment with peginterferon alfa and ribavirin. Since this treatment regimen will remain the backbone of antiviral treatment for the coming years, these adverse events still need to be managed. No studies have been performed to investigate the best treatment options for these side effects. Widely used is acetaminophen prior to the time of the injection which helps relieving flu-like side effects. After some weeks these adverse events generally resolve, however, fatigue, loss of appetite and loss of energy will remain important side effects which can affect adherence to therapy. Most patients have weight loss during treatment, adequate nutrition and hydration may help to improve fatigue and weight loss.

Psychiatric side effects

Neuropsychiatric side effects are very common during antiviral therapy with peginterferon alfa and ribavirin. During treatment several symptoms such as impaired cognition, agitation, irritability and depressive symptoms can occur (39-42). Approximately one third of treated patients develop a major depression or suffers from clinically significant depressive symptoms during antiviral treatment (43-44). The large prevalence of neuropsychiatric side can partly be contributed to pre-existing psychiatric morbidities and substance abuse. Another explanation for depressive symptoms is that patients infected with chronic hepatitis C have reduced tryptophan levels compared to healthy controls. Tryptophan levels can return to physiological levels after successful antiviral treatment (45). Peginterferon alfa based treatment itself probably causes tryptophan depletion leading to a worsening of depressive symptoms during antiviral therapy (46). Several studies have investigated the use of selective serotonin re-uptake inhibitors (SSRI's) for the prevention of the development of depression (47-50). Prophylactic treatment with citalopram or escitalopram is not supported by the findings of these studies. However SSRI's are highly effective in the treatment of peginterferon alfa induced depression. Further studies should be aimed to identify subgroups of patients with a high risk of developing depressive symptoms which could benefit from prophylactic treatment with SSRI's during antiviral treatment.

Dermatologic side effects

Frequently occurring dermatologic side effects include alopecia, dermatitis, pruritus, dry skin and injection site reactions (51). Most of these mild skin reactions can be treated with topical corticosteroids, anti-pruritic crèmes and hydrating lotions. Alopecia occurs in approximately 20% of patients and is completely reversible in nearly all cases (52). Less frequent dermatologic adverse events include exacerbation of lichen planus, erythematous and maculopapular rash, sweating, psoriasis, urticaria, eczema, nail disorders and abnormal hair texture (51, 53-55). Patients with pre-existing psoriasis may experience a psoriatic flare during treatment. These patients should be managed in conjunction with a dermatologist. In case they do not respond to anti-psoriatic therapy, treatment discontinuation is inevitable (55). Very rare but severe skin reactions include toxic epidermal necrolysis, Stephen-Johnson syndrome, angioedema, extensive erythema multiforme, cutaneous sarcoidosis, linear immunoglobulin A bullous dermatosis and porphyria cutanea tarda (53-54). In case these skin reactions occur treatment should be discontinued immediately.

Hematologic abnormalities

Anemia

Anemia is one of the most common adverse events leading to dose reductions and discontinuations of peginterferon alfa and ribavirin. Anemia is caused partly by a strong bone marrow suppression of peginterferon alfa. The other mechanism underlying the anemia is ribavirin induced hemolysis. Ribavirin is taken up by erythrocytes and activated to ribavirin triphosphate. Erythrocytes are unable to hydrolyze ribavirin triphosphate, which results in entrapment of ribavirin triphosphate in erythrocytes leading to 60-fold greater concentrations in erythrocytes compared to plasma concentrations (56). The largest drop in hemoglobin levels occur in the first 8 weeks of treatment with peginterferon alfa and ribavirin followed by a gradual stabilisation of hemoglobin levels and a rapid return to baseline levels after treatment discontinuation. The mean maximum decline in hemoglobin levels in different studies varies from 2.5 to 3.7 g/dL. Approximately 9 to 28% of patients will have a drop in hemoglobin below 10g/dL and thus require dose modifications of ribavirin (4-6, 21, 57-59).

The extent of this treatment induced anemia varies greatly between treated individuals. Recently, genetic variants of inosine triphosphatase have been found to be strongly associated with ribavirin induced anemia and the number of dose reductions of ribavirin. The polymorphisms rs1127354 and rs7270101 were significantly associated with hemoglobin reduction at week 4. The minor alleles of both variants protected against Hb decline and this association was strengthened when both variants were combined. Patients with the minor alleles on both variants had lower rates of anemia, and ribavirin dose reductions were observed. No association between SVR and these protective variants could be found (60-62). Other patient characteristics which are associated with the amount of ribavirin induced

hemolytic anemia are female gender, age >60, higher ribavirin dose, Asian race and a reduced creatinine clearance (63-65).

Peginterferon alfa and ribavirin induced anemia leads to an increase of complaints of fatigue, dyspnea d'effort and can significantly impair quality of life. To our knowledge, an increase in cardiac ischaemia during peginterferon/ribavirin has never been observed.

Adding telaprevir to the current treatment regimen leads to an increase of hemoglobin decline with 0.5-1.0g/dL (15, 17, 66). In the Boceprevir studies 49% of patients had a hemoglobin drop larger than 100g/dL (16). For this reason management of anemia during antiviral treatment for chronic hepatitis C will become even more important.

Treatment induced anemia leads to dose reductions of ribavirin and a decrease in compliance to therapy. In many clinical trials dose reductions of ribavirin were performed in case of a hemoglobin concentration < 10g/dL (20). It is however known that dose reductions can compromise treatment efficacy (9). Blood transfusions help to increase the haemoglobin levels temporarily, however multiple blood transfusions can lead to iron overload and the occurrence of irregular antibodies. The use of epoetin alfa during treatment with peginterferon alfa and ribavirin to prevent anemia has been investigated thoroughly. Addition of 40000 units of epoetin alfa in patients with Hb levels ≤ 12 g/dL lead to an increase of Hb levels with 2.8 g/dL compared to 0.4 g/dL in the placebo arm ($p < 0.0001$). In epoetin alfa arm in 83% of patients ribavirin dosages of at least 800mg could be maintained compared to 54% of patients in the placebo arm (67). These results were confirmed by a larger randomized trial. After 8 weeks of 40000 units epoetin alfa subcutaneously once weekly ribavirin dosages were maintained in 88% of patients compared to 60% of patients receiving placebo ($p < 0.001$). Furthermore mean quality of life scores significantly improved on all domains of the linear analog scale assessment and on 7 of the 8 domains of the short form-36. Epoetin alfa was well tolerated with nausea and headache as the most common side effects (68). In a post hoc analysis of the same study cohort, mean health quality of life scores of patients with anemia during antiviral treatment were significantly lower than those of both the general population and patients who had other chronic conditions. Hemoglobin level increases after the addition epoetin alfa were strongly associated with an improved quality of life with the largest improvements in patients with the greatest hemoglobin increases (69). Another study investigated the effect of EPO and higher doses of ribavirin on SVR rates. This study contained 3 treatment arms: standard dose ribavirin (13.3 mg/kg) without epoetin alfa, standard dose ribavirin with epoetin alfa and high dose ribavirin (15.2 mg/kg) in combination with epoetin alfa. All arms were in combination with peginterferon alfa-2b. Patients receiving standard dose ribavirin and epoetin alfa had less anemia (defined as Hb 10g/dL) and less dose reductions of ribavirin. However SVR rates did not differ significantly between groups (29% vs. 19% in the placebo and epoetin alfa arm respectively). The SVR rate in patients receiving high dose ribavirin and epoetin alfa was significantly higher compared to the groups with standard dose ribavirin (49% vs. 29% and

19%, $p < 0.05$) (70). In conclusion, the use of epoetin alfa increases quality of life and reduces the need for dose reductions. However, an increased SVR rate has never been shown.

Neutropenia

Peginterferon alfa induced bone marrow suppression causes a rapid decline in absolute neutrophil counts. The mean decline in absolute neutrophil count from baseline to nadir ranges from 34-68% during peginterferon alfa and ribavirin therapy. The strongest decline can be found in the first 4 weeks followed by a gradual stabilisation. The median time to the lowest neutrophil count (ANC) ranges from 4-14 weeks. Approximately 30% of patients develop an ANC below $750/\mu\text{L}$ and 2 to 6.5% of patients develop an ANC below $500/\mu\text{L}$ (71-74). The addition of boceprevir to standard of care might increase rates of neutropenia. When all the boceprevir containing arms are combined from the SPRINT 1 trial, the proportion of patients with an ANC below $750/\mu\text{L}$ is 22% compared to 15% of patients receiving standard of care (16). In the SPRINT 2 trial 24 and 25% of patients in the boceprevir containing arms had ANC between $500-750/\mu\text{L}$ compared to 14% of patients receiving standard of care. The proportion of patients with ANC below $500/\mu\text{L}$ was comparable between boceprevir containing arms and standard of care (6 and 8% vs. 4%) (28).

Current guidelines recommend dose reductions when ANC drops below $750/\mu\text{L}$ and treatment discontinuation when ANC drops below 500 or $375/\mu\text{L}$ (75). These guidelines are based on findings of large cohort studies of infections in oncology patients receiving chemotherapy. These studies conclude that the risk of infection is increased in patients who develop grade 4 neutropenia ($<500/\mu\text{L}$) with the largest risk of infection in patients with an $\text{ANC} < 100/\mu\text{L}$. However, in these patients, other factors such as mucosal damage, the effects of commensal flora, and the alteration of organ function caused by the underlying disease also contribute to the risk of infection. In patients with chronic hepatitis C these factors are mostly absent. Several studies have investigated the correlation between ANC and infections. Although an increased incidence of infections exists during antiviral therapy, none of these studies could demonstrate a correlation between neutrophil counts and infections. Infection rates ranged from 4-23% (71-74, 76-77). Most of these infections were mild, however, some may lead to discontinuation of therapy or hospital admission. In one study investigating the risk factors for infection during antiviral treatment, older age and baseline hyperglycemia were significantly associated with an increased risk of infection. Chronic obstructive pulmonary disease, compensated cirrhosis and on treatment neutropenia were not associated with an increased incidence of infections (72).

The use of granulocyte colony-stimulating factor has been shown to significantly increase leucocytes and absolute neutrophil count and thereby prevent dose reductions during treatment with peginterferon alfa and ribavirin in some studies (78-80). Younossi et al. found a positive effect on SVR rates in patients using growth factors (darbepoetin alfa and filgrastim). However, this study was small and the effect on infection rate during antiviral treatment was

not investigated (80). Considering the fact that absolute neutrophil counts below 750/ μ L are not associated with an increased infection rate during antiviral treatment, the benefit of adding G-CSF to peginterferon alfa and ribavirin therapy can be questioned.

In conclusion, an increased incidence of infections exists in patients treated with peginterferon alfa and ribavirin, however infection risk is not correlated with the absolute neutrophil count and the use of growth colony stimulating factors is questionable. Changes of guidelines should be considered. A possible alternative guideline for neutropenia would be to maintain peginterferon alfa dose as long as absolute neutrophil counts remain above 375/ μ L in case the patient does not have signs of a bacterial infection. In case of an absolute neutrophil count below 375/ μ L, peginterferon alfa dose should be reduced in steps of 25% until ANC remains stable above 375/ μ L.

Thrombocytopenia

The proportion of patients developing severe thrombocytopenia (grade 3 or 4) during treatment with peginterferon alfa and ribavirin is not as large as patients developing neutropenia but is still considerable. Grade 3 thrombocytopenia (platelet count <50000/ μ L) occurs in 3-9% of patients treated with peginterferon alfa and ribavirin and a fraction of patients develops grade 4 thrombocytopenia (platelet count <25000/ μ L) (4-6, 81). However, cirrhotic patients, who need antiviral therapy the most, are often thrombocytopenic due to increased sequestration of thrombocytes in the enlarged spleen and an impaired thrombopoietin production (82-83). Thrombocytes further decrease after initiation of therapy with peginterferon alfa and ribavirin due to bone marrow suppression.

Recently a study on the occurrence of thrombocytopenia and the risk of bleeding during peginterferon alfa and ribavirin treatment has been published. In a cohort of 321 patients 30 patients developed grade 3 thrombocytopenia. Cirrhotic patients were more likely to develop grade 3 thrombocytopenia than non-cirrhotic patients (35.3% vs. 2.4%, $p < 0.001$). A total of 48 bleedings were observed in 27 patients. Having grade 3 thrombocytopenia was strongly associated with the occurrence of bleedings. However, all bleedings reported in patients with grade 3 thrombocytopenia or worse were classified as mild (no specific treatment or dose reduction of peginterferon alfa). Only 1 bleeding was defined as severe (gastrointestinal bleeding with hemoglobin decline), however this bleeding occurred in a patient with a platelet count above 50000/ μ L. The authors concluded that no severe bleedings occurred in patients with grade 3 thrombocytopenia and peginterferon alfa/ribavirin treatment was generally safe in this specific group. Furthermore patients with baseline thrombocytopenia should not be excluded from antiviral therapy (81).

Eltrombopag, an orally active thrombopoietin-receptor agonist, has been studied in several diseases associated with thrombocytopenia. McHutchison et al. performed a fase 2 study to investigate the effect of eltrombopag in chronic hepatitis C patients with baseline thrombocytopenia (platelet counts between 20000 and 70000/ μ L). Patients were randomly assigned to

receive eltrombopag (30, 50 or 75mg daily) or placebo. The primary endpoint was a platelet count of 100000/ μ L or more after 4 weeks of treatment. After 4 weeks 75%, 79% and 95% of patients receiving 30, 50 and 75mg daily achieved the primary endpoint compared to 0% of patients receiving placebo. In case of platelet counts \geq 100000 antiviral treatment with peginterferon alfa and ribavirin was initiated. Twelve weeks of treatment was completed in 36%, 53% and 65% of patients receiving 30, 50 and 75 mg of eltrombopag respectively compared to 6% of patients receiving placebo. The authors concluded that eltrombopag increases platelet count in patients with cirrhosis related thrombocytopenia, thereby permitting the initiation of antiviral therapy. Eltrombopag was generally safe; however, eltrombopag is known to cause thrombosis and cataract (84). Furthermore it is expensive and will drive up the costs of HCV treatment even further. Eltrombopag could be a solution for patients with severe thrombocytopenia; however, further studies are required to investigate the effect of eltrombopag on the prevention of bleeding. Furthermore one trial investigating the safety and efficacy of eltrombopag prior to elective invasive procedures in patients with thrombocytopenia was terminated early due to an unexpected number of patients developing a portal vein thrombosis. In conclusion, grade 4 thrombocytopenia is rare during antiviral therapy, although it is more common in patients with liver cirrhosis. Grade 4 thrombocytopenia is associated with an increase in bleedings but major bleedings almost never occur. Eltrombopag could be a solution for patients with grade 4 thrombocytopenia.

A possible alternative for the current guidelines for thrombocytopenia would be to maintain peginterferon alfa dose when platelet counts remain stable above 25000/ μ L as long as the patient does not have signs of active bleeding. Peginterferon alfa dose should be temporarily discontinued when platelet counts drop below 25000/ μ L and could be restarted at a lower dose when platelet counts rise above 25000/ μ L again.

CONCLUSION

Peginterferon alfa and ribavirin have many side effects which lead to impaired treatment adherence and dose reductions of both drugs. Adequate treatment of side effects is crucial in order to maintain the optimal doses and thereby achieve the highest chances of a successful treatment outcome. Adding telaprevir or boceprevir to the current treatment regimen will increase chances of SVR with a possibility of shorter treatment duration. However, peginterferon/ribavirin will remain the backbone of anti HCV treatment for the coming decade and the addition of telaprevir or boceprevir will lead to an increase in adverse events and therefore side effect management remains crucial in order to optimize treatment adherence.

REFERENCES

1. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997;349:825-832.
2. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001;345:41-52.
3. Verna EC, Brown RS, Jr. Hepatitis C virus and liver transplantation. *Clin Liver Dis* 2006;10:919-940.
4. 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.
5. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-355.
6. 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.
7. Fried MW. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002;36:S237-244.
8. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology* 2003;124:1711-1719.
9. Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, Lok AS, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007;132:103-112.
10. Lange CM, Sarrazin C, Zeuzem S. Review article: specifically targeted anti-viral therapy for hepatitis C - a new era in therapy. *Aliment Pharmacol Ther* 2010;32:14-28.
11. Lindenbach BD, Evans MJ, Syder AJ, Wolk B, Tellinghuisen TL, Liu CC, Maruyama T, et al. Complete replication of hepatitis C virus in cell culture. *Science* 2005;309:623-626.
12. Kim JL, Morgenstern KA, Lin C, Fox T, Dwyer MD, Landro JA, Chambers SP, et al. Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide. *Cell* 1996;87:343-355.
13. Kim JL, Morgenstern KA, Griffith JP, Dwyer MD, Thomson JA, Murcko MA, Lin C, et al. Hepatitis C virus NS3 RNA helicase domain with a bound oligonucleotide: the crystal structure provides insights into the mode of unwinding. *Structure* 1998;6:89-100.
14. Deore RR, Chern JW. NS5B RNA dependent RNA polymerase inhibitors: the promising approach to treat hepatitis C virus infections. *Curr Med Chem* 2010;17:3806-3826.
15. Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009;360:1839-1850.
16. Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naïve patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010;376:705-716.
17. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-1838.
18. McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, et al. Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010;362:1292-1303.
19. Kieffer TL, Kwong AD, Picchio GR. Viral resistance to specifically targeted antiviral therapies for hepatitis C (STAT-Cs). *J Antimicrob Chemother* 2010;65:202-212.

20. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009;49:1335-1374.
21. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009;361:580-593.
22. Reesink HW, Zeuzem S, Weegink CJ, Forestier N, van Vliet A, van de Wetering de Rooij J, McNair L, et al. Rapid decline of viral RNA in hepatitis C patients treated with VX-950: a phase Ib, placebo-controlled, randomized study. *Gastroenterology* 2006;131:997-1002.
23. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, et al. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011;364:2417-2428.
24. Ira M, Jacobson JGM, Geoffrey M, Dusheiko,, Adrian M, Di Bisceglie RR, Natalie H, Bzowej,, Patrick Marcellin AJM, Leif Bengtsson, Ann Marie, Dunne NA, Shelley George, Robert S. Kauffman,, Zeuzem S. Telaprevir in combination with peginterferon and ribavirin in genotype 1 HCV treatment-naive patients: final results of phase 3 ADVANCE study. . *Hepatology* 2010;52:427A.
25. Pawlotsky JM. The results of Phase III clinical trials with telaprevir and boceprevir presented at the Liver Meeting 2010: a new standard of care for hepatitis C virus genotype 1 infection, but with issues still pending. *Gastroenterology* 2011;140:746-754.
26. Sherman K, Flamm S, Afdhal N, Nelson DR, Sulkowski MS, Everson T, Fried MW, et al. Telaprevir in combination with peginterferon alfa-2a and ribavirin for 24 or 48 weeks in treatment-naive genotype 1 HCV patients who achieved an extended rapid virological response: final results of phase 3 ILLUMINATE study. *Hepatology* 2010;52:401A.
27. Bacon B. HCV Respond-2 final results: High sustained virologic response among genotype 1 previous non-responders and relapsers to peginterferon/ribavirin when re-treated with boceprevir plus pegintron (peginterferon alfa-2b)/ribavirin. *Hepatology (suppl)* 2010;52:430A.
28. Poordad F MCJ, Bacon BR, . Boceprevir combined with peginterferon alfa-2b/ribavirin for treatment-naive patients with HCV genotype 1: SPRINT-2 final results. . *Hepatology* 2010;Volume 52:2.
29. Terrault N, Cooper C, Balart LA, Larrey L, Box TD, Yoshida EM, Lawitz E, et al. Phase II randomised, partially-blind, parallelgroup study of oral danoprevir (RG7227) with peginterferon alfa-2a (PEGASYS®) plus ribavirin in treatment naive genotype 1patients with CHC: results of a planned week 12 interim analysis of the ATLAS study. *Hepatology* 2010;52:335A.
30. Manns MP, Gane EJ, Rodriguez-Torres M, Albrecht D, Stoehr, Yeh CT, Marcellin P, et al. Sustained viral response (SVR) rates in genotype 1 treatment naïve patients with chronic hepatitis C (CHC) infection treated with vaniprevir (MK-7009), a NS3/4A protease inhibitor, in combination with pegylated interferon alfa-2a and ribavirin for 28 days. *Hepatology* 2010;52:361A.
31. de Bruijne J, Bergmann JF, Reesink HW, Weegink CJ, Molenkamp R, Schinkel J, Tong X, et al. Antiviral activity of narlaprevir combined with ritonavir and pegylated interferon in chronic hepatitis C patients. *Hepatology* 2010;52:1590-1599.
32. Reesink HW, Fanning GC, Abou Farha K, Weegink C, Van Vliet A, van 't Klooster G, Lenz O, et al. Rapid HCV-RNA Decline With Once Daily TMC435: A Phase I Study in Healthy Volunteers and Hepatitis C Patients. *Gastroenterology* 2010;138:913-921.
33. Pockros P, Nelson D, Godofsky E, Rodriguez-Torres M, Everson GT, Fried MW, Ghalib R, et al. High relapse rate seen at week 72 for patients treated with R1626 combination therapy. *Hepatology* 2008;48:1349-1350.
34. Pockros PJ, Nelson D, Godofsky E, Rodriguez-Torres M, Everson GT, Fried MW, Ghalib R, et al. R1626 plus peginterferon Alfa-2a provides potent suppression of hepatitis C virus RNA and significant antiviral synergy in combination with ribavirin. *Hepatology* 2008;48:385-397.
35. Kronenberger B, Zeuzem S. Future treatment options for HCV: double, triple, what is the optimal combination? *Best Pract Res Clin Gastroenterol* 2008;22:1123-1136.

36. Jensen DM, Wedemeyer H, Herring RW, Rodriguez-Torres M, Bzowej NH, Pockros P, Vierling JM, et al. High rates of early viral response, promising safety profile and lack resistance-related breakthrough in HCV GT1/4 patients treated with RG7128 plus pegIFN alfa-2a (40KD)/RBV: planned week 12 interim analysis from the PROPEL study. *Hepatology* 2010;52:360A
37. Zeuzem S, buggisch P, Agarwal K, Manns MP, Marcellin P, Foster GR, Sereni D, et al. Dual, triple, and quadruple combination treatment with a protease inhibitor (GS-9256) and a polymerase inhibitor (GS-9190) alone and in combination with ribavirin (RBV) or PegIFN/RBV for up to 28 days in treatment-naive, genotype 1 HCV subjects. *Hepatology* 2010;52:400A.
38. Reddy KR, Shiffman ML, Morgan TR, Zeuzem S, Hadziyannis S, Hamzeh FM, Wright TL, et al. Impact of ribavirin dose reductions in hepatitis C virus genotype 1 patients completing peginterferon alfa-2a/ribavirin treatment. *Clin Gastroenterol Hepatol* 2007;5:124-129.
39. Dieperink E, Willenbring M, Ho SB. Neuropsychiatric symptoms associated with hepatitis C and interferon alpha: A review. *Am J Psychiatry* 2000;157:867-876.
40. Raison CL, Borisov AS, Broadwell SD, Capuron L, Woolwine BJ, Jacobson IM, Nemeroff CB, et al. Depression during pegylated interferon-alpha plus ribavirin therapy: prevalence and prediction. *J Clin Psychiatry* 2005;66:41-48.
41. Raison CL, Demetrashvili M, Capuron L, Miller AH. Neuropsychiatric adverse effects of interferon-alpha: recognition and management. *CNS Drugs* 2005;19:105-123.
42. Schaefer M, Schmidt F, Folwaczny C, Lorenz R, Martin G, Schindlbeck N, Heldwein W, et al. Adherence and mental side effects during hepatitis C treatment with interferon alfa and ribavirin in psychiatric risk groups. *Hepatology* 2003;37:443-451.
43. Kraus MR, Schafer A, Faller H, Csef H, Scheurlen M. Psychiatric symptoms in patients with chronic hepatitis C receiving interferon alfa-2b therapy. *J Clin Psychiatry* 2003;64:708-714.
44. Lang JP, Melin P, Ouzan D, Rotily M, Fontanges T, Marcellin P, Chousterman M, et al. Pegylated interferon-alpha2b plus ribavirin therapy in patients with hepatitis C and psychiatric disorders: results of a cohort study. *Antivir Ther* 2010;15:599-606.
45. Zignego AL, Cozzi A, Carpenedo R, Giannini C, Rosselli M, Biagioli T, Aldinucci A, et al. HCV patients, psychopathology and tryptophan metabolism: analysis of the effects of pegylated interferon plus ribavirin treatment. *Dig Liver Dis* 2007;39 Suppl 1:S107-111.
46. Comai S, Cavalletto L, Chemello L, Bernardinello E, Ragazzi E, Costa CV, Bertazzo A. Effects of PEG-interferon alpha plus ribavirin on tryptophan metabolism in patients with chronic hepatitis C. *Pharmacol Res* 2011;63:85-92.
47. Diez-Quevedo C, Masnou H, Planas R, Castellvi P, Gimenez D, Morillas RM, Martin-Santos R, et al. Prophylactic treatment with escitalopram of pegylated interferon alfa-2a-induced depression in hepatitis C: a 12-week, randomized, double-blind, placebo-controlled trial. *J Clin Psychiatry* 2011;72:522-528.
48. Kraus MR, Schafer A, Schottker K, Keicher C, Weissbrich B, Hofbauer I, Scheurlen M. Therapy of interferon-induced depression in chronic hepatitis C with citalopram: a randomised, double-blind, placebo-controlled study. *Gut* 2008;57:531-536.
49. Morasco BJ, Rifai MA, Loftis JM, Indest DW, Moles JK, Hauser P. A randomized trial of paroxetine to prevent interferon-alpha-induced depression in patients with hepatitis C. *J Affect Disord* 2007;103:83-90.
50. Raison CL, Woolwine BJ, Demetrashvili MF, Borisov AS, Weinreib R, Staab JP, Zajecka JM, et al. Paroxetine for prevention of depressive symptoms induced by interferon-alpha and ribavirin for hepatitis C. *Aliment Pharmacol Ther* 2007;25:1163-1174.
51. Hashimoto Y, Kanto H, Itoh M. Adverse skin reactions due to pegylated interferon alpha 2b plus ribavirin combination therapy in a patient with chronic hepatitis C virus. *J Dermatol* 2007;34:577-582.

52. Kartal ED, Alpat SN, Ozgunes I, Usluer G. Reversible alopecia universalis secondary to PEG-interferon alpha-2b and ribavirin combination therapy in a patient with chronic hepatitis C virus infection. *Eur J Gastroenterol Hepatol* 2007;19:817-820.
53. Grossmann Sde M, Teixeira R, de Aguiar MC, do Carmo MA. Exacerbation of oral lichen planus lesions during treatment of chronic hepatitis C with pegylated interferon and ribavirin. *Eur J Gastroenterol Hepatol* 2008;20:702-706.
54. Sulkowski MS, Cooper C, Hunyady B, Jia J, Ogurtsov P, Peck-Radosavljevic M, Shiffman ML, et al. Management of adverse effects of Peg-IFN and ribavirin therapy for hepatitis C. *Nat Rev Gastroenterol Hepatol* 2011;8:212-223.
55. Yurci A, Guven K, Torun E, Gursoy S, Baskol M, Akgun H, Ozbakir O, et al. Pyoderma gangrenosum and exacerbation of psoriasis resulting from pegylated interferon alpha and ribavirin treatment of chronic hepatitis C. *Eur J Gastroenterol Hepatol* 2007;19:811-815.
56. De Franceschi L, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, Noventa F, et al. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. *Hepatology* 2000;31:997-1004.
57. Jacobson IM, Brown RS, Jr., Freilich B, Afdhal N, Kwo PY, Santoro J, Becker S, et al. Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. *Hepatology* 2007;46:971-981.
58. Jensen DM, Marcellin P, Freilich B, Andreone P, Di Bisceglie A, Brandao-Mello CE, Reddy KR, et al. Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. *Ann Intern Med* 2009;150:528-540.
59. Balan V, Schwartz D, Wu GY, Muir AJ, Ghalib R, Jackson J, Keeffe EB, et al. Erythropoietic response to anemia in chronic hepatitis C patients receiving combination pegylated interferon/ribavirin. *Am J Gastroenterol* 2005;100:299-307.
60. Suzuki F, Suzuki Y, Akuta N, Sezaki H, Hirakawa M, Kawamura Y, Hosaka T, et al. Influence of ITPA polymorphisms on decreases of hemoglobin during treatment with pegylated interferon, ribavirin, and telaprevir. *Hepatology* 2011;53:415-421.
61. Thompson AJ, Fellay J, Patel K, Tillmann HL, Naggie S, Ge D, Urban TJ, et al. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. *Gastroenterology* 2010;139:1181-1189.
62. Thompson AJ, Santoro R, Piazzolla V, Clark PJ, Naggie S, Tillmann HL, Patel K, et al. Inosine triphosphatase genetic variants are protective against anemia during antiviral therapy for HCV2/3 but do not decrease dose reductions of RBV or increase SVR. *Hepatology* 2010.
63. Nachnani JS, Rao GA, Bulchandani D, Pandya PK, Alba LM. Predictors of hematological abnormalities in patients with chronic hepatitis C treated with interferon and ribavirin. *Ann Hematol* 2010;89:121-125.
64. Nomura H, Tanimoto H, Kajiwara E, Shimono J, Maruyama T, Yamashita N, Nagano M, et al. Factors contributing to ribavirin-induced anemia. *J Gastroenterol Hepatol* 2004;19:1312-1317.
65. Reau N, Hadziyannis SJ, Messinger D, Fried MW, Jensen DM. Early predictors of anemia in patients with hepatitis C genotype 1 treated with peginterferon alfa-2a (40KD) plus ribavirin. *Am J Gastroenterol* 2008;103:1981-1988.
66. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-1838.
67. Dieterich DT, Wasserman R, Brau N, Hassanein TI, Bini EJ, Bowers PJ, Sulkowski MS. Once-weekly epoetin alfa improves anemia and facilitates maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alfa. *Am J Gastroenterol* 2003;98:2491-2499.

68. Afdhal NH, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, Wright T, et al. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. *Gastroenterology* 2004;126:1302-1311.
69. Pockros PJ, Shiffman ML, Schiff ER, Sulkowski MS, Younossi Z, Dieterich DT, Wright TL, et al. Epoetin alfa improves quality of life in anemic HCV-infected patients receiving combination therapy. *Hepatology* 2004;40:1450-1458.
70. Shiffman ML, Salvatore J, Hubbard S, Price A, Sterling RK, Stravitz RT, Luketic VA, et al. Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. *Hepatology* 2007;46:371-379.
71. Antonini MG, Babudieri S, Maida I, Baiguera C, Zanini B, Fenu L, Dettori G, et al. Incidence of Neutropenia and Infections During Combination Treatment of Chronic Hepatitis C with Pegylated Interferon Alfa-2a or Alfa-2b Plus Ribavirin. *Infection* 2008.
72. Roomer R, Hansen BE, Janssen HL, de Knegt RJ. Risk factors for infection during treatment with peginterferon alfa and ribavirin for chronic hepatitis C. *Hepatology* 2010;52:1225-1231.
73. Soza A, Everhart JE, Ghany MG, Doo E, Heller T, Promrat K, Park Y, et al. Neutropenia during combination therapy of interferon alfa and ribavirin for chronic hepatitis C. *Hepatology* 2002;36:1273-1279.
74. Yang JF, Hsieh MY, Hou NJ, Dai CY, Huang JF, Lin ZY, Chen SC, et al. Bacterial infection and neutropenia during peginterferon plus ribavirin combination therapy in patients with chronic hepatitis C with and without baseline neutropenia in clinical practice. *Aliment Pharmacol Ther* 2009;29:1000-1010.
75. Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver D. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009;49:1335-1374.
76. Cooper CL, Al-Bedwawi S, Lee C, Garber G. Rate of infectious complications during interferon-based therapy for hepatitis C is not related to neutropenia. *Clin Infect Dis* 2006;42:1674-1678.
77. Puoti M, Babudieri S, Rezza G, Viale P, Antonini MG, Maida I, Rossi S, et al. Use of pegylated interferons is associated with an increased incidence of infections during combination treatment of chronic hepatitis C: a side effect of pegylation? *Antivir Ther* 2004;9:627-630.
78. Koirala J, Gandotra SD, Rao S, Sangwan G, Mushtaq A, Htwe TH, Adamski A, et al. Granulocyte colony-stimulating factor dosing in pegylated interferon alpha-induced neutropenia and its impact on outcome of anti-HCV therapy. *J Viral Hepat* 2007;14:782-787.
79. Koskinas J, Zacharakis G, Sidiropoulos J, Elefsiniotis J, Savvas S, Kotsiou S, Kountouras D, et al. Granulocyte colony stimulating factor in HCV genotype-1 patients who develop Peg-IFN-alpha2b related severe neutropenia: a preliminary report on treatment, safety and efficacy. *J Med Virol* 2009;81:848-852.
80. Younossi ZM, Nader FH, Bai C, Sjogren R, Ong JP, Collantes R, Sjogren M, et al. A phase II dose finding study of darbepoetin alpha and filgrastim for the management of anaemia and neutropenia in chronic hepatitis C treatment. *J Viral Hepat* 2008;15:370-378.
81. Roomer R, Hansen BE, Janssen HL, de Knegt RJ. Thrombocytopenia and the risk of bleeding during treatment with peginterferon alfa and ribavirin for chronic hepatitis C. *J Hepatol* 2010;53:455-459.
82. Afdhal N, McHutchison J, Brown R, Jacobson I, Manns M, Poordad F, Weksler B, et al. Thrombocytopenia associated with chronic liver disease. *J Hepatol* 2008;48:1000-1007.
83. Peck-Radosavljevic M, Wichlas M, Homoncik-Kraml M, Kreil A, Hofer H, Jessner W, Gangl A, et al. Rapid suppression of hematopoiesis by standard or pegylated interferon-alpha. *Gastroenterology* 2002;123:141-151.
84. Cuker A. Toxicities of the thrombopoietic growth factors. *Semin Hematol* 2010;47:289-298.

**RISK FACTORS FOR INFECTION
DURING TREATMENT WITH
PEGINTERFERON ALFA AND
RIBAVIRIN FOR CHRONIC HEPATITIS C**

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ABSTRACT

Neutropenia during treatment with peginterferon alfa and ribavirin for chronic hepatitis C (HCV) is a common cause for dose reductions of peginterferon alfa. These reductions are performed to prevent bacterial and fungal infections, which are common during HCV treatment and attributed to neutropenia. The aims of this study were to investigate the occurrence of infections and their relation to neutropenia and to find potential risk factors for infections during HCV treatment.

In this single center cohort study 2876 visits of 321 patients treated with peginterferon alfa and ribavirin were evaluated for neutropenia, infections, dose reductions and potential risk factors for infection during HCV treatment.

Baseline mean absolute neutrophil count (ANC) was 3420 cells/ μ L, 16 patients had baseline ANC below 1500 cells/ μ L. During treatment neutropenia defined as ANC <750 cells/ μ L was observed in 95 patients (29.7%) and ANC < 375/ μ L was observed in 16 patients (5%). Ninety-six infections were observed in 70 patients (21.8%). Thirteen infections (13.5%) were defined as severe. Infections were not correlated with neutropenia during treatment. Dose reductions did not lead to a decreased infection rate. In the multivariate logistic regression analysis age older than 55 (OR 2.06, CI 1.19-3.56, $p=0.01$) and baseline hyperglycemia (OR 2.17 95% CI 1.15-4.10, $p=0.016$) were associated with an increased risk of infection during HCV treatment. Cirrhosis and chronic obstructive pulmonary disease (COPD) were no risk factors for infections.

Conclusion

Bacterial infections during treatment with peginterferon alfa and ribavirin are not associated with neutropenia. Older patients and patients with poorly controlled diabetes mellitus are more at risk to develop infections during HCV treatment.

INTRODUCTION

Chronic HCV infection is a major cause of cirrhosis, hepatocellular carcinoma and end stage liver disease (1). Treatment of chronic HCV infection with peginterferon alfa and ribavirin leads to a sustained virological response in 42-80% of patients depending on HCV genotype (2-4). Unfortunately, treatment with peginterferon alfa and ribavirin is associated with many side effects. One of these side effects is peginterferon alfa induced bone marrow suppression which leads to a significant decline in blood cells, particularly in white blood cells. During therapy a decline of 30-50% in absolute neutrophil counts is common and approximately 20% of patients develop neutrophil counts below 750/ μ L. The development of neutropenia is the most common cause for dose reductions of peginterferon alfa or discontinuation of therapy (5). These dose reductions are performed to reduce the risk of bacterial and fungal infections. Infections occur in 4-23% of patients and although most are mild, some may lead to hospital admission or discontinuation of therapy (6-10). Guidelines for dose reductions for neutropenia are based on studies investigating infection rates in oncologic patients receiving chemotherapy. In these studies the risk of infection was considered increased when neutrophil counts dropped below 500/ μ L, with the greatest risk of infection with neutrophil counts below 100/ μ L (11).

However, earlier studies investigating the correlation between neutropenia and infections never found a significant correlation, although grade 4 neutropenia was rare in these studies (7, 9, 10). Furthermore limited data is available about which factors are responsible for the increased incidence of bacterial and fungal infections.

For this reason we conducted a study to investigate which factors are associated with the occurrence of bacterial and fungal infections and to investigate the relationship between neutrophil counts and infections.

PATIENTS AND METHODS

Patients

In this cohort study we included all patients treated with peginterferon alfa-2a or 2b and ribavirin for chronic HCV infection between 2000 and 2009. Data was obtained from a total of 321 patients treated with peginterferon alfa-2a or 2b and ribavirin. Of these patients, 218 were treated within a standard of care protocol. The remaining 103 patients were treated within clinical studies: 86 patients participated in 3 clinical trials and received standard of care with peginterferon alfa-2a or 2b plus weight-based ribavirin. The remaining 17 patients received a peginterferon induction regimen with either peginterferon alfa-2a (270-360 μ g weekly) for 24 weeks or peginterferon alfa-2b (2.0-3.0 μ g/kg weekly) for 24 weeks followed by 48 weeks peginterferon alfa and daily weight based ribavirin (12). In patients who were treated within

a standard of care protocol peginterferon alfa dose reductions were carried out at discretion of the treating physician. Patient characteristics e.g. age, physical condition, virological response and co-morbidities were taken into account when considering a dose reduction. In case of neutropenia patients were monitored more frequent. All study protocols of the clinical trials stated that dose reductions should be performed according to product labels: reduction of peginterferon alfa dose to 75% when ANC dropped below 750/ μL and temporary discontinuation of therapy when neutrophil counts dropped below 375/ μL . However, in some patients these guidelines were not applied and these patients were treated at the discretion of the treating physician as well. In patients who participated in randomized clinical trials, dose reductions of peginterferon alfa for neutropenia were performed according to product labels: reduction of peginterferon alfa dose to 75% when ANC dropped below 750/ μL and temporary discontinuation of therapy when neutrophil counts dropped below 375/ μL . Patients who received conventional interferon or patients with an HIV or HBV co-infection were excluded from the analysis. All clinical trials had approval from the local ethics committee and all patients who participated in these studies provided written informed consent.

Data acquisition

We obtained data on baseline characteristics gender, age, race, BMI, Metavir score, genotype, previous interferon based treatment, platelet count, absolute neutrophil counts (ANC), hemoglobin, bilirubin, albumin, glucose levels, the presence of COPD, history of heroin use, smoking and the presence of diabetes mellitus. During therapy all patients visited the outpatient clinic in 1 to 6 weeks intervals depending on study protocol and clinical condition. At every visit platelet counts, ANC and hemoglobin concentrations were determined and patients were screened for infections, dose reductions and discontinuation of therapy. Neutropenia was defined as a neutrophil count below 1500/ μL , moderate neutropenia as <750/ μL and severe neutropenia as <375/ μL . Neutrophil counts were assessed at levels of 750/ μL or 375/ μL because these levels are the usual thresholds to reduce the peginterferon alfa dose or to discontinue therapy. Infections were diagnosed using blood tests, cultures, urinalysis and X-rays. Infections were defined as mild when patients were treated with antibiotics, dose of peginterferon alfa was reduced or no treatment was given. Infections were defined as severe when patients were admitted to a hospital, treatment was discontinued or the patient died. Correlation between infections and ANC was done using ANC of the preceding visits.

Statistical analysis

Chi square and Fisher's exact test were used for the comparison of dichotomous variables. Student t tests were used for the comparison of continuous variables. Linear logistic regression analysis was used to determine which baseline factors were associated with baseline neutrophil counts. To assess the risk of having neutrophil counts below 750/ μL or 375/ μL and to assess the risk of infections during treatment, multiple regression analyses were performed

treating each visit as an observation, with correction for the fact that each patient had multiple visits and multiple events. Neutrophil counts were analyzed as dichotomous variables (neutrophil counts less or more than $750\mu\text{L}$ and $375/\mu\text{L}$). $P < 0.05$ was considered statistically significant. All statistical tests were two-tailed. SPSS 15.0 statistical package (SPSS, Chicago, IL, USA) and SAS 9.2 PROC GENMOD (SAS institute, Cary, NC) with the application of the repeated statement were used for this analysis.

RESULTS

A total of 2876 visits of 321 patients treated with peginterferon alfa and ribavirin were included in the analysis. Baseline characteristics of patients are summarized in table 1. Cirrhosis was present in 68 patients (21%), COPD in 29 (9%) patients and diabetes mellitus in 27 (8%) patients.

Table 1. Baseline characteristics

Number of patients	321
Age (mean, range in years)	46 (14-70)
Gender (Male/Female)	213/108
Race (N, proportion)	
Caucasian	234 (73%)
Asian	42 (13%)
Black	19 (6%)
Other	26 (8%)
BMI (mean, range in kg/m^2)	26 (16-39)
Histology* (metavir score)	
F0-1	83/265 (31%)
F2	91/265 (34%)
F3	37/265 (14%)
F4	54/265 (21%)
Diagnosis of cirrhosis by ultrasound	68/321 (21%)
HCV Genotype	
1	147 (46%)
2	45 (14%)
3	110 (34%)
4	19 (6%)
Platelet count (mean, range in thousands/ μL)	191 (23-381)
Absolute neutrophil count (mean, range in cells/ μL)	3420 (640-10900)
Hemoglobin (mean, range in mmol/L)	9.2 (6.4-11.2)
COPD	29/321 (9%)
Diabetes mellitus	27/321 (8%)
History of heroin use	157/321 (49%)
Smoking	182/295 (57%)
Peginterferon induction regimen	17/321 (5%)
Peginterferon alfa 2a or 2b (a/b)	226/95
Treatment duration (median, range in weeks)	25 (3-79)

Liverbiopsies were available in 265 patients.

Neutrophil counts

Mean neutrophil count at baseline was 3423/ μL ranging from 640 to 10900/ μL with 16 patients having neutrophil counts below 1500/ μL . Caucasians had higher baseline neutrophil counts than non-Caucasians (3550/ μL vs. 3121/ μL , $p=0.034$) and patients with COPD had higher baseline neutrophil counts than patients without COPD (4247/ μL vs. 3297/ μL , $p=0.019$). Other initial factors which were significantly associated with low baseline neutrophil counts were increasing age, low platelet count, low haemoglobin and albumin concentration (univariate analysis). In the multivariate analysis low platelet count ($p<0.001$) and haemoglobin concentration ($p=0.03$) as well as increasing age ($p=0.03$) were significantly correlated with low neutrophil counts. The presence of COPD was associated with high baseline neutrophil counts ($p<0.001$) in the multivariate analysis.

Mean neutrophil count drop was 68% (range, 0-91%) to a mean neutrophil count of 1098/ μL ranging from 170/ μL to 6800/ μL (fig 1). Median time of reaching lowest neutrophil count was 14 weeks. Neutrophil counts below 750/ μL were observed in 95 patients (29.7%) and 16 patients (5%) developed neutrophil counts below 375/ μL . There was no significant difference in mean neutrophil count drop between patients treated with peginterferon alfa 2a and 2b: 2417/ μL vs. 2108/ μL respectively ($p=0.076$). Patients receiving peginterferon alfa induction therapy did not have a larger decline in neutrophil counts than patients receiving standard dose (2049 vs. 2342, $p=0.39$). The presence of COPD (OR 0.233, 0.059-0.916, $p=0.037$),

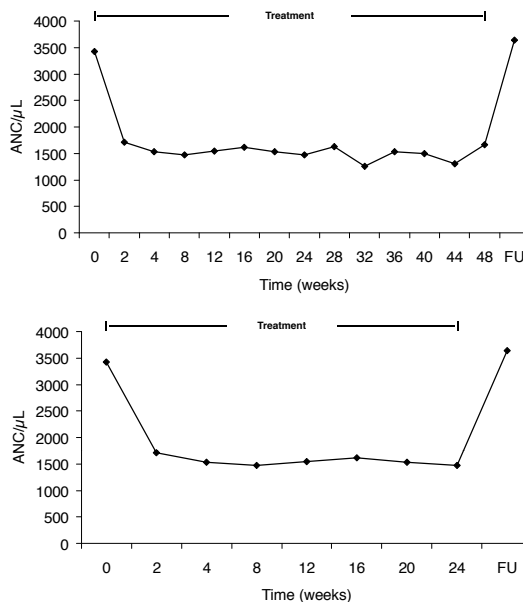


Fig. 1. ANCs during treatment with peginterferon alfa and ribavirin in patients (A) following a 48-week regimen (above) and (B) following a 24-week regimen (below).

bodyweight (per 10kg, OR 0.937, 0.891-0.986, $p=0.012$), baseline neutrophil count (OR 0.48, 0.332-0.695, $p<0.001$) and baseline platelet count (OR 0.989, 0.982-0.995, $p<0.001$) were significantly associated with not having neutrophil counts below $750/\mu\text{L}$ in the multivariate logistic regression analysis.

Infections

A total of 96 infections were diagnosed in 70 patients (21.8%). Types of infections are listed in table 2a. Infections defined as 'other infections' included phlebitis, meningitis (severe), and lymphangitis. Thirteen infections in 13 patients were defined as severe; treatment was discontinued in 9 cases and 8 patients required hospital admission (table 2b). The mean age of these patients was 50 years (range 36-68). One patient, a 63 year old male with diabetes mellitus and cirrhosis developed a gastrointestinal infection followed by sepsis. Despite antibiotic treatment infection did not resolve and the patient died eventually.

Table 2a. Type of infections in 70 of 321 (22%) patients treated with peginterferon alfa and ribavirin for chronic HCV.

Type of infection	Number (%)	Severe infection (%)
Urinary tract	21 (22)	2
Upper respiratory tract	19 (20)	0
Head, ears, eyes, nose or throat	21 (22)	0
Dermatological	16 (17)	1
Pulmonary	12 (13)	7
Gastrointestinal	4 (4)	2
Other	3 (3)	1
	96 (100)	13 (14)

Table 2b. Severe infections in 13 patients

#	Sex	Age	Type of infection	Comorbidity	Lowest ANC/ μL	Action taken	Outcome
1	M	47	Pneumonia	None	6560	Disc. / adm.	Resolved
2	V	68	Pyelonefritis	DM / Cirrhosis	750	Disc. / adm.	Resolved
3	M	49	GI infection	DM	1090	Admission	Resolved
4	M	38	Pneumonia	None	2900	Discontinuation	Resolved
5	M	63	GI infection / sepsis	DM / Cirrhosis	580	Disc. / adm.	Death
6	V	46	Pneumonia	None	550	Admission	Resolved
7	M	54	Erysipelas	Cirrhosis	510	Discontinuation	Resolved
8	M	58	Pneumonia	COPD	3000	Discontinuation	Resolved
9	M	38	Meningitis	None	1280	Disc. / adm.	Resolved
10	V	54	UWI / sepsis	Cirrhosis	630	Disc. / adm.	Resolved
11	M	43	Pneumonia	COPD	1660	Discontinuation	Resolved
12	V	57	Pneumonia	DM / Cirrhosis / COPD	920	Admission	Resolved
13	M	36	Pneumonia	None	1000	Discontinuation	Resolved

Disc. : Discontinuation

Adm. : Hospital admission

Dose reductions and discontinuations

Peginterferon alfa dose was reduced 83 times in 53 patients. Per protocol dose reductions in patients receiving peginterferon alfa induction therapy were excluded. Dose reductions were performed 41 times due to neutropenia in 21 patients (6.5%), 19 times due to thrombocytopenia in 12 patients (3.7%) and 2 times due to anemia in 2 patients (0.6%). In 4 patients peginterferon alfa dose was reduced due to infections and in 17 patients for other reasons (e.g. intolerance and depression). Treatment was discontinued in 27 patients, in 9 patients due to infection and due to neutropenia in 1 patient. Patients who underwent a dose reduction, regardless of the reason, did not have less infections in the remaining treatment period after the reduction compared to patients without dose reductions: 22% (53/268) vs. 20.8% (11/53), $p=0.839$.

In patients with a lowest ANC $<750/\mu\text{L}$ peginterferon alfa dose was reduced 33 times: 20 times due to neutropenia and 13 times for other reasons (e.g. thrombocytopenia or intolerance). One patient had an ANC $>750/\mu\text{L}$ at time of reduction for neutropenia. The remaining 62 patients did not undergo a dose reduction. Of these patients 21 patients were treated within a clinical trial. Median ANC at time of first reduction for neutropenia was $450/\mu\text{L}$ and median lowest ANC in patients without a reduction was $600/\mu\text{L}$. Patients with neutropenia who underwent a dose reduction (for any reason) did not have less infections in the remaining treatment period after reduction compared to neutropenic patients without dose reductions: 21.2% (7/33) vs. 25.8% (16/62), $p=0.858$. Median time of infection after a dose reduction was 15 weeks (1-36 weeks).

Of these patients 16 had a lowest ANC $<375/\mu\text{L}$, in this group peginterferon alfa dose was discontinued in 1 patient. In 11 patients peginterferon alfa dose was reduced for neutropenia and in 3 patients for other reasons. One patient did not undergo a dose reduction for neutropenia. This patient was a 33 year old male without co-morbidities. His lowest ANC was $280/\mu\text{L}$ and no infections occurred in this patient.

Risk factors for infections

Variables which were associated with having infections during HCV treatment are listed in table 3. In this univariate analysis all visits were taken into account with correction of repeated measurements. Age was a significant risk factor for infection in the univariate analysis (per 10 year OR 1.42, 95% CI 1.05 – 1.92, $p=0.02$). The infection risk rapidly increased after the age of 55 years (figure 2). Forty-three patients were aged older than 55 years. Infections occurred in 41.9 % of these patients compared to 18.7% in patients younger than 55 years of age ($p=0.001$). Patients with diabetes mellitus (DM) had a higher infection rate than patients without DM (20.4% vs. 37.0%, $p=0.045$). However, since not all patients who were diagnosed with DM were hyperglycemic at baseline, we also looked at patients with baseline hyperglycemia, regardless of DM diagnosis. Hyperglycemia was defined as a non-fasting glucose concentration larger than the upper limit of normal (7.8 mmol/l). This difference was even

larger (42.3% vs. 20.7%, $p=0.014$). Longer treatment duration was significantly associated with an increased risk of infection (per week OR 1.02, 95% CI 1.01 – 1.04, $p=0.008$).

No risk factors could be identified for severe infections. Infection rates were similar in patients who developed moderate neutropenia ($<750/\mu\text{L}$) compared to patients who remained above $750/\mu\text{L}$ (21% vs. 22%, $p=0.79$). The infection rate after visits with neutrophil counts $<750/\mu\text{L}$ was 3% compared to 4.2% after visits with neutrophil counts $>750/\mu\text{L}$ ($p=0.34$). Among the 16 patients who developed severe neutropenia ($<375/\mu\text{L}$), 19% developed an infection in the remaining treatment period after the occurrence of severe neutropenia compared to 22% of patients who did not develop severe neutropenia ($p=0.76$). No infections occurred during visits when neutrophil counts were $<375/\mu\text{L}$ at the preceding visit. When only cirrhotic patients were included in this analysis, infection rates were similar in patients who did develop moderate and severe neutropenia compared to those who did not ($p=0.76$ and 0.42 respectively). The mean ANC was $2202/\mu\text{L}$ during visits with infections compared to $1750/\mu\text{L}$ during visits without infections ($p=0.001$). The risk of infection was equal during all treatment weeks ($p=0.37$) and with the total time of ANC $<750/\mu\text{L}$ ($p=0.38$). The infection rate in cirrhotic patients was higher than in non-cirrhotic patients, however this difference was not significant (29.4% vs. 19.8%, $p=0.08$).

In the multivariate analysis for the risk of infections only age older than 55 years (OR 2.06, CI 1.19-3.56, $p=0.01$) and baseline hyperglycemia (OR 2.17 95% CI 1.15-4.10, $p=0.016$) were significantly associated with infections during treatment with peginterferon alfa and ribavirin.

Table 3. Variables associated with infections during peginterferon and ribavirin treatment. With correction for repeated measurements.

Variables	Odds ratio (95% CI)	P
Age (per 10 years)	1.42 (1.05 – 1.92)	0.02
Age older than 55	2.48 (1.45 – 4.25)	0.001
Sex (female)	1.42 (0.70 – 1.98)	0.54
BMI (kg/m ²)	1.02 (0.97 – 1.08)	0.47
Weight (kg)	1.00 (0.99 – 1.02)	0.77
Caucasian vs. Non Caucasian	0.72 (0.43 – 1.08)	0.22
COPD	1.54 (0.77 – 3.02)	0.21
Cirrhosis	1.29 (0.75 – 2.21)	0.36
Diabetes mellitus	2.29 (1.00 – 5.27)	0.045
Smoking	1.16 (0.67 – 1.98)	0.6
Peginterferon alfa 2a vs. 2b	1.14 (0.67 – 1.99)	0.63
Baseline hyperglycemia	2.55 (1.31 – 4.97)	0.006
Neutrophil count at time of infection	2.11 (1.34 – 3.34)	0.001
Neutrophil count ($<750/\mu\text{L}$) at visit prior to infection	0.70 (0.34 – 1.44)	0.33
Neutrophil count ($<750/\mu\text{L}$) two visits prior to infection	1.19 (0.66 – 2.14)	0.55
Cumulative time of ANC $<750/\mu\text{L}$ (per 4 weeks)	1.05 (0.94 – 1.18)	0.38
Total treatment duration (weeks)	1.02 (1.01 – 1.04)	0.008
Treatment week	1.01 (0.99 – 1.02)	0.37

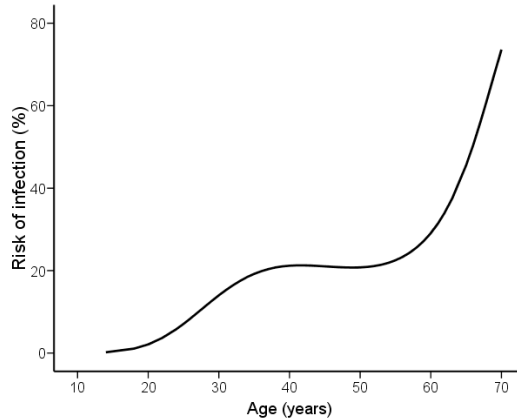


Fig. 2. Risk of infection according to age. A rapid increase was seen in patients >55 years of age.

DISCUSSION

This cohort study gives a detailed description of risk factors for infections during antiviral therapy other than neutropenia. Furthermore this is the largest study to date investigating the correlation between neutropenia and infections with a large amount of patients having grade 4 neutropenia (<500/ μ L).

In this cohort of 321 patients treated with peginterferon alfa and ribavirin for chronic hepatitis C we found 96 infections in 70 patients (22 %). Thirteen infections were defined as severe and one patient died. Patients who developed moderate or severe neutropenia did not have higher rates of infections than patients without neutropenia. These results are comparable with the results of other studies investigating the relationship between absolute neutrophil counts and infections (6-10). In these studies infection rates ranged from 4.3 to 29.7% of patients and also no correlation between absolute neutrophil counts and infections was found. However, the percentage of patients with grade 3 or 4 neutropenia was much higher in our cohort. This effect might be caused by the low amount of dose reductions for neutropenia executed in this cohort (6.5% of all patients) and the inclusion of patients with baseline neutropenia. To date product labels of peginterferon alfa and many guidelines for HCV treatment still recommend dose reductions of peginterferon alfa when absolute neutrophil counts drop below 750/ μ L and discontinuation of therapy when absolute neutrophil counts drop below 500 or 375/ μ L. These recommendations are to prevent bacterial or fungal infections. It is known that dose reductions compromise treatment efficacy and especially for difficult-to-treat patients maintaining optimal peginterferon alfa dose is most crucial (13, 14).

In this cohort patients with neutropenia who underwent a dose reduction did not have fewer infections than neutropenic patients without dose reductions. However these results should be interpreted with caution because dose reductions were performed at discretion of

the treating physician. Patients who underwent dose reductions could therefore be more at risk to develop infections.

Guidelines for dose reductions are based on findings of large cohort studies of infections in oncologic patients receiving chemotherapy. These studies conclude that the risk of infection is increased in patients who develop grade 4 neutropenia ($<500/\mu\text{L}$) with the greatest risk of infections in patients with absolute neutrophil counts $<100/\mu\text{L}$ (11, 15-18). However, in these patients, other factors such as mucosal damage, the effects of commensal flora and alteration of organ function caused by the underlying disease, play an important role in the risk of infection as well. These factors are absent in clinically stable patients with chronic hepatitis C. Based on these facts it is reasonable to assume that HCV patients receiving antiviral treatment are less immunocompromised than oncologic patients receiving chemotherapy. Furthermore from the results of our study and other studies we can conclude that the level of neutropenia induced by treatment with peginterferon alfa and ribavirin in chronic HCV patients is not associated with an increased risk of bacterial and fungal infections.

Still, an increased incidence of infections does exist in patients receiving antiviral treatment with peginterferon alfa/ribavirin combination therapy. And although not related to absolute neutrophil counts, there must be a cause of the increased infection rate. One hypothesis is that peginterferon alfa alters neutrophil function. Giorgini and colleagues investigated neutrophil function during HCV treatment (19). An increase in oxidative burst and chemotaxis of neutrophils during treatment compared to baseline was demonstrated. To date, limited data are available on this subject and more studies are required to explore the effects of interferon on neutrophil function.

We also investigated patient characteristics which might contribute to the risk of infection. Cirrhotic patients had more infections than non cirrhotic patients, although the difference was small and not significant. This might be due to the fact that only patients with compensated cirrhosis were treated. These results are in agreement with the results of the groups of Cooper and Antonini (6, 7). Both studies did not find an increased infection risk in patients with Child A cirrhosis. Others found an increased rate of infections in cirrhotic patients; 3 out of 20 cirrhotic patients (15%) compared to 6 out of 187 non cirrhotic patients. Both the numbers of cirrhotic patients and infections however, were small in this study. Carrion et al. investigated the safety and efficacy of treatment with peginterferon alfa and ribavirin in patients with child B & C cirrhosis awaiting liver transplantation. They found a high incidence of severe bacterial infections and their conclusion was to avoid antiviral therapy in patients with Child B & C cirrhosis (20).

There was a very strong correlation between age and the risk of infection. Risk of infections remained stable until approximately the age of 55 and then rapidly increased (figure 2). This phenomenon can be explained by the decline in immune function seen with aging. Both the innate and the adaptive immunity are affected. For neutrophils particularly, the phagocytic ability and superoxide production are reduced in elderly patients (21).

At last, we investigated the effect of diabetes mellitus on the occurrence of infections. We found a significant higher infection rate in patients diagnosed with diabetes mellitus. We also investigated the infection rate in patients with baseline hyperglycemia regardless of the diagnosis of diabetes mellitus. In this group of patients the infection rate was even larger. This can be explained by the fact that besides the vascular insufficiency, which is often present in diabetic patients, high glucose levels can lead to an impaired leukocyte function. Chemotaxis, phagocytosis, intracellular bacterial activity, cell-mediated immunity, opsonisation and adherence to vascular endothelium are all depressed in diabetic patients with hyperglycemia (22, 23).

In conclusion, levels of neutropenia induced by peginterferon alfa are not associated with an increased risk of infection in patients with compensated HCV infection. Recommendations in guidelines and product labels might therefore be overly cautious. Elderly patients and patients with poorly controlled glucose levels are more at risk to develop infections during treatment with peginterferon and ribavirin, therefore caution is warranted in these patient groups.

REFERENCES

1. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997;349:825-832.
2. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncalves 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.
3. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-355.
4. 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.
5. Fried MW. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002;36:S237-244.
6. Antonini MG, Babudieri S, Maida I, Baiguera C, Zanini B, Fenu L, Dettori G, et al. Incidence of Neutropenia and Infections During Combination Treatment of Chronic Hepatitis C with Pegylated Interferon Alfa-2a or Alfa-2b Plus Ribavirin. *Infection* 2008.
7. Cooper CL, Al-Bedwawi S, Lee C, Garber G. Rate of infectious complications during interferon-based therapy for hepatitis C is not related to neutropenia. *Clin Infect Dis* 2006;42:1674-1678.
8. Puoti M, Babudieri S, Rezza G, Viale P, Antonini MG, Maida I, Rossi S, et al. Use of pegylated interferons is associated with an increased incidence of infections during combination treatment of chronic hepatitis C: a side effect of pegylation? *Antivir Ther* 2004;9:627-630.
9. Soza A, Everhart JE, Ghany MG, Doo E, Heller T, Promrat K, Park Y, et al. Neutropenia during combination therapy of interferon alfa and ribavirin for chronic hepatitis C. *Hepatology* 2002;36:1273-1279.
10. Yang JF, Hsieh MY, Hou NJ, Dai CY, Huang JF, Lin ZY, Chen SC, et al. Bacterial infection and neutropenia during peginterferon plus ribavirin combination therapy in patients with chronic hepatitis C with and without baseline neutropenia in clinical practice. *Aliment Pharmacol Ther* 2009;29:1000-1010.
11. Collantes RS, Younossi ZM. The use of growth factors to manage the hematologic side effects of PEG-interferon alfa and ribavirin. *J Clin Gastroenterol* 2005;39:S9-13.
12. Bergmann JF, Vrolijk JM, van der Schaar P, Vroom B, van Hoek B, van der Sluys Veer A, de Vries RA, et al. Gamma-glutamyltransferase and rapid virological response as predictors of successful treatment with experimental or standard peginterferon-alpha-2b in chronic hepatitis C non-responders. *Liver Int* 2007;27:1217-1225.
13. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002;123:1061-1069.
14. Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, Lok AS, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007;132:103-112.
15. Bodey GP, Buckley M, Sathe YS, Freireich EJ. Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. *Ann Intern Med* 1966;64:328-340.
16. Viscoli C. The evolution of the empirical management of fever and neutropenia in cancer patients. *J Antimicrob Chemother* 1998;41 Suppl D:65-80.
17. Viscoli C. Management of infection in cancer patients. studies of the EORTC International Antimicrobial Therapy Group (IATG). *Eur J Cancer* 2002;38 Suppl 4:S82-87.

18. Viscoli C, Varnier O, Machetti M. Infections in patients with febrile neutropenia: epidemiology, microbiology, and risk stratification. *Clin Infect Dis* 2005;40 Suppl 4:S240-245.
19. Giorgini A, Capsoni F, Podda M, Lleo A, Battezzati PM, Ongari AM, Selmi C, et al. Treatment with PEG-interferon and ribavirin for chronic hepatitis C increases neutrophil and monocyte chemotaxis. *Ann N Y Acad Sci* 2009;1173:847-857.
20. Carrion JA, Martinez-Bauer E, Crespo G, Ramirez S, Perez-del-Pulgar S, Garcia-Valdecasas JC, Navasa M, et al. Antiviral therapy increases the risk of bacterial infections in HCV-infected cirrhotic patients awaiting liver transplantation: A retrospective study. *J Hepatol* 2009;50:719-728.
21. Weiskopf D, Weinberger B, Grubeck-Loebenstien B. The aging of the immune system. *Transpl Int* 2009;22:1041-1050.
22. Delamaire M, Maugeudre D, Moreno M, Le Goff MC, Allanic H, Genetet B. Impaired leucocyte functions in diabetic patients. *Diabet Med* 1997;14:29-34.
23. Llorente L, De La Fuente H, Richaud-Patin Y, Alvarado-De La Barrera C, Diaz-Borjon A, Lopez-Ponce A, Lerman-Garber I, et al. Innate immune response mechanisms in non-insulin dependent diabetes mellitus patients assessed by flow cytometry. *Immunol Lett* 2000;74:239-244.

**THROMBOCYTOPENIA AND THE RISK
OF BLEEDING DURING TREATMENT
WITH PEGINTERFERON ALFA AND
RIBAVIRIN FOR CHRONIC HEPATITIS C**

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ABSTRACT

Background

Chronic HCV patients with baseline thrombocytopenia are often excluded from treatment with peginterferon alfa and ribavirin or undergo many dose reductions of peginterferon alfa. The aim of this study was to investigate the correlation between thrombocytopenia and the occurrence of bleedings during antiviral treatment for HCV infection.

Methods

In this single center cohort study 2876 visits of 321 patients treated with peginterferon alfa and ribavirin were evaluated for thrombocytopenia, bleedings and dose reductions during HCV treatment.

Results

Mean platelet count at baseline was 207000/ μ L for non-cirrhotic patients (n= 253) and 132000/ μ L for cirrhotic patients (n= 68). Mean platelet drop was 42% from 191000 to 113100/ μ L (range 8000-284000/ μ L). Severe thrombocytopenia (platelet counts <50000/ μ L) was observed in 30 patients (9.3%) at 166 visits and 9 patients developed platelet counts <25000/ μ L at 15 visits. Forty-eight bleedings were observed in 27 patients (8.4%). Only 1 bleeding, due to gastrointestinal angiodysplasia, was defined as severe. However, this patient did not have severe thrombocytopenia at time of bleeding. During visits, patients reported more minor bleedings when platelet counts were <50000/ μ L compared to visits with platelet counts \geq 50000/ μ L (11.4% vs. 1.1%, $P<0.001$). In the multivariate analysis only platelet count <50000/ μ L was a significant predictor of bleeding ($p<0.001$).

Conclusion

Severe bleedings did not occur in patients with platelet counts below 50000/ μ L; based on these findings, treatment with peginterferon alfa and ribavirin appears to be safe in patients with platelet counts below 50000/ μ L although platelet counts below 25000/ μ L were rare.

INTRODUCTION

Chronic HCV infection is a major cause of cirrhosis, hepatocellular carcinoma and end stage liver disease (1). HCV treatment with peginterferon alfa and ribavirin leads to sustained virological responses in 42-80% of patients depending on HCV genotype (2-4). Unfortunately, treatment with peginterferon alfa and ribavirin is associated with many side effects (5). One of these side effects is thrombocytopenia which accounts for a considerable number of peginterferon alfa dose reductions to prevent (major) bleedings (5, 6). These dose reductions compromise treatment efficacy (7, 8). Product labels of peginterferon alfa advise to reduce peginterferon alfa dose when platelet counts drop below 50000/ μL (grade 3 thrombocytopenia) and to stop treatment when platelet counts drop below 25000/ μL (grade 4 thrombocytopenia). Furthermore, patients with baseline platelet counts below 80000/ μL are often excluded from antiviral therapy. Unfortunately cirrhotic patients, who need antiviral therapy the most, are often thrombocytopenic and therefore excluded from antiviral therapy or undergo many dose reductions. It is however questionable whether a platelet count of 50000/ μL is an appropriate threshold to reduce the peginterferon alfa dose. Studies investigating the threshold for platelet transfusion in patients with acute leukemia receiving induction chemotherapy concluded that the risk of major bleeding in clinically stable patients was only increased when platelet counts dropped below 10000/ μL , or even below 5000/ μL (9). While platelet counts lower than 20000/ μL are very rare during treatment with peginterferon alfa and ribavirin. To date, limited data is available about the clinical significance of thrombocytopenia and the risk of bleeding during treatment with peginterferon alfa and ribavirin. For this reason we conducted a study to assess the risk of (major) bleedings and their association with thrombocytopenia during antiviral treatment for chronic hepatitis C with peginterferon alfa and ribavirin.

PATIENTS AND METHODS

Patients

In this study we included all patients treated with peginterferon alfa-2a or 2b and ribavirin between 2000 and 2009. Data was obtained from 218 patients treated with peginterferon alfa-2a or 2b and ribavirin within a standard of care protocol. In addition, 103 patients treated within clinical studies were included in the analysis: 86 patients participated in 3 clinical trials and received standard of care with peginterferon alfa-2a or 2b plus weight-based ribavirin. The remaining 17 patients received a peginterferon induction regimen with either peginterferon alfa-2a (270-360 μg weekly) for 24 weeks or peginterferon alfa-2b (2.0-3.0 $\mu\text{g}/\text{kg}$ weekly) for 24 weeks followed by 48 weeks peginterferon alfa and daily weight based ribavirin (10). During therapy all patients visited the outpatient clinic in 1 to 6 weeks intervals depending on study protocol and clinical condition. At every visit platelet counts, absolute neutrophil counts

(ANC) and hemoglobin concentrations were determined and patients were asked if bleeding had occurred in the period from their last outpatient clinic visit. In patients who participated in randomized clinical trials, dose reductions of peginterferon alfa for thrombocytopenia were performed conform product labels: reduction of peginterferon alfa dose to 75% when platelet counts dropped below 50000/ μL and temporary discontinuation of therapy when platelet counts dropped below 25000/ μL . In patients who were treated within a standard of care protocol peginterferon alfa dose reductions were carried out at discretion of the treating physician. In general the product labels were followed, although in some patients peginterferon alfa dose was maintained when platelet counts remained stable above 25000/ μL . Patients who received conventional interferon or patients with an HIV or HBV co-infection were excluded from the analysis. All clinical trials had approval from the local ethics committee and all patients who participated in these studies provided written informed consent.

Data acquisition

We obtained data on baseline characteristics gender, age, race, BMI, Metavir score, genotype, previous interferon based treatment, platelet counts, absolute neutrophil counts, hemoglobin, bilirubin and albumin concentrations, the presence of hemophilia, the use of anticoagulants and antiplatelet therapy. Thrombocytopenia was defined as a peripheral platelet count below 150000/ μL . During therapy thrombocytopenia was assessed at levels of 50000 and 25000/ μL , since these levels are the usual thresholds for dose reductions or discontinuation of peginterferon alfa / ribavirin therapy. Bleedings were defined as minor (no specific treatment or dose reduction) or severe (hospital admission, requirement of platelet transfusion, permanent disability or death).

Statistics

Chi square and Fisher's exact test were used for the comparison of dichotomous variables. Student t tests were used for the comparison of continuous variables. We performed a linear logistic regression analysis to determine which baseline factors were influencing baseline platelet counts. To assess the risk of having platelets counts below 50000/ μL and the risk of bleeding during treatment, multiple regression analyses were performed treating each visit as an observation. Then we corrected for the fact that each patient had multiple visits and multiple events. Platelet counts were both analyzed as continuous and dichotomous variables (platelet count less or more than 50000/ μL). $P < 0.05$ was considered statistically significant. All statistical tests were two-tailed. SPSS 15.0 statistical package (SPSS, Chicago, IL, USA) and SAS 9.2 PROC genmod (SAS institute, Cary, NC) with the application of the repeated statement were used for this analysis.

RESULTS

A total of 321 patients treated with peginterferon alfa and ribavirin were included in the analysis. Of these patients 2876 visits during treatment were recorded. The mean time between visits was 4.1 weeks. Baseline characteristics of patients are summarized in table 1. Cirrhosis was present in 68 patients (21%).

Table 1. Baseline characteristics

Number of patients	321
Age (mean, range in years)	46 (14-70)
Gender (Male/Female)	213/108
Race (N, proportion)	
Caucasian	234 (73%)
Asian	42 (13%)
Black	19 (6%)
Other	26 (8%)
BMI (mean, range in kg/m ²)	26 (16-39)
Histology (metavir score)	
F0-1	91/289 (32%)
F2	94/289 (33%)
F3	37/289 (12%)
F4	68/321 (21%)
HCV Genotype	
1	147 (46%)
2	45 (14%)
3	110 (34%)
4	19 (6%)
Platelet count (mean, range in thousands/ μ L)	191 (23-381)
Absolute neutrophil count (mean, range in cells/ μ L)	3420 (640-10900)
Hemoglobin (mean, range in mmol/L)	9.2 (6.4-11.2)
Prothrombin time (mean, range in sec)	12.8 (9.3-40.9)
Albumin (mean, range in gram/L)	43.5 (31-52)
Total bilirubin (mean, range in μ mol/L)	11 (2-34)
Use of anticoagulants	15 (5%)
The presence of hemophilia	17 (5%)
Peginterferon induction regimen	17 (5%)
Peginterferon alfa 2a or 2b (a/b)	226/95
Treatment duration (median, range in weeks)	25 (3-79)

Platelet counts at baseline

Data on baseline platelet counts were available in 303 patients. Mean platelet count at baseline was 191000/ μ L, ranging from 23000-381000/ μ L with 14 patients having baseline platelet counts below 80000/ μ L. Cirrhotic patients had lower baseline platelet counts than non-cirrhotic patients (207000/ μ L vs. 132000/ μ L, respectively ($p < 0.001$)). Males had lower platelet counts than females 184000/ μ L vs. 204000/ μ L ($p = 0.009$). Furthermore baseline platelet counts were significantly correlated with baseline neutrophil counts, baseline bilirubin

and albumin concentrations and prothrombin time. In the multivariate analysis only the presence of cirrhosis ($p < 0.001$) and male gender ($p = 0.033$) were significantly correlated with low platelets at baseline.

Changes in platelet counts during treatment

Platelet counts decreased by an average of 42% (SD 17%) from 191000 to 113100/ μL , ranging from 8000 to 284000/ μL (figure 1). Median time of reaching lowest platelet count was 12 weeks after initiation of treatment. As expected, patients with high baseline platelet counts had larger absolute platelet drops. However, relative decreases did not differ significantly. Patients treated with peginterferon alfa-2a had a larger decrease in platelet counts compared to patients treated with peginterferon alfa-2b, 82800/ μL vs. 66300/ μL ($p = 0.002$). There was no difference in platelet drop between patients treated with an induction regimen or a standard dose of peginterferon (74000/ μL vs. 78100/ μL , $p = 0.696$). In the multivariate analysis high baseline platelet count ($p < 0.001$) and peginterferon alfa 2a ($p < 0.001$) were significantly correlated with the decline in platelet counts.

Platelet counts below 150000/ μL were observed during 1600 visits in 251 patients (78.2%) and 135 patients (42.1%) developed a platelet count below 100000/ μL in 733 visits. Grade 3 thrombocytopenia defined as a platelet count below 50000/ μL was observed during 166 visits in 30 patients (9.3%), and 9 patients (2.8%) had a platelet count below 25000/ μL (grade 4) during 15 visits (table 2). Rates of platelet counts below 50000/ μL were significantly higher in cirrhotic patients compared to non-cirrhotic patients (35.3% vs. 2.4% ($P < 0.001$)). In the multivariate analysis baseline platelet count and cirrhosis were associated with having at least one platelet count below 50000/ μL (per 10000/ μL ; OR 0.931, CI 0.895-0.968, $p < 0.001$ and OR 15.906, CI 1.005-34.694, $p = 0.049$).

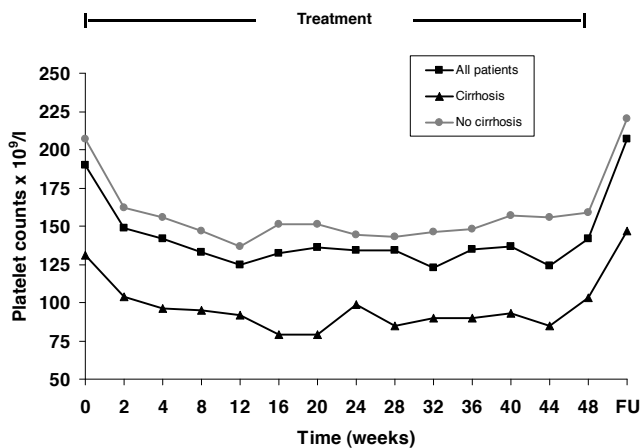


Fig. 1. Changes in platelet counts during treatment with peginterferon alfa and ribavirin of all patients.

Table 2. Platelet counts and the occurrence of bleedings during treatment with peginterferon and ribavirin

Platelet count / μL	Number of patients* (%)	Number of visits**	Number of visits with bleedings (%)
> 150000	70 (21.8)	1200	6 (0.5)
100000 – 150000	116 (36.1)	867	9 (1.0)
50000 – 100000	105 (32.7)	567	14 (2.5)
25000 – 50000	21 (6.5)	151	14 (9.3)
< 25000	9 (2.8)	15	5 (33.3)

*Number of patients with lowest platelet count in platelet category

** Platelet counts were missing at 76 visits

Patients with baseline platelet counts below 80000/ μL

Fourteen patients had baseline platelet counts below 80000/ μL (range 23000-79000). The patient with a baseline platelet count of 23000/ μL had been treated with peginterferon alfa and ribavirin before; this treatment was discontinued due to osteomyelitis of the mandibula. During his first treatment platelet counts were low but remained stable, for this reason retreatment was initiated. The other 13 patients had baseline platelet counts between 50000-80000/ μL . Platelets dropped to a median platelet count of 26500/ μL (figure 2). One patient had a platelet count drop to 8000/ μL , this patient was treated with a platelet transfusion and treatment was discontinued. This 35 year old female patient had a history of bone marrow transplantation because of acute myeloid leukaemia after which she developed chronic persistent thrombocytopenia.

Four of 14 patients (29%) with baseline platelet counts below 80000/ μL achieved SVR and 5 had minor bleedings, no severe bleedings occurred.

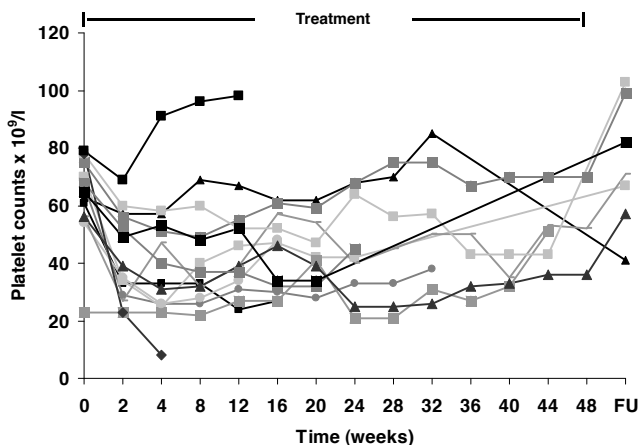


Fig. 2. Platelet count drops during treatment of all 14 patients with baseline platelet counts <80,000/l. Platelet counts remained above 20,000/l except for one patient. This patient was a young female with a history of bone marrow transplantation.

Reductions and discontinuations

Peginterferon alfa dose was reduced 19 times due to thrombocytopenia in 12 patients (3.7%). Median platelet count at time of first reduction was 35000/ μ L (range 8000-59000/ μ L). Median time until first reduction was 7 weeks (range 2-19 weeks) after initiation of treatment. In 18 patients (5.6%) with platelet counts below 50000/ μ L no dose reductions of peginterferon alfa were performed. In these patients platelet counts remained stable between 25000 and 50000/ μ L. In 2 patients, treatment with peginterferon alfa and ribavirin was discontinued due to thrombocytopenia; one patient had persistent thrombocytopenia despite of dose reductions, the other is mentioned above. One patient discontinued treatment due to severe bleeding/ anaemia (see below).

Bleedings

A total of 48 bleedings were observed in 27 patients during antiviral treatment. Types of bleeding are shown in table 3. All bleedings were defined as mild except for one gastrointestinal bleeding. In this patient, five weeks after initiation of antiviral treatment haemoglobin concentration dropped from 5.7 mmol/l to 2.6 mmol/l in two weeks time. Platelet counts were 67000/ μ L and 65000/ μ L respectively. He was admitted to the hospital and was treated with a blood transfusion. Antiviral treatment was discontinued. Several angiodysplastic abnormalities were found in the jejunum on double balloon enteroscopy. After successful treatment with APC, retreatment was initiated without any significant bleedings.

Table 3. Type of bleeding in 27 out of 321 patients treated with peginterferon and ribavirin

Type of bleeding	Number (%)	Severe bleeding
Epistaxis	25 (52)	0
Gingival	13 (27)	0
Hematoma	4 (8)	0
Gastrointestinal	2 (4)	1
Hyper menorrhoea	2 (4)	0
Vaginal	2 (4)	0
Total	48 (100)	1

Bleedings were more often reported during visits with platelet counts below 50000/ μ L than during visits with platelet counts \geq 50000/ μ L, 11.4% vs. 1.1% ($p < 0.001$). Mean platelet count at time of bleeding was 80800/ μ L (range 8000-262000/ μ L) compared to 144800/ μ L (21000-422000/ μ L) during visits when no bleeding occurred ($p < 0.001$). Cirrhotic patients had more bleedings than non-cirrhotic patients; 19.1% vs. 5.5% ($p < 0.001$). Two patients had platelet counts below 20000/ μ L at 3 visits, during all these visits bleeding was reported (epistaxis and gingival bleeding). Variables that were associated with the risk of bleeding are listed in table 4. The occurrence of bleeding was not influenced by treatment duration ($p = 0.45$). The effect of platelet counts (squared) on the risk of bleeding is plotted in figure 3. In the multivariate analysis only platelet count < 50000 / μ L was significantly associated with the risk of bleeding (OR 7.51, CI 2.17-26.0, $p < 0.001$).

Table 4. Odds ratios for the occurrence of bleedings during treatment (univariate analysis)

Variable	OR (CI 95%)	P value
Age (10 years)	1.30 (0.92-1.84)	0.14
BMI (kg/m ²)	0.95 (0.85-1.07)	0.39
Female gender	1.67 (0.67-4.13)	0.27
Caucasian vs. non Caucasian	0.78 (0.29-2.06)	0.61
Cirrhosis	4.71 (1.93-11.5)	0.0007
Peginterferon alfa 2b vs. 2a	0.48 (0.19-1.24)	0.13
Use of anti coagulants	1.59 (0.21-11.8)	0.65
Pre-treatment albumin (gram/L)	0.84 (0.76-0.94)	0.0015
Pre-treatment prothrombin time (sec)	1.07 (0.98-1.17)	0.13
Pre-treatment bilirubin (mmol/L)	1.09 (1.05-1.14)	<0.0001
Platelet count squared (per 10000/ μ L decrease)	1.51 (1.24-1.85)	<0.0001
Platelet count < 50000/ μ L	7.51 (2.17-26.0)	0.0001
Treatment duration (months)	0.97 (0.89-1.05)	0.45

Odds ratios adjusted for treatment duration were similar.

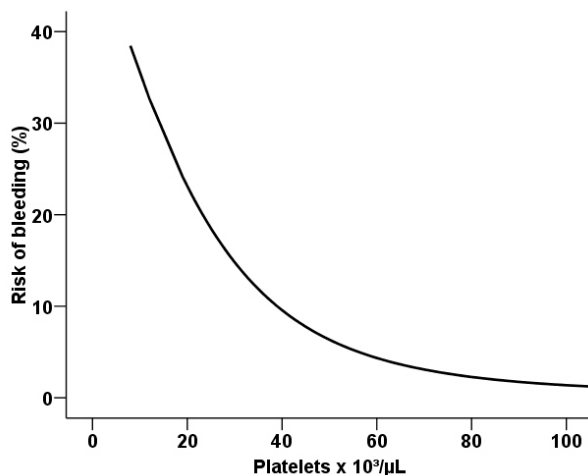


Fig. 3. The risk of bleeding according to platelet counts during therapy.

DISCUSSION

This cohort study is the first to investigate the risk of bleedings and their relation with platelet counts during treatment with peginterferon alfa and ribavirin. In this cohort of 321 patients treated with peginterferon alfa and ribavirin we found a mean platelet drop of 42%. Thirty patients (9.3%) had platelet counts below 50000/ μ L and only two patients had platelet count below 20000/ μ L. Forty-eight bleedings were observed in 27 patients. All bleedings but one were minor and the only severe bleeding occurred in a patient without severe thrombocytopenia. Although there was a strong relationship between platelet counts and the occurrence of

minor bleedings (figure 3), no major bleedings occurred in patients with platelet counts below 50000/ μ L.

Earlier studies reported a 30-50% drop in platelet counts after initiation of treatment with peginterferon alfa and ribavirin. Approximately 3-6% of patients developed platelet counts below 50000/ μ L during treatment (2-4). Except for the report of two serious bleedings in one study (3), no data is available on bleedings during treatment with peginterferon alfa and ribavirin.

To prevent (major) bleedings, product labels recommend dose reductions when platelet counts fall below 50000/ μ L and discontinuation of peginterferon alfa when platelet counts fall below 25000/ μ L. Dose reductions however, compromise treatment efficacy and especially for patients infected with genotype 1, maintaining the optimal peginterferon alfa and ribavirin dose is crucial for achieving a sustained virological response (7, 8). In this cohort 12 of 30 patients with platelet counts below 50000/ μ L underwent a dose reduction or discontinuation of peginterferon alfa dose. In the remaining 18 patients, who were treated outside of clinical trials, peginterferon alfa dose was maintained at discretion of treating physician. In these patients virological response and stability of platelet counts were also taken into account when a dose reduction was considered. A possible alternative for the current guideline would be to maintain peginterferon alfa dose when platelet counts remain stable above 25000/ μ L and to temporarily discontinue when platelet counts drop below 25000/ μ L. Peginterferon alfa could be restarted at a lower dose when platelet counts rise above 25000/ μ L again. However, confirmation of our findings by larger cohort studies or prospective randomised controlled trials is needed before revision of the current guidelines would be taken into consideration.

Furthermore, some cirrhotic patients for whom viral clearance is most crucial, have very low baseline platelet counts (<80000/ μ L) caused by splenomegaly and impaired thrombopoietin production (11, 12). These patients are often excluded from antiviral therapy or undergo many dose reductions. In our study fourteen patients had baseline platelet counts below 80000/ μ L of whom 12 patients had proven cirrhosis. None of these patients developed platelet counts below 20000/ μ L. Minor bleedings occurred in five patients and four patients achieved SVR. These patients would have been excluded from antiviral therapy when current guidelines would have been followed.

In this study major bleedings did not occur in patients with grade 3 or 4 thrombocytopenia. These results are supported by other studies investigating the relation between bleedings and platelet counts (9, 13-17). The first study investigating the quantitative correlation between platelet counts and bleedings in patients undergoing intensive chemotherapy for acute leukaemia showed that that serious bleedings were rare with platelet counts above 20000/ μ L (17). Other studies investigating the threshold for platelet transfusions in patients receiving chemotherapy for acute leukaemia showed that the risk of major bleedings was not increased when platelet counts remained above 5000/ μ L in clinically stable patients. In fact, major bleedings were not only related to platelet counts but primarily to other risk factors

for bleeding associated with chemotherapy such as disseminated intravascular coagulation (DIC), sepsis and heparin therapy (9, 13-15). In a similar study investigating the threshold for platelet transfusions in patients with gynaecologic malignancies, no major bleedings were observed in 32 patients with 100 episodes of severe thrombocytopenia (platelet count $<20000/\mu\text{L}$). Minor bleeding occurred in 27% of episodes with severe thrombocytopenia. The most likely explanation for the absence of major bleedings is that these patients did not have any risk factor for bleeding such as DIC, sepsis or heparin therapy (16). Although oncologic patients receiving chemotherapy are not entirely comparable with chronic HCV patients, it is reasonable to assume that HCV patients without decompensated cirrhosis do not have a larger bleeding risk than these patients.

To date, studies are carried out to evaluate the effect of a new orally active thrombopoietin-receptor agonist, eltrombopag. In the first study, patients with platelet counts between 20000 and 70000/ μL received a pre-treatment with eltrombopag to facilitate treatment with peginterferon alfa and ribavirin. Platelet counts increased in a dose dependent manner. After antiviral therapy was initiated, platelet counts decreased but remained above baseline values (18). Eltrombopag was generally safe, it is however known to cause cataract and thrombosis. Furthermore it is expensive and the already high costs of HCV treatment will be driven up even further. Although eltrombopag could be a solution for patients with severe thrombocytopenia, further studies are required to investigate the effect of eltrombopag on the prevention of bleeding.

In conclusion, no severe bleedings occurred in patients with platelet counts below 50000/ μL treated with peginterferon alfa and ribavirin for chronic hepatitis C. Based on these results HCV treatment of patients with platelet counts below 50000/ μL appears to be safe although the number of patients with platelet counts below 25000/ μL was low. Second, HCV treatment was safe in patients with baseline thrombocytopenia and based on these results they should not be excluded from antiviral therapy. However, larger studies are needed to confirm the findings of this study.

REFERENCES

1. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997;349:825-832.
2. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-355.
3. 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.
4. 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.
5. Fried MW. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002;36:S237-244.
6. Collantes RS, Younossi ZM. The use of growth factors to manage the hematologic side effects of PEG-interferon alfa and ribavirin. *J Clin Gastroenterol* 2005;39:S9-13.
7. Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, Lok AS, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007;132:103-112.
8. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002;123:1061-1069.
9. Gmur J, Burger J, Schanz U, Fehr J, Schaffner A. Safety of stringent prophylactic platelet transfusion policy for patients with acute leukaemia. *Lancet* 1991;338:1223-1226.
10. Bergmann JF, Vrolijk JM, van der Schaar P, Vroom B, van Hoek B, van der Sluys Veer A, de Vries RA, et al. Gamma-glutamyltransferase and rapid virological response as predictors of successful treatment with experimental or standard peginterferon-alpha-2b in chronic hepatitis C non-responders. *Liver Int* 2007;27:1217-1225.
11. Afdhal N, McHutchison J, Brown R, Jacobson I, Manns M, Poordad F, Weksler B, et al. Thrombocytopenia associated with chronic liver disease. *J Hepatol* 2008;48:1000-1007.
12. Peck-Radosavljevic M, Wichlas M, Pidlich J, Sims P, Meng G, Zacherl J, Garg S, et al. Blunted thrombopoietin response to interferon alfa-induced thrombocytopenia during treatment for hepatitis C. *Hepatology* 1998;28:1424-1429.
13. Slichter SJ. Relationship between platelet count and bleeding risk in thrombocytopenic patients. *Transfus Med Rev* 2004;18:153-167.
14. Friedmann AM, Sengul H, Lehmann H, Schwartz C, Goodman S. Do basic laboratory tests or clinical observations predict bleeding in thrombocytopenic oncology patients? A reevaluation of prophylactic platelet transfusions. *Transfus Med Rev* 2002;16:34-45.
15. Heckman KD, Weiner GJ, Davis CS, Strauss RG, Jones MP, Burns CP. Randomized study of prophylactic platelet transfusion threshold during induction therapy for adult acute leukemia: 10,000/microL versus 20,000/microL. *J Clin Oncol* 1997;15:1143-1149.
16. Fanning J, Hilgers RD, Murray KP, Bolt K, Aughenbaugh DM. Conservative management of chemotherapeutic-induced thrombocytopenia in women with gynecologic cancers. *Gynecol Oncol* 1995;59:191-193.
17. Gaydos LA, Freireich EJ, Mantel N. The quantitative relation between platelet count and hemorrhage in patients with acute leukemia. *N Engl J Med* 1962;266:905-909.

18. McHutchison JG, Dusheiko G, Shiffman ML, Rodriguez-Torres M, Sigal S, Bourliere M, Berg T, et al. Eltrombopag for thrombocytopenia in patients with cirrhosis associated with hepatitis C. *N Engl J Med* 2007;357:2227-2236.

**DISCORDANCE BETWEEN DIFFERENT
HCV RNA ASSAYS FOR WEEK 24
HCV RNA DETERMINATION DURING
PEGINTERFERON ALFA/RIBAVIRIN
TREATMENT FOR CHRONIC HEPATITIS C**

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ABSTRACT

Background

The development of more sensitive HCV RNA assays may necessitate re-evaluation of stopping rules, e.g. HCV RNA-negativity at week 24 during treatment with peginterferon alfa and ribavirin for chronic hepatitis C. The aim of this study was to assess discordance between week 24 HCV RNA test results of two Polymerase Chain reaction (PCR) based assays (Amplicor and TaqMan) and the Transcription Mediated Amplification (TMA) HCV RNA qualitative assay.

Methods

Eighty-nine week 24 samples which were negative using PCR based assays during treatment were retested with the TMA qualitative assay to investigate discordance between tests results. All week 24 samples were HCV RNA negative by Amplicor or by TaqMan.

Results

Forty-six out of 89 (52%) patients achieved sustained virological response (SVR). Viral breakthrough or relapse occurred in 43 patients (48%). All 89 HCV RNA negative week 24 samples were retested with the qualitative TMA assay. Eleven out of 89 samples had detectable HCV RNA (12%). All patients with detectable HCV RNA experienced breakthrough or relapse (negative predictive value (NPV) 100%). Forty-six of 78 patients with undetectable HCV RNA at week 24 using the TMA assay achieved SVR. This resulted in a positive predictive value (PPV) of 59% for the TMA assay compared to a PPV of 52% of the PCR based assays.

Conclusion

All patients with detectable HCV RNA at week 24 using the TMA assay eventually relapsed. Based on these results the use of this more sensitive HCV RNA assay could lead to the prevention of unnecessary treatment

INTRODUCTION

The current standard treatment for chronic hepatitis C (HCV) is a 24 or 48 week regimen with peginterferon alfa and ribavirin (1-3) (PEGIFN/RBV). This treatment regimen is being monitored by determination of HCV RNA levels before, during and after HCV treatment. Quantitative HCV RNA assays are carried out to assess the viral load at baseline and at week 12. Qualitative assays are being performed to determine whether HCV RNA is undetectable at week 24, at the end of treatment (EOT) and 24 weeks after EOT. Current guidelines recommend discontinuation of therapy if HCV RNA decline is less than 2 logs at week 12 or when HCV RNA is still detectable at week 24 of therapy. These recommendations are based on negative predictive values (NPV) of nearly 100% of a less than 2 log drop at week 12 and HCV RNA-positivity at week 24 (3-6).

The most commonly used techniques for the detection of HCV RNA are target amplification as applied in PCR and transcription mediated amplification (TMA) (7-8). Two examples of PCR based assays which are often used for the detection of HCV RNA during treatment are the COBAS® AmpliPrep/ COBAS® AMPLICOR HCV TEST V2.0, lower limit of detection (LLOD) 20 IU/ml (Amplicor) and the COBAS® AmpliPrep/ COBAS® TaqMan® HCV test v1.0, LLOD 15 IU/ml (TaqMan) (9-10). Both tests are from Roche Diagnostics, Almere, the Netherlands. Detectable HCV RNA with these PCR based assays at week 24 will lead to discontinuation of therapy. There is however a significant amount of patients with undetectable HCV RNA at this time point who will eventually have a viral relapse after completion of therapy (4).

The LLOD of the VERSANT® TMA HCV RNA qualitative assay (Siemens Medical Solutions Diagnostics, Eindhoven, The Netherlands) is lower (5.3 IU/ml) than the LLOD of the PCR based assays (15-20 IU/ml) (11). These differences in LLOD could lead to discordance in week 24 HCV RNA test results and thus in differences between PPVs. Furthermore it is unclear whether a negative test result at week 24 obtained by the TMA assay has the same NPV as a negative test result obtained by the less sensitive PCR based assays. In case of comparable NPVs, the most sensitive assay would prevent PEG-IFN/RBV related side effects and costs associated with treatment due to unnecessary continuation of HCV therapy.

For this reason we retested week 24 HCV RNA samples that had been tested during antiviral therapy with the PCR based assays Amplicor or TaqMan with a qualitative HCV RNA assay to investigate discordance between test results and to determine the negative predictive value of the TMA.

METHODS

Sample selection

A total of 89 week 24 samples were included in the analysis. Week 24 samples of 77 patients were selected from a single center cohort of 321 patients with chronic hepatitis C treated with PEGIFN/RBV between 2000 and 2009 (12-13). The remaining 12 samples were selected from a cohort study investigating continuous interferon alfa-2b administration in combination with ribavirin. All selected patients received a 24, 48 or 72 week treatment regimen depending on HCV genotype, viral load, response and previous treatment. Selected patients were HCV RNA positive at week 4 and HCV RNA negative at week 24 of treatment. Serum samples dated from 2001 through 2009 and were all stored frozen at -80 °C. During therapy HCV RNA was determined using the Amplicor and the TaqMan. Serum sample testing was performed according to the manufacturer's instructions.

Retesting HCV RNA with the VERSANT TMA HCV RNA qualitative assay

All 89 PCR negative week 24 samples were retested with the TMA assay according to the manufacturer's instructions. The sensitivity is independent of the different HCV genotypes. The principle is based on amplification of target RNA, afterwards 1 detection probe per copy RNA is bound. The assay needs 500 µl plasma or serum, the lower detection limit is 5.3 IU/ml. Specificity of this assay of 99.6% minimizes the risk of false positive results (11).

Statistical analysis

Prediction of therapy outcome was done by using NPV and PPV. PPVs of week 24 HCV RNA negativity were calculated using all patients, including patients with undetectable HCV RNA at week 12 of treatment.

RESULTS

Samples

A total of 89 week 24 samples were included in the analysis. Baseline characteristics of patients are summarized in table 1. Fifty-five patients (61.8%) had genotype 1, 5 (5.6%) had genotype 2, 20 (22.5%) had genotype 3 and 9 patients (10.1%) had genotype 4. All patients were HCV RNA-positive at week 4 of antiviral treatment. At week 12, 23 patients were HCV RNA-negative by PCR, 49 patients were positive but did not have quantifiable HCV RNA and 11 patients had quantifiable HCV RNA ranging from 630 to 12300 IU/ml. In 6 patients week 12 HCV RNA was missing. All patients were HCV RNA-negative at week 24 of antiviral treatment tested with the Amplicor (77 patients) and Taqman (12 patients) PCR tests. Virological break-

Table 1. Baseline characteristics

Variable	
Number of patients	89
Age (mean, range in years)	45.6 (22-69)
Gender (male/female)	66/23
BMI (mean, range in kg/m ²)	26.8 (19.6-36)
Genotype (1,2,3 & 4)	55, 5, 20, 9
Cirrhosis	16 (18%)
Previous interferon based treatment	26 (29.2%)
Peginterferon induction therapy	3 (3.4%)
Baseline viral load (log IU/ml)	6.61
Early virological response (HCV RNA negative at wk 12)	23 (25.9%)
Treatment duration (24, 48, 72 weeks)	19, 65, 5

through or relapse occurred in 43 patients (48%) and 46 patients (52%) achieved an SVR. Nineteen patients were treated for 24 weeks, 65 for 48 weeks and 5 patients for 72 weeks.

Retesting with the TMA HCV RNA qualitative assay

All 77 week 24 HCV RNA samples tested with the Amplicor were retested with the TaqMan. No discordance was found between the Amplicor and TaqMan PCR based assays. All 89 week 24 samples were retested with the TMA assay. Eleven out of 89 samples retested with the TMA based assay were HCV RNA-positive (12%). Two HCV RNA-positive patients were treated for 24 weeks and 9 patients for 48 weeks. Nine patients with detectable HCV RNA using the TMA assay had a virological relapse after treatment and 2 patients had a virological breakthrough during treatment. One of the 9 relapsers had undetectable HCV RNA 24 weeks after the end of treatment (EOT) with the TMA assay, however HCV RNA was detectable again one year after EOT. In the remaining 78 samples retested with the TMA assay HCV RNA could not be detected. Of these patients 32 (41%) had a virological relapse and 46 (59%) achieved SVR. The minimum follow up of these patients was at least one year after end of treatment. The PPV of undetectable HCV RNA at week 24 determined by the TMA assay was 59% (46/78) compared to 52% (46/89) for the PCR based assays (table 2). The NPV of a positive HCV RNA test result for virological relapse was 100% (11/11).

Table 2. Positive predictive values of undetectable HCV RNA of 3 different assays.

HCV RNA assay	SVR / Total	PPV (%)
PCR Amplicor negative*	41 / 77	53.2%
PCR TaqMan negative**	46 / 89	51.7%
TMA negative	46 / 78	58.9%

*PCR Amplicor was performed during therapy of 77 patients treated with peginterferon alfa and ribavirin.

**PCR TaqMan was performed during therapy of patients treated with continuous interferon alfa and ribavirin; furthermore all samples were retested with the TaqMan. No discordance was found between TaqMan and Amplicor.

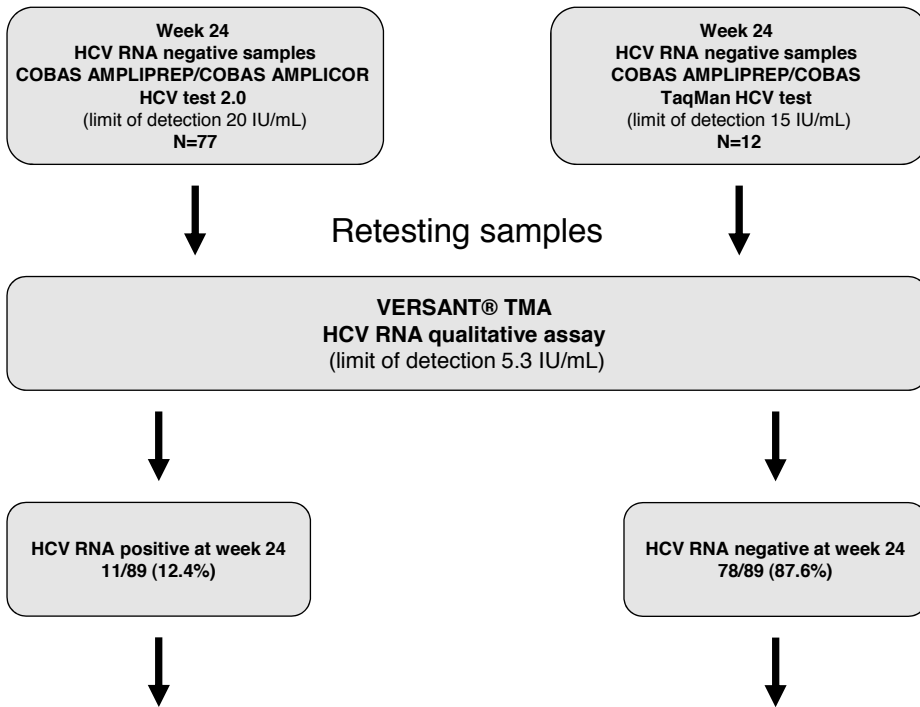


Figure 1. Study design

DISCUSSION

This study investigates discordance between the TMA HCV RNA qualitative assay with a LLOD of 5.3 IU/ml and two PCR based assays with a minimal follow up of at least one year. In this cohort we found discordance in 12% of the test results. All patients with detectable HCV RNA at week 24 using the TMA assay experienced a viral relapse or breakthrough after or during treatment. Eventually all patients became HCV RNA-positive and thus the NPV of detectable HCV RNA at week 24 using the TMA assay was 100% in this study. Furthermore, the PPV of a negative TMA test result was somewhat higher than the PPV of a negative PCR test result; 59 vs. 52%, although we should mention that these PPVs are based on all patients, including patients with undetectable HCV RNA at week 12.

Earlier studies reported on detectable HCV RNA by TMA assays in EOT samples who were HCV RNA-negative when tested with PCR based assays. With the exception of a few cases all patients with a positive TMA test result had a viral relapse after treatment cessation (14-17).

Morishima and colleagues retested PCR negative on treatment HCV RNA samples with the TMA assay, at that time the LLOD was 9.6 IU/ml (18). They found a discordance of 19.9% between the PCR based and the TMA assay in week 24 samples. Six patients (9%) with a

negative PCR and a positive TMA at week 24 eventually achieved a sustained virological response. These patients however, had a negative TMA test result on week 20 of therapy. Patients with detectable HCV RNA at both time points all experienced viral relapse. They concluded that a single positive TMA test result could not rule out SVR. However, the NPV of a positive TMA test result was still high: 91%. Furthermore, they mention in their discussion that a delayed relapse after 24 weeks of follow up could not be ruled out. Another study of Morishima and colleagues showed that some PCR negative/TMA positive samples were TMA negative on repeat testing. Discordant TMA test results occurred in patients with HCV RNA levels fluctuating around the LLOD and were strongly associated with viral breakthrough or relapse (19).

In conclusion, based on these results the NPV of a positive TMA test result on week 24 is still 100%. The use of the more sensitive TMA assay could lead to the prevention of unnecessary treatment and thereby the associated side effects and costs.

REFERENCES

1. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-355.
2. 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.
3. 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.
4. Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Goncales FL, Jr., Haussinger D, et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. *J Hepatol* 2005;43:425-433.
5. Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver D. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009;49:1335-1374.
6. 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.
7. Sarrazin C, Hendricks DA, Sedarati F, Zeuzem S. Assessment, by transcription-mediated amplification, of virologic response in patients with chronic hepatitis C virus treated with peginterferon alpha-2a. *J Clin Microbiol* 2001;39:2850-2855.
8. Sarrazin C. Highly sensitive hepatitis C virus RNA detection methods: molecular backgrounds and clinical significance. *J Clin Virol* 2002;25 Suppl 3:S23-29.
9. Barbeau JM, Goforth J, Caliendo AM, Nolte FS. Performance characteristics of a quantitative TaqMan hepatitis C virus RNA analyte-specific reagent. *J Clin Microbiol* 2004;42:3739-3746.
10. Martinot-Peignoux M, Khiri H, Leclere L, Maylin S, Marcellin P, Halfon P. Clinical performances of two real-time PCR assays and bDNA/TMA to early monitor treatment outcome in patients with chronic hepatitis C. *J Clin Virol* 2009;46:216-221.
11. Gorrin G, Friesenhahn M, Lin P, Sanders M, Pollner R, Eguchi B, Pham J, et al. Performance evaluation of the VERSANT HCV RNA qualitative assay by using transcription-mediated amplification. *J Clin Microbiol* 2003;41:310-317.
12. Roomer R, Hansen BE, Janssen HL, de Knegt RJ. Risk factors for infection during treatment with peginterferon alfa and ribavirin for chronic hepatitis C. *Hepatology* 2010;52:1225-1231.
13. Roomer R, Hansen BE, Janssen HL, de Knegt RJ. Thrombocytopenia and the risk of bleeding during treatment with peginterferon alfa and ribavirin for chronic hepatitis C. *J Hepatol* 2010;53:455-459.
14. Comanor L, Anderson F, Ghany M, Perrillo R, Heathcote EJ, Sherlock C, Zitron I, et al. Transcription-mediated amplification is more sensitive than conventional PCR-based assays for detecting residual serum HCV RNA at end of treatment. *Am J Gastroenterol* 2001;96:2968-2972.
15. Sarrazin C, Teuber G, Kokka R, Rabenau H, Zeuzem S. Detection of residual hepatitis C virus RNA by transcription-mediated amplification in patients with complete virologic response according to polymerase chain reaction-based assays. *Hepatology* 2000;32:818-823.
16. Gerotto M, Dal Pero F, Bortoletto G, Ferrari A, Pistis R, Sebastiani G, Fagioli S, et al. Hepatitis C minimal residual viremia (MRV) detected by TMA at the end of Peg-IFN plus ribavirin therapy predicts post-treatment relapse. *J Hepatol* 2006;44:83-87.

17. Desombere I, Van Vlierberghe H, Couvent S, Clinckspoor F, Leroux-Roels G. Comparison of qualitative (COBAS AMPLICOR HCV 2.0 versus VERSANT HCV RNA) and quantitative (COBAS AMPLICOR HCV monitor 2.0 versus VERSANT HCV RNA 3.0) assays for hepatitis C virus (HCV) RNA detection and quantification: impact on diagnosis and treatment of HCV infections. *J Clin Microbiol* 2005;43:2590-2597.
18. Morishima C, Morgan TR, Everhart JE, Wright EC, Shiffman ML, Everson GT, Lindsay KL, et al. HCV RNA detection by TMA during the hepatitis C antiviral long-term treatment against cirrhosis (Halt-C) trial. *Hepatology* 2006;44:360-367.
19. Morishima C, Morgan TR, Everhart JE, Wright EC, Apodaca MC, Gretch DR, Shiffman ML, et al. Interpretation of positive transcription-mediated amplification test results from polymerase chain reaction-negative samples obtained after treatment of chronic hepatitis C. *Hepatology* 2008;48:1412-1419.

CONTINUOUS INTERFERON ALFA-2B INFUSION IN COMBINATION WITH RIBAVIRIN FOR CHRONIC HEPATITIS C IN TREATMENT EXPERIENCED PATIENTS

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ABSTRACT

Background

Sustained virological response (SVR) rates in previous nonresponders to peginterferon alfa and ribavirin for chronic hepatitis C (HCV) remain low (~10%). We hypothesize that continuous subcutaneous delivery of fully potent interferon (IFN) alfa-2b via an external pump will lead to stable blood concentrations and thereby prevent subtherapeutic trough levels associated with viral breakthrough. The aims of the study were to assess safety, tolerability and virological response in patients who previous peginterferon alfa/ribavirin nonresponders.

Methods

We randomized 30 HCV genotype 1 (n=24) and 4 (n=6) patients to receive 6, 9 or 12 MU IFN alfa-2b daily by continuous subcutaneous administration using an insulin pump (Medtronic MiniMed 508) in combination with ribavirin (1000-1600mg) for 48 weeks .

Results

The magnitude of viral decline in the 12 MU group after 4 weeks of treatment was 2.67 log HCV RNA compared to 1.21 and 1.27 log HCV RNA in the 9 and 6 MU group respectively ($p=0.001$). In the intention-to-treat analysis SVR rate was 20% (6/30). The per-protocol SVR rate was 25% (6/24) of which 4 out of 6 patients in the high-dose arm achieved SVR.

Adverse events appeared dose-dependent, were mostly mild to moderate and typical of IFN therapy. Five patients developed irritation and/or abscesses at the injection site. Six serious adverse events were reported in 5 patients.

Conclusions

Continuous delivery of IFN alfa-2b can induce a strong dose-dependent viral suppression. This could be an effective approach in conjunction with, or as lead-in therapy prior to treatment with a direct antiviral agent.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is one of the leading causes of cirrhosis, hepatocellular carcinoma and end stage liver disease (1). The current standard of care for chronic HCV infection is a 24 or 48 week regimen with peginterferon (PEGIFN) alfa and ribavirin (RBV). This treatment regimen leads to sustained viral responses in 42-82% of patients depending on host factors and viral genotype (2-4). Unfortunately, 50-60% of patients with genotype 1 and 4 do not respond to this treatment regimen, which has led to a continuous increase in the pool of patients non responsive to this treatment regimen. Retreatment sustained virological response (SVR) rates of these patients range between 4 and 15%. Increasing this percentage is considered to be a great challenge (5-10).

Pegylation of interferon (IFN) alfa has improved the pharmacokinetic profile of conventional IFN by maintaining constant blood levels. This enabled once-weekly dosing and resulted in higher response rates. However, it has been shown that the volume of distribution due to pegylation is considerably restricted, decreasing biological activity and potentially decreasing treatment efficacy (11). Continuous exposure to IFN alfa could potentially overcome this hindrance by providing sustained and constant levels of a fully potent protein. We hypothesize that constant levels could induce a stable viral suppression and prevent side effects associated with peaks after injection as well as subtherapeutic drug levels associated with troughs.

The continuous infusion of IFN alfa has been studied in several small phase 1 studies. A significant decrease in serum ALT was observed in one study and continuous infusion of interferon was safe and well tolerated. However, these studies were small and treatment length was inadequate (12-14).

In this pilot study we aim to investigate efficacy, safety and feasibility of continuous subcutaneous infusion of IFN alfa-2b in combination with weight-based RBV (15.2 mg/kg) for 48 weeks in patients who previously failed to respond to PEGIFN alfa and RBV.

METHODS

This study, referred to as the SCIN-C study (Subcutaneous Continuous Interferon alfa-2b infusion in Chronic Hepatitis C Previous Nonresponders) was an investigator initiated study, sponsored by the Foundation of Liver and Gastrointestinal Disorders (SLO, Rotterdam, the Netherlands). Financial support and infusion devices were obtained from Medtronic Inc. (Minneapolis, Mn, USA).

Study design

The study was a single center, randomized, open label, dose finding study with 3 treatment arms. We randomly assigned 24 genotype 1 and 6 genotype 4 patients in a 1:1:1 ratio to

receive 6, 9 or 12 MU IFN alfa-2b per day by continuous subcutaneous infusion in combination with daily RBV (figure 1). Stratified random assignment was used to balance genotype distribution. RBV dosage was weight-based: 1000 mg for patients ≤ 65 kg, 1200mg for 65-80 kg, 1400 mg for >80 -100kg and 1600mg for >100 kg. Patients visited the outpatient clinic at time of screening, at baseline, during treatment (week 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 40, 44 and 48) and during post-treatment follow up (week 52, 60 and 72). In the event of grade 3 adverse events IFN alfa-2b dose was reduced by 1.5 MU/day. In case of an ANC $<750/\mu\text{L}$ (absolute neutrophil count) or a platelet count $<50000/\mu\text{L}$ IFN alfa-2b dose was reduced to 75% of the initial dose. This dose reduction was not performed in the first 12 weeks of therapy or in case of HCV RNA positivity after 12 weeks of therapy, considering the negative impact of dose reductions, on virological response. In case of ANC $<375/\mu\text{L}$ or a platelet count $<25000/\mu\text{L}$ IFN alfa-2b dose was reduced to 50% of the initial dose regardless of viral load or duration of therapy. When hemoglobin concentration dropped below 5.0 mmol/L IFN alfa-2b dose was reduced to 75%, in case of a drop below 4.0 mmol/L RBV dose was reduced to 10mg/kg daily and patients were treated with blood transfusions.

Patients

Patients were considered eligible for enrollment in this study if they were between 18 and 60 years of age, had chronic hepatitis C infection genotype 1 or 4, were unresponsive to previous PEGIFN/RBV therapy and had persistently elevated serum ALT or histological evidence of continuing or progressive fibrosis. Non-response to previous therapy was defined as null response (a less than 2 log drop at week 12 during previous therapy), partial response (HCV RNA positivity at week 24), breakthrough (viral breakthrough during therapy) or relapse (viral relapse after therapy). Duration of previous treatment was required to be at least 3 months. All patients had detectable HCV RNA by a polymerase chain reaction (PCR) assay. Patients were excluded if they had signs of decompensated liver disease, evidence of hepatocellular carcinoma (hepatic imaging performed within 3 months prior to screening), other acquired or inherited liver diseases, co-infection with HIV or chronic hepatitis B, significant pulmonary, cardiovascular or renal dysfunction, malignancies in the previous 5 years, history of seizure disorder, uncontrolled thyroid disease, psychiatric disorders, the presence of immunological disorders, pregnancy, breast feeding, and/or active substance abuse (I.V. drugs or >80 grams of alcohol per day).

Continuous subcutaneous infusion of interferon alfa-2b

For subcutaneous infusion of IFN alfa-2b the MiniMed® insulin pump (Medtronic, Minneapolis, Mn, USA) was used. At time of screening, patients received instructions regarding pump handling and operation. At baseline these instructions were repeated and patients were asked to demonstrate how to handle the Minimed® pump. If patients encountered problems during treatment regarding pump handling, they were instructed to call one of the investigators.

Patients received 5 reservoirs with IFN alfa-2b for every 2 weeks on therapy. Reservoirs were replaced every 3 days.

Assessment of safety and feasibility

Safety was assessed by physical examination, laboratory tests and recording of adverse events at every visit during and after therapy. At every outpatient clinic visit patients were asked if any problems regarding to pump handling had occurred. Furthermore the daily IFN alfa-2b dose administration was checked using an automated dosing registry within the pump and patients registered the daily IFN dose and RBV dose on drug accountability forms

Assessment of virological response

HCV RNA was measured at every visit from baseline until week 12, at week 24, 36, 48 and at every visit during follow up using the VERSANT® HCV RNA 3.0 quantitative assay (branched DNA, lower limit of quantification 615 IU/ml). In case of unquantifiable HCV RNA a qualitative assay (Cobas AmpliCor / Cobas TaqMan HCV test v1.0, lower limit of detection <15 IU/ml) was used for the detection of HCV RNA. Treatment was discontinued in patients with detectable HCV RNA at week 24. Virological endpoints were viral decline during therapy and HCV RNA negativity at week 48 and after 24 weeks of follow up.

Interferon level determination

IFN alfa-2b concentrations were determined by application of a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) method (BMS216INST, Bender Medsystems Diagnostics GmbH, Vienna, Austria).

IL28B genotype determination

The IL28b SNP rs12979860 variants were determined using competitive allele-specific PCR (KASP; KBioscience Hoddesdon, UK).

Statistical analysis

Chi square and Fisher's exact tests were used to compare adverse events and virological responses between 3 different treatment arms. Both parametric and nonparametric tests were performed to compare continuous variables. Linear regression was used to analyze viral decline and virological responses during treatment. Continuous variables are expressed as means \pm SD or medians (range) where appropriate. All statistical tests were two-sided, and a *P* value < .05 was considered to be statistically significant.

SPSS version 15.0 was used for all statistical analysis (SPSS Inc., Chicago, IL, USA).

RESULTS

Patients

Patients were enrolled between July 2007 and November 2008. A total of 32 were assessed for eligibility and 30 patients were randomly assigned to 1 of 3 treatment arms (Figure 1). End of follow up data are available in 26 patients (87%) (Figure 2). Baseline characteristics of patients are summarized in table 1. No significant differences between treatment arms were observed. Patients were predominantly Caucasian (93%) and were infected with HCV genotype 1 (80%). Twenty patients were classified as null or partial responders during previous therapy and 10 patients experienced viral breakthrough during or viral relapse after their previous treatment. IL28B SNP rs12979860 was determined in 29 patients. As expected most patients had the intermediate or poor response variants CT and TT and only 3 patients had the favorable genotype CC. No significant differences between the 3 treatment arms were found.

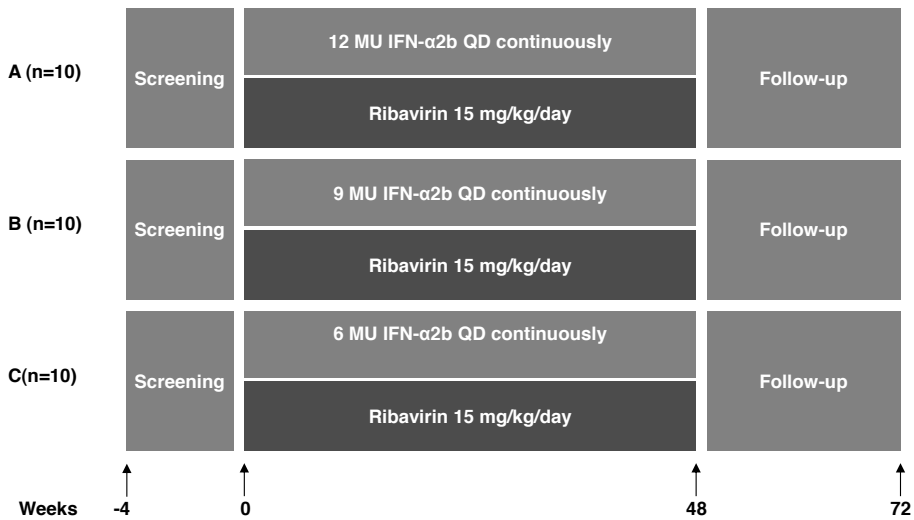


Fig. 1. Study design. Treatment was discontinued if HCV RNA was detectable at week 24 of treatment using the COBAS TaqMan HCV RNA assay (LLOD<15 IU/ml)

Safety and tolerability

Adverse events, hematologic abnormalities, dose reductions and treatment discontinuations are listed in table 2. All patients had at least one adverse event. In the 12 MU group 5.8% of adverse events were classified as severe compared to 3.3% and 2.2% of adverse events in the 9 and 6 MU groups, respectively ($p=0.230$). Twenty-four infections occurred in 17 patients. Injection site reactions occurred in 21 patients. Four of these reactions were defined as small skin abscesses at the injection site, all of which improved rapidly after drainage. Severe injection site reactions mostly occurred in the first months of this study probably

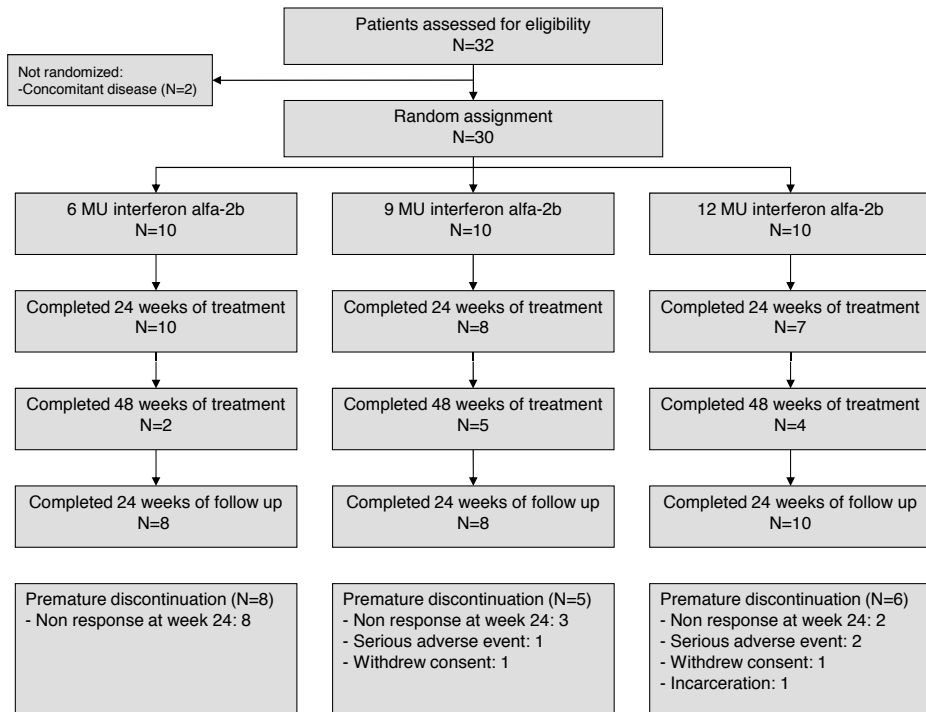


Fig. 2. Study flow diagram.

due to less experience regarding replacement of the infusion sets. In 5 patients injection site reactions were defined as severe and in 2 patients as a serious adverse event (SAE) due to hospital admission. One of these 2 hospitalized patients was diagnosed with cellulitis and antiviral treatment was discontinued temporarily. Some weeks later this patient developed a hyperglycemia induced seizure, the patient's second SAE, and treatment was discontinued permanently. This patient suffered from diabetes mellitus and had not been compliant with insulin therapy. Other SAE's included community acquired pneumonia, diarrhea and an upper respiratory tract infection. In total 6 SAE's occurred in 5 patients; 4 in the 12 MU and 1 in the 9 MU group ($p=0.027$). All SAE's resolved after temporary or permanent discontinuation of treatment. Four out of 5 patients with SAE's had cirrhosis. Dose reductions due to hematologic abnormalities or adverse events were performed in 5 out of 30 patients (17%): 3 patients from the 12 MU group and 2 patients from the 9 MU group. In 6 patients treatment was discontinued prematurely (4 patients from the 12 MU group and 2 patients from the 9 MU group, $p=0.12$). Reasons for discontinuation were the occurrence of an SAE in 3 patients, withdrawal of consent in 2 patients and due to incarceration in 1 patient.

Table 1. Baseline characteristics

Variable	Total	6MU	9MU	12MU	P-value
	N=30	N=10	N=10	N=10	
Male (%)	22 (73)	7 (70%)	6 (60%)	9 (90%)	0.303
Age (years)	46.9	46.1	48.2	46.5	0.685
Caucasian/Black/Asian	27/1/2	8/1/1	9/0/1	10/0/0	0.399
Genotype 1 vs. 4	24/6	8/2	8/2	8/2	1.000
Cirrhosis (%)	13 (43)	3 (30)	3 (30)	7 (70)	0.123
IL28B genotype (rs12979860)					
CC/CT/TT	3/20/6	1/6/2	1/6/3	1/8/1	0.863
BMI in kg/m ² (mean)	27.5	26.6	28.7	27.2	0.664
Response to previous therapy					0.980
-Non response week 12*	9	2	4	3	
-Non response week 24**	11	4	3	4	
-Breakthrough	3	1	1	1	
-Relapse	7	3	2	2	
Mean log HCV RNA	5.45	5.25	5.67	5.46	0.397
Albumin in g/L	42.9	42	44.7	42	0.371
Bilirubin in μ mol/L	12.0	10.2	12.5	13.2	0.790
Prothrombin time in seconds	12.2	11.7	12.1	12.7	0.267
ALT in U/L	88.3	88.9	88	88	0.717
GGT in U/L	113.5	151.3	81.9	107.2	0.245
Hemoglobin in mmol/L	9.3	9.2	9.5	9.4	0.889
ANC in cells / μ L mean	3.1	3.0	2.8	3.3	0.571
Platelet count in thousands / μ L	177	183	192	158	0.249

*defined as null responder

**defined as partial responder

Interferon levels

IFN levels increased dose-dependently reaching peak levels between 48 hours and 1 week of treatment followed by continuous steady state levels during the remaining treatment period. Mean IFN levels at week 4 were 344 pg/ml, 264 pg/ml and 225 pg/ml in the 12, 9 and 6 MU group respectively. Between patients a great inter individual variability was observed. A weak negative correlation was found between IFN levels and viral load ($r=-0.297$, $p<0,001$).

Viral kinetics

The magnitude of viral decline in the 12 MU group after 4 weeks of treatment was 2.67 log HCV RNA compared to 1.21 and 1.27 log HCV RNA in the 9 and 6 MU groups respectively ($p=0.001$, figure 3). After 12 weeks viral decline was 3.57 log HCV RNA in the 12 MU group compared to 2.8 log HCV RNA in the 9 MU group and 1.91 log HCV RNA in the 6 MU group ($p=0.075$). In the multivariate linear regression analysis IFN alfa-2b dose ($p=0.004$) and the IL28b variant ($p=0.017$) were significantly associated with viral decline at week 4.

Table 2. Dose reductions, discontinuations, adverse events and laboratory abnormalities

Variable	Total	12 MU	9MU	6MU
	N=30	N=10	N=10	N=10
Dose reductions	5	3	2	0
Discontinuations	6	4	2	0
Serious adverse events	6	5	1	0
Infections	24	6	13	5
Adverse events				
-Fatigue	28	8	10	8
-Myalgia	20	8	6	6
-Headache	20	6	9	5
-Nausea	18	7	8	3
-Loss of appetite	18	7	5	6
-Fever	17	5	6	6
-Injection site reaction	17	7	5	5
-Pruritus	13	5	6	2
-Weight loss	13	6	4	3
-Alopecia	13	6	4	3
-Depression	13	4	6	3
-Abdominal pain	12	5	5	2
-Diarrhea	12	4	3	5
-Emotional disorder	11	3	4	4
-Dyspnea	11	6	4	1
-Cough	11	3	6	2
-Concentration impairment	9	5	1	3
-Arthralgia	9	3	4	2
-Rash	9	4	4	1
-Dry skin	9	4	4	1
-Dizziness	9	2	2	5
-Irritability	7	2	1	4
-Back pain	7	2	3	2
-Vomiting	6	3	3	0
-Dry mouth	6	2	3	1
-Obstipation	6	3	3	0
-Epistaxis	6	4	2	0
Other	33	11	9	13
Hematologic abnormalities				
-Anemia*	14	4	5	5
-Neutropenia**	15	5	5	5
-Thrombocytopenia***	6	2	1	3

* Defined as Hb <6.2 mmol/L

** Defined as ANC <750/ μ L*** Defined as Platelet count <50000/ μ L

Viral response

In the intention-to-treat analysis 6 out of 30 (20%) patients achieved SVR. Three out of 20 (15%) previous null or partial responders (1 from the 9 MU group and 2 from the 12 MU group) achieved SVR. The remaining 3 patients who achieved SVR were patients with a viral breakthrough during, or relapse after previous antiviral therapy (1 from the 6 Mu group and 2

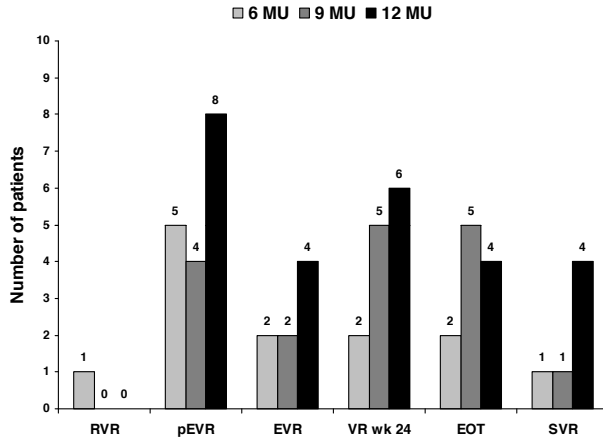


Fig. 3. Virological responses during therapy per treatment arm

RVR: rapid virological response, defined as HCV RNA <15 IU/ml at week 4; pEVR: partial early virological response, defined as > 2 log drop of HCV RNA at week 12; EVR: complete early virological response, defined as HCV RNA <15 IU/ml at week 12; VR24: virological response at week 24, defined as HCV RNA <15 IU/ml; EOT: end of treatment response, defined as HCV RNA <15 IU/ml at end of treatment; SVR: sustained virological response, defined as HCV RNA <15 IU/ml at 24 weeks after treatment discontinuation.

from the 12 MU group). Of the 6 SVR patients 3 had cirrhosis (all from the 12 MU group). Five out of 24 (21%) genotype 1 patients and 1 out of 6 (17%) genotype 4 patients achieved SVR.

In the per-protocol analysis the SVR rate was 6 out of 24 (25%). Of the 6 patients (67%) treated per-protocol in the 12 MU group, 4 achieved SVR compared to 1 out of 10 (10%) and 1 out of 8 (12.5%) in the 6 and 9 MU group respectively ($p=0.042$).

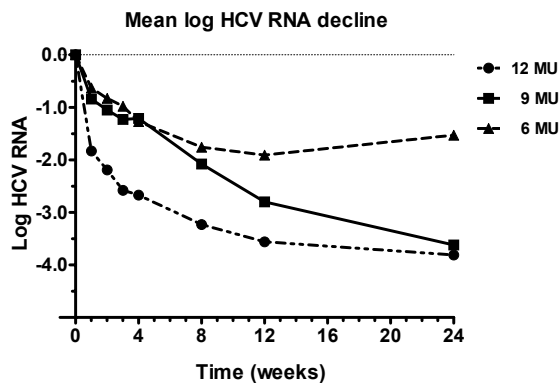


Fig. 4. Viral decline during therapy

The magnitude of viral decline in the 12 MU group after 4 weeks of treatment was 2.67 log HCV RNA compared to 1.21 and 1.27 log HCV RNA in the 9 and 6 MU group respectively ($p=0.001$).

Virological response rates are shown in figure 4. At week 4, one patient (from the 6 MU group) had undetectable HCV RNA. In the 12, 9 and 6 MU group respectively, 4, 2 and 2 patients had undetectable HCV RNA at week 12 ($p=0.384$). Eight out of 10 patients from the 12 MU group became HCV RNA negative during therapy compared to 5/10 (50%) and 2/10 (20%) in the 9 and 6 MU group respectively ($p=0.027$). Two patients of these patients were HCV negative before week 24 (week 8 and 12) but had to stop treatment due to an SAE. Importantly, 10 out of 20 patients with a previous null or partial response to antiviral therapy became HCV RNA negative during therapy (HCV RNA <15 IU/ml).

All patients with the IL28b rs12979860 CC variant became HCV RNA negative whereas only 50% of patients with the CT (10/20) and TT variants (3/6) became HCV RNA negative ($p=0.423$). In patients who achieved SVR IL28B rs12979860 variant was CC in 2 patients (one in each of the 6 and 9 MU groups) and CT in 4 patients (all from the 12 MU group). The third CC patient had a cEVR (complete early virological response), however treatment was stopped in this patient in week 21 due to an SAE. In the 12 MU group, virological responses were independent of IL28b genotype.

None of the patients who had less than a 2 log decline of HCV RNA at week 4 achieved SVR and thus the negative predictive value (NPV) was 100%. Thirteen out of 27 patients (48%) had detectable HCV RNA at week 12 of antiviral therapy and none of these patients achieved an SVR (NPV 100%).

DISCUSSION

This pilot study is the first to investigate safety and efficacy of continuous infusion of high daily doses of IFN alfa-2b in combination with RBV for a full 48 weeks in chronic hepatitis C patients who previously failed therapy. The rationale of continuous subcutaneous infusion of IFN alfa-2b was firstly to optimize response by maintaining constant high IFN alfa blood concentrations enabling continuous viral suppression and secondly prevention of side effects associated with IFN peaks which may occur with weekly injections of PEG-IFN alfa.

Our most important finding was that delivery of IFN alfa-2b in combination with RBV resulted in a strong dose-dependent viral suppression. At week 4 two-thirds of patients from the 12 MU group had unquantifiable HCV RNA and at week 12 all patients from this group had unquantifiable HCV RNA. Furthermore, 50% of previous null and partial responders became HCV RNA negative during treatment. Six out of 30 patients (20%) achieved SVR. The per-protocol SVR rate was 25% with 4 out of 6 patients (67%) from the 12 MU group achieving SVR.

This strong viral suppression of high dose continuous subcutaneous IFN-2b infusion could play an important role within the recently proposed concept of lead-in therapy prior to triple therapy with PEGIFN alfa, RBV and a direct antiviral agent. In these studies investigating

direct antiviral agents, a rapid virological decline during a PEGIFN alfa/RBV lead-in was crucial to achieve SVR and prevent virological breakthrough or relapse (15-18). Short-term high dose continuous IFN alfa infusion could help to achieve this necessary rapid viral decline and therefore this concept of continuous IFN-2b alfa could play an important role in the new era of direct antiviral agents. Especially patients with a known null or partial response to previous therapy with peginterferon alfa and ribavirin could benefit from this treatment concept.

In addition, this study reports on the predictiveness of SNPs near the IL28b gene in previous non-responders. As expected most patients had the rs12979860 CT and TT variants that are associated with decreased responsiveness to PEGIFN/RBV therapy whereas only 3 patients had the favorable CC variant of this SNP. An important finding was that the strong viral suppression in patients treated with 12 MU of IFN alfa-2b was independent of IL28b genotype. These results suggest that the lack of innate IFN responsiveness seen in this group of patients can be overcome by high doses of continuous IFN alfa-2b infusion. Patients with the CC genotype had a more pronounced virological response compared to CT and TT patients. Response rates between patients with CT and TT variants were comparable.

Side effects were typically IFN related and severity increased as dose increased. Weekly peaks of side effects did not occur, however intensity of side effects appeared comparable to treatment with PEGIFN alfa and RBV. Five patients developed skin abscesses and severe injection site reactions. These reactions occurred only in the early phase of the study and could be prevented later on in the study by adequate instructions for replacement of the injection cannula (infusion sets). Most serious adverse events occurred in cirrhotic patients, for this reason caution is warranted in this patient group. To prevent infectious complications antibiotic prophylactic therapy should be considered. Dose reductions and discontinuation occurred only in the 9 and 12 MU groups. The most common reasons for dose reductions were adverse events and hematologic abnormalities.

Interferon levels were measured throughout the study, all patients reached steady state levels in the first weeks of treatment. The pharmacokinetic profile of continuous infusion of interferon alfa differs from that of treatment with peginterferon alfa-2a or 2b and ribavirin, which causes peak and trough concentrations. A study investigating pharmacokinetics of peginterferon alfa 2a and 2b showed that undetectable trough concentrations of peginterferon alfa-2b can occur in some patients (19). This phenomenon did not occur in our study.

A great interindividual variability of interferon alfa concentrations exists between patients. For this reason it is difficult to use these concentrations for prediction of treatment outcome. To our knowledge, to date, no data have been published on the predictiveness of (peg)interferon alfa concentrations on treatment outcome.

Approximately 50-60% of genotype 1 patients are non-responsive to treatment with PEGIFN alfa and RBV for chronic hepatitis C. Several treatment options to cure these difficult-to-treat patients have been investigated. Retreatment of genotype 1 patients who previously failed PEGIFN alfa and RBV therapy with PEGIFN alfa-2b and weight based RBV lead to

SVR rates up to 11% (8). Comparable SVR rates were achieved with retreatment of true non-responders with consensus IFN and RBV (5). Studies investigating PEGIFN induction therapy could not demonstrate an increase in SVR rates. However, early virological response rates were increased in patients receiving induction therapy (6-7, 9-10). The highest SVR rates were achieved when combining a PEGIFN alfa-2a/RBV induction regimen with an extension of the treatment duration to 72 weeks (7). Sixteen percent of these patients, who were nonresponsive to previous therapy with PEGIFN alfa and RBV, achieved SVR.

In our study, treatment with IFN alfa-2b and RBV by continuous infusion led to an intention-to-treat SVR rate of 20%, this was nearly twice as high compared to the SVR rate of retreatment with PEGIFN alfa and RBV reported so far (8). A limitation of this study is the lack of a control arm. However, retreating previous null or partial responders to peginterferon and ribavirin with the same regimen, will have limited chances of SVR.

In conclusion, continuous delivery of high doses of IFN alfa-2b with a pump device can be carried out successfully in this difficult-to-treat population. If side effects are managed adequately, continuous delivery of IFN alfa-2b can induce a strong dose-dependent viral suppression leading to improved SVR rates. Short-term continuous IFN treatment could also be an effective approach in conjunction with or as lead-in therapy prior to treatment with a direct antiviral agent, especially in difficult-to-treat populations.

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REFERENCES

1. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997;349:825-832.
2. 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.
3. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-355.
4. 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.
5. Bacon BR, Shiffman ML, Mendes F, Ghalib R, Hassanein T, Morelli G, Joshi S, et al. Retreating chronic hepatitis C with daily interferon alfacon-1/ribavirin after nonresponse to pegylated interferon/ribavirin: DIRECT results. *Hepatology* 2009;49:1838-1846.
6. Bergmann JF, Vrolijk JM, van der Schaar P, Vroom B, van Hoek B, van der Sluys Veer A, de Vries RA, et al. Gamma-glutamyltransferase and rapid virological response as predictors of successful treatment with experimental or standard peginterferon-alpha-2b in chronic hepatitis C non-responders. *Liver Int* 2007;27:1217-1225.
7. Jensen DM, Marcellin P, Freilich B, Andreone P, Di Bisceglie A, Brandao-Mello CE, Reddy KR, et al. Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. *Ann Intern Med* 2009;150:528-540.
8. Poynard T, Colombo M, Bruix J, Schiff E, Terg R, Flamm S, Moreno-Otero R, et al. Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. *Gastroenterology* 2009;136:1618-1628 e1612.
9. Roberts SK, Weltman MD, Crawford DH, McCaughan GW, Sievert W, Cheng WS, Rawlinson W, et al. Impact of high-dose peginterferon alfa-2A on virological response rates in patients with hepatitis C genotype 1: a randomized controlled trial. *Hepatology* 2009;50:1045-1055.
10. 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.
11. Zeuzem S, Welsch C, Herrmann E. Pharmacokinetics of peginterferons. *Semin Liver Dis* 2003;23 Suppl 1:23-28.
12. Schenker S, Cutler D, Finch J, Tamburro CH, Afrime M, Sabo R, Bay M. Activity and tolerance of a continuous subcutaneous infusion of interferon-alpha2b in patients with chronic hepatitis C. *J Interferon Cytokine Res* 1997;17:665-670.
13. Carreno V, Tapia L, Ryff JC, Quiroga JA, Castillo I. Treatment of chronic hepatitis C by continuous subcutaneous infusion of interferon-alpha. *J Med Virol* 1992;37:215-219.
14. Tong M, Hassanein T, Van Antwerp W, Olek E, Murphy B. Delivery of consensus interferon by continuous infusion for the treatment of chronic hepatitis C: A pilot viral kinetic study in nonresponder patients. *Hepatology* 2003;38:308.
15. Bacon B. HCV Respond-2 final results: High sustained virologic response among genotype 1 previous non-responders and relapsers to peginterferon/ribavirin when re-treated with boceprevir plus pegintron (peginterferon alfa-2b)/ribavirin. *Hepatology (suppl)* 2010;52:430A.
16. Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010;376:705-716.

17. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-1838.
18. McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, et al. Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010;362:1292-1303.
19. Bruno R, Sacchi P, Ciappina V, Zochetti C, Patruno S, Maiocchi L, Filice G. Viral dynamics and pharmacokinetics of peginterferon alpha-2a and peginterferon alpha-2b in naive patients with chronic hepatitis c: a randomized, controlled study. *Antivir Ther* 2004;9:491-497.

**VIRAL KINETICS AND IMMUNE
ACTIVATION DURING CONTINUOUS
SUBCUTANEOUS ADMINISTRATION OF
HIGH DOSE INTERFERON ALFA-2B IN
TREATMENT EXPERIENCED CHRONIC
HEPATITIS C PATIENTS**

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ABSTRACT

Background

Retreatment outcome of chronic HCV-infected patients with peginterferon/ribavirin (PegIFN/RBV) is generally poor after previous non-response or relapse.

Aim

To compare viral kinetics and markers of immune activation between treatment-experienced HCV infected patients receiving two different dosages of continuously administered IFN alfa-2b, which resulted in a significant difference in viral response at week 4.

Methods

Patients were randomized to receive 12 MU IFN (n=10) or 9 MU IFN (n=10) per day by continuous subcutaneous administration combined with weight-based RBV. HCV RNA levels, IFN levels, the enzymatic activity of 2,5-oligoadenylate synthetase (2,5-OAS) and levels of neopterin and beta2-microglobulin were measured in serum at T=0, 4, 8, 12, 24, 48, 72, 96 hours and at week 1, 2, 3, 4 after start of treatment. Sustained virological response was based on HCV RNA negativity 24 weeks post-treatment.

Results

A typical biphasic viral decline was demonstrated with a significant stronger median HCV RNA decline at day 2 (-1.57 log vs. -0.59 log; p=0.006) and week 4 (-2.58 log vs. -0.84 log; p=0.017) in patients receiving 12 MU IFN per day versus 9 MU IFN per day, respectively. The significant stronger HCV RNA decline in the 12 MU dose group was reflected by higher neopterin peak levels at day 2 and higher steady levels up to week 4 (3.75 ng/ml vs. 2.40 ng/ml; p=0.027). No significant differences were found between the two dose groups with regard to serum IFN levels, 2,5-OAS activity and beta2-microglobulin levels. All patients that achieved SVR (4 patients in the 12MU dose group vs. 1 patient in the 9 MU dose group) had a more than 2 log viral decline at week 4.

Conclusion

A strong HCV RNA decline at week 4 can be induced by high dose continuous IFN therapy in patients who failed previous PegIFN/RBV. A more than 2 log viral decline at week 4 is essential for achieving SVR. Strong viral suppression is associated with high neopterin levels.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major public health problem leading to liver fibrosis, cirrhosis and hepatocellular carcinoma (1). An estimated 180 million people are infected worldwide (2). It is expected that HCV related mortality will continue to increase over the next two decades (3). The current standard of care with pegylated interferon-alfa (PegIFN) and ribavirin (RBV) results in sustained virological response (SVR) in approximately 40-50% of genotype 1 infected patients (4-6). Phase III clinical trials with protease inhibitors (telaprevir and boceprevir) have shown that SVR rates can increase up to 80% in treatment-naïve patients and up to 65% in treatment-experienced patients (7-10). These direct antiviral agents show strong, rapid viral declines and are expected to enter clinical practice soon, but need to be combined with PegIFN (and RBV), stressing the continued importance of achieving immunological control during anti-HCV therapy.

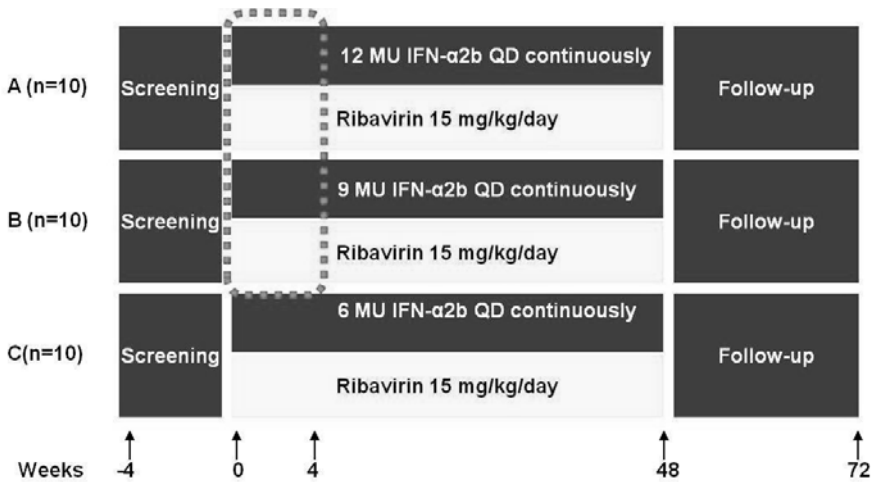
Viral kinetic analyses have provided insight into different response types during (Peg)IFN-based therapy and are important for prediction of SVR (11). Responders to therapy show a typical biphasic decline of HCV RNA with a rapid first phase lasting for approximately 24-48 hours followed by a slower second phase (12-15). Subsequently, marked elevation is seen of several serum markers that are known to reflect the induction of antiviral activity and systemic immune activation, including 2,5-oligoadenylate synthetase (2,5-OAS) activity, neopterin and beta2-microglobulin levels (16-19). The levels of these IFN-induced markers are indicative of the antiviral efficacy of (Peg)IFN and have been implicated as factors determining the clinical outcome of therapy (18, 20).

In the present study, we sought to analyze differences between IFN-inducible markers in treatment-experienced patients with chronic hepatitis C who were treated with continuous subcutaneous IFN infusion in our earlier randomized SCIN-C trial (21). The rationale for continuous IFN delivery by an external pump included achievement of sustained and constant levels of the IFN protein, resulting in increased antiviral activity and biologic potency. This randomized trial showed a significant stronger mean decline in viral load at week 4 in patients receiving continuously 12 MU standard IFN per day combined with weight based RBV (-2.67 log HCV RNA) versus 9 MU (-1.21 log HCV RNA) and 6 MU per day (-1.27 log HCV RNA), respectively. In the current analysis we sought to explain differences in viral decline between patients in the two highest dose groups (12 MU and 9 MU standard IFN per day) based on early viral kinetics, markers of immune activation and IFN levels.

METHODS

Study design

The characteristics of the chronic hepatitis C patients who participated in this study have been described in detail before (21). This was a single center, randomized, open label, dose finding study and was performed in accordance with Good Clinical Practice and the World Medical Association Declaration of Helsinki, after approval by the institutional review board. We randomly assigned 24 genotype 1 and 6 genotype 4 patients in a 1:1:1 ratio to receive 12, 9 or 6 MU standard IFN- α 2b per day by continuous subcutaneous infusion in combination with daily weight-based RBV (approximately 15 mg/kg). Patients were treated for 48 weeks, but discontinued therapy at week 24 when HCV RNA positive. All patients provided written informed consent before participating in any study-related activity. For the ancillary study the two cohorts of chronic HCV infected patients receiving 12 MU or 9 MU IFN per day were evaluated for viral kinetics and markers of immune response (Figure 1). All patients in the main study were asked to participate in the ancillary study which included extra serum sample collections during the first week at T=4, 8, 12, 24, 48, 72, 96 hours after onset of therapy. Informed consent for extra sample collections during the first week of therapy was obtained in 7 out of 10 patients in the 12 MU dose group and 8 out of 10 patients in the 9 MU dose group.



The study period of this ancillary analysis is marked in red.

Figure 1. Study design SCIN-C.

Patients

Patients were considered eligible for enrollment in this study if they were between 18 and 60 years of age, had chronic hepatitis C infection genotype 1 or 4, were unresponsive to previous PegIFN/RBV therapy and had persistently elevated serum ALT or histological evidence of continuing or progressive fibrosis. Non-response to previous therapy was defined as null response (a less than 2 log drop at week 12 during previous therapy), partial response (HCV RNA positivity at week 24), breakthrough (viral breakthrough during therapy) or relapse (viral relapse after therapy). Duration of previous treatment was required to be at least 3 months. All patients had detectable HCV RNA in serum by a polymerase chain reaction (PCR) assay. Patients were excluded if they had signs of decompensated liver disease, evidence of hepatocellular carcinoma (hepatic imaging performed within 3 months prior to screening), other acquired or inherited liver diseases, co-infection with HIV or chronic hepatitis B, significant pulmonary, cardiovascular or renal dysfunction, malignancies in the previous 5 years, history of seizure disorder, uncontrolled thyroid disease, psychiatric disorders, the presence of immunological disorders, pregnancy, breast feeding, and/or active substance abuse (I.V. drugs or >80 grams of alcohol per day).

Viral assessment

Serum samples were collected at T=0, 4, 8, 12, 24, 48, 72, 96 hours and at week 1, 2, 3, 4 after start of treatment. Sustained virological response was based on HCV RNA negativity 24 weeks post-treatment. HCV RNA measurements were performed by the VERSANT® HCV RNA 3.0 quantitative assay (branched DNA, lower limit of quantification 615 IU/ml). In case of undetectable HCV RNA 24 weeks post-treatment a qualitative assay (Cobas Amplicor / Cobas TaqMan HCV test v1.0, lower limit of detection <15 IU/ml) was used for the detection of HCV RNA.

Interferon level determination

Interferon levels were assessed at the same timepoints as HCV RNA levels. IFN alfa-2b concentrations were determined by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) method (Bender Medsystems Diagnostics GmbH, Vienna, Austria).

Serum markers of immune activation

Activity of 2,5-oligoadenylate synthetase (2,5-OAS) activity, neopterin and beta2-microglobulin levels were assessed at the same timepoints as HCV RNA levels and IFN levels. Neopterin and beta2-microglobulin levels were analyzed by ELISA (DRG Diagnostics, Marburg, Germany). Activity of 2,5-OAS was analyzed by radioimmunoassay (Eiken, Tokyo, Japan).

Statistics

No formal sample size calculations for this study were performed. The determination of the sample size was based on empirical considerations rather than statistical justification. The sample size of 10 patients in each dose group was considered appropriate for this type of study. Both parametric and nonparametric tests were performed to compare continuous variables. Continuous variables are expressed as means or medians where appropriate. All statistical tests were two-sided, and a p-value less than 0.05 was considered to be statistically significant. SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and SAS 9.2 (location) were used for all statistical analyses.

RESULTS

Patients

Baseline patient characteristics have been described previously and were comparable between both groups (21). In the 12 MU dose group 90% of patients were male versus 60% in the 9 MU dose group. The mean age was 46.5 versus 48.2, respectively. Median baseline viral load was 5.39 log versus 5.78 log HCV RNA, respectively. In the 12 MU dose group one patient discontinued therapy after 2 weeks. Adverse events were typically IFN/RBV related and have been described previously (21).

HCV RNA levels

A typical biphasic viral decline was seen in most patients. The strongest decline was seen in patients receiving 12 MU IFN per day (Figure 2). Approximately 48 hours after onset of therapy

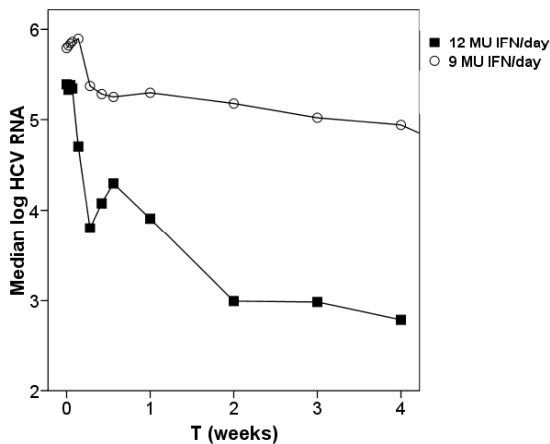


Figure 2. Viral decline according to dose.

the strongest first phase decline was seen, followed by a much slower second phase decay (both dose groups). The median HCV RNA drop at day 2 was 1.57 log in 12 MU dose group versus 0.59 log in the 9 MU dose group ($p=0.006$). The median HCV RNA drop at week 4 was 2.58 log in the 12 MU dose group versus 0.84 log in the 9 MU dose group ($p=0.017$). SVR was achieved in 4 patients in the 12 MU dose group and in 1 patient in the 9 MU dose group. All patients achieving SVR after 48 weeks of therapy had more than 2 log decline of HCV RNA at week 4.

Interferon levels

Serum IFN- α levels peaked between T=48 hours and week 1 followed by steady-state (Figure 3). A stronger IFN peak was seen in patients receiving 9 MU IFN/day at day 3 (329 pg/ml versus 188 pg/ml, $p=0.49$), but IFN levels continued to rise in the 12 MU group and maximum levels were reached between week 2 and 4. The highest serum IFN levels at week 4 were reached in patients receiving 12 MU IFN/day with median IFN levels of 294 pg/ml, versus 157 pg/ml in patients receiving 9 MU IFN/day ($p=0.093$). Although IFN levels were quickly measurable in all patients, there was some inter-individual variability. We could not detect any differences in IFN patterns between individual patients in the 12 MU dose group and the 9 MU dose group and between patients within these group that went on to achieve SVR (Figure 4).

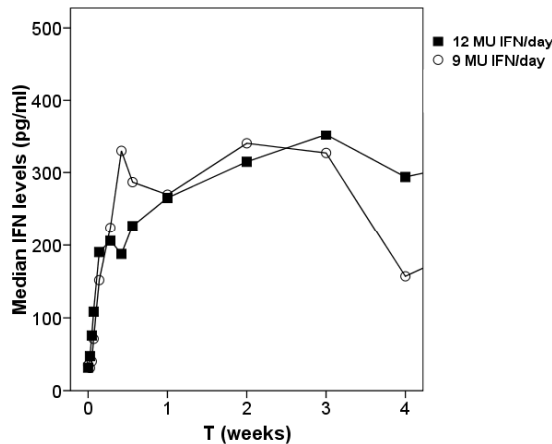


Figure 3. Interferon levels according to dose.

Induction of 2,5-OAS, neopterin and beta2-microglobulin

Most patients had undetectable or low levels of 2,5-OAS, neopterin and beta2-microglobulin at baseline and now significant differences were seen between the two dose groups at start of treatment. Activity of 2,5-OAS peaked between T=24 hours and week 1 (Figure 5A). Patients receiving 12 MU per day showed steady state of 2,5-OAS activity after this peak, while in

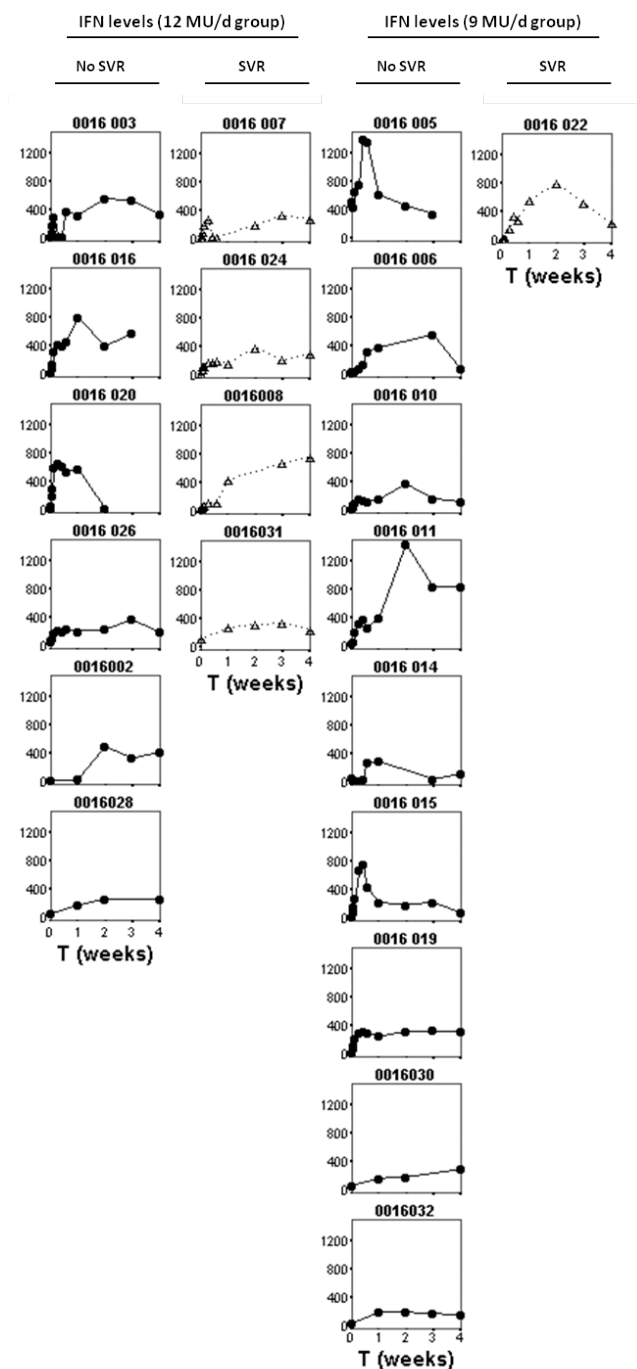


Figure 4. Individual interferon levels (pg/ml)

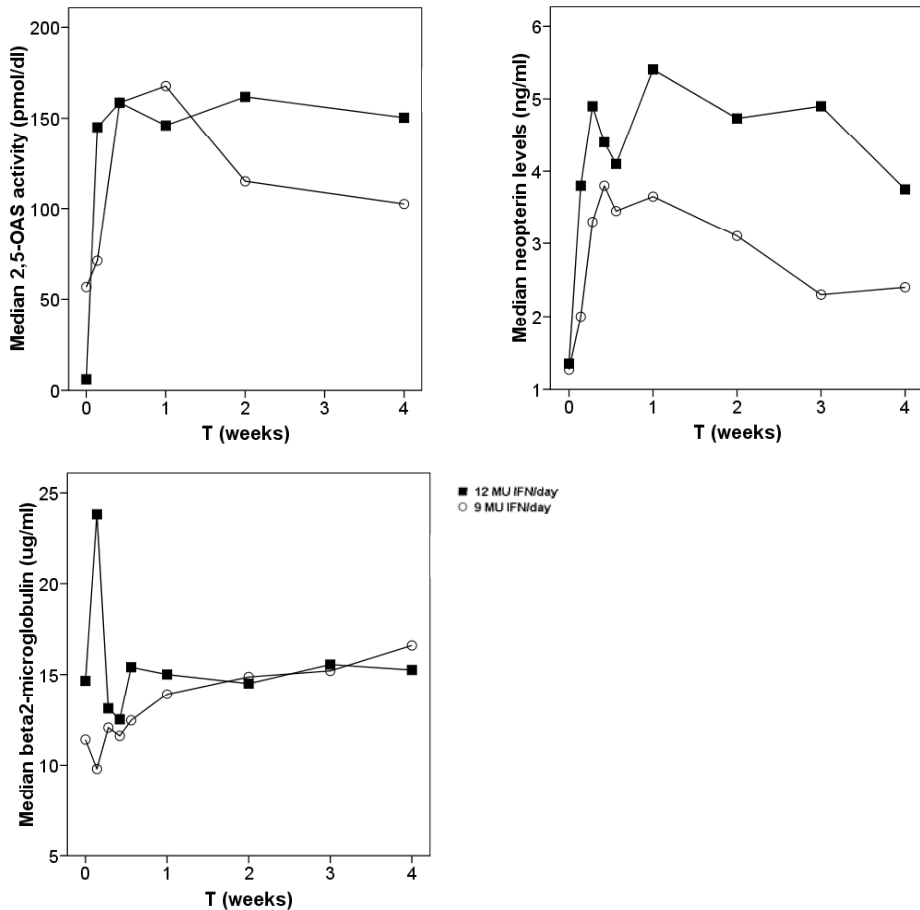


Figure 5

A. 2,5-OAS activity.

B. Neopterin levels.

C. Beta2-microglobulin levels.

patients receiving 9 MU per day this peak was followed by slow decline resulting in lower 2,5-OAS activity at week 4 (158 vs. 102 pmol/dl; $p=0.43$). Neopterin level increased within 24 hours in most patients but higher peak levels between day 2 and 3 were seen in patients receiving 12 MU per day (Figure 5B). Following the neopterin peak, a gradual decline was seen of neopterin levels, resulting in significant higher levels at week 4 in the 12 MU dose group (3.75 vs. 2.40 ng/ml; $p=0.027$). Beta2-microglobulin levels increased moderately in all patients (Figure 5C).

The only significant difference that was seen, with regard to these markers of immune activation, were higher neopterin levels in the 12 MU dose group. However, on an individual

basis we could not detect a specific pattern in neopterin levels between patients receiving 12 MU or 9 MU IFN and those patients within these group that went on to achieve SVR (Figure 6).

DISCUSSION

In this study we show that a strong HCV RNA decline at week 4 can be induced by continuous subcutaneous administration of 12 MU IFN alfa-2b per day combined with weight-based RBV in patients who failed previous PegIFN/RBV therapy. A significant stronger HCV RNA decline during the first 4 weeks was seen in patients receiving 12 MU IFN per day versus 9 MU IFN per day. All patients that achieved SVR (5 out of 20) had a more than 2 log viral decline at week 4.

The original pilot study was designed to investigate safety and efficacy of high dose IFN by continuous subcutaneous administration (21). We hypothesized that constant high levels of unmodified IFN might improve viral suppression and avoid adverse events associated with serum IFN peaks during thrice weekly (or daily) administration of standard IFN or once weekly administration of PegIFN.

Initial viral kinetic studies of HCV showed that administration of IFN produces a biphasic decline in viral load (12, 14, 22, 23). The first phase is dose dependent and shows a rapid decrease in serum HCV RNA concentration of 0.5-2.0 log. The slope of the first phase of viral decline reflects IFN sensitivity. The second phase begins 24-48 hours after onset of treatment and reflects immune-mediated clearance of HCV infected cells. Pegylation of IFN improved viral response rates compared with standard IFN due to an improved pharmacokinetic profile, allowing once weekly dosing with stable blood concentrations throughout the dosing interval (4, 6). A similar biphasic viral decline was seen with PegIFN during the first weeks of therapy (24). Viral rebound between 48 and 72 hours after start of treatment occurs in some patients and has been associated with decreased serum PegIFN concentrations (25, 26). In our study, this typical biphasic HCV RNA decline was seen in most patients receiving continuous IFN. However, a significant difference in viral kinetics was seen between patients receiving 12 MU IFN per day versus 9 MU IFN per day. This difference was predominantly caused by a stronger first phase decline at day 2, but also due to a steeper second phase in patients receiving 12 MU IFN per day. Based on IFN levels we could not explain the difference between both dosing groups. Especially during the first phase of viral decline higher peak IFN levels in serum were seen in the 9 MU dose group, in contrast with what would be expected. Notably, viral rebound after the first phase of viral decline was seen in the 12 MU dose group despite continuous IFN administration. Interestingly, a relation was seen between neopterin levels (as marker of immune activation) and viral decline. Higher neopterin peak levels were achieved at day 2 in the 12 MU dose group compared to the 9 MU dose group, followed by higher levels during the first 4 weeks of therapy. The viral rebound after day 2 in the 12 MU dose group was also reflected by temporarily decreased neopterin levels. Although neopterin is

regarded as a biomarker for activation of the cellular immune system, and its concentration is elevated in several diseases including chronic HCV infection, its role as an antiviral effector molecule is still unclear (27). Importantly, despite the observation that patients receiving 12 MU IFN demonstrated more potent viral decline as compared to patients receiving 9 MU, no differences were found with regard to 2,5-OAS activity and beta2-microglobulin levels between the two dose groups. This is surprising since serum levels of these markers are known to reflect systemic antiviral activity induced by IFN. In order to identify in detail which antiviral effector molecules, such as members of the IFN stimulated genes (ISG) family, are differentially induced by the 12 MU dose and not the 9 MU dose, future studies will include gene profiling during the course of treatment by microarray analysis of peripheral blood collected from patients participating in this study (28).

This is the first study to evaluate viral kinetics in HCV infected previous non-responders during continuous subcutaneous administration of high dose IFN in combination with RBV. We demonstrated a biphasic viral decline, as described before during treatment with unmodified and pegylated IFN. The significant stronger HCV RNA decline in the 12 MU dose group was reflected by higher neopterin levels.

Understanding viral kinetics in hepatitis C remains of utmost importance as viral decay at week 4 is still the most important predictor of achieving SVR. Current new triple therapies including direct antiviral agents show much stronger early viral decline than seen with PegIFN/RBV. However, PegIFN induced immune control will remain a cornerstone of anti-HCV therapy. Continuous high dose IFN administration combined with RBV and a direct antiviral agent might further improve triple therapy outcome, especially in previous non-responders or relapsers.

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REFERENCES

1. Seeff LB. Natural history of chronic hepatitis C. *Hepatology* 2002;36:S35-46.
2. Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001;345:41-52.
3. Deuffic-Burban S, Poynard T, Sulkowski MS, Wong JB. Estimating the future health burden of chronic hepatitis C and human immunodeficiency virus infections in the United States. *J Viral Hepat* 2007;14:107-115.
4. 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.
5. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;140:346-355.
6. 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.
7. Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med*;364:1207-1217.
8. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med*;364:2405-2416.
9. Poordad F, McCone J, Jr., Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med*;364:1195-1206.
10. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, et al. Telaprevir for retreatment of HCV infection. *N Engl J Med*;364:2417-2428.
11. Perelson AS, Herrmann E, Micol F, Zeuzem S. New kinetic models for the hepatitis C virus. *Hepatology* 2005;42:749-754.
12. Bekkering FC, Brouwer JT, Leroux-Roels G, Van Vlierberghe H, Elewaut A, Schalm SW. Ultra-rapid hepatitis C virus clearance by daily high-dose interferon in non-responders to standard therapy. *J Hepatol* 1998;28:960-964.
13. Cotler SJ, Layden JE, Neumann AU, Jensen DM. First phase hepatitis c viral kinetics in previous nonresponders patients. *J Viral Hepat* 2003;10:43-49.
14. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 1998;282:103-107.
15. 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;23:366-371.
16. Giannelli G, Antonelli G, Fera G, Dianzani F, Schiraldi O. 2',5'-Oligoadenylate synthetase activity as a responsive marker during interferon therapy for chronic hepatitis C. *J Interferon Res* 1993;13:57-60.
17. Hoffmann G, Wirleitner B, Fuchs D. Potential role of immune system activation-associated production of neopterin derivatives in humans. *Inflamm Res* 2003;52:313-321.
18. Schenker S, Cutler D, Finch J, Tamburro CH, Affrime M, Sabo R, Bay M. Activity and tolerance of a continuous subcutaneous infusion of interferon-alpha2b in patients with chronic hepatitis C. *J Interferon Cytokine Res* 1997;17:665-670.
19. Wejstal R, Norkrans G, Weiland O, Schvarcz R, Fuchs D, Wachter H, Fryden A, et al. Lymphocyte subsets and beta 2-microglobulin expression in chronic hepatitis C/non-A, non-B. Effects of interferon-alpha treatment. *Clin Exp Immunol* 1992;87:340-345.

20. Kim KI, Kim SR, Sasase N, Taniguchi M, Harada S, Kinoshita K, Kim SH, et al. 2'-,5'-Oligoadenylate synthetase response ratio predicting virological response to PEG-interferon-alpha2b plus ribavirin therapy in patients with chronic hepatitis C. *J Clin Pharm Ther* 2006;31:441-446.
21. Roomer R, Bergmann JF, Boonstra A, Hansen BE, Haagmans B, Kwadijk-de Gijssel S, Van Vuuren AJ, et al. Continuous subcutaneous administration of high-dose interferon alfa-2b combined with ribavirin in chronic hepatitis C patients: a dose-finding and safety study in treatment-experienced patients. *Antivir Ther* 2011;in press.
22. 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;26:226-231.
23. Layden-Almer JE, Layden TJ. Viral kinetics in hepatitis C virus: special patient populations. *Semin Liver Dis* 2003;23 Suppl 1:29-33.
24. Zeuzem S, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, Colucci G, et al. Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon alpha2a. *Gastroenterology* 2001;120:1438-1447.
25. Buti M, Sanchez-Avila F, Lurie Y, Stalgis C, Valdes A, Martell M, Esteban R. Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin. *Hepatology* 2002;35:930-936.
26. Formann E, Jessner W, Bennett L, Ferenci P. Twice-weekly administration of peginterferon-alpha-2b improves viral kinetics in patients with chronic hepatitis C genotype 1. *J Viral Hepat* 2003;10:271-276.
27. Wilmer A, Nolchen B, Tilg H, Herold M, Pechlaner C, Judmaier G, Dietze O, et al. Serum neopterin concentrations in chronic liver disease. *Gut* 1995;37:108-112.
28. Sadler AJ, Williams BR. Interferon-inducible antiviral effectors. *Nat Rev Immunol* 2008;8:559-568.

SPERM DNA INTEGRITY IS NOT AFFECTED BY TREATMENT WITH PEGINTERFERON ALFA AND RIBAVIRIN FOR CHRONIC HEPATITIS C

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Submitted for publication.

ABSTRACT

Background and aims

Limited data are available on the effect of treatment with peginterferon alfa and ribavirin on human semen quality. We conducted a study to investigate the effects of chronic hepatitis C and treatment with peginterferon alfa and ribavirin on spermatogenesis and sperm DNA integrity.

Methods

Serum and semen samples of 23 hepatitis C patients were collected before, during and after treatment. Seminal and endocrinological parameters and sperm DNA integrity (expressed as DNA fragmentation index) were analyzed.

Results

Baseline oligozoospermia (sperm concentration $<15 \times 10^6/l$) and asthenospermia ($<32\%$ moving spermatozoa) both occurred in 9 patients (39%). Median seminal volume decreased significantly during treatment (1.6 ml to 0.9 ml, $p=0.005$). No significant changes in progressive motility and sperm concentration were found. A significant increase of luteinizing hormone ($p=0.011$) but not of follicle stimulating hormone ($p=0.241$) was seen during treatment. Median DNA fragmentation index of hepatitis C patients before treatment was comparable with that of 22 healthy controls: 19.2% (range 2.6-42.8%) vs. (15.8%, range 6.4-25.7, $p=0.51$). Median DNA fragmentation index was higher in methadone users, 32.2% vs. 12.6% ($p=0.039$) and in patients using antipsychotics (olanzapine and risperidon); 34.0% vs. 12.7%, $p=0.01$. Sperm DNA integrity was not altered during treatment and follow-up.

Conclusions

Semen abnormalities were found in a relatively large proportion of hepatitis C patients, but treatment did not lead to further impairment of sperm quality. Sperm DNA integrity, which is associated with poor reproductive outcome and with a higher miscarriage risk, was not altered by treatment with peginterferon alfa and ribavirin.

INTRODUCTION

A treatment regimen with peginterferon alfa and ribavirin (PEGIFN/RBV) is the standard of care for chronic hepatitis C virus (HCV) infection and despite the development of successful direct antiviral agents, this treatment combination will remain the backbone of antiviral therapy against HCV (1). In animals, RBV has repeatedly been proven to be teratogenic. Malformations of limbs, spine, ribs, eyes and central nervous system have been shown in rodents in addition to reductions in sperm count and alterations in morphology of spermatozoa (2-3) after exposure to RBV. Contradicting results have been found in studies investigating the effect of interferon alfa on semen quality (4-5). In women, most reports on direct maternal exposure to PEGIFN/RBV or RBV alone prior to or during pregnancy describe normal pregnancies (6-9), however there are some reports of miscarriage, elective terminations and birth defects (10-11). Little is known about the effects on offspring of males treated with PEGIFN and RBV. Again, there are some reports of miscarriage but most pregnancies resulted in normal live born infants (12-14). In a prospective observational cohort study pregnant women who were exposed both directly and indirectly to RBV, were followed till delivery and their live born infants were followed for one year. In this study the incidence of birth defects after both direct and indirect RBV exposure did not significantly differ from that in the general population, but enrolment was too short to obtain the required sample size (10).

It is however known that RBV is detectable in semen during antiviral treatment (15) and because to date the potential mutagenic effects of RBV cannot be excluded, guidelines strongly recommend double contraception during and until 7 months after treatment for male patients and their female partners (16-17). It is however questionable whether this is necessary.

There is some evidence that a proportion of HCV patients have semen abnormalities prior to treatment and that semen quality further deteriorates during the first weeks of treatment (15, 18). However, limited data are available on semen quality during follow up and no data are available on sperm DNA integrity (sperm DNA damage) induced by PEGIFN/RBV treatment.

Sperm concentration and sperm motility are important parameters for male fertility. Sperm DNA integrity is associated with a lower spontaneous conception rate and possibly with a higher miscarriage risk (19-20). In addition, luteinising hormone stimulates the production of testosterone and FSH activates spermatogenesis. Serum levels of inhibin B are positively correlated with the number of spermatozoa produced (21) and are negatively correlated with the FSH levels. Serum concentrations of free testosterone (non SHBG-bound testosterone) reflect the functioning of Leydig cells in the testis.

We conducted a study to investigate the occurrence of semen abnormalities in patients with HCV infection and to investigate the effect of PEGIFN/RBV treatment on spermatogenesis, sperm DNA integrity and endocrinological parameters prior to, during and after treatment.

METHODS

Study design

This study is a single center observational study. Treatment naïve male patients with a chronic HCV infection for whom antiviral therapy with PEGIFN/RBV was planned could be included in the study. Patients with a hepatitis B and human immunodeficiency virus (HIV) co-infection were excluded from participation.

Semen analysis

Semen samples were obtained at baseline, week 12, 24, 48 and 24 weeks after treatment discontinuation (FU24). Semen samples were obtained by masturbation at the Andrology unit after at least 3 days of abstinence. Semen samples were analyzed on volume, concentration and motility according to WHO standard criteria (22). Sperm motility was assessed by categorizing the spermatozoa in 2 groups: progressively motile and non-progressively motile spermatozoa. The first group contains active moving spermatozoa. The last group contains cells which don't move at all or cells that move but remain at the same position. Sperm morphology was not included in the study protocol. Since this semen parameter is considered an unreliable marker to determine fertility due to the great inter- and intra observer variability. However, data on morphology was available in some of the patients.

Sperm DNA integrity analysis

Sperm DNA integrity was measured using the sperm chromatin structure assay (SCSA). The SCSA was performed as described by Evenson and Jost (23), using a FACS cytometer (Becton Dickinson, San Jose, CA). In brief, frozen samples were quickly thawed, diluted to a concentration of $1-2 \times 10^6$ sperm cells/ml, exposed to acid detergent solution, and stained with acridine orange. Data collection of the fluorescence pattern in 5000 cells was performed at 3 minutes after acid treatment. Debris, bacteria and leukocytes were gated out during acquisition as recommended (23). The extent of DNA damage is expressed as the DNA fragmentation index (DFI). Cell Quest Pro and Winlist software were used to calculate the DFI of each sample. All samples were measured in duplicate. A DFI larger than 30% is considered abnormal. Median DFI values and dichotomized values (DFI < 30% and DFI \geq 30%) were used in the analyses. DFI data were compared with DFI data of 22 proven fertile men who donated a semen sample before vasectomy.

Serum analysis

Serum samples were collected at baseline, week 12, 24, 48 and at FU24. Luteinising hormone (LH), follicle stimulating hormone (FSH), free testosterone and inhibin B were determined to investigate testicular function before, during and after treatment. Levels of free testosterone were calculated as described by de Ronde et al. (24). The methods used to estimate hormone

levels were luminescence-based immunometric assays for LH, FSH and SHBG (Immulite Siemens DPC, Los Angeles, CA, USA), coated tube radio immunoassay for testosterone (Siemens DPC) and an enzyme-immunometric assay for inhibin B (Diagnostic Systems Laboratory, Webster, TX, USA).

Statistical analysis

Fisher's exact tests were used to compare dichotomized variables. Continuous variables were expressed as medians with range. Wilcoxon signed rank tests and Mann-Whitney U tests were performed to compare continuous variables. A p-value below 0.05 was considered statistically significant. In case of the comparison of baseline samples with on-treatment and follow-up samples a correction for multiple testing was made and thus a p-value below 0.0125 was considered statistically significant. Multivariate linear regression was applied to determine which baseline factors influenced sperm DNA integrity at baseline. SPSS version 17.0 (SPSS inc., Chicago, IL) was used for this analysis.

RESULTS

Patients

A total of 23 male patients were included in the analysis. Baseline samples were available in 23 patents. On-treatment and follow up samples form 19 patients were available. The remaining 4 patients withdrew informed consent after providing a baseline semen and blood sample. Baseline characteristics are summarized in table 1. Seven patients used methadone and 2 patients smoked cannabis on a regular basis. Other co-medication included selective serotonin reuptake inhibitors, benzodiazepines and anti-psychotics which were used in 9 patients. All patients were naïve to antiviral treatment and were treated with peginterferon

Table 1. Baseline characteristics

Number of patients	23
Age (mean, range in years)	43 (22-62)
BMI (mean, range in kg/m ²)	24 (17-31)
Cirrhosis (metavir score)	1
HCV Genotype	
1	13 (57%)
2	4 (17%)
3	6 (4%)
Methadone	7/23 (30%)
Smoking	19/23 (89%)
Treatment duration 24/48 weeks	12/11
Low seminal volume	10 (43%)
Oligozoospermia	9 (39%)
Asthenozoospermia	9 (39%)

alfa-2a and RBV (10-15 mg/kg), except for 7 patients, who received high dose RBV (25-29 mg/kg). Two patients were treated for 12 weeks, 13 patients for 24 weeks and 4 patients for 48 weeks. Data on semen samples taken at week 48 are not shown in the figures because only 4 samples were available. Sustained virological response (SVR) was achieved in 11 patients.

Semen analysis

At baseline semen abnormalities were common. Ten out of 23 patients (43%) had a low seminal volume (seminal volume <1.5ml), 9 patients (39%) had oligozoospermia (sperm concentration <15 x 10⁶/ml) and 9 patients (39%) had asthenozoospermia (progressive motility <32%). Data

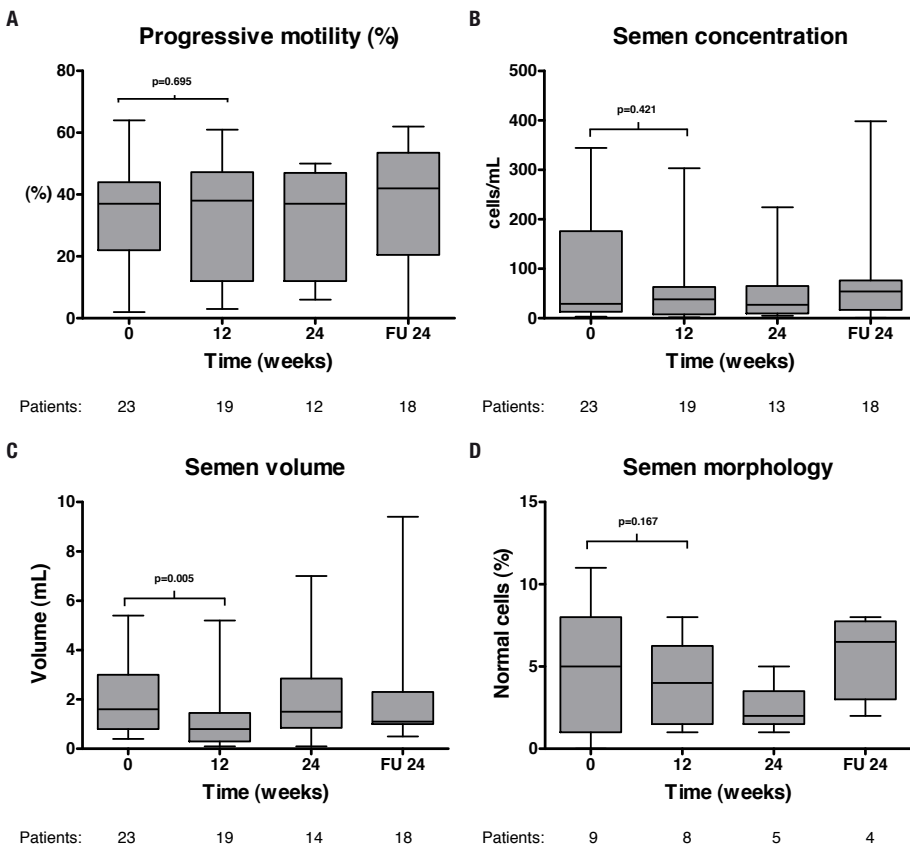


Fig. 1

Progressive motility (A), semen concentration (B), seminal volume (C) and seminal morphology (D) during treatment (median, range)

Seminal volume was significantly decreased significantly at week 12 ($p=0.005$), but not at week 24. No significant changes in sperm concentration and progressive motility were observed during treatment. A non-significant decrease in spermatozoa with normal morphology was observed during treatment

on sperm morphology were available in 9 patients; 4 out of 9 (44%) had teratozoospermia (defined as <4% normal spermatozoa) at baseline.

Seminal volume was significantly decreased at week 12 of treatment ($p=0.005$) but not at week 24 and 48 (figure 1a). Median sperm concentration at baseline was $29 \times 10^6/\text{ml}$ (range $3\text{-}344 \times 10^6/\text{ml}$). Median sperm concentrations at week 12, 24, 48 and FU24 were 38, 27, 37 and $54 \times 10^6/\text{ml}$ respectively. The median percentage of progressive motility at baseline was 37% (range 2-64%). Median progressive motility percentages at week 12, 24, 48 and FU24 were 37%, 39%, 42% and 43% respectively. Eleven out of 19 patients (58%) had a decrease in sperm concentration and 10 out of 19 had a decrease in progressive motility during treatment, however both sperm concentrations and progressive motility were not significantly altered (figure 1b and c). At baseline median percentage of normal spermatozoa was 5% (range 0-11%). During treatment percentage of normal spermatozoa decreased, however this decrease was not significant ($p=0.167$) (figure 1d). Semen parameters during treatment did not significantly differ between patients receiving high dose or standard dose RBV.

Sperm DNA integrity analysis

Data on sperm DNA integrity were available in 18 patients. At baseline 4 of 18 patients (22%) had a DFI >30%, which is considered abnormal. Median DFI at baseline was 19.2% (range 2.6 - 42.8%), 15.4% (range 1.5- 45.6%) at week 12, 17.5% (6.6 - 35.5%) at week 24, 13.1% (range 2.8 - 28%) at week 48 and 17.6% (range 2.6 - 73.5%) at week FU24. DNA fragmentation index did not significantly change during treatment and follow up (figure 2). The median DFI of 18 HCV patients at baseline did not significantly differ from the median DFI of 22 proven fertile healthy controls: 19.2%, range 2.6 - 42.8% vs. 15.8%, range 6.4-25.7 ($p=0.51$).

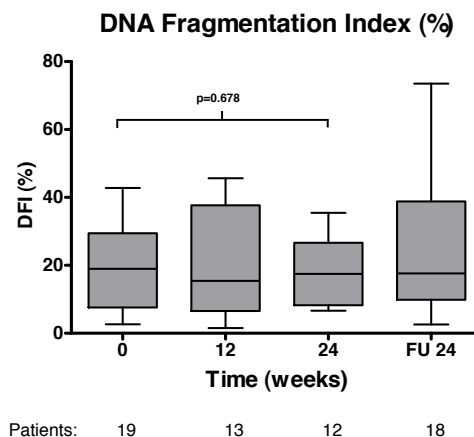


Fig. 2 Sperm DNA integrity expressed as the DNA fragmentation index (DFI) during treatment (median, range) No significant changes in sperm DNA integrity were observed during treatment and during follow up

Endocrinological parameters

Changes in gonadotrophic hormones, free testosterone and inhibin B are shown in figure 3. A slight increase in both FSH (figure 3a) and LH (figure 3b) was seen during treatment which was significant for LH ($p=0.011$) but not for FSH ($p=0.241$). During follow up LH and FSH returned to baseline levels. There was a trend towards a decline of free testosterone and inhibin B (figure 3c and 3d) during treatment, however these declines did not reach statistical significance ($p=0.074$ and 0.138).

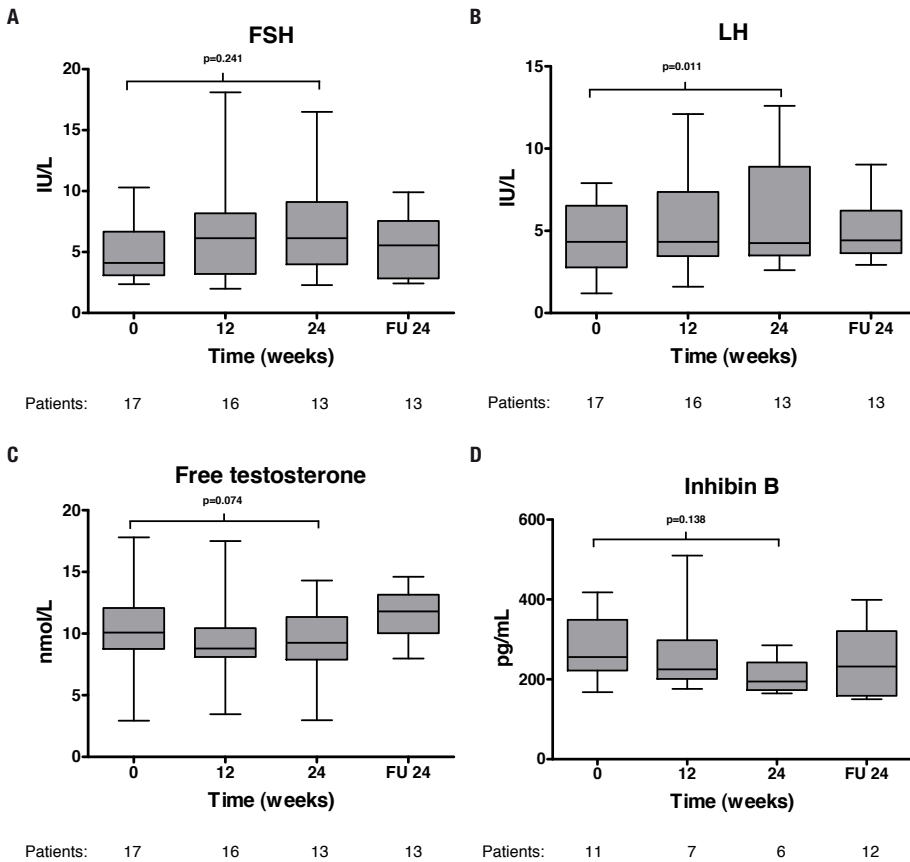


Fig. 3 Follicle stimulating hormone (FSH) (A), luteinizing hormone (LH) (B), free testosterone (C) and inhibin B (D) during treatment (median, range)

A significant increase in LH was observed during treatment ($p=0.011$). FSH was not significantly influenced by peginterferon alfa and ribavirin treatment ($p=0.241$). A decrease in free testosterone and Inhibin B was observed during treatment with a trend towards significance for testosterone ($p=0.074$)

Factors influencing semen parameters and sperm DNA integrity

At baseline, methadone users had a higher DFI compared to patients not using methadone, 32.2% vs. 12.6% ($p=0.039$) and DFI was higher in patients using antipsychotics (olanzapine and risperidon) compared to non-users (34.0% vs. 12.7%, $p=0.01$) (figure 4). In a multivariate linear regression model both variables remained significant ($p=0.035$ for methadone and $p=0.005$ for antipsychotics). Sperm DNA integrity was significantly correlated with progressive motility ($r= -0.674$, $p<0.001$). There was no difference in sperm volume, concentration and motility as well as sperm DNA integrity between baseline and follow up in patients who achieved SVR (table 2). Other factors influencing sperm concentration and progressive motility could be identified. Cannabis use was not associated with increased DFI.

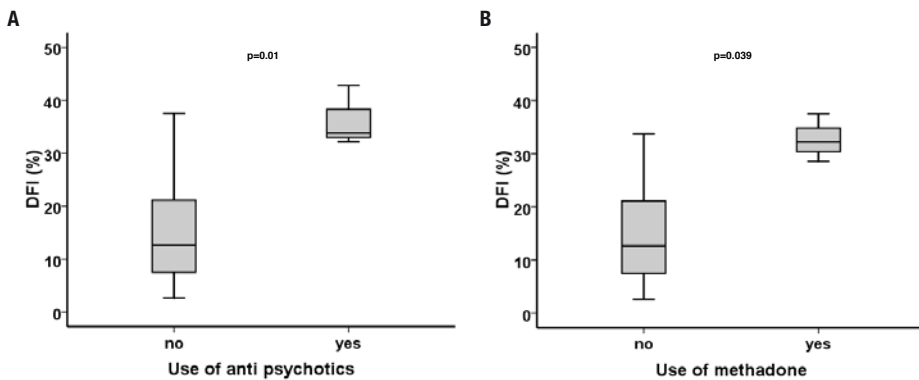


Fig. 4

DFI in patients using antipsychotics (A) methadone (B) (median, range)

DFI was significantly increased in patients using methadone and antipsychotics

Table 2. Median semen parameters at baseline and during follow up in both SVR ($n=10$) and non SVR patients ($n=9$).

SVR	Parameters	Baseline	Follow up	P
	Volume	2.0	1.9	0.79
	Concentration	26.0×10^6	51.0×10^6	0.11
	Motility	38.0%	44.0%	0.84
	Sperm DNA integrity	12.6%	18.0%	0.11
Non-SVR				P
	Volume	1.5	1.10	0.25
	Concentration	60.5×10^6	59.0×10^6	0.31
	Motility	22.5%	36.0%	0.18
	Sperm DNA integrity	21.9%	17.2%	0.35

DISCUSSION

This study is the first to report on semen abnormalities, endocrinological parameters as well as sperm DNA integrity in HCV patients prior, during and after antiviral treatment with PEGIFN/RBV. In this study approximately 40% of HCV patients had semen abnormalities at baseline. Sperm concentration and motility, which are predictors of male fertility, did not significantly change during treatment with PEGIFN/RBV and during follow up. We did find a significant decrease of semen volume at week 12 of treatment, which could possibly be explained by a decrease in sexual arousal during antiviral treatment (25). We also report on semen morphology, however, this parameter is considered unreliable because of a great inter- and intra- observer variability and secondly, this parameter is a poor predictor of fertility. For this reason morphology is no longer part of the standard semen analysis at our Andrology unit and data was not available in most patients. A decline in the amount of spermatozoa with normal morphology was observed during treatment, this decline however, was not significant.

Sperm DNA integrity is currently the most reliable predictor of spontaneous pregnancies and the occurrence of miscarriage. Potentially, it may be associated with the risk of birth defects. Sperm DNA damage, expressed as DFI, is increased in patients with various forms of cancer, treatment with radiotherapy and several andrological abnormalities (20, 26). The median DFI of HCV patients did not significantly differ from healthy controls and during therapy the DFI levels were not significantly altered. These findings are in disagreement with a case report on sperm DNA integrity during PEGIFN/RBV therapy for HCV (27). In this report about a patient receiving viraferon and RBV for chronic HCV, DFI levels increased during therapy and returned to baseline levels after treatment discontinuation.

At last we investigated alterations in gonadotrophic and gonadal hormones during antiviral treatment. An increase in FSH and LH was observed during treatment together with a decrease, although not significant, in free testosterone and inhibin B (down regulator of FSH). All endocrinological parameters returned to baseline levels during follow up. These results indicate a compensated minimal deterioration of testicular function during treatment with PEGIFN/RBV treatment. However, differences are small and should be interpreted with caution.

A possible explanation for semen abnormalities could be a direct effect of the virus on spermatogenesis (18). However, a decrease in the number of patients with semen abnormalities after achieving SVR would then be expected (table 2). This was not the case in our study. Another explanation could be that semen abnormalities are not related to the virus but to confounding factors often seen in HCV patients like the use of methadone, cocaine, alcohol or specific medication (28-29). In our study DFI levels were significantly higher in methadone users and in patients who used antipsychotics. Despite the strong correlation between DFI levels and progressive motility, the use of methadone and antipsychotics were not associated with decreased progressive motility.

Our findings are not entirely in agreement with previous findings of Hofer et al. (15). They found a significant decrease in sperm concentration at week 4 and a significant decrease in normal cell morphology at week 12 in a cohort of 15 patients. No significant changes in motility were found. They concluded that antiviral treatment leads to substantial alterations in both quantitative and qualitative parameters. However, changes in semen quality were only minimal, not consistent and only measured at week 4, 12 and 24 of treatment and not during follow up. Furthermore, sperm DNA integrity, a more reliable parameter of semen quality and teratogenicity, was not assessed. An interesting finding of this study was the presence of RBV in sperm. They found a twofold higher concentration in semen compared to serum concentrations at week 4 and 12 of antiviral treatment. Unfortunately data on RBV concentrations during follow up are still lacking and the clinical significance of detectable RBV in semen also remains unclear. In case RBV is transmittable to female partners then blood concentrations would be extremely low due to the small volume of semen samples.

In conclusion, sperm DNA integrity in HCV patients is comparable with healthy controls and is not affected by treatment with PEGIFN/RBV. Based on these findings and the findings of birth registry trials it would be questionable whether double contraception until 7 months after treatment cessation, or even during treatment, is necessary for male patients. Semen abnormalities were present in a proportion of HCV patients and considering the fact that these abnormalities did not resolve after achieving SVR, a plausible alternative cause for these abnormalities could be the confounding factors found in HCV patients, such as the use of methadone or antipsychotics.

REFERENCES

1. Gane E. Future hepatitis C virus treatment: interferon-sparing combinations. *Liver Int* 2011;31 Suppl 1:62-67.
2. Narayana K, D'Souza UJ, Rao KP. Effect of ribavirin on epididymal sperm count in rat. *Indian J Physiol Pharmacol* 2002;46:97-101.
3. Narayana K, D'Souza UJ, Seetharama Rao KP. Ribavirin-induced sperm shape abnormalities in Wistar rat. *Mutat Res* 2002;513:193-196.
4. Hibi H, Yokoi K, Yamamoto M. Effects of alpha-interferon on sperm production, concentration, and motility in the rat. *Int J Urol* 1997;4:603-607.
5. Ulusoy E, Cayan S, Yilmaz N, Aktas S, Acar D, Doruk E. Interferon alpha-2b may impair testicular histology including spermatogenesis in a rat model. *Arch Androl* 2004;50:379-385.
6. Hegenbarth K, Maurer U, Kroisel PM, Fickert P, Trauner M, Stauber RE. No evidence for mutagenic effects of ribavirin: Report of two normal pregnancies. *American Journal of Gastroenterology* 2001;96:2286-2287.
7. Labarga P, Pinilla J, Cachorro I, Ruiz Y. Infant of 22 months of age with no anomalies born from a HCV- and HIV-infected mother under treatment with pegylated interferon, ribavirin and antiretroviral therapy during the first 16 weeks of pregnancy. *Reprod Toxicol* 2007;24:414-416.
8. Mishkin D, Deschenes M. Conception soon after discontinuing interferon/ribavirin therapy: a successful outcome. *Am J Gastroenterol* 2001;96:2285-2286.
9. Rezvani M, Koren G. Pregnancy outcome after exposure to injectable ribavirin during embryogenesis. *Reprod Toxicol* 2006;21:113-115.
10. Roberts SS, Miller RK, Jones JK, Lindsay KL, Greene MF, Maddrey WC, Williams IT, et al. The Ribavirin Pregnancy Registry: Findings after 5 years of enrollment, 2003-2009. *Birth Defects Res A Clin Mol Teratol* 2010;88:551-559.
11. Valentin M, Ducarme G, Yver C, Vuillard E, Belarbi N, Renier D, Luton D. Trigenocephaly and valproate: a case report and review of literature. *Prenat Diagn* 2008;28:259-261.
12. Bianca S, Ettore G. Male periconceptual ribavirin-interferon alpha-2b exposure with no adverse fetal effects. *Birth Defects Res A Clin Mol Teratol* 2003;67:77-78.
13. De Santis M, Carducci B, Cavaliere AF, De Santis L, Lucchese A, Straface G, Caruso A. Paternal exposure to ribavirin: pregnancy and neonatal outcome. *Antivir Ther* 2003;8:73-75.
14. Maddrey WC. Safety of combination interferon alfa-2b/ribavirin therapy in chronic hepatitis C-relapsed and treatment-naive patients. *Semin Liver Dis* 1999;19 Suppl 1:67-75.
15. Hofer H, Donnerer J, Sator K, Stauer K, Scherzer TM, Dejaco C, Sator M, et al. Seminal fluid ribavirin level and functional semen parameters in patients with chronic hepatitis C on antiviral combination therapy. *J Hepatol* 2010;52:812-816.
16. <http://www. Roche.nl/fmfiles/re7123011/PRODUCTEN/Copegus/Copegus-200mg-VII-62-101101.pdf>.
17. http://www.schering-plough.nl/binaries/Pati_ntenbijijsluiter_Rebetol_tcm_169-189839.pdf.
18. Durazzo M, Premoli A, Di Bisceglie C, Bertagna A, Faga E, Birolì G, Manieri C, et al. Alterations of seminal and hormonal parameters: An extrahepatic manifestation of HCV infection? *World J Gastroenterol* 2006;12:3073-3076.
19. Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, de Angelis P, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. *Hum Reprod* 1999;14:1039-1049.
20. Smit M, Romijn JC, Wildhagen MF, Weber RF, Dohle GR. Sperm chromatin structure is associated with the quality of spermatogenesis in infertile patients. *Fertil Steril* 2010;94:1748-1752.
21. Pierik FH, Vreeburg JT, Stijnen T, De Jong FH, Weber RF. Serum inhibin B as a marker of spermatogenesis. *J Clin Endocrinol Metab* 1998;83:3110-3114.
22. WHO. WHO laboratory manual for the examination and processing of human semen. 2010.

23. Evenson D, Jost L. Sperm chromatin structure assay is useful for fertility assessment. *Methods Cell Sci* 2000;22:169-189.
24. de Ronde W, van der Schouw YT, Muller M, Grobbee DE, Gooren LJ, Pols HA, de Jong FH. Associations of sex-hormone-binding globulin (SHBG) with non-SHBG-bound levels of testosterone and estradiol in independently living men. *J Clin Endocrinol Metab* 2005;90:157-162.
25. van Rooijen JH, Slob AK, Gianotten WL, Dohle GR, van der Zon AT, Vreeburg JT, Weber RF. Sexual arousal and the quality of semen produced by masturbation. *Hum Reprod* 1996;11:147-151.
26. Smit M, van Casteren NJ, Wildhagen MF, Romijn JC, Dohle GR. Sperm DNA integrity in cancer patients before and after cytotoxic treatment. *Hum Reprod* 2010;25:1877-1883.
27. Pecou S, Moinard N, Walschaerts M, Pasquier C, Daudin M, Bujan L. Ribavirin and pegylated interferon treatment for hepatitis C was associated not only with semen alterations but also with sperm deoxyribonucleic acid fragmentation in humans. *Fertil Steril* 2009;91:933 e917-922.
28. Madhusoodanan S, Parida S, Jimenez C. Hyperprolactinemia associated with psychotropics—a review. *Hum Psychopharmacol* 2010;25:281-297.
29. Ragni G, De Lauretis L, Bestetti O, Sghedoni D, Gambaro V. Gonadal function in male heroin and methadone addicts. *Int J Androl* 1988;11:93-100.

SUMMARY AND DISCUSSION
SAMENVATTING EN DISCUSSIE



SUMMARY AND DISCUSSION

In the last two decades great strides have been made in the treatment of chronic hepatitis C. To date, up to 70% of patients can be treated successfully by inducing a sustained virological response with peginterferon alfa and ribavirin. However, there are some viral, host and treatment factors which reduce the probability of a successful treatment outcome (1). Patients infected with HCV genotype 1, which is the most common genotype in Europe, Northern America and Japan or patients infected with genotype 4, which is more common in Egypt, have a reduced chance of SVR compared to patients infected with genotype 2 and 3. On the other hand, several host factors, such as fibrosis stage, weight, sex, age, race and IL28b genotype all influence the chance of success of antiviral treatment (2). At last treatment adherence also plays an important role in the chance of achieving an SVR. Especially for patients with a low a-priori chance of viral response, maximum adherence is crucial to achieve a sustained virological response (3).

Unfortunately this treatment regimen is associated with many side effects which impair adherence and lead to dose reductions (4). Recently developed direct acting antivirals significantly increase SVR rates in patients with HCV genotype 1 (5-6). However, these new agents also have side effects which will make therapy even harder to endure. In **chapter 1** of this thesis an overview of side effects and their management of both the current treatment regimen and the possible future regimens is given.

Optimizing guidelines for dose reductions

One of the most common side effects of peginterferon alfa/ribavirin treatment are cytopenias such as neutropenia, thrombocytopenia and anemia. Approximately 20% of patients develop a neutrophil count below 750/ μ L which is the most common reason for peginterferon alfa dose reductions (7). Furthermore guidelines recommend temporary discontinuation in case of an absolute neutrophil count below 375/ μ L. As mentioned previously dose reductions impair treatment efficacy and for this reason preventing them is crucial to achieve a sustained virological response.

In **chapter 2** we investigated which factors are associated with the occurrence of infections and whether infections are associated with peginterferon alfa induced neutropenia. In this retrospective cohort study of 321 patients 96 infections were found in 70 patients (22%) and 95 patients (30%) developed an absolute neutrophil count below 750/ μ L. No association between infections and absolute neutrophil counts below 750/ μ L or 375/ μ L could be found. The only factors which were associated with an increased risk of infection were baseline hyperglycemia and older age. Another interesting finding of this study was that patients with neutropenia who underwent a dose reduction did not have fewer infections than neutropenic patients without dose reductions. However these results should be interpreted with caution because dose reductions were performed at discretion of the treating physician. In **chapter 3**

we investigated whether patients with treatment induced thrombocytopenia were more at risk to develop (major) bleedings during antiviral treatment. According to current guidelines peginterferon alfa dose should be reduced in case of a platelet count below 50000/ μL and should be temporary discontinued in case of a platelet count below 25000/ μL . In this retrospective cohort study, the same cohort of 321 patients was used. A total of 30 (9%) patients developed platelet counts below 50000/ μL and 9 patients below 25000/ μL . Forty-eight bleedings were observed in 27 patients (8.4%). Only 1 bleeding, due to gastrointestinal angiodysplasia, was defined as severe. However, this patient did not have severe thrombocytopenia at the time of bleeding. Bleedings were strongly correlated with platelet counts $<50000/\mu\text{L}$. However, no severe bleedings occurred in patients with platelet counts $<50000/\mu\text{L}$. In this cohort 12 of 30 patients with platelet counts below 50000/ μL underwent a dose reduction or discontinuation of peginterferon alfa dose. In the remaining 18 patients, who were treated outside of clinical trials, peginterferon alfa dose was maintained at discretion of treating physician. In these patients virological response and stability of platelet counts were also taken into account when a dose reduction was considered.

Based on the findings in **chapters 2 and 3** we conclude that guidelines are too overly strict and should be adjusted. A possible alternative for the current guidelines for thrombocytopenia would be to maintain peginterferon alfa dose when platelet counts remain stable above 25000/ μL as long as the patient does not have signs of active bleeding. Peginterferon alfa dose should be temporarily discontinued when platelet counts drop below 25000/ μL and could be restarted at a lower dose when platelet counts rise above 25000/ μL again. For neutropenia the peginterferon alfa dose could be maintained as long as absolute neutrophil counts remain above 375/ μL in case the patient does not have signs of a bacterial infection. In case of an absolute neutrophil count below 375/ μL , peginterferon alfa dose should be reduced in steps of 25% until ANC remains stable above 375/ μL .

Optimizing guidelines for stopping rules

On-treatment measurement of viral kinetics is crucial to predict the probability of a sustained virological response. A significant proportion of patients does not have an adequate response to treatment with peginterferon alfa and ribavirin defined as a <2 log drop in HCV RNA at week 12 or HCV RNA positivity at week 24 (7-8). The negative predictive value of a partial or null response ranges between 97 and 100% and thus treatment can be stopped in these patients. These stopping rules have been based on studies using HCV RNA assays with a lower limit of detection (LLOD) of 50 IU/ml (9). Nowadays commonly used HCV RNA assays have a LLOD of 15-20 IU/ml (10-11) and even assays with a LLOD as low as 5 IU/ml are currently available (12). These differences in LLOD could lead to discordance in week 24 HCV RNA test results and thus in differences between positive predictive values (PPV). Furthermore it is unclear whether a negative test result at week 24 obtained by the TMA assay has the same negative predictive value (NPV) as a negative test result obtained by the less sensitive

PCR based assays. In case of comparable NPVs, the most sensitive assay should be used in order to prevent peginterferon alfa/ribavirin related side effects and costs due to unnecessary continuation of HCV therapy. In **chapter 4** two PCR based assays (TaqMan and Amplicor) with a lower limit of detection of 15 and 20 IU/ml respectively and a TMA based assay (Versant) with LLOD of 5.3 IU/ml were compared. In this study a total 89 week 24 HCV RNA samples were tested with the two PCR based assays and the TMA assay. No discordance was found between the two PCR based assays. Discordance between the PCR based assays and the TMA assay was found in 11 out of 89 patients (12%). All patients with detectable HCV RNA at week 24 using the TMA assay experienced a viral relapse or breakthrough after or during treatment and thus the NPV of detectable HCV RNA at week 24 using the TMA assay was 100% in this study. Furthermore, the PPV of a negative TMA test result was somewhat higher than the PPV of a negative PCR test result; 59 vs. 52%, although we should mention that these PPVs are based on all patients, including patients with undetectable HCV RNA at week 12. Based on the results of this study the use of the more sensitive TMA assay could lead to the prevention of unnecessary treatment and thereby the associated side effects and costs.

Optimizing treatment response in previous non-responders

As mentioned previously, 50-60% of patients with genotype 1 and 4 do not respond to treatment with peginterferon alfa and ribavirin, which has led to a continuous increase in the pool of patients non responsive to therapy. Retreating these patients with the same regimen leads to sustained virological response (SVR) rates between 4 and 15% (13-18). Increasing this percentage is considered to be a great challenge.

Pegylation of interferon (IFN) alfa has improved the pharmacokinetic profile of conventional IFN by maintaining constant blood levels. This enabled once-weekly dosing and resulted in higher response rates. However, it has been shown that the volume of distribution due to pegylation is considerably restricted, decreasing biological activity and potentially decreasing treatment efficacy (19). Continuous exposure to IFN alfa could potentially overcome this hindrance by providing sustained and constant levels of a fully potent protein. We hypothesize that constant levels could induce a stable viral suppression and prevent side effects associated with peaks after injection as well as subtherapeutic drug levels associated with troughs. In **chapter 5 and 6** we investigated the efficacy, safety and feasibility of continuous subcutaneous infusion of IFN alfa-2b in combination with weight-based ribavirin (15.2 mg/kg) for 48 weeks in patients who previously failed to respond to peginterferon alfa and ribavirin. The most important finding was that continuous delivery of IFN alfa-2b in combination with ribavirin resulted in a strong dose-dependent viral suppression. At week 4 two-thirds of patients from the 12 MU group had HCV RNA levels below 615 IU/ml and at week 12 all patients from this group had HCV RNA below 615 IU/ml. Furthermore, 50% of previous null and partial responders became HCV RNA negative during treatment. This strong viral suppression of high dose continuous subcutaneous IFN-2b infusion could play an important role within the

recently proposed concept of lead-in therapy prior to triple therapy with PEGIFN alfa, RBV and a direct antiviral agent. In these studies investigating direct antiviral agents, a rapid virological decline during a PEGIFN alfa/RBV lead-in was crucial to achieve SVR and prevent virological breakthrough or relapse (5, 20-22). Short-term high dose continuous IFN alfa infusion could help to achieve this necessary rapid viral decline and therefore this concept of continuous IFN-2b alfa could play an important role in the new era of direct antiviral agents. Especially patients with a known null or partial response to previous therapy with peginterferon alfa and ribavirin could benefit from this treatment concept.

Interferon levels were measured throughout the study, all patients reached steady state levels in the first weeks of treatment. The pharmacokinetic profile of continuous infusion of interferon alfa differs from that of treatment with peginterferon alfa-2a or 2b and ribavirin, which causes peak and trough concentrations. A study investigating pharmacokinetics of peginterferon alfa 2a and 2b showed that undetectable trough concentrations of peginterferon alfa-2b can occur in some patients (23). This phenomenon did not occur in our study. A great interindividual variability of interferon alfa concentrations was measured between patients. For this reason it is difficult to use these concentrations for the prediction of treatment outcome. To our knowledge, to date, no data have been published on the predictiveness of (peg)interferon alfa concentrations on treatment outcome.

Several markers for immune activation and viral kinetics in the early phase of treatment were measured in a subset of patients participating in this study (**chapter 6**). A typical biphasic viral decline was seen in most patients. The strongest decline was seen in patients receiving 12 MU interferon per day. The immune markers neopterin, 2,5 OAS and beta-2-microglobulin increased in all patients with no significant differences between treatment groups except for higher neopterin levels in patients receiving 12 MU of continuous interferon alfa-2b. No significant correlations could be found between viral decline and markers for immune activation.

Spermatotoxicity of peginterferon alfa and ribavirin

In animals, ribavirin has repeatedly been proven to be teratogenic. Malformations of limbs, spine, ribs, eyes and central nervous system have been shown in rodents in addition to reductions in sperm count and alterations in morphology of spermatozoa (24-25) after exposure to ribavirin. Contradicting results have been found in studies investigating the effect of interferon alfa on semen quality (26-27). Little is known about the effects on offspring of males treated with peginterferon alfa and ribavirin. There are some reports of miscarriage but most pregnancies resulted in normal live born infants (28-30). In a prospective observational cohort study pregnant women who were exposed both directly and indirectly to ribavirin, were followed till delivery and their live born infants were followed for one year. In this study, the incidence of birth defects after both direct and indirect ribavirin exposure did not significantly differ from that in the general population, but enrolment was too short to obtain the required sample size (31).

In **chapter 7** the occurrence of semen abnormalities in patients with HCV infection and the effect of peginterferon alfa/ribavirin treatment on spermatogenesis, sperm DNA integrity and endocrinological parameters prior to, during and after treatment are described. Approximately 40% of HCV patients had semen abnormalities at baseline. Sperm concentration and motility, which are predictors of male fertility, did not significantly change during treatment with peginterferon alfa/ribavirin and during follow up.

Sperm DNA integrity is currently the most reliable predictor of spontaneous pregnancies and the occurrence of miscarriage. Potentially, it may be associated with the risk of birth defects. Sperm DNA damage, expressed as DFI, is increased in patients with various forms of cancer, treatment with radiotherapy and several andrological abnormalities (32-33). The median DFI of HCV patients did not significantly differ from healthy controls and during therapy the DFI levels were not significantly altered. Minimal changes in gonadotrophic and gonadal hormones were observed during antiviral treatment. An increase in FSH and LH was observed during treatment together with a decrease, although not significant, in free testosterone and inhibin B (down regulator of FSH). All endocrinological parameters returned to baseline levels during follow up. These results indicate a compensated minimal deterioration of testicular function during treatment with peginterferon alfa/ribavirin treatment. However, differences are small and should be interpreted with caution.

A possible explanation for semen abnormalities could be a direct effect of the virus on spermatogenesis (34). Another explanation could be that semen abnormalities are not related to the virus but to confounding factors often seen in HCV patients like the use of methadone, cocaine, alcohol or specific medication (35-36). In our study DFI levels were significantly higher in methadone users and in patients who used antipsychotics. Based on the findings of this study and based on the findings of birth registry trials it would be questionable whether double contraception until 7 months after treatment cessation, or even during treatment, is necessary for male patients.

CONCLUSION

Although great progress in optimizing response rates of peginterferon alfa and ribavirin for chronic hepatitis C have been made, still 30% of patients fail to respond to this treatment regimen. Factors associated with treatment failure are viral genotype, several host factors, and treatment adherence. Recently developed direct acting antiviral agents will increase the the number of successful treatment outcomes. Unfortunately, these agents cannot be given as monotherapy and are only successful when given in combination with peginterferon alfa and ribavirin. For this reason the combination of peginterferon alfa and ribavirin will remain the backbone of antiviral therapy for chronic hepatitis C in the following years. Management of side effects and adequate utilization of dose reduction rules will remain of utmost importance

to maximize treatment outcome. Conclusions from this thesis are that treatment of patients with thrombocytopenia and neutropenia is safe and therefore these patients should not be excluded from antiviral therapy. When treatment is initiated, the most sensitive qualitative HCV RNA assay should be used to monitor virological response at week 24 to prevent unnecessary treatment and thereby the associated costs and side effects. For patients with the lowest probability of response to triple therapy with peginterferon alfa, ribavirin and a direct acting antiviral agent, high dose continuous interferon alfa infusion could be a possible option to increase the probability of successful treatment outcome.

REFERENCES

1. Moraes Coelho HS, Villela-Nogueira CA. Predictors of response to chronic hepatitis C treatment. *Ann Hepatol* 2010;9 Suppl:54-60.
2. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;461:399-401.
3. Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, Lok AS, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007;132:103-112.
4. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology* 2003;124:1711-1719.
5. Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naïve patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010;376:705-716.
6. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-1838.
7. 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.
8. Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Goncales FL, Jr., Haussinger D, et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. *J Hepatol* 2005;43:425-433.
9. 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.
10. Barbeau JM, Goforth J, Caliendo AM, Nolte FS. Performance characteristics of a quantitative TaqMan hepatitis C virus RNA analyte-specific reagent. *J Clin Microbiol* 2004;42:3739-3746.
11. Martinot-Peignoux M, Khiri H, Leclere L, Maylin S, Marcellin P, Halfon P. Clinical performances of two real-time PCR assays and bDNA/TMA to early monitor treatment outcome in patients with chronic hepatitis C. *J Clin Virol* 2009;46:216-221.
12. Gorrin G, Friesenhahn M, Lin P, Sanders M, Pollner R, Eguchi B, Pham J, et al. Performance evaluation of the VERSANT HCV RNA qualitative assay by using transcription-mediated amplification. *J Clin Microbiol* 2003;41:310-317.
13. Bacon BR, Shiffman ML, Mendes F, Ghalib R, Hassanein T, Morelli G, Joshi S, et al. Retreating chronic hepatitis C with daily interferon alfacon-1/ribavirin after nonresponse to pegylated interferon/ribavirin: DIRECT results. *Hepatology* 2009;49:1838-1846.
14. Bergmann JF, Vrolijk JM, van der Schaar P, Vroom B, van Hoek B, van der Sluys Veer A, de Vries RA, et al. Gamma-glutamyltransferase and rapid virological response as predictors of successful treatment with experimental or standard peginterferon-alpha-2b in chronic hepatitis C non-responders. *Liver Int* 2007;27:1217-1225.
15. Jensen DM, Marcellin P, Freilich B, Andreone P, Di Bisceglie A, Brandao-Mello CE, Reddy KR, et al. Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. *Ann Intern Med* 2009;150:528-540.
16. Poynard T, Colombo M, Bruix J, Schiff E, Terg R, Flamm S, Moreno-Otero R, et al. Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. *Gastroenterology* 2009;136:1618-1628 e1612.

17. Roberts SK, Weltman MD, Crawford DH, McCaughan GW, Sievert W, Cheng WS, Rawlinson W, et al. Impact of high-dose peginterferon alfa-2A on virological response rates in patients with hepatitis C genotype 1: a randomized controlled trial. *Hepatology* 2009;50:1045-1055.
18. 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.
19. Zeuzem S, Welsch C, Herrmann E. Pharmacokinetics of peginterferons. *Semin Liver Dis* 2003;23 Suppl 1:23-28.
20. Bacon B. HCV Respond-2 final results: High sustained virologic response among genotype 1 previous non-responders and relapsers to peginterferon/ribavirin when re-treated with boceprevir plus pegintron (peginterferon alfa-2b)/ribavirin. *Hepatology (suppl)* 2010;52:430A.
21. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-1838.
22. McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, et al. Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010;362:1292-1303.
23. Bruno R, Sacchi P, Ciappina V, Zochetti C, Patruno S, Maiocchi L, Filice G. Viral dynamics and pharmacokinetics of peginterferon alpha-2a and peginterferon alpha-2b in naive patients with chronic hepatitis c: a randomized, controlled study. *Antivir Ther* 2004;9:491-497.
24. Narayana K, D'Souza UJ, Rao KP. Effect of ribavirin on epididymal sperm count in rat. *Indian J Physiol Pharmacol* 2002;46:97-101.
25. Narayana K, D'Souza UJ, Seetharama Rao KP. Ribavirin-induced sperm shape abnormalities in Wistar rat. *Mutat Res* 2002;513:193-196.
26. Hibi H, Yokoi K, Yamamoto M. Effects of alpha-interferon on sperm production, concentration, and motility in the rat. *Int J Urol* 1997;4:603-607.
27. Ulusoy E, Cayan S, Yilmaz N, Aktas S, Acar D, Doruk E. Interferon alpha-2b may impair testicular histology including spermatogenesis in a rat model. *Arch Androl* 2004;50:379-385.
28. Bianca S, Ettore G. Male periconceptional ribavirin-interferon alpha-2b exposure with no adverse fetal effects. *Birth Defects Res A Clin Mol Teratol* 2003;67:77-78.
29. De Santis M, Carducci B, Cavaliere AF, De Santis L, Lucchese A, Straface G, Caruso A. Paternal exposure to ribavirin: pregnancy and neonatal outcome. *Antivir Ther* 2003;8:73-75.
30. Maddrey WC. Safety of combination interferon alfa-2b/ribavirin therapy in chronic hepatitis C-relapsed and treatment-naive patients. *Semin Liver Dis* 1999;19 Suppl 1:67-75.
31. Roberts SS, Miller RK, Jones JK, Lindsay KL, Greene MF, Maddrey WC, Williams IT, et al. The Ribavirin Pregnancy Registry: Findings after 5 years of enrollment, 2003-2009. *Birth Defects Res A Clin Mol Teratol* 2010;88:551-559.
32. Smit M, Romijn JC, Wildhagen MF, Weber RF, Dohle GR. Sperm chromatin structure is associated with the quality of spermatogenesis in infertile patients. *Fertil Steril* 2010;94:1748-1752.
33. Smit M, van Casteren NJ, Wildhagen MF, Romijn JC, Dohle GR. Sperm DNA integrity in cancer patients before and after cytotoxic treatment. *Hum Reprod* 2010;25:1877-1883.
34. Durazzo M, Premoli A, Di Bisceglie C, Bertagna A, Faga E, Biroli G, Manieri C, et al. Alterations of seminal and hormonal parameters: An extrahepatic manifestation of HCV infection? *World J Gastroenterol* 2006;12:3073-3076.
35. Madhusoodanan S, Parida S, Jimenez C. Hyperprolactinemia associated with psychotropics-a review. *Hum Psychopharmacol* 2010;25:281-297.
36. Ragni G, De Lauretis L, Bestetti O, Sghedoni D, Gambaro V. Gonadal function in male heroin and methadone addicts. *Int J Androl* 1988;11:93-100.

SAMENVATTING EN DISCUSSIE

In de laatste twee decennia is er een grote vooruitgang geboekt in de behandeling van chronische hepatitis C. Tot op heden kan 70% van de patiënten succesvol worden behandeld door een blijvende virologische response te induceren met peginterferon alfa en ribavirine. Er zijn echter virale en patientgebonden factoren die de kans op een succesvolle behandeling sterk verminderen (1). Patiënten die besmet zijn met HCV genotype 1, het meest voorkomende genotype in Europa, Noord-Amerika en Japan of patiënten besmet met genotype 4, dat vaker in Egypte voorkomt, hebben een verminderde kans op een blijvende virologische response dan patiënten besmet met genotype 2 en 3. Naast het virus spelen ook patient-gebonden factoren, zoals de mate van leverfibrose (verlittekening), gewicht, geslacht, leeftijd, ras en bepaalde genetische factoren een rol in de kans op een succesvolle antivirale behandeling. Onlangs is ontdekt dat een bepaalde puntmutatie nabij het interleukine-28b gen de sterkste voorspeller is van een succesvolle antivirale behandeling (2). Tenslotte is er nog een derde factor die een belangrijke rol speelt in de kans op succes, namelijk het daadwerkelijk krijgen van meer dan 80% van de totale dosering voor meer dan 80% van de geplande behandelingsduur. Met name voor patiënten met een lage a priori kans op een succesvolle behandeling is een optimale behandeling van cruciaal belang voor het bereiken van een blijvende virologische response (3).

Helaas is de behandeling met peginterferon alfa en ribavirine geassocieerd met veel bijwerkingen die vaak tot dosisverlagingen leiden, hierdoor is de meest optimale behandeling vaak niet mogelijk (4).

Gelukkig zijn er in de laatste jaren nieuwe antivirale middelen ontwikkeld voor patiënten met genotype 1 die de kans op een blijvende response aanzienlijk verhogen (5-6). Helaas hebben deze nieuwe middelen ook weer veel bijwerkingen waardoor de behandeling nog moeilijker te verdragen wordt. In **hoofdstuk 1** van dit proefschrift wordt een overzicht gegeven van de bijwerkingen van zowel de huidige behandelcombinatie als de toekomstige behandelcombinaties.

Optimalisering van richtlijnen voor dosisverlagingen

Een paar van de meest voorkomende bijwerkingen van de behandelcombinatie peginterferon alfa en ribavirine zijn neutropenie (laag aantal afweercellen), trombocytopenie (laag aantal bloedplaatjes) en anemie (laag aantal rode bloedcellen). Ongeveer 20% van de patiënten ontwikkelt een absoluut neutrofielen getal onder de 750/ μ L, wat de meest voorkomende reden is voor het verlagen van de peginterferon alfa dosis (7). Deze dosisverlaging wordt verricht om bacteriele infecties te voorkomen. Bij een absoluut neutrofielen getal onder de 375/ μ L dient de peginterferon alfa zelfs tijdelijk te worden onderbroken. Echter, zoals eerder vermeld, leiden dosisverlagingen tot een minder effectieve behandeling. Om deze reden is het cruciaal om het

aantal dosisverlagingen tot een minimum te beperken om een zo groot mogelijke kans op een blijvende virologische response te creëren.

Bacteriele infecties komen veel voor tijdens de behandeling van hepatitis C. In **hoofdstuk 2** hebben wij onderzocht of peginterferon alfa geïnduceerde neutropenia daadwerkelijk leidt tot meer bacteriele infecties en welke andere factoren eventueel gerelateerd zijn aan het ontstaan van deze infecties. In deze retrospectieve cohortstudie van 321 patiënten zijn er 96 infecties gevonden in 70 patiënten (22%). Daarnaast ontwikkelden 95 patiënten (30%) een absoluut neutrofielen getal onder de 750/ μL . Verrassend was dat hyperglycemie (diabetes mellitus) en oudere leeftijd de enige factoren waren die sterk geassocieerd waren met het ontstaan van infecties en dat er geen enkele relatie was met het neutrofielengetal en het ontstaan van infecties. Een tweede interessante bevinding van deze studie was dat patiënten met neutropenie die een dosisverlaging ondergingen niet minder infecties ontwikkelden dan patiënten met neutropenie die geen dosisverlaging ondergingen. Deze resultaten moeten echter met de nodige voorzichtigheid worden geïnterpreteerd omdat dosis verlagingen werden uitgevoerd op basis van de klinische conditie van de patient en dus bestaat er een kans dat een dosisverlaging niet werd uitgevoerd in fitte patienten die op voorhand een lagere kans op een eventuele infectie hadden. In **hoofdstuk 3** hebben we onderzocht of patiënten met peginterferon alfa geïnduceerde trombocytopenie meer risico hadden op het ontwikkelen van (ernstige) bloedingen tijdens de behandeling. Volgens de huidige richtlijnen dient de peginterferon alfa dosis te worden verlaagd in het geval van trombocyten onder de 50000/ μL en moet peginterferon alfa worden gestopt bij trombocyten onder de 25000/ μL . In deze studie is gebruikt gemaakt van hetzelfde cohort van 321 patiënten. In totaal ontwikkelden 30 (9%) patiënten een matig ernstige trombocytopenie (bloedplaatjes lager dan 50000/ μL) en 9 patiënten ontwikkelden een ernstige trombocytopenie (bloedplaatjes lager dan 25000/ μL). In totaal werden er 48 bloedingen in 27 patiënten waargenomen (8%). Slechts 1 bloeding, een darmbloeding, werd gedefinieerd als ernstig. De betreffende patiënt had echter geen matig ernstige trombocytopenie op het moment van bloeden. Er was wel degelijk een sterke correlatie tussen bloedingen en matige of ernstige trombocytopenie. Deze bloedingen waren echter allen als mild gedefinieerd. In dit cohort ondergingen 12 van de 30 patiënten met een trombocytengetal onder de 50000/ μL een dosisverlaging of een staking van de behandeling van de peginterferon alfa dosis. In de resterende 18 patiënten, werd de peginterferon alfa dosis niet verlaagd. Bij deze patienten werd het belang van een virologische response en het stabiele trombocytengetal ook meegenomen in de overweging.

Gebaseerd op de bevindingen van de **hoofdstukken 2 en 3** zijn de richtlijnen voor dosisreducties momenteel te voorzichtig en verminderen de kans op een optimale behandeling. Om deze reden moeten de richtlijnen worden aangepast. Een mogelijk alternatief voor de huidige richtlijnen voor trombocytopenie zou zijn om de peginterferon alfa dosering te handhaven bij een trombocytengetal boven 25000/ μL zo lang de patiënt geen tekenen van een actieve bloeding heeft. Bij een trombocytengetal onder de 25000/ μL zou de peginterferon alfa tijdelijk

gestopt moeten worden totdat de trombocyten weer boven de 25000/ μ L zijn gekomen, vervolgens kan de peginterferon alfa met een lagere dosering worden hervat. Bij een neutropenie kan de peginterferon alfa dosis gehandhaafd blijven zolang het absoluut neutrofielen getal boven 375/ μ L blijft in het geval de patiënt geen aanwijzingen voor een bacteriële infectie heeft. In het geval van een absoluut neutrofielen getal onder de 375/ μ L, moet de peginterferon alfa dosis worden verminderd in stappen van 25% totdat het absoluut neutrofielen getal stabiel boven de 375/ μ L blijft.

Optimaliseren van regels voor stopmomenten

Het meten van de virale kinetiek tijdens de behandeling is cruciaal voor het voorspellen van de kans op een succesvolle behandeling. Een aanzienlijk deel van de patiënten heeft slechts een gedeeltelijke response of geen response (non-response) op de behandeling. Hiervan is sprake als de virale daling op week 12 minder dan 2 log in HCV RNA is of wanneer het HCV RNA nog aantoonbaar is op week 24 van de behandeling (7-8). De negatieve voorspellende waarde van een gedeeltelijke response of een non-response ligt tussen de 97 en 100%. Het is dus niet zinvol door te gaan met de behandeling bij deze patiënten. Deze negatief voorspellende waarden en de stopregels zijn gebaseerd op studies die HCV RNA testen gebruikten met een detectie ondergrens (LLOD) van 50 IU/ml (9). De HCV RNA tests die tegenwoordig veel gebruikt worden hebben echter een detectie ondergrens van 15 tot 20 IU/ml (10-11) en zelfs testen met een LLOD van 5 IU/ml zijn momenteel beschikbaar (12). Deze verschillen in detectiegrenzen kunnen leiden tot verschillende uitkomsten bij het meten van het HCV RNA op week 24 van de behandeling. Hoe gevoeliger de test, hoe groter de kans dat het HCV RNA nog aantoonbaar is en hoe groter de kans is dat de behandeling op week 24 gestopt wordt. Het is echter onduidelijk of het nog steeds terecht is om de behandeling te stoppen bij het gebruik van een gevoeliger test. Mocht dit wel het geval zijn dan zou altijd de meest gevoelige test gebruikt moeten worden om onnodige behandeling en de daarbij komende kosten en bijwerkingen te voorkomen.

In **hoofdstuk 4** zijn twee PCR gebaseerde testen (TaqMan en Amplicor) met ondergrenzen van respectievelijk 15 en 20 IU/ml en een TMA gebaseerde test (Versant) met een ondergrens van 5.3 IU/ml met elkaar vergeleken. In deze studie zijn 89 week 24 HCV RNA samples getest met zowel de twee PCR testen als de TMA test. Er werden geen verschillen in uitkomsten gevonden tussen de twee PCR gebaseerde testen. Echter 11 van de 89 samples (12%) waren wel positief wanneer zij werden hertest met de veel gevoeliger TMA test. Geen enkele patient met aantoonbaar HCV RNA op week 24 gedetecteerd met de TMA test behaalde uiteindelijk een succesvolle virale response. De negatief voorspellende waarde van aantoonbaar HCV RNA met de meest gevoelige test was in deze studie dus nog steeds 100%. Bovendien was de positief voorspellende waarde van een ondetecteerbaar HCV RNA hoger dan de positief voorspellende waarde van een negatief testresultaat behaald met de minder gevoelige PCR test; namelijk 59 vs. 52%. Gebaseerd op de resultaten van deze studie moet altijd de meest

gevoelige HCV RNA test gebruikt worden om onnodige behandeling en de daarbij horende bijwerkingen en kosten te voorkomen.

Optimaliseren van de behandeling in eerdere non-responders

Zoals al eerder genoemd reageert ongeveer 50 tot 60% van de patiënten met genotype 1 en 4 reageren niet op behandeling met peginterferon alfa en ribavirine. Dit heeft geleid tot een grote groep van non-responders. Wanneer deze groep nog eens behandeld wordt met dezelfde behandelcombinatie behaalt ongeveer 4 tot 15% een blijvende virologische response (13-18). Verhoging van dit percentage wordt beschouwd als de belangrijkste uitdaging op dit moment.

Pegylering van interferon alfa heeft het farmacokinetische profiel van conventioneel interferon verbeterd door constantere bloedspiegels van interferon vergeleken met regulier interferon alfa te bewerkstelligen. Naast de hogere response percentages hoeft peginterferon alfa nog maar 1 keer per week toegediend te worden in plaats van 3 keer per week. Een nadeel van pegylering is echter dat het verdelingsvolume en de biologische activiteit aanzienlijk minder zijn dan bij conventioneel interferon alfa en daarmee mogelijk ook de virale effectiviteit (19). Continue toediening van regulier interferon alfa zou deze belemmering kunnen opheffen door middel van het creëren van constante spiegels van regulier interferon alfa met een maximale biologische activiteit. Wij veronderstellen dat constante concentraties van interferon alfa leiden tot een constante onderdrukking van het virus en dat bijwerkingen die normaal voorkomen bij de wekelijkse peginterferon alfa geïnduceerde concentratiepieken alsmede de potentiële subtherapeutische concentratiedalen aan het einde van de week kunnen worden voorkomen.

Hoofdstuk 5 en 6 gaan over de effectiviteit, veiligheid en haalbaarheid van continue subcutane toediening van interferon alfa in combinatie met ribavirine voor 48 weken bij patiënten die eerder niet voldoende gereageerd hebben op de standaard behandeling met peginterferon alfa en ribavirine. De belangrijkste bevinding van dit onderzoek was dat continue toediening van interferon alfa in combinatie met ribavirine resulteerde in een sterke dosis afhankelijke virale onderdrukking. Na 4 weken behandeling had tweederde van de patiënten uit de hoge dosis groep (12MU) HCV RNA concentraties onder de 615 IU/ml en op week 12 had alle patiënten uit deze groep een HCV RNA concentratie onder de 615 IU/ml. Bovendien werd 50% van de patiënten die eerder helemaal niet of nauwelijks reageerde op de behandeling tijdens deze behandeling HCV RNA negatief. Deze sterke virale onderdrukking van hoge dosis continue subcutane interferon alfa toediening kan een belangrijke rol spelen tijdens toekomstige behandelingschema's met één van de nieuwe antivirale middelen. In de studies die deze middelen onderzochten werd voordat dit middel werd toegevoegd eerst 4 weken behandeld met alleen peginterferon alfa en ribavirine. Dit om het virus alvast wat af te zwakken waardoor de kans op resistentie voor het nieuwe middel (boceprevir) kleiner werd. Patiënten die tijdens deze zogenaamde 'lead-in' fase nauwelijks daling hadden, hadden ook een veel kleinere kans op een succesvolle behandeling. Hoge dosis continue interferon alfa zou, gezien

de sterke virale daling die het kan induceren, in deze groep slecht reagerende patienten van grote betekenis kunnen zijn (5, 20-22).

Tijdens deze studie werden ook interferon concentraties gemeten. In alle patiënten werden uiteindelijk stabiele concentraties bereikt. Dit farmacokinetische profiel verschilt dus van peginterferon alfa, waarbij pieken en dalen worden gezien. Een onderzoek naar farmacokinetiek van peginterferon alfa toonde aan dat bij sommige patienten peginterferon alfa concentraties aan het einde van de week (vlak voor de volgende injectie) niet meer meetbaar waren (23). Bij de continue toediening van interferon alfa in deze studie kwam dit bij geen enkele patient voor. Wel werd er een grote variabiliteit van interferon alfa concentraties gemeten tussen de patiënten. Om deze reden is het moeilijk om deze concentraties te gebruiken voor een eventuele voorspelling van een succesvolle behandeling. Voor zover bekend zijn er ook geen studies waarin peginterferon alfa concentraties bruikbaar waren voor de voorspelling van een succesvolle behandeling.

Verder is de virale kinetiek en verschillende markers voor immunosuppressie gemeten in de vroege fase van de studie in een subgroep van patiënten (**hoofdstuk 6**). Een typische bifasische virale daling was te zien bij de meeste patiënten. De sterkste daling was te zien in patienten die de hoogste dosering (12 MU) ontvingen. De markers voor immunosuppressie: neopterine, 2,5 OAS en bèta-2-microglobuline waren bij alle patiënten verhoogd zonder significante verschillen tussen de verschillende behandelgroepen met uitzondering van hogere neopterine concentraties in patiënten die 12 MU ontvingen. Geen significante correlaties konden worden gevonden tussen de virale daling en de markers voor immunosuppressie.

Spermatotoxiciteit van peginterferon alfa en ribavirine

In dierexperimenten is herhaaldelijk bewezen dat ribavirine teratogeen is. Aangeboren afwijkingen van ledematen, de wervelkolom, ribben, ogen en het centrale zenuwstelsel zijn geobserveerd bij knaagdieren. Daarnaast was het aantal spermacellen minder en werden er afwijkingen gevonden in de morfologie van spermacellen (24-25) na blootstelling aan ribavirine. Tegenstrijdige resultaten zijn gevonden in studies die onderzoek deden naar het effect van interferon alfa op de sperma kwaliteit (26-27). Er is weinig bekend over de effecten op het nageslacht van mannen die behandeld werden met peginterferon alfa en ribavirine. Er zijn enkele publicaties over miskramen na indirecte blootstelling van de vrucht via de man. De meeste gerapporteerde zwangerschappen resulteerden echter in normale gezonde baby's (28-30). In een prospectieve observationele cohort studie naar zwangere vrouwen die zowel direct als indirect werden blootgesteld aan ribavirine was de incidentie van aangeboren afwijkingen niet hoger dan de incidentie van aangeboren afwijkingen in de algemene bevolking. Het aantal geïncludeerde vrouwen was echter wel te klein voor de vooraf berekende powercalculatie (31).

Hoofdstuk 7 beschrijft het voorkomen van sperma afwijkingen bij patiënten met chronische hepatitis C en het effect van de behandeling met peginterferon alfa en ribavirine op de

spermatogenese, sperma DNA integriteit en endocrinologische parameters. Ongeveer 40% van de hepatitis C patiënten hadden voorafgaand aan de behandeling al sperma afwijkingen. Echter spermaconcentratie en motiliteit (beweeglijkheid), belangrijke voorspellers van de mannelijke vruchtbaarheid, veranderden niet tijdens de behandeling met peginterferon alfa en ribavirine. Ook na de behandeling was er geen significante verandering te zien.

Sperma DNA integriteit is momenteel een van de meest betrouwbare voorspellers van spontane zwangerschappen of de kans op een miskraam. Sperma DNA integriteit wordt uitgedrukt in de DNA fragmentatie index (DFI). Het DFI is verhoogd bij patiënten met verschillende vormen van kanker, patiënten die bestraling hebben ondergaan of bij patiënten met verschillende andrologische afwijkingen (32-33). De mediane DFI van hepatitis C patiënten verschilde niet aanzienlijk van gezonde patiënten zonder hepatitis C. Verder veranderden de DFI waardes niet significant tijdens de behandeling met peginterferon alfa en ribavirine. Wel werden er minimale veranderingen in de gonadotrofe en gonadale hormonen waargenomen tijdens de antivirale behandeling. Er werd een toename van zowel FSH en LH geobserveerd gecombineerd met een daling, hoewel niet significant, in het vrije testosteron en het inhibine B (downregulator van FSH). Na het staken van de behandeling herstelden alle endocrinologische parameters tot het niveau van voor de behandeling. Deze resultaten wijzen erop dat er sprake was van een gecompenseerde minimale verslechtering van de testiculaire functie tijdens behandeling met peginterferon alfa en ribavirine. Echter, de verschillen zijn klein en moeten derhalve met voorzichtigheid geïnterpreteerd worden.

Een mogelijke verklaring voor de sperma afwijkingen zou een direct effect van het virus op de spermatogenese kunnen zijn (34). Een andere mogelijke verklaring zou kunnen zijn dat de sperma afwijkingen niet gerelateerd zijn aan de chronische hepatitis C maar aan andere factoren die vaak voorkomen bij hepatitis C gebruik zoals het gebruik van methadon, cocaïne, alcohol of specifieke medicatie (35-36). In onze studie waren DFI waardes significant hoger in methadon gebruikers en bij patiënten die antipsychotica gebruikten. Op basis van de bevindingen van deze studie en op basis van de bevindingen van de observationele cohort studie bij zwangere vrouwen die zowel direct als indirect zijn blootgesteld aan peginterferon alfa en ribavirine is het maar de vraag of dubbele anticonceptie tot 7 maanden na het staken van de behandeling wel echt nodig is.

CONCLUSIE

Hoewel er grote vooruitgang is geboekt in het optimaliseren van de behandeling van chronische hepatitis C met peginterferon alfa en ribavirine, reageert nog steeds ongeveer 30% van de patiënten niet op deze behandelcombinatie. Factoren van invloed op de kans van slagen op deze behandeling zijn het virale genotype, patientgebonden factoren en het geven van een optimale dosering. Recent ontwikkelde antivirale middelen zullen het percentage succesvolle

behandeling nog verder laten toenemen. Helaas, kunnen deze middelen niet als monotherapie gegeven worden en zijn ze alleen succesvol in combinatie met peginterferon alfa en ribavirine. Peginterferon alfa en ribavirine blijven daarom de basis voor antivirale therapie in de komende jaren. Het adequaat behandelen van bijwerkingen en het correct reguleren van de optimale dosering blijft daarom cruciaal voor een succesvolle uitkomst van de therapie. Verder blijkt uit dit proefschrift dat de behandeling van patiënten met trombocytopenie en neutropenie veilig is en daarom hoeft men deze groep patiënten niet te onthouden van antivirale therapie. Ook kunnen de regels voor dosisreducties vanwege trombocytopenie en neutropenie enigszins versoepeld worden. Tijdens de behandeling is het gebruik van de meest gevoelige HCV RNA test zeer belangrijk om onnodig lange behandeling en de daarmee geassocieerde kosten en bijwerkingen te voorkomen. Voor patiënten met de laagste kans op een succesvolle response op therapie met peginterferon alfa, ribavirine en een direct antiviraal middel, zou een continue toegediende hoge dosis interferon alfa een mogelijke optie kunnen zijn om de kans op een succesvolle behandeling te verhogen.

REFERENTIES

1. Moraes Coelho HS, Villela-Nogueira CA. Predictors of response to chronic hepatitis C treatment. *Ann Hepatol* 2010;9 Suppl:54-60.
2. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;461:399-401.
3. Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, Lok AS, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007;132:103-112.
4. Russo MW, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology* 2003;124:1711-1719.
5. Kwo PY, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, et al. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naïve patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010;376:705-716.
6. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-1838.
7. 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.
8. Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Goncalves FL, Jr., Haussinger D, et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. *J Hepatol* 2005;43:425-433.
9. 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.
10. Barbeau JM, Goforth J, Caliendo AM, Nolte FS. Performance characteristics of a quantitative TaqMan hepatitis C virus RNA analyte-specific reagent. *J Clin Microbiol* 2004;42:3739-3746.
11. Martinot-Peignoux M, Khiri H, Leclere L, Maylin S, Marcellin P, Halfon P. Clinical performances of two real-time PCR assays and bDNA/TMA to early monitor treatment outcome in patients with chronic hepatitis C. *J Clin Virol* 2009;46:216-221.
12. Gorrin G, Friesenhahn M, Lin P, Sanders M, Pollner R, Eguchi B, Pham J, et al. Performance evaluation of the VERSANT HCV RNA qualitative assay by using transcription-mediated amplification. *J Clin Microbiol* 2003;41:310-317.
13. Bacon BR, Shiffman ML, Mendes F, Ghalib R, Hassanein T, Morelli G, Joshi S, et al. Retreating chronic hepatitis C with daily interferon alfacon-1/ribavirin after nonresponse to pegylated interferon/ribavirin: DIRECT results. *Hepatology* 2009;49:1838-1846.
14. Bergmann JF, Vrolijk JM, van der Schaar P, Vroom B, van Hoek B, van der Sluys Veer A, de Vries RA, et al. Gamma-glutamyltransferase and rapid virological response as predictors of successful treatment with experimental or standard peginterferon-alpha-2b in chronic hepatitis C non-responders. *Liver Int* 2007;27:1217-1225.
15. Jensen DM, Marcellin P, Freilich B, Andreone P, Di Bisceglie A, Brandao-Mello CE, Reddy KR, et al. Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. *Ann Intern Med* 2009;150:528-540.
16. Poynard T, Colombo M, Bruix J, Schiff E, Terg R, Flamm S, Moreno-Otero R, et al. Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. *Gastroenterology* 2009;136:1618-1628 e1612.

17. Roberts SK, Weltman MD, Crawford DH, McCaughan GW, Sievert W, Cheng WS, Rawlinson W, et al. Impact of high-dose peginterferon alfa-2A on virological response rates in patients with hepatitis C genotype 1: a randomized controlled trial. *Hepatology* 2009;50:1045-1055.
18. 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.
19. Zeuzem S, Welsch C, Herrmann E. Pharmacokinetics of peginterferons. *Semin Liver Dis* 2003;23 Suppl 1:23-28.
20. Bacon B. HCV Respond-2 final results: High sustained virologic response among genotype 1 previous non-responders and relapsers to peginterferon/ribavirin when re-treated with boceprevir plus peginteron (peginterferon alfa-2b)/ribavirin. *Hepatology (suppl)* 2010;52:430A.
21. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;360:1827-1838.
22. McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, et al. Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010;362:1292-1303.
23. Bruno R, Sacchi P, Ciappina V, Zochetti C, Patruno S, Maiocchi L, Filice G. Viral dynamics and pharmacokinetics of peginterferon alpha-2a and peginterferon alpha-2b in naive patients with chronic hepatitis c: a randomized, controlled study. *Antivir Ther* 2004;9:491-497.
24. Narayana K, D'Souza UJ, Rao KP. Effect of ribavirin on epididymal sperm count in rat. *Indian J Physiol Pharmacol* 2002;46:97-101.
25. Narayana K, D'Souza UJ, Seetharama Rao KP. Ribavirin-induced sperm shape abnormalities in Wistar rat. *Mutat Res* 2002;513:193-196.
26. Hibi H, Yokoi K, Yamamoto M. Effects of alpha-interferon on sperm production, concentration, and motility in the rat. *Int J Urol* 1997;4:603-607.
27. Ulusoy E, Cayan S, Yilmaz N, Aktas S, Acar D, Doruk E. Interferon alpha-2b may impair testicular histology including spermatogenesis in a rat model. *Arch Androl* 2004;50:379-385.
28. Bianca S, Ettore G. Male periconceptional ribavirin-interferon alpha-2b exposure with no adverse fetal effects. *Birth Defects Res A Clin Mol Teratol* 2003;67:77-78.
29. De Santis M, Carducci B, Cavaliere AF, De Santis L, Lucchese A, Straface G, Caruso A. Paternal exposure to ribavirin: pregnancy and neonatal outcome. *Antivir Ther* 2003;8:73-75.
30. Maddrey WC. Safety of combination interferon alfa-2b/ribavirin therapy in chronic hepatitis C-relapsed and treatment-naive patients. *Semin Liver Dis* 1999;19 Suppl 1:67-75.
31. Roberts SS, Miller RK, Jones JK, Lindsay KL, Greene MF, Maddrey WC, Williams IT, et al. The Ribavirin Pregnancy Registry: Findings after 5 years of enrollment, 2003-2009. *Birth Defects Res A Clin Mol Teratol* 2010;88:551-559.
32. Smit M, Romijn JC, Wildhagen MF, Weber RF, Dohle GR. Sperm chromatin structure is associated with the quality of spermatogenesis in infertile patients. *Fertil Steril* 2010;94:1748-1752.
33. Smit M, van Casteren NJ, Wildhagen MF, Romijn JC, Dohle GR. Sperm DNA integrity in cancer patients before and after cytotoxic treatment. *Hum Reprod* 2010;25:1877-1883.
34. Durazzo M, Premoli A, Di Bisceglie C, Bertagna A, Faga E, Biroli G, Manieri C, et al. Alterations of seminal and hormonal parameters: An extrahepatic manifestation of HCV infection? *World J Gastroenterol* 2006;12:3073-3076.
35. Madhusoodanan S, Parida S, Jimenez C. Hyperprolactinemia associated with psychotropics-a review. *Hum Psychopharmacol* 2010;25:281-297.
36. Ragni G, De Lauretis L, Bestetti O, Sghedoni D, Gambaro V. Gonadal function in male heroin and methadone addicts. *Int J Androl* 1988;11:93-100.

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BIBLIOGRAFIE

1. Bergmann JF, Slavenburg S, Roomer R, de Knecht RJ, Drenth JP. Rationale and design of the virological response and ribavirin dosage (VIRID) study in hepatitis. *Neth J Med* 2008;66:44-45.
2. Roomer R, de Knecht RJ. Hepatitis C. In: NVH cursusboek klinische Hepatologie 2008.
3. Slavenburg S, Lamers MH, Roomer R, de Knecht RJ, van Oijen MG, Drenth JP. Current clinical care compared with new Dutch guidelines for hepatitis C treatment. *Neth J Med* 2009;67:177-181.
4. Roomer R, de Knecht RJ. Hepatitis C, present standard of care. In: NVH cursusboek klinische hepatologie 2009.
5. Roomer R, Hansen BE, Janssen HL, de Knecht RJ. Risk factors for infection during treatment with peginterferon alfa and ribavirin for chronic hepatitis C. *Hepatology* 2010;52:1225-1231.
6. Roomer R, Hansen BE, Janssen HL, de Knecht RJ. Thrombocytopenia and the risk of bleeding during treatment with peginterferon alfa and ribavirin for chronic hepatitis C. *J Hepatol* 2010;53:455-459.
7. Roomer R, de Knecht RJ. Hepatitis C, huidige standaardbehandeling. In: NVH cursusboek klinische Hepatologie 2010.
8. Roomer R, van Vuuren AJ, Schutten M, Heijens A, Janssen HL, de Knecht RJ. Discordance between HCV RNA assays for week 24 HCV RNA determination during pegylated interferon-alpha/ribavirin treatment for chronic hepatitis C. *Antivir Ther* 2011;16:771-774.
9. Roomer R, Bergmann JF, Boonstra PA, Hansen BE, Haagmans BL, Kwadijk-de Gijssel S, van Vuuren AJ, de Knecht RJ, Janssen HLA. Continuous interferon alfa-2b infusion in combination with ribavirin for chronic hepatitis C in treatment experienced patients. *Antivir Ther* 2011, accepted for publication.
10. Bergmann JF, Roomer R, Haagmans BL, Hansen BE, de Knecht RJ, Janssen HLA, Boonstra PA. Viral kinetics and immune activation during continuous subcutaneous administration of high dose interferon alfa-2b in treatment experienced chronic hepatitis C patients. Submitted.
11. Roomer R, Bezemer G, van Brakel J, Romijn JC, Hansen BE, de Jong FH, Dohle GR, Janssen HLA, de Knecht RJ. Sperm DNA integrity is not affected by treatment with peginterferon alfa and ribavirin for chronic hepatitis C. Submitted.

CURRICULUM VITAE

Robert Roomer werd geboren op 30 januari 1983 te 's Gravenhage. In 2001 behaalde hij zijn VWO diploma aan het Alfrink College te Zoetermeer. Hieropvolgend startte hij met de opleiding Geneeskunde aan de Erasmus Universiteit te Rotterdam. Na het verrichten van zijn afstudeeronderzoek in Suriname behaalde hij zijn doctoraal examen in 2005. Na het behalen van het artsexamen in oktober 2007 begon hij met zijn promotieonderzoek naar de optimalisering van hepatitis C behandeling onder begeleiding van Rob de Knecht en Harry Janssen. In maart 2011 won hij de "NVH Young Hepatologist Award" voor beste klinisch hepatologisch wetenschappelijk artikel van 2010. Sinds april 2011 is hij in opleiding tot Maag- Darm- Leverarts met dr. R.A. de Man als opleider. De vooropleiding Interne geneeskunde volgt hij in het Reinier de Graaf Gasthuis in Delft (opleider E.F.M. Posthuma) evenals de eerste 2 van de 4 jaar maag-, darm- en leverziekten (opleider dr. J.T. Brouwer). Hij woont samen met Sarah van der Heide in Rotterdam.

Summary of PhD training and teaching

Name PhD student: Robert Roomer

PhD period: 2007-2011

Erasmus MC Department: Gastroenterology
and Hepatology

Promotor: Prof. Dr. H.L.A. Janssen

1. PhD training

	Year	Workload
General courses		
Classical methods of data analysis. Netherlands Institute for Health Sciences, Rotterdam, the Netherlands.	2009	104 hours
Presentations and workshops		
Infections during treatment with peginterferon and Ribavirin for chronic hepatitis C is not associated with Neutropenia. 60th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), Boston	2009	36 hours
Sperm DNA integrity is not affected by treatment with peginterferon alfa and ribavirin for chronic hepatitis C. Annual meeting of the Netherlands Association of Hepatology, Veldhoven 2010	2010	12 hours
Discordance between different HCV RNA assays for Week 24 HCV RNA determination during peginterferon alfa/ribavirin treatment for chronic hepatitis C', Annual meeting of the Netherlands Association of Hepatology, Veldhoven 2010	2010	12 hours
Poster presentations		
Discordance between HCV RNA week 24 test results of three different HCV RNA assays during treatment of chronic hepatitis C: optimization of treatment length using the most sensitive HCV RNA assay. 60th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), Boston	2009	24 hours
Thrombocytopenia during treatment with peginterferon and ribavirin for chronic hepatitis C is not associated with severe bleedings'60th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), Boston	2009	36 hours
Week 24 HCV RNA determination during treatment with peginterferon alfa and ribavirin for chronic hepatitis C using the most sensitive HCV RNA assay prevents unnecessary treatment, 61st Annual meeting of the American Association for the Study of Liver Diseases (AASLD), Boston	2010	24 hours
Continuous subcutaneous administration of high dose interferon alfa 2b combined with ribavirin in chronic hepatitis C patients: a dose finding and safety study in treatment experienced patients, 61st Annual meeting of the American Association for the Study of Liver Diseases (AASLD), Boston	2010	36 hours
Sperm DNA integrity is not affected by treatment with peginterferon alfa and ribavirin for chronic hepatitis C, (Presidential poster of distinction) 61st Annual meeting of the American Association for the Study of Liver Diseases (AASLD), Boston	2010	36 hours

	Year	Workload
International conferences		
43rd Annual Meeting of the European Association for the Study of the Liver (EASL). Milan, Italy.	2008	28 hours
44th Annual Meeting of the European Association for the Study of the Liver (EASL). Copenhagen, Denmark.	2009	28 hours
The Liver Meeting 2009, 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America.	2009	28 hours
45th Annual Meeting of the European Association for the Study of the Liver (EASL). Vienna, Austria.	2010	28 hours
The Liver Meeting 2010, 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA, United States of America.	2010	28 hours
Attended seminars and workshops		
De sterkste schakel Hepatitis B en C	2007	2 hours
5th Post-AASLD symposium. Rotterdam, the Netherlands.	2007	2 hours
Hepatitis Masterclass. Utrecht, the Netherlands	2008	18 hours
Eerste Lagerhuisdebat Hepatitis B en C. Amsterdam, the Netherlands.	2008	2 hours
6th Post-AASLD symposium. Rotterdam, the Netherlands.	2008	2 hours
Abdominal Ultrasound Course, Dutch Liver Week, Soestduinen, The Netherlands	2009	16 hours
Cursus Effectief presenteren van wetenschappelijk onderzoek.	2009	8 hours
Tweede Lagerhuisdebat Hepatitis B en C. Amsterdam, the Netherlands.	2009	2 hours
7th Post-AASLD symposium. Rotterdam, the Netherlands.	2009	2 hours
Federa Medisch Wetenschappelijke dag	2010	8 hours
8th Post-AASLD symposium. Rotterdam, the Netherlands.	2010	2 hours
Derde Lagerhuisdebat Hepatitis B en C. Utrecht, the Netherlands.	2010	2 hours

2. Teaching

	Year	Workload
Lecturing		
Chronic hepatitis C, Oost Europa cursus, Isala klinieken, Zwolle	2008	12 hours
Diagnosis and treatment of chronic hepatitis C. Third year Erasmus MC medical students participating in a 4-week Gastroenterology and Hepatology training program. Rotterdam, the Netherlands.	2009	4 hours
Behandeling van chronische hepatitis C, ROIG onderwijs, Erasmus MC.	2010	4 hours

