Neuropsychiatric and other side effects of peginterferon-based therapy of chronic hepatitis C infection

Geert Bezemer

Colofon

Layout and printing: Kunstdrukkerij Mercurius

Illustration: Edvard Munch, Melancholy, 1892
·Volgens de oude Grieken werd de gemoedstoestand van mensen bepaald door het (on)evenwicht tussen vier lichaamssappen of, in het Latijn, humores: bloed (Grieks haima, Latijn sanguis), gele gal (Grieks xanthè cholè), zwarte gal (Grieks melaina cholè) en slijm (Grieks phlegma). Een teveel aan zwarte gal werd verondersteld neerslachtigheid, introversie en depressies te veroorzaken. Nog steeds worden neerslachtige stemmingen aangeduid met de termen melancholie en zwartgalligheid.· Wikipedia ·

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CONTENTS

General Introduction

The Hepatitis C virus (HCV) was identified in 1989, after extensive research, (1-2) as a cause of the so-called non-A non-B hepatitis. HCV is an RNA virus belonging to the genus Hepacivirus in the family of Flaviviridae. Six major different genotypes of the virus are discerned (3). HCV is transmitted via blood-blood contact, and consequently, the majority of HCV patients in western countries are infected by the intravenous use of drugs or by receiving blood transfusion before 1992, the year when screening tests became available. World wide more than 170 million people are chronically infected with HCV (4).
After an acute infection with HCV only about twenty percent of the people is able to clear the virus, the others become chronically infected. Of the patients with a chronic HCV infection, about 20 percent develops progressive fibrosis developing into cirrhosis, ultimately leading to end-stage liver disease and hepatocellular carcinoma (5-6). Successful eradication of the virus prevents progression of the liver disease and leads to improved survival (7).

Treatment of chronic HCV

The endogeneous cytokine interferon-alfa (IFN) has, amongst others, antiviral effects. Peginterferon-alfa (PEGIFN) is the pegylated form of IFN. At this moment, combination therapy with PEGIFN and ribavirin (RBV) is standard treatment of chronic HCV. RBV has weak antiviral effect when given as monotherapy, but has a synergistic effect in combination with (PEG)IFN. The precise underlying mechanism is still unclear, and several theories are being proposed including a direct antiviral effect by RBV acting as a mutagen through its incorporation into the HCV genome, subsequently resulting in error catastrophe (8-9), or indirect by modulating the expression of interferon-stimulated genes (10-12). Monotherapy with IFN leads to eradication of the virus (sustained virological response; SVR) in about 15 % of patients, IFN combined with RBV induces an SVR in about 40 % of patients (13-14), whereas current combination treatment with PEGIFN and RBV is capable of inducing SVR in approximately 55 % (15-16). New developed anti-viral agents like telaprevir and boceprevir show promising results, but for optimal response rates combination therapy of these agents with PEGIFN and RBV still is necessary (17-18). Patient-related factors influencing SVR include genotype, gender, race, insulin resistance, single nucleotide polymorphism of IL-28B (19), but most notably also adherence to therapy.
Patients who fail to receive more then 80 % of the dosage of PEGIFN and RBV during more then 80% of the expected treatment time had 50 % lower rates of SVR (20). However treatment with PEGIFN and RBV leads to a broad spectrum of, sometimes severe,

side effects in the majority of patients for which dose reductions and even discontinuation of therapy is often inescapable. These severe and the most common side effects are briefly discussed below.

Side effects of PEGIFN and RBV

Disturbance of auto-immunity Introduction of PEGIFN and RBV can induce systemic autoimmune diseases such as sarcoidosis, systemic lupus erythematosus, rheumatoid arthritis and diabetes mellitus (21-25). In addition, possibly involving the same pathogenesis, various skin abnormalities are described such as eczema, psoriasis and pyoderma gangrenosum as side effect of PEGIFN and RBV (26-27). Again possibly as a manifestation of immune disturbance, pneumonitis is one of the broad spectrum of pulmonary problems PEGIFN and RBV can give (28).

Signaling of the central nervous system (PEG)IFN and RBV have – via several pathways - effects on the central nervous system. Immediately after the start of treatment, this results in flu-like symptoms including fever, malaise and pains in bones, joints and muscles. As a more subacute extension of this flu-like syndrome, treatment with (PEG)IFN is often associated with enduring malaise, lethargy and bodily pains. Central effects of cytokines therapy also manifest in psychiatric side effects including depression and even suicide, anxiety, increased irritability, sleeping disorders and impaired concentration (29). Neurological problems like parkinsonism (30), Bell's palsy (31) and tinnitus (32) are also reported. Fatigue and decreased appetite with weight loss are common.

Inhibition of cell proliferation Bone marrow depression due to (PEG)IFN is common resulting in anemia, thrombopenia and neutropenia with risk of bleeding (33) and infection (34). As RBV is associated with hemolysis, blood transfusion is sometimes necessary. Thinning of hair and alopecia are frequently observed (35). Gastro-intestinal complaints such as diarrhoea, nausea and vomiting are frequently reported and might also be related to inhibition of cell proliferation.

Cardiovascular complications Arrhythmia, cardiomyopathy and myocardial infarction are rare but can be fatal (36-37). Retinopathy, mostly asymptomatic, is quite commonly caused by PEGIFN, but also thrombosis of the retinal vein is described (38-39).

The above mentioned side effects frequently necessitate premature termination of treatment; Psychiatric side effects are the major reason of discontinuation of (PEG)IFN and RBV in registration trials (13, 40-41).

Aim and outline of the thesis

Dose reduction or cessation of therapy negatively impacts the efficacy of antiviral treatment and psychiatric side effects are the most frequent reason for dose reduction or cessation of therapy. In response, to prevent suboptimal treatment due to PEGIFN-induced psychopathology, one can closely monitor the psychiatric status of the patients on antiviral therapy and treat the emerging psychiatric disturbance, for instance in close cooperation with a psychiatrist. When adopting that strategy, it would be helpful, to identify patients at increased risk for developing cytokine-induced psychiatric side effects. Another strategy (if proven feasible and effective) would be to try to prevent the occurrence of psychiatric side effects, e.g. by psychopharmacological prophylactic treatment. Finally, PEGIFN-induced psychopathology can also be conceived as an 'experiment of nature' which provides an opportunity to study the pathophysiology of depression, anger, anxiety and sickness behavior.

The main focus of this thesis is on PEGIFN-induced psychopathology.

Chapter 1 reviews its current clinical features and its biochemical background.

SSRI's (Selective Serotonin Reuptake Inhibitors) are proven to be effective in the treatment of PEGIFN-induced psychopathology (42-44), but the effect of prophylactic treatment is still unclear. **Chapter 2** discusses the prophylactic treatment with the SSRI escitalopram compared to placebo in the prevention of psychiatric side effects in a randomized clinical trial.

Chapter 3 describes in a substudy of the previously mentioned randomized clinical trial the predictors of psychopathology to identify patient patients at risk for cytokine-induced psychopathology.

PEGIFN induces changes in the cytokine network, the hypothalamic-pituitary-adrenal axis, the hypothalamic-pituitary-thyroid axis and in the brain serotonin (5-HT) function (45). **Chapter 4 and 5** describes the peripheral parameters reflecting the brain metabolism before, during and after treatment with (PEG)IFN and RBV together with clinical parameters of psychiatric disturbances.

In animals, RBV has repeatedly been proven to be teratogenic (46-47). Little is known about the effects on offspring of males treated with PEGIFN and RBV. There are some reports of miscarriage but most pregnancies resulted in normal live born infants (48-50). It is however known that RBV is detectable in semen during antiviral treatment (51) 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. It is however questionable whether this is necessary. **Chapter 6** describes not only changes in sperm motility and morphology during and after treatment with PEGIFN and RBV, but also sperm DNA integrity. Sperm DNA integrity is currently the most reliable predictor of spontaneous pregnancies and the occurrence of miscarriage.

Patients chronically infected with hepatitis C virus (HCV) have a decreased health related quality of life (HRQL) compared to the general population (52). Treatment of chronic HCV with (PEG)IFN and RBV further diminishes HRQL due to its side effects. However, in case of successful treatment, an improvement of HRQL-scores is observed (53-55). **Chapter 7** describes the results of an international, multicenter, randomized, controlled study in which a variety of factors associated with HRQL at baseline (treatment naïve) and the long-term effects on HRQL after a course of treatment with (PEG)IFN are studied.

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 Apr 21;244(4902):359-62.
2. Houghton M. The long and winding road leading to the identification of the hepatitis C virus. J Hepatol. 2009 Nov;51(5):939-48.
3. Kuiken C, Simmonds P. Nomenclature and numbering of the hepatitis C virus. Methods Mol Biol. 2009;510:33-53.
4. Hepatitis C--global prevalence (update). Wkly Epidemiol Rec. 1999 Dec 10;74(49):425-7.
5. Massard J, Ratziu V, Thabut D, Moussalli J, Lebray P, Benhamou Y, et al. Natural history and predictors of disease severity in chronic hepatitis C. J Hepatol. 2006;44(1 Suppl):S19-24.
6. Fattovich G, Pantalena M, Zagni I, Realdi G, Schalm SW, Christensen E. Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. Am J Gastroenterol. 2002 Nov;97(11):2886-95.
7. Veldt BJ, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, et al. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. Ann Intern Med. 2007 Nov 20;147(10):677-84.
8. Crotty S, Maag D, Arnold JJ, Zhong W, Lau JY, Hong Z, et al. The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. Nat Med. 2000 Dec;6(12):1375-9.
9. Crotty S, Cameron CE, Andino R. RNA virus error catastrophe: direct molecular test by using ribavirin. Proc Natl Acad Sci U S A. 2001 Jun 5;98(12):6895-900.
10. Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, et al. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. Hepatology. 2007 Nov;46(5):1548-63.
11. Zhang Y, Jamaluddin M, Wang S, Tian B, Garofalo RP, Casola A, et al. Ribavirin treatment up-regulates antiviral gene expression via the interferon-stimulated response element in respiratory syncytial virus-infected epithelial cells. J Virol. 2003 May;77(10):5933-47.
12. Feld JJ, Lutchman GA, Heller T, Hara K, Pfeiffer JK, Leff RD, et al. Ribavirin improves early responses to peginterferon through improved interferon signaling. Gastroenterology. 2010 Jul;139(1):154-62 e4.
13. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med. 1998 Nov 19;339(21):1485-92.
14. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet. 1998 Oct 31;352(9138):1426-32.
15. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001 Sep 22;358(9286):958-65.
16. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, 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 Mar 2;140(5):346-55.
17. McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, et al. Telaprevir for previously treated chronic HCV infection. N Engl J Med. 2010 Apr 8;362(14):1292-303.
18. Pawlotsky JM. The Results of Phase III Clinical Trial 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 Jan 19.
19. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet. 2009 Oct;41(10):1105-9.

20. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology. 2002 Oct;123(4):1061-9.
21. Adla M, Downey KK, Ahmad J. Hepatic sarcoidosis associated with pegylated interferon alfa therapy for chronic hepatitis C: case report and review of literature. Dig Dis Sci. 2008 Oct;53(10):2810-2.
22. Yan KK, Dinihan I, Freiman J, Zekry A. Sarcoidosis presenting with granulomatous uveitis induced by pegylated interferon and ribavirin therapy for Hepatitis C. Intern Med J. 2008 Mar;38(3):207-10.
23. Ho V, McLean A, Terry S. Severe systemic lupus erythematosus induced by antiviral treatment for hepatitis C. J Clin Rheumatol. 2008 Jun;14(3):166-8.
24. Yang D, Arkfeld D, Fong TL. Development of anti-CCP-positive rheumatoid arthritis following pegylated interferon-alpha2a treatment for chronic hepatitis C infection. J Rheumatol. 2010 Aug 1;37(8):1777.
25. Yamazaki M, Sato A, Takeda T, Komatsu M. Distinct clinical courses in type 1 diabetes mellitus induced by peg-interferon-alpha treatment for chronic hepatitis C. Intern Med. 2010;49(5):403-7.
26. Veldt BJ, Schalm SW, Janssen HL. Severe allergic eczema due to pegylated alpha-interferon may abate after switching to daily conventional alpha-interferon. J Clin Gastroenterol. 2007 Apr;41(4):432.
27. Yurci A, Guven K, Torun E, Gursoy S, Baskol M, Akgun H, 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 Sep;19(9):811-5.
28. Slavenburg S, Heijdra YF, Drenth JP. Pneumonitis as a consequence of (peg)interferon-ribavirin combination therapy for hepatitis C: a review of the literature. Dig Dis Sci. 2010 Mar;55(3):579-85.
29. Van Gool AR, Kruit WH, Engels FK, Stoter G, Bannink M, Eggermont AM. Neuropsychiatric side effects of interferon-alfa therapy. Pharm World Sci. 2003 Feb;25(1):11-20.
30. Almeida CM, Galvao Mde L, Ferreira PL, Braga WS. Interferon-induced Parkinsonism in a patient with chronic hepatitis C. Arq Neuropsiquiatr. 2009 Sep;67(3A):715-6.
31. Barut S, Karaer H, Oksuz E, Eken AG, Basak AN. Bell's palsy and choreiform movements during peginterferon alpha and ribavirin therapy. World J Gastroenterol. 2009 Aug 7;15(29):3694-6.
32. Johnson K, Sargent LA, Galizio C, Ubogu EE. Interferon-alpha-2b/ribavirin-induced vestibulocochlear toxicity with dysautonomia in a chronic hepatitis C patient. Eur J Gastroenterol Hepatol. 2008 Nov;20(11):1110-4.
33. 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 Sep;53(3):455-9.
34. 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 Oct;52(4):1225-31.
35. 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 Sep;19(9):817-20.
36. Sonnenblick M, Rosin A. Cardiotoxicity of interferon. A review of 44 cases. Chest. 1991 Mar;99(3):557-61.
37. Condat B, Asselah T, Zanditenas D, Estampes B, Cohen A, O'Toole D, et al. Fatal cardiomyopathy associated with pegylated interferon/ribavirin in a patient with chronic hepatitis C. Eur J Gastroenterol Hepatol. 2006 Mar;18(3):287-9.
38. d'Alteroche L, Majzoub S, Lecuyer AI, Delplace MP, Bacq Y. Ophthalmologic side effects during alpha-interferon therapy for viral hepatitis. J Hepatol. 2006 Jan;44(1):56-61.
39. Goncalves LL, Farias AQ, Goncalves PL, D'Amico EA, Carrilho FJ. Branch retinal vein thrombosis and visual loss probably associated with pegylated interferon therapy of chronic hepatitis C. World J Gastroenterol. 2006 Jul 28;12(28):4602-3.
40. Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. N Engl J Med. 1998 Nov 19;339(21):1493-9.

41. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002 Sep 26;347(13):975-82.
42. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med. 2001 Mar 29;344(13):961-6.
43. Kraus MR, Schafer A, Scheurlen M. Paroxetine for the prevention of depression induced by interferon alfa. N Engl J Med. 2001 Aug 2;345(5):375-6.
44. Kraus MR, Schafer A, Schottker K, Keicher C, Weissbrich B, Hofbauer I, et al. Therapy of interferon-induced depression in chronic hepatitis C with citalopram: a randomised, double-blind, placebo-controlled study. Gut. 2008 Apr;57(4):531-6.
45. Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2005 Feb;29(2):201-17.
46. Narayana K, D'Souza UJ, Rao KP. Effect of ribavirin on epididymal sperm count in rat. Indian J Physiol Pharmacol. 2002 Jan;46(1):97-101.
47. Narayana K, D'Souza UJ, Seetharama Rao KP. Ribavirin-induced sperm shape abnormalities in Wistar rat. Mutat Res. 2002 Jan 15;513(1-2):193-6.
48. Bianca S, Ettore G. Male periconceptional ribavir-ininterferon alpha-2b exposure with no adverse fetal effects. Birth Defects Res A Clin Mol Teratol. 2003 Jan;67(1):77-8.
49. De Santis M, Carducci B, Cavaliere AF, De Santis L, Lucchese A, Straface G, et al. Paternal exposure to ribavirin: pregnancy and neonatal outcome. Antivir Ther. 2003 Feb;8(1):73-5.
50. 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.
51. Hofer H, Donnerer J, Sator K, Staufer K, Scherzer TM, Dejaco C, et al. Seminal fluid ribavirin level and functional semen parameters in patients with chronic hepatitis C on antiviral combination therapy. J Hepatol. 2010 Jun;52(6):812-6.
52. Foster GR, Goldin RD, Thomas HC. Chronic hepatitis C virus infection causes a significant reduction in quality of life in the absence of cirrhosis. Hepatology. 1998 Jan;27(1):209-12.
53. Bonkovsky HL, Woolley JM. Reduction of health-related quality of life in chronic hepatitis C and improvement with interferon therapy.The Consensus Interferon Study Group. Hepatology (Baltimore, Md. 1999 Jan;29(1):264-70.
54. Ware JE, Jr., Bayliss MS, Mannocchia M, Davis GL. Health-related quality of life in chronic hepatitis C: impact of disease and treatment response. The Interventional Therapy Group. Hepatology. 1999 Aug;30(2):550-5.
55. McHutchison JG, Ware JE, Jr., Bayliss MS, Pianko S, Albrecht JK, Cort S, et al. The effects of interferon alpha-2b in combination with ribavirin on health related quality of life and work productivity. J Hepatol. 2001 Jan;34(1):140-7.

Psychopathology in hepatitis C: its clinical features and underlying biochemical mechanisms

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ABSTRACT

Patients with a chronic hepatitis C virus (HCV) infection are frequently troubled by psychiatric symptoms like depression, anxiety, anger and cognitive impairment. These symptoms already exist in the natural history of the disease and frequently worsen during treatment with peginterferon-alpha (PEGIFN). This review discusses the existing data on these symptoms and the different theories on their etiology in the natural history of the disease as well as the psychopathology induced by PEGIFN. Psychopathology due to PEGIFN has a major impact on patients; however rates of observed psychopathology differ largely due to several reasons. In treatment-naïve patients symptoms appear to be related not only to the frequent problematic social background of the HCV patient, but also to the viral infection itself. Subsequently, theories on the biochemical mechanism of PEGIFN-induced psychiatric disturbances are described. Not only changes in serotonin and its precursor tryptophan play a role, but for instance also the formation of toxic metabolites and alteration of the other monoaminergic neurotransmitters like dopamine and (nor)adrenaline. Finally management strategies are discussed. Within these strategies serotonin reuptake inhibitors play a key role in the multidisciplinary management of the HCV patients suffering from the psychiatric symptoms caused by both the infection and its treatment.

INTRODUCTION

More than 170 million people worldwide are chronically infected with hepatitis C virus (HCV) (1). This chronic infection can lead to liver fibrosis, eventually progressing to cirrhosis with complications such as liver failure, portal hypertension and hepatocellular carcinoma (2-3). Successful eradication of the virus prevents progression of liver disease and leads to improved survival (4-5). To eradicate the virus (as indicated by a sustained virological response: SVR) treatment with pegylated interferon-alpha (PEGIFN) and ribavirin (RBV) is now standard of care with SVR rates of 50-60 % (6-7). In the very near future new antiviral agents will be added to this standard of care. These protease inhibotors VICTRELIS (boceprevir) and INCIVO (telaprevir) will augment treatment responses, but (for now) will not replace the use of PEGIFN and RBV (8-11).

The treatment with PEGIFN and RBV is hampered by, sometimes severe, side effects such as induction of auto-immune diseases, pancytopenia with infection and bleeding, but also psychopathology such as depression, anxiety, irritability and impaired concentration. The addition of the new agents may shorten treatment duration in selected cases, but probably will not decrease the frequencies of side effects, as these new agents cause for example dysgeusia, rash with pruritus and anemia. Notably, already in earlier stages of the disease and without receiving cytokine-based treatment, patients chronically infected with HCV are thought to have increased levels of both somatic and neuropsychiatric complaints, possibly in part as a direct result of the viral infection.

The clinical features of these neuropsychiatric symptoms in treatment naïve patients and in patients treated with PEGIFN will be discussed in this review. Subsequently, theories on the biochemical mechanism of these PEGIFN-induced psychiatric disturbances and management strategies will be described.

Psychopathology in the natural history of HCV

Several studies have shown impaired quality of life and mood disturbances in patients with a chronic liver disease (12-13), more detailed in those with a viral hepatitis (14) and especially in patients with a chronic HCV infection (15-16). In a population of veterans with HCV (n=783), 34 % had symptoms of moderate to severe depression and 37 % used antidepressants (15). In a similar HCV population (n=62) high scores were observed on questionnaires assessing anger (50%), anxiety (30.6 %) and depression (22.6%) (16).

There are several theories to explain this phenomenon. As a major part of these patients

are infected via intravenous drug use, social factors and background are supposed to play an important role (17-18). However in a population of HCV patients without a background of drug abuse and with a stable social background the presence of neuropsychiatric symptoms and impaired mood (depression and anxiety) was increased compared to healthy controls (19). Therefore, some authors suggest that the social stigmatization that HCV patients experience could contribute (20-21) or that processes or self-perception (awareness of having a chronic infectious) disease would play an important role (22).

Several observations support the theory that HCV itself is responsible for these psychopathological symptoms and decrease in quality of life. The presence of a chronic infection possibly induces a state of so called sickness behavior, characterized among other by depressive symptoms, fatigue, sleep disturbance and concentration impairment (23). Possibly in line with this, patients with HCV display lower levels of tryptophan (TRP), the precursor of serotonin (5-HT) compared to healthy controls, which might lead to mood lowering (24). In addition cerebral proton magnetic resonance spectroscopy showed alterations in HCV patients, suggesting altered monoaminergic neurotransmission (25). Finally, several studies demonstrated the presence of hepatitis C virus in the brain and cerebral spinal fluid, which also might compromise cerebral function and give rise to psychiatric symptoms (26-30). Resuming, psychopathology in the untreated HCV-patient is quite common and seems not only caused by psychosocial factors but also by the (chronic) presence of the virus itself.

Psychopathology due to PEGIFN

The endogenous cytokine IFN has (direct) antiviral, immuno-modulatory, and anti-angiogenic effects in the human body. Immunotherapy with exogenous administered IFN started in the eighties in patients with Hepatitis B and C infection but also in patients with melanoma, renal cell carcinoma and chronic myeloid leukemia. Almost every HCV patient experiences side effects of this treatment (at least 90 %) and about 10 % develops a serious adverse event (31-32).

(PEG)IFN-induced psychopathology is one of the best-documented psychiatric side effects of medication. However, interpretation problems remain. For instance, most studies lack a control group not treated with (PEG)IFN, so it is not completely clear to which degree the reported side effects can be attributed to the direct physiological effects of the cytokine. At least a part of the observed psychopathology will be preexisting, as mentioned before,

and one can imagine that many patients with HCV, due to their personal history, are more psychologically vulnerable to the stress induced by the treatment. In addition different studies have used different methods: not only concerning the individual assessing the symptoms (e.g. hepatologist, oncologist, psychiatrist, researcher or the patient himself), but also with regard to the instruments used to rate the psychopathology. In addition, it is not self-evident if e.g. symptoms of fatigue should be viewed as somatic side effects (for instance due to anemia and hypothyroidism) or as part of a depressive syndrome. Probably as a consequence, percentages of observed psychopathology differ largely in studies in HCV-patients treated with (PEG)IFN, varying from 10-38 % of (major) depression (33-36), but when the whole cluster of psychiatric complaints is taken together (depression, irritability, anxiety and impaired cognitive functioning) percentages can increase to 57.7 % (37-39). To note the clinical relevance of these side effects, in registration trials of (PEG)IFN, psychopathology was the major reason for discontinuation of antiviral therapy and even suicides were reported (37, 40-41). Also in the registration trials of the new antiviral agents suicidal attempts and even suicides are reported (10-11).

Theories on biochemical mechanism of PEGIFN-induced psychopathology

Several theories on the biochemical routes and mechanisms by which (PEG)IFN signals the brain and evokes psychopathology have been proposed. The most important ones are discussed here. Probably, after injection of the exogenous cytokine, cascades of other cytokines are activated, that signal the brain via the vagal nerve (after activation of cytokine receptors on the nerve) and via the blood. Maybe, IFN or cytokines induced by IFN cross the blood-brain-barrier and subsequently impact on neural systems via cytokine receptors in the brain, such as those for Interleukin (IL)-1 (23). Alternatively, at the blood-brain-barrier, compounds like nitric oxide (NO) and prostaglandins might be induced that diffuse in the brain parenchyma. Furthermore, lowering of the plasma concentrations of the amino acid tryptophan (TRP) might affect neurotransmission in the brain and hormonal changes might play a role. Finally shortage of co-factors, the production of neurotoxic compounds and changes in NO might play a role. The proposed biochemical effector mechanisms are discussed shortly below.

1) Changes in the hormonal status and stress regulation Thyroid dysfunction due to (PEG)IFN is not uncommon and thyroid dysfunction and especially hypothyroidism is associated with depression (42-43). However, a clear association between levels of thyroid hormone

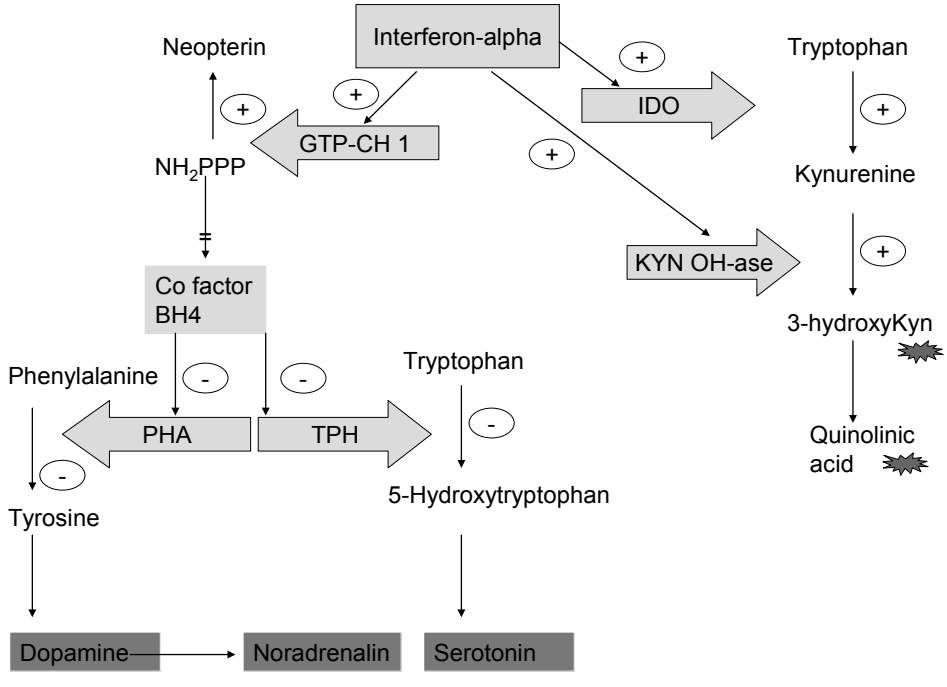
and clinical symptoms in hepatitis C-patients during (PEG)IFN therapy could not be established (44). The hypothalamic-pituitary-adrenal (HPA) axis is involved in stress regulation and dysregulation of the HPA-axis probably plays an important role in the pathogenesis of mood disorders. Also, the hypothalamic-pituitary-adrenal (HPA) axis is disturbed by (PEG)IFN treatment and accordingly, this might play a role in IFN-induced psychiatric disturbance (45-48).

2) Changes in the cytokine network Depressive disorders are associated with a proinflammatory status (23). The pro inflammatory cytokine IL-6 appears to play an important role in the pathophysiology of depression (49-51), is proven to be increased by (PEG)IFN and its concentration has even been found to be correlated with depressive symptoms and anxiety (52-53). Remarkably Bull et al. reported an association between the type of IL-6 polymorphism of patients and the PEGIFN-induced depressive symptoms (54).

3) Changes in brain monoaminergic activity (See Figure 1) As changes in monoaminergic (amongst others serotonergic and dopaminergic) neurotransmission are thought to underlie mood and anxiety disorders, selective serotonin reuptake inhibitors (SSRIs) are thought to enhance serotonergic neurotransmission. Indeed SSRIs are effective against IFN-induced depression, and IFN-induced psychiatric side effects have been attributed to changes monoaminergic neurotransmission. IFN-based treatment impacts on several parameters related to 5-HT function. For instance, two studies showed a relationship between depressive symptoms during IFN treatment and the polymorphisms in the 5-HT transporter gene (54-55). Platelet 5-HT levels are correlated with levels of 5-HT in the brain (56-57) and platelet 5-HT levels decrease during treatment with PEGIFN (58). TRP depletion (TRP is the precursor of 5-HT) is known to induce depression and anxiety in vulnerable persons (59). Possibly in line with this, plasma concentrations of TRP decrease due to (PEG)IFN and this decrease is found to be correlated in some, but not all studies, with depressive symptoms but probably also with anxiety and irritability (60-62). This decrease in TRP is thought to be induced by stimulation of indoleamine-2,3-dioxygenase by (PEG)IFN which degrades TRP to kynurenin (KYN) (63). A small study by Schaefer et al. reported an improvement in psychiatric complaints in patients with insufficient response to selective serotonin reuptake inhibitors (SSRI's), with the addition of TRP (64). Furthermore, guanosine-triphosphatecyclohydrolase-1, an enzyme responsible for the production of neopterin and tetrahydrobiopterin (BH4), is also stimulated by (PEG)IFN. BH4 plays a fundamental role in the biosynthesis of the monoamines dopamine, noradrenaline,

Figure 1 | Hypothesis on effects of administration of interferon-alpha (IFN) on pathways in the brain. IFN stimulates a) the enzyme indoleamine-2,3-dioxygenase (IDO) that converts tryptophan into kynurenine, b) the enzyme kynurenine hydroxylase (KYN OH-ase) that hydroxylates kynurenine to the neurotoxic 3-hydroxykynurenine, c) the enzyme guanosine-triphosphate-cyclohydrolase-1 (GTP-CH1) that stimulates the formation of 7,8-dihydroneopterin triphosphate (NH2PPP) from GTP. In conditions of immune activation, the synthesis and release of neopterin by activated macrophages/microglia is initiated at the expense of tetrahydrobiopterin (BH4). BH4 is an important co-factor of phenylalanine-hydroxylase (PHA) that hydroxylates phenylalanine to tyrosine, the precursor of the bioactive dopamine and noradrenaline. BH4 is also co-factor of tryptophan-hydroxylase (TPH) which converts tryptophan to 5-hydroxytryptophan, the precursor of the bioactive serotonin. Shortage of BH4 will change levels op dopamine, noradrenaline and serotonin.

(Reprinted and adapted from 'Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms; Capuron L, Schroecksnadel S, Féart C, Aubert A, Higueret D, Barberger-Gateau P, Layé S, Fuchs D; Biol Psychiatry. 2011 Jul 15;70(2):175-82 with permission from Elsevier).



adrenaline, and 5-HT (65-67) and changes in BH4 are related to mood disorders (68-69). Neopterin levels are already elevated in HCV patients compared to the general population (70-71) and further increase due to PEGIFN administration (72-73) possibly leads to BH4 depletion. Higher serum neopterin levels have been related to depression (74).

(PEG)IFN also alters nitric oxide (NO) synthesis (75-76), which in turn might alter the regulation of monoaminergic neurotransmission (77-78). Nitrate concentrations rose in

patients treated with IFN who developed a depression within four weeks after start of treatment (79). NO-synthesis is reflected by the ratio of the amino acid citrulline to arginine and this ratio decreases in patients with melanoma treated with PEGIFN (80). Finally, changes in BH4 will cause changes in NO-synthesis (81).

4) Changes in neurotoxic metabolites (See Figure 1) Cytokines induced by IFN also induce the enzyme kynurenine hydroxylase, and therefore treatment with (PEG)IFN could lead to an increase of the production of neurotoxic metabolites of KYN such as 3-hydroxykynurenine and quinolinic acid (82-83). A recent study showed changes in KYN levels and not of TRP in cerebrospinal fluid during treatment with IFN and found these changes to be correlated with depressive symptoms (84).

As an overarching theory, the induction of the sickness behaviour has been proposed to explain the psychiatric side effects of IFN-based therapy. Sickness behaviour is thought to be a common, inborn and coordinated set of adaptive behavioural changes that develop in individuals during the course of an infection or trauma, facilitating coping with the infection or trauma. Sickness behaviour in animals includes fever, lethargy, social withdrawal, loss of appetite and hyperalgesia. This behaviour would facilitate cure and counteract further spread of the infectious agent in the group. Proinflammatory cytokines (especially IL-1 and IL-6) induce this sickness behaviour and are usually terminated by endogenous anti-inflammatory molecules. In HCV patients chronic sickness behaviour could be seen due to the persistent presence of the infection, like in other groups of patients with a chronic infection or cancer. The administration of the exogenous cytokine (PEG)IFN then will maintain and even further increase symptoms of sickness behaviour, resembling symptoms of depression and other psychiatric symptoms.

Management strategies

A previous or actual history of psychiatric disturbance appears to be a predictor for PEGIFN-induced psychopathology (85-86), however not all studies confirm this (87-88). Current guidelines on treatment of HCV with PEGIFN and RBV state the need for a careful psychiatric assessment before start of treatment (6-7) with the, still present, exclusion of treatment of PEGIFN and RBV in patients with an untreated or poorly controlled psychiatric illness. A recent study showed however that with a routine medical interview preexistent psychiatric disturbances like depression can easily be overlooked, so the authors advocate a standardized manner for screening of psychopathology with (self report) questionnaires (89).

If patients with a preexisting psychiatric illness are screened and treated for (PEG)IFN induced psychopathology then they do not differ in adherence, drop-out rate or even response to therapy compared to patients without psychiatric history (86, 90-95). Also active use of illicit drugs is no longer an absolute contraindication for antiviral therapy (6). This group has a high rate of coexisting psychopathology already before treatment (95-97), so their psychopathology should also be discerned and treated carefully.

A multidisciplinary approach is needed for all groups patients to obtain good results: integrated care with a hepatologist, psychiatrist and trained nurses leads to more initiation of treatment, prevention and adequate treatment of PEGIFN-induced mood disturbances and in that way improves adherence to treatment (91, 93), also in patients without psychiatric history (98).

In research on screening for psychiatric disturbance prior to antiviral treatment a variety of observer-based and self-reporting rating scales is used, but comparative studies are lacking. The use of a combination of scales and scales focussing not only on depression, but also on anger, anxiety and other psychiatric symptoms is possibly most valuable. These scales are not only useful before the start of treatment with PEGIFN, but also during treatment. They provide a standardized manner to screen for psychopathology, such that these symptoms can be detected and treated (if necessary) early. Informing patients before treatment with PEGIFN about the symptoms they can develop will help the patients to recognize and cope with these complaints.

The key medical treatment option for psychiatric side effects is the use of SSRIs. SSRIs are proven to be effective in the treatment of PEGIFN-induced psychopathology (33, 99-100). Musselman et al. showed a beneficial effect of prophylactic use of an SSRI to prevent depression in oncology patients treated with IFN (99). In hepatitis C patients conflicting data about prophylactic use of SSRIs are reported. Kraus et al. (101) and Schaefer et al. (102) showed a beneficial effect of SSRIs in patients retreated with IFN with a previous IFN-induced depression or previous psychiatric medical history. In a randomized, placebo-controlled trial also in treatment naïve patients treated with peginterferon and ribavirine prophylactic use of SSRI's had a beneficial effect on several symptoms like sadness, hostile feelings and inner tension and also on the incidence of a major depression according the current DSM guidelines (103). Other, smaller studies in treatment-naive patients did not show this beneficial effect (104-106), or only in selected patients (107).

In summary, psychopathology in HCV patients is a common phenomenon and becomes a major problem when these patients are treated with PEGIFN. The extent of these symptoms and the underlying biochemical mechanisms become increasingly clear. Awareness of psychopathology in this setting with accurate screening and multidisciplinary treatment of (severe) psychiatric disturbances with a low threshold to use SSRIs is needed.

REFERENCES

1. Hepatitis C--global prevalence (update). Wkly Epidemiol Rec. 1999 Dec 10;74(49):425-7.
2. Fattovich G, Pantalena M, Zagni I, Realdi G, Schalm SW, Christensen E. Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. Am J Gastroenterol. 2002 Nov;97(11):2886-95.
3. Massard J, Ratziu V, Thabut D, Moussalli J, Lebray P, Benhamou Y, et al. Natural history and predictors of disease severity in chronic hepatitis C. J Hepatol. 2006;44(1 Suppl):S19-24.
4. Veldt BJ, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, et al. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. Ann Intern Med. 2007 Nov 20;147(10):677-84.
5. Backus LI, Boothroyd DB, Phillips BR, Belperio P, Halloran J, Mole LA. A Sustained Virologic Response Reduces Risk of All-Cause Mortality in Patients With Hepatitis C. Clin Gastroenterol Hepatol. 2011 Mar 11.
6. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology. 2009 Apr;49(4):1335-74.
7. Craxi A. EASL Clinical Practice Guidelines: Management of hepatitis C virus infection. J Hepatol. 2011 Feb 28.
8. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, et al. Telaprevir for retreatment of HCV infection. N Engl J Med. 2011 Jun 23;364(25):2417-28.
9. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med. 2011 Jun 23;364(25):2405-16.
10. Poordad F, McCone J, Jr., Bacon BR, Bruno S, Manns MP, Sulkowski MS, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med. 2011 Mar 31;364(13):1195-206.
11. Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med. 2011 Mar 31;364(13):1207-17.
12. Weissenborn K, Bokemeyer M, Krause J, Ennen J, Ahl B. Neurological and neuropsychiatric syndromes associated with liver disease. AIDS. 2005 Oct;19 Suppl 3:S93-8.
13. Gutteling JJ, Duivenvoorden HJ, Busschbach JJ, de Man RA, Darlington AS. Psychological determinants of health-related quality of life in patients with chronic liver disease. Psychosomatics. 2010 Mar;51(2):157-65.
14. Ozkan M, Corapcioglu A, Balcioglu I, Ertekin E, Khan S, Ozdemir S, et al. Psychiatric morbidity and its effect on the quality of life of patients with chronic hepatitis B and hepatitis C. Int J Psychiatry Med. 2006;36(3):283-97.
15. Nelligan JA, Loftis JM, Matthews AM, Zucker BL, Linke AM, Hauser P. Depression comorbidity and antidepressant use in veterans with chronic hepatitis C: results from a retrospective chart review. J Clin Psychiatry. 2008 May;69(5):810-6.
16. Rowan PJ, Al-Jurdi R, Tavakoli-Tabasi S, Kunik ME, Satrom SL, El-Serag HB. Physical and psychosocial contributors to quality of life in veterans with hepatitis C not on antiviral therapy. J Clin Gastroenterol. 2005 Sep;39(8):731-6.
17. Wilson MP, Castillo EM, Batey AM, Sapyta J, Aronson S. Hepatitis C and depressive symptoms: psychological and social factors matter more than liver injury. Int J Psychiatry Med. 2010;40(2):199-215.
18. Hauser W, Zimmer C, Schiedermaier P, Grandt D. Biopsychosocial predictors of health-related quality of life in patients with chronic hepatitis C. Psychosom Med. 2004 Nov-Dec;66(6):954-8.
19. Lowry D, Coughlan B, McCarthy O, Crowe J. Investigating health-related quality of life, mood and neuropsychological test performance in a homogeneous cohort of Irish female hepatitis C patients. J Viral Hepat. 2010 May;17(5):352-9.
20. Zacks S, Beavers K, Theodore D, Dougherty K, Batey B, Shumaker J, et al. Social stigmatization and hepatitis C virus infection. J Clin Gastroenterol. 2006 Mar;40(3):220-4.
21. Zickmund S, Ho EY, Masuda M, Ippolito L, LaBrecque DR. "They treated me like a leper". Stigmatization and the quality of life of patients with hepatitis C. J Gen Intern Med. 2003 Oct;18(10):835-44.

22. Wessely S, Pariante C. Fatigue, depression and chronic hepatitis C infection. Psychol Med. 2002 Jan;32(1):1-10.
23. Dantzer R. Cytokine, sickness behavior, and depression. Immunol Allergy Clin North Am. 2009 May;29(2):247-64.
24. Cozzi A, Zignego AL, Carpendo R, Biagiotti T, Aldinucci A, Monti M, et al. Low serum tryptophan levels, reduced macrophage IDO activity and high frequency of psychopathology in HCV patients. J Viral Hepat. 2006 Jun;13(6):402-8.
25. Weissenborn K, Ennen JC, Bokemeyer M, Ahl B, Wurster U, Tillmann H, et al. Monoaminergic neurotransmission is altered in hepatitis C virus infected patients with chronic fatigue and cognitive impairment. Gut. 2006 Nov;55(11):1624-30.
26. Laskus T, Radkowski M, Adair DM, Wilkinson J, Scheck AC, Rakela J. Emerging evidence of hepatitis C virus neuroinvasion. AIDS. 2005 Oct;19 Suppl 3:S140-4.
27. Forton DM, Allsop JM, Cox IJ, Hamilton G, Wesnes K, Thomas HC, et al. A review of cognitive impairment and cerebral metabolite abnormalities in patients with hepatitis C infection. AIDS. 2005 Oct;19 Suppl 3:S53-63.
28. Maggi F, Giorgi M, Fornai C, Morrica A, Vatteroni ML, Pistello M, et al. Detection and quasispecies analysis of hepatitis C virus in the cerebrospinal fluid of infected patients. J Neurovirol. 1999 Jun;5(3):319-23.
29. Laskus T, Radkowski M, Bednarska A, Wilkinson J, Adair D, Nowicki M, et al. Detection and analysis of hepatitis C virus sequences in cerebrospinal fluid. J Virol. 2002 Oct;76(19):10064-8.
30. Radkowski M, Wilkinson J, Nowicki M, Adair D, Vargas H, Ingui C, et al. Search for hepatitis C virus negative-strand RNA sequences and analysis of viral sequences in the central nervous system: evidence of replication. J Virol. 2002 Jan;76(2):600-8.
31. Dusheiko G. Side effects of alpha interferon in chronic hepatitis C. Hepatology. 1997 Sep;26(3 Suppl 1):112S-21S.
32. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med. 2009 Aug 6;361(6):580-93.
33. Kraus MR, Schafer A, Scheurlen M. Paroxetine for the prevention of depression induced by interferon alfa. N Engl J Med. 2001 Aug 2;345(5):375-6.
34. Horikawa N, Yamazaki T, Izumi N, Uchihara M. Incidence and clinical course of major depression in patients with chronic hepatitis type C undergoing interferon-alpha therapy: a prospective study. Gen Hosp Psychiatry. 2003 Jan-Feb;25(1):34-8.
35. Hauser P, Khosla J, Aurora H, Laurin J, Kling MA, Hill J, et al. A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. Mol Psychiatry. 2002;7(9):942-7.
36. Miyaoka H, Otsubo T, Kamijima K, Ishii M, Onuki M, Mitamura K. Depression from interferon therapy in patients with hepatitis C. Am J Psychiatry. 1999 Jul;156(7):1120.
37. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med. 1998 Nov 19;339(21):1485-92.
38. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001 Sep 22;358(9286):958-65.
39. 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 Jun;64(6):708-14.
40. Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. N Engl J Med. 1998 Nov 19;339(21):1493-9.
41. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002 Sep 26;347(13):975-82.

42. Dalgard O, Bjoro K, Hellum K, Myrvang B, Bjoro T, Haug E, et al. Thyroid dysfunction during treatment of chronic hepatitis C with interferon alpha: no association with either interferon dosage or efficacy of therapy. J Intern Med. 2002 May;251(5):400-6.
43. Jackson IM. The thyroid axis and depression. Thyroid. 1998 Oct;8(10):951-6.
44. Loftis JM, Wall JM, Linardatos E, Benvenga S, Hauser P. A quantitative assessment of depression and thyroid dysfunction secondary to interferon-alpha therapy in patients with hepatitis C. J Endocrinol Invest. 2004 Jul-Aug;27(7):RC16-20.
45. Capuron L, Raison CL, Musselman DL, Lawson DH, Nemeroff CB, Miller AH. Association of exaggerated HPA axis response to the initial injection of interferon-alpha with development of depression during interferon-alpha therapy. Am J Psychiatry. 2003 Jul;160(7):1342-5.
46. Claes SJ. CRH, stress, and major depression: a psychobiological interplay. Vitam Horm. 2004;69:117-50.
47. Raison CL, Rye DB, Woolwine BJ, Vogt GJ, Bautista BM, Spivey JR, et al. Chronic interferon-alpha administration disrupts sleep continuity and depth in patients with hepatitis C: association with fatigue, motor slowing, and increased evening cortisol. Biol Psychiatry. 2010 Nov 15;68(10):942-9.
48. Gisslinger H, Svoboda T, Clodi M, Gilly B, Ludwig H, Havelec L, et al. Interferon-alpha stimulates the hypothalamic-pituitary-adrenal axis in vivo and in vitro. Neuroendocrinology. 1993 Mar;57(3):489-95.
49. Maes M, Meltzer HY, Bosmans E, Bergmans R, Vandoolaeghe E, Ranjan R, et al. Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. J Affect Disord. 1995 Aug 18;34(4):301-9.
50. Maes M, Bosmans E, De Jongh R, Kenis G, Vandoolaeghe E, Neels H. Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. Cytokine. 1997 Nov;9(11):853-8.
51. Sluzewska A, Rybakowski J, Bosmans E, Sobieska M, Berghmans R, Maes M, et al. Indicators of immune activation in major depression. Psychiatry Res. 1996 Oct 16;64(3):161-7.
52. Bonaccorso S, Puzella A, Marino V, Pasquini M, Biondi M, Artini M, et al. Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. Psychiatry Res. 2001 Dec 15;105(1-2):45-55.
53. Wichers MC, Kenis G, Leue C, Koek G, Robaeys G, Maes M. Baseline immune activation as a risk factor for the onset of depression during interferon-alpha treatment. Biol Psychiatry. 2006 Jul 1;60(1):77-9.
54. Bull SJ, Huezo-Diaz P, Binder EB, Cubells JF, Ranjith G, Maddock C, et al. Functional polymorphisms in the interleukin-6 and serotonin transporter genes, and depression and fatigue induced by interferon-alpha and ribavirin treatment. Mol Psychiatry. 2009 Dec;14(12):1095-104.
55. Pierucci-Lagha A, Covault J, Bonkovsky HL, Feinn R, Abreu C, Sterling RK, et al. A functional serotonin transporter gene polymorphism and depressive effects associated with interferon-alpha treatment. Psychosomatics. 2010 Mar;51(2):137-48.
56. Fisar Z, Kalisova L, Paclt I, Anders M, Vevera J. Platelet serotonin uptake in drug-naive depressive patients before and after treatment with citalopram. Psychiatry Res. 2008 Nov 30;161(2):185-94.
57. McCloskey DJ, Postolache TT, Vittone BJ, Nghiem KL, Monsale JL, Wesley RA, et al. Selective serotonin reuptake inhibitors: measurement of effect on platelet function. Transl Res. 2008 Mar;151(3):168-72.
58. Schafer A, Scheurlen M, Seufert J, Keicher C, Weissbrich B, Rieger P, et al. Platelet serotonin (5-HT) levels in interferon-treated patients with hepatitis C and its possible association with interferon-induced depression. J Hepatol. 2010 Jan;52(1):10-5.
59. Young SN, Smith SE, Pihl RO, Ervin FR. Tryptophan depletion causes a rapid lowering of mood in normal males. Psychopharmacology (Berl). 1985;87(2):173-7.
60. Bonaccorso S, Marino V, Puzella A, Pasquini M, Biondi M, Artini M, et al. Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. J Clin Psychopharmacol. 2002 Feb;22(1):86-90.
61. Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M, Dantzer R. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. Mol Psychiatry. 2002;7(5):468-73.

62. Russo S, Kema IP, Haagsma EB, Boon JC, Willemse PH, den Boer JA, et al. Irritability rather than depression during interferon treatment is linked to increased tryptophan catabolism. Psychosom Med. 2005 Sep-Oct;67(5):773-7.
63. Byrne GI, Lehmann LK, Kirschbaum JG, Borden EC, Lee CM, Brown RR. Induction of tryptophan degradation in vitro and in vivo: a gamma-interferon-stimulated activity. J Interferon Res. 1986 Aug;6(4):389-96.
64. Schaefer M, Winterer J, Sarkar R, Uebelhack R, Franke L, Heinz A, et al. Three cases of successful tryptophan add-on or monotherapy of hepatitis C and IFNalpha-associated mood disorders. Psychosomatics. 2008 Sep-Oct;49(5):442-6.
65. Neurauter G, Schrocksnadel K, Scholl-Burgi S, Sperner-Unterweger B, Schubert C, Ledochowski M, et al. Chronic immune stimulation correlates with reduced phenylalanine turnover. Curr Drug Metab. 2008 Sep;9(7):622-7.
66. Widner B, Ledochowski M, Fuchs D. Interferon-gamma-induced tryptophan degradation: neuropsychiatric and immunological consequences. Curr Drug Metab. 2000 Sep;1(2):193-204.
67. Thony B, Auerbach G, Blau N. Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochem J. 2000 Apr 1;347 Pt 1:1-16.
68. Hashimoto R, Mizutani M, Ohta T, Nakazawa K, Nagatsu T. Changes in plasma tetrahydrobiopterin levels of depressives in depressive and remission phases: reconfirmed by measurement with an internal standard. Neuropsychobiology. 1994;29(2):57-60.
69. Hoekstra R, van den Broek WW, Fekkes D, Bruijn JA, Mulder PG, Pepplinkhuizen L. Effect of electroconvulsive therapy on biopterin and large neutral amino acids in severe, medication-resistant depression. Psychiatry Res. 2001 Sep 20;103(2-3):115-23.
70. Schennach H, Schoenitzer D, Fuchs D. Association between chronic hepatitis C virus infection and increased neopterin concentrations in blood donations. Clin Chem. 1998 Oct;44(10):2225-6.
71. Grungreiff K, Reinhold D, Ansorge S. Serum concentrations of sIL-2R, IL-6, TGF-beta1, neopterin, and zinc in chronic hepatitis C patients treated with interferon-alpha. Cytokine. 1999 Dec;11(12):1076-80.
72. Fuchs D, Norkrans G, Wejstal R, Reibnegger G, Weiss G, Weiland O, et al. Changes of serum neopterin, beta 2-microglobulin and interferon-gamma in patients with chronic hepatitis C treated with interferon-alpha 2b. Eur J Med. 1992 Jul-Aug;1(4):196-200.
73. Glue P, Fang JW, Rouzier-Panis R, Raffanel C, Sabo R, Gupta SK, et al. Pegylated interferon-alpha2b: pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. Clin Pharmacol Ther. 2000 Nov;68(5):556-67.
74. Celik C, Erdem M, Cayci T, Ozdemir B, Ozgur Akgul E, Kurt YG, et al. The association between serum levels of neopterin and number of depressive episodes of major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2010 Mar 17;34(2):372-5.
75. Bogdan C. Nitric oxide and the immune response. Nat Immunol. 2001 Oct;2(10):907-16.
76. MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. Annu Rev Immunol. 1997;15:323-50.
77. Kiss JP. Role of nitric oxide in the regulation of monoaminergic neurotransmission. Brain Res Bull. 2000 Aug;52(6):459-66.
78. Prast H, Philippu A. Nitric oxide as modulator of neuronal function. Prog Neurobiol. 2001 May;64(1):51-68.
79. Suzuki E, Yoshida Y, Shibuya A, Miyaoka H. Nitric oxide involvement in depression during interferon-alpha therapy. Int J Neuropsychopharmacol. 2003 Dec;6(4):415-9.
80. Fekkes D, Bannink M, Kruit WH, Van Gool AR, Mulder PG, Sleijfer S, et al. Influence of pegylated interferon-alpha therapy on plasma levels of citrulline and arginine in melanoma patients. Amino Acids. 2007 Jan;32(1):121-6.
81. Daff S. NO synthase: structures and mechanisms. Nitric Oxide. 2010 Aug 1;23(1):1-11.
82. Myint AM, Kim YK, Verkerk R, Scharpe S, Steinbusch H, Leonard B. Kynurenine pathway in major depression: evidence of impaired neuroprotection. J Affect Disord. 2007 Feb;98(1-2):143-51.

83. Wichers MC, Koek GH, Robaeys G, Verkerk R, Scharpe S, Maes M. IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. Mol Psychiatry. 2005 Jun;10(6):538-44.
84. Raison CL, Dantzer R, Kelley KW, Lawson MA, Woolwine BJ, Vogt G, et al. CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. Mol Psychiatry. 2010 Apr;15(4):393-403.
85. Scalori A, Apale P, Panizzuti F, Mascoli N, Pioltelli P, Pozzi M, et al. Depression during interferon therapy for chronic viral hepatitis: early identification of patients at risk by means of a computerized test. Eur J Gastroenterol Hepatol. 2000 May;12(5):505-9.
86. Ho SB, Nguyen H, Tetrick LL, Opitz GA, Basara ML, Dieperink E. Influence of psychiatric diagnoses on interferon-alpha treatment for chronic hepatitis C in a veteran population. Am J Gastroenterol. 2001 Jan;96(1):157-64.
87. Pariante CM, Orru MG, Baita A, Farci MG, Carpiniello B. Treatment with interferon-alpha in patients with chronic hepatitis and mood or anxiety disorders. Lancet. 1999 Jul 10;354(9173):131-2.
88. Van Thiel DH, Friedlander L, Molloy PJ, Fagiuoli S, Kania RJ, Caraceni P. Interferon-alpha can be used successfully in patients with hepatitis C virus-positive chronic hepatitis who have a psychiatric illness. Eur J Gastroenterol Hepatol. 1995 Feb;7(2):165-8.
89. Leutscher PD, Lagging M, Buhl MR, Pedersen C, Norkrans G, Langeland N, et al. Evaluation of depression as a risk factor for treatment failure in chronic hepatitis C. Hepatology. 2010 Aug;52(2):430-5.
90. Silverman BC, Kim AY, Freudenreich O. Interferon-induced psychosis as a "psychiatric contraindication" to hepatitis C treatment: a review and case-based discussion. Psychosomatics. 2010 Jan;51(1):1-7.
91. Castera L, Constant A, Henry C, Champbenoit P, Bernard PH, De Ledinghen V, et al. Impact on adherence and sustained virological response of psychiatric side effects during peginterferon and ribavirin therapy for chronic hepatitis C. Aliment Pharmacol Ther. 2006 Oct 15;24(8):1223-30.
92. Pariante CM, Landau S, Carpiniello B. Interferon alfa-induced adverse effects in patients with a psychiatric diagnosis. N Engl J Med. 2002 Jul 11;347(2):148-9.
93. Knott A, Dieperink E, Willenbring ML, Heit S, Durfee JM, Wingert M, et al. Integrated psychiatric/medical care in a chronic hepatitis C clinic: effect on antiviral treatment evaluation and outcomes. Am J Gastroenterol. 2006 Oct;101(10):2254-62.
94. Schaefer M, Hinzpeter A, Mohmand A, Janssen G, Pich M, Schwaiger M, et al. Hepatitis C treatment in "difficult-to-treat" psychiatric patients with pegylated interferon-alpha and ribavirin: response and psychiatric side effects. Hepatology. 2007 Oct;46(4):991-8.
95. Fireman M, Indest DW, Blackwell A, Whitehead AJ, Hauser P. Addressing tri-morbidity (hepatitis C, psychiatric disorders, and substance use): the importance of routine mental health screening as a component of a comanagement model of care. Clin Infect Dis. 2005 Apr 15;40 Suppl 5:S286-91.
96. Johnson ME, Fisher DG, Fenaughty A, Theno SA. Hepatitis C virus and depression in drug users. Am J Gastroenterol. 1998 May;93(5):785-9.
97. Golub ET, Latka M, Hagan H, Havens JR, Hudson SM, Kapadia F, et al. Screening for depressive symptoms among HCV-infected injection drug users: examination of the utility of the CES-D and the Beck Depression Inventory. J Urban Health. 2004 Jun;81(2):278-90.
98. Neri S, Bertino G, Petralia A, Giancarlo C, Rizzotto A, Calvagno GS, et al. A multidisciplinary therapeutic approach for reducing the risk of psychiatric side effects in patients with chronic hepatitis C treated with pegylated interferon alpha and ribavirin. J Clin Gastroenterol. 2010 Oct;44(9):e210-7.
99. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med. 2001 Mar 29;344(13):961-6.
100. Kraus MR, Schafer A, Schottker K, Keicher C, Weissbrich B, Hofbauer I, et al. Therapy of interferon-induced depression in chronic hepatitis C with citalopram: a randomised, double-blind, placebo-controlled study. Gut. 2008 Apr;57(4):531-6.

101. Kraus MR, Schafer A, Al-Taie O, Scheurlen M. Prophylactic SSRI during interferon alpha re-therapy in patients with chronic hepatitis C and a history of interferon-induced depression. J Viral Hepat. 2005 Jan;12(1):96-100.
102. Schaefer M, Schwaiger M, Garkisch AS, Pich M, Hinzpeter A, Uebelhack R, et al. Prevention of interferon-alpha associated depression in psychiatric risk patients with chronic hepatitis C. J Hepatol. 2005 Jun;42(6):793-8.
103. de Knegt RJ, Bezemer G, Van Gool AR, Drenth JP, Hansen BE, Droogleever Fortuyn HA, et al. Randomised clinical trial: escitalopram for the prevention of psychiatric adverse events during treatment with peginterferon-alfa-2a and ribavirin for chronic hepatitis C. Aliment Pharmacol Ther. 2011 Dec;34(11-12):1306-17.
104. Morasco BJ, Loftis JM, Indest DW, Ruimy S, Davison JW, Felker B, et al. Prophylactic antidepressant treatment in patients with hepatitis C on antiviral therapy: a double-blind, placebo-controlled trial. Psychosomatics. 2010 Sep-Oct;51(5):401-8.
105. 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 Nov;103(1-3):83-90.
106. Diez-Quevedo C, Masnou H, Planas R, Castellvi P, Gimenez D, Morillas RM, 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. 2010 Oct 5.
107. Raison CL, Woolwine BJ, Demetrashvili MF, Borisov AS, Weinreib R, Staab JP, et al. Paroxetine for prevention of depressive symptoms induced by interferon-alpha and ribavirin for hepatitis C. Aliment Pharmacol Ther. 2007 May 15;25(10):1163-74.

Randomized clinical trial:
escitalopram for the prevention of psychiatric adverse events during treatment with peginterferon-alfa-2a and ribavirin for chronic hepatitis C

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SUMMARY

Background Treatment of hepatitis C with peginterferon and ribavirin is associated with psychiatric side effects, frequently necessitating dose reduction or therapy cessation.

Aim: We assessed the efficacy of prophylactic escitalopram to prevent psychiatric side-effects during peginterferon and ribavirin treatment in a randomized, double-blind, placebo-controlled trial.

Methods 79 hepatitis C patients were treated with peginterferon and ribavirin. Patients received escitalopram (n=40, 10 mg) or placebo (n=39), which was initiated together with peginterferon and ribavirin. Primary outcomes were an increase of 2 points or more on the items reported sadness, inner tension and impaired concentration of the Montgomery-Asberg Depression Rating Scale, and hostile feelings of the Brief Anxiety Scale. Secondary outcome was the development of depression diagnosed by the Mini International Neuropsychiatric Interview. Measurements were performed at baseline, week 4, 12 and 24 during antiviral treatment, and 24 weeks thereafter.

Results The incidence of psychiatric side effects was significantly lower in patients treated with escitalopram compared to placebo for all primary and secondary outcomes, except for impaired concentration: reported sadness 27.5 vs. 48.7% (p=0.052), inner tension 17.5 vs. 38.5% (p=0.038), impaired concentration 55.0 vs. 66.7% (p=0.288) and hostile feelings 22.5 vs 43.6% (p=0.046) (escitalopram vs. placebo, X^2-test). The sum-scores of all four endpoints showed an overall beneficial effect of excitalopram (p=0.009, Mann-Whitney U test). Depression occurred in 12.5% of the patients in the escitalopram-group vs. 35.9% in the placebo-group (p=0.015, X^2-test).

Conclusions Prophylactic treatment with escitalopram is effective in the prevention of psychiatric side effects during interferon-based treatment of hepatitis C.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major cause of liver cirrhosis and hepatocellular carcinoma, and the most common indication for liver transplantation in Europe and the United States (1). The current standard therapy with peginterferon (PEGIFN) and ribavirin (RBV) yields sustained virological response rates (SVR) of 50-80% (2-4). However, treatment with standard interferon or PEGIFN is associated with the development of severe psychiatric side effects, most notably depression. In addition, increased hostile feelings (5), feelings of malaise and fatigue (6), concentration difficulties (7-8) and anxiety (9-10) are recognized psychiatric adverse effects.

The psychiatric side effects negatively affect quality of life during treatment and lead to early dose reduction, treatment interruption or even treatment cessation. This threatens treatment success because continued use of at least 80% of the prescribed dosage for at least 80% of the planned treatment duration, is crucial to the achievement of a sustained viral response (11). A variety of case reports and open-label studies (12) and one randomized-controlled study (13), indicate that selective serotonin inhibitors (SSRIs) alleviate overt depression developed during PEGIFN therapy. Consequently, one can adopt the strategy to closely monitor patients for the development of depression and start subsequent antidepressant treatment. However, prophylactic treatment could also be considered because this might not only be effective against the development of a full-blown depressive disorder, but also against the development of subthreshold psychiatric symptoms (e.g. depressed mood without the presence of a full-blown depressive disorder or irritability per se). Both effects will potentially result in improved quality of life during treatment for all patients, improved treatment adherence and possibly improved response rates.

We designed a randomized, double-blind, placebo-controlled trial studying the effects of prophylactic SSRI treatment on the development of psychiatric side effects of antiviral therapy in HCV. The primary aim was to investigate whether prophylactic treatment with escitalopram during treatment with PEGIFN and RBV was able to prevent psychiatric symptoms to a clinically significant degree (defined as an increase of two points on observer-based rating scales reflecting anxiety, loss of concentration, depression (Montgomery and Asberg Depression Rating Scale: MADRS), and hostile feelings (BAS: Brief Anxiety Scale). As secondary aim, we compared the incidence of syndromal depression (defined as major depression according to DSM IV criteria) according to current classification (14) between the two treatment groups.

METHODS

Setting and participants

Patients between 18-70 years of age, with chronic hepatitis C as shown by detectable serum HCV-RNA, with hepatitis C genotype 1 to 4, with an indication for antiviral therapy according to current clinical HCV guidelines, and who had provided written informed consent were considered eligible for the study. The following exclusion criteria were used: presence of contra-indications for antiviral therapy, abnormal values for thyroid stimulating hormone, a concurrent psychiatric axis I diagnosis according to DSM-IV-criteria (such as a major depressive episode, bipolar disorder or psychotic disorder), concurrent use of psychotropic drugs (such as monoamine-oxydase-inhibitors, St John's worth, lithium, serotonin-agonists and anti-epileptics), a history or other evidence of severe illness or malignancy and any other condition which would make the patient, according to the opinion of the investigator, unsuitable for the study.

The human medical ethics committees of the participating centers approved the study, the trial was registered at www.clinicaltrials.gov (Clinical Trials.gov Identifier: NCT00133276) and the study was performed according to the Declaration of Helsinki and ICH-GCP guidelines. All patients were well aware that during the study anti-depressants could be given in the absence of concurrent psychiatric disease and/or absence of a psychiatric history.

Antiviral treatment and follow-up

Antiviral therapy consisted of peginterferon alfa-2a (Pegasys®, Roche) at a dose of 180 microgram weekly subcutaneously for 24 or 48 weeks depending on genotype and response to treatment together with ribavirin (Copegus®, Roche). At the start of the trial patients with genotype 2 and 3 were treated with standard 800 mg RBV per day, however as the importance of RBV became more evident, patients with genotype 2 and 3 were also offered the same weight-based dose given to genotype 1and 4 patients. Control visits during treatment were scheduled at week 0 (baseline), week 2, 4, 8, 12, 16, 20 and 24. Week 24 indicated the end of this study. Depending on genotype and virological response treatment was continued according to international guidelines. Based on these guidelines total treatment duration was 24 weeks in genotypes 2 and 3, and 48 weeks in genotypes 1 and 4. When, in genotype 1 and 4 patients, there was no early virological response at week 12 (less than 2 log drop) or when HCV-RNA was still positive at week 24, antiviral treatment was stopped prematurely. The concomitant use of paracetamol, methadone and benzodiazepines was allowed. Twenty-four weeks after completion of antiviral therapy, a follow-up visit was scheduled.

Randomization and intervention

Patients were randomly assigned to use either escitalopram or placebo, for a period of 26 weeks, in identical looking tablets initiated together with antiviral treatment (escitalopram and placebo were provided by Lundbeck). The dose of escitalopram was 5 mg daily during the first two weeks, 10 mg once daily until week 24 and thereafter 5 mg during 2 weeks. A biostatistician made a randomization list, yielding 50 numbers with placebo and 50 numbers with verum in blocks of four. The coded list remained at the pharmacy and the clinical research department during the course of the study. The study medication and placebo were stored at the Pharmacy Department of the Erasmus MC, Rotterdam, the Netherlands and delivered in blisters of 10 tablets. Patients, investigators and treating clinicians were blind to the use of escitalopram or placebo.

Outcomes and follow-up

The primary outcome measure was pre-defined as an increase at one of the time points during treatment of two points or more compared to baseline on the items reported sadness (depressed mood), inner tension and concentration difficulties of the Montgomery–Asberg Depression Rating Scale (MADRS) or on the item hostile feelings of the Brief Anxiety Scale (BAS). The secondary outcome measure was pre-defined as the presence of syndromal depression as diagnosed by the Mini International Neuropsychiatric Interview (M.I.N.I). Patients were censored after achievement of the event.

Psychiatric evaluation took place at baseline and at 4, 12 and 24 weeks after the start of treatment, and at 24 weeks after completion of antiviral therapy. No assessments took place in case PEGIFN and RBV were stopped prematurely (e.g. because of viral non-response).

One of the investigators (GB) performed the psychiatric ratings after extensive training.

In case of emerging clinically significant psychiatric disorders, referral to the outpatient clinic of one of the psychiatric departments of the participating centers was arranged. In other instances, e.g. when depressive disorder developed at the end of the antiviral treatment and was expected to abate after cessation of antiviral therapy, management was done by the treating clinicians. The study code was broken only when clinically deemed necessary by the participating and coordinating center e.g. in order to treat with open-label antidepressant therapy. The investigator performing the psychiatric evaluations was blinded to the status of patients who required breaking of the blind.

The presence of a major depressive episode was confirmed with the relevant module of

the M.I.N.I., which is a short structured diagnostic interview for DSM-IV-TR and ICD-10 (15) psychiatric disorders and was designed to perform a short but accurate structured psychiatric interview (16). Depressive symptoms were assessed using the MADRS, a clinician rated depression scale and one of the most commonly used symptom severity scales in depression (17), consisting of 10 items each of which is scored from 0 to 6. In addition, the BAS was used specifically for the item hostility in order to assess hostile feelings (18). For confirmation, as self-report questionnaires, the Beck Depression Inventory (BDI) and the Symptom Check List-90 (SCL-90) were used. The BDI is a well-validated and reliable 21-item self-report questionnaire designed to measure depressive symptoms (19). Five somatic items (e.g. measuring fatigue and loss of appetite) of the BDI were also recorded. The SCL-90 was used to assess if other domains of psychic functioning besides mood were affected. The SCL-90 is a well-validated, multidimensional self-report symptom inventory, designed to assess various dimensions of psychopathology, including hostile feelings (20).

Statistical analysis

Based on literature of PEGIFN-induced psychopathology we estimated an incidence of the primary outcome measure of 50% in the placebo group. A reduction to 30% due to prophylactic use of escitalopram was expected. The number of patients needed was calculated using two-sided X^2-test for equal proportions and equal group size (nQuery Advisor 4.0 software, Virgina Commonwealth University, Richmond, VA, USA). For a power of 0.80 at X^2=0.05, 37 patients per arm were required. To account for potential non-evaluability, 80 patients were included.

Analysis was performed on intention-to-treat basis. The study population was defined as all patients in whom baseline psychiatric evaluation was performed and who received at least one dose of study medication in combination with one dose of PEGIFN and RBV. Study groups were defined according to their initial assignment (placebo or escitalopram). Data from all different evaluation points of the two study groups were incorporated in the analysis as long as patients were still on antiviral treatment at that point in time, irrespective if they were still using study medication, received open-label SSRI treatment or used no study medication or SSRI at all. In case of non-evaluability, the patient was censored at that time point. To test for differences between patients with escitalopram or placebo the Chi-square test of the primary outcome measures was used. Furthermore a total sum-score of the four primary outcome measures was calculated and compared with the non-parametric Mann-Whitney U-test.

The relation between patient characteristics at baseline and the primary and secondary outcomes were examined by a univariate score-test. The independence of these factors was assessed by multiple logistic regression analysis.

Mean differences of the crude observed item scores or sum scores of the BAS, MADRS, BDI and SCL-90 over the evaluation time-points during treatment (week 4, 12, 24) were analyzed with repeated measurement analysis using an unstructured covariance matrix of the time. At the time of follow-up these scores were compared between the two treatment groups with student t-test.

The level for statistical significance was set at $p<0.05$.

RESULTS

Study population

Eighty-five patients were screened and 80 patients met the eligibility criteria for the study and were randomized. Of the patients considered non-eligible, one had a history of an antidepressant-induced manic episode, one had a current depressive episode according to the M.I.N.I., one was co-infected with Human Immunodeficiency Virus, one used prohibited medication, and one reported severe psychosocial problems which were expected to hamper treatment adherence. After randomization, one patient declined treatment, so 79 patients participated in the study. Patients were enrolled between May 2005 and June 2007; 57 in Erasmus MC Rotterdam, 19 in Radboud University Nijmegen Medical Center and 3 in Academic Medical Center Amsterdam.

Baseline characteristics of the 79 patients are summarized in Table 1. In the escitalopram group, slightly more females participated and less (substance-induced) depressive episodes were reported; otherwise baseline characteristics were comparable. As diagnosed with the M.I.N.I., 24 (30%) patients had a history of one or more depressive episodes: 8 (20%) in the escitalopram group, and 16 (41%) in the placebo group ($p=0.042$, Pearson Chi-Square). Eleven patients (14%) reported having overdosed or attempted suicide. Twenty-nine patients (37%) reported a history of psychosis, mostly related to the use of illicit drugs such as cocaine and amphetamines. None of them was actively psychotic at entrance of the study. Twenty-eight patients (35%) had been hospitalized in mental health and/or addiction centers. No recurrences of psychosis were observed during the study. At the time of inclusion 22 (28%) patients had an ongoing contact with either mental health

services or addiction services. Twelve (15%) were daily users of benzodiazepines and 10 (13%) were using methadone.

Of the whole group, 47 patients (59%) were born in the Netherlands, 40 patients (51%) were employed or housewife/househusband, 33 (42%) were on a form of social benefit, one was retired (1.3%) and 5 (6.3%) had no source of income. Fifty-four patients (68%) lived with a partner and/or with children, 17 (22%) were living single and 8 (10%) were hospitalized or detained.

Figure 1 | Trial profile

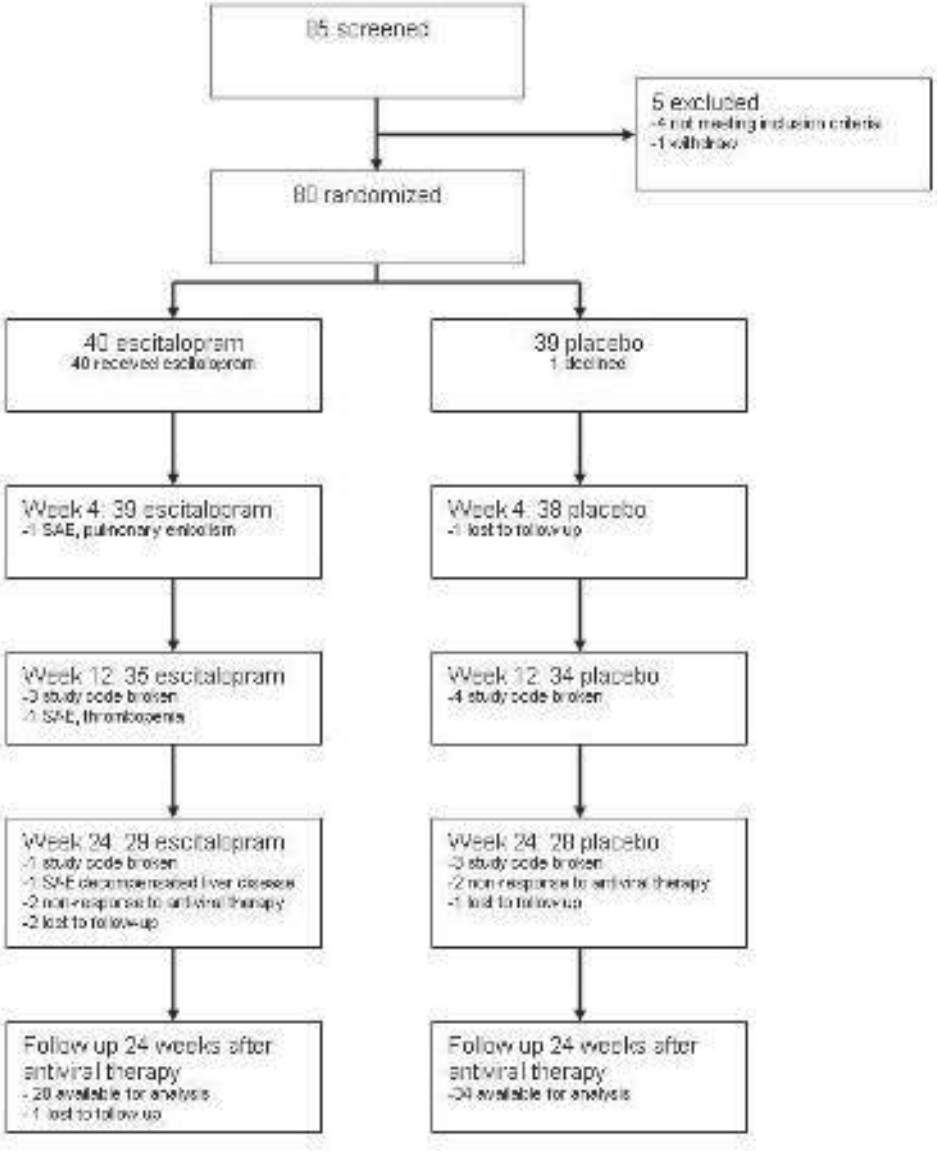
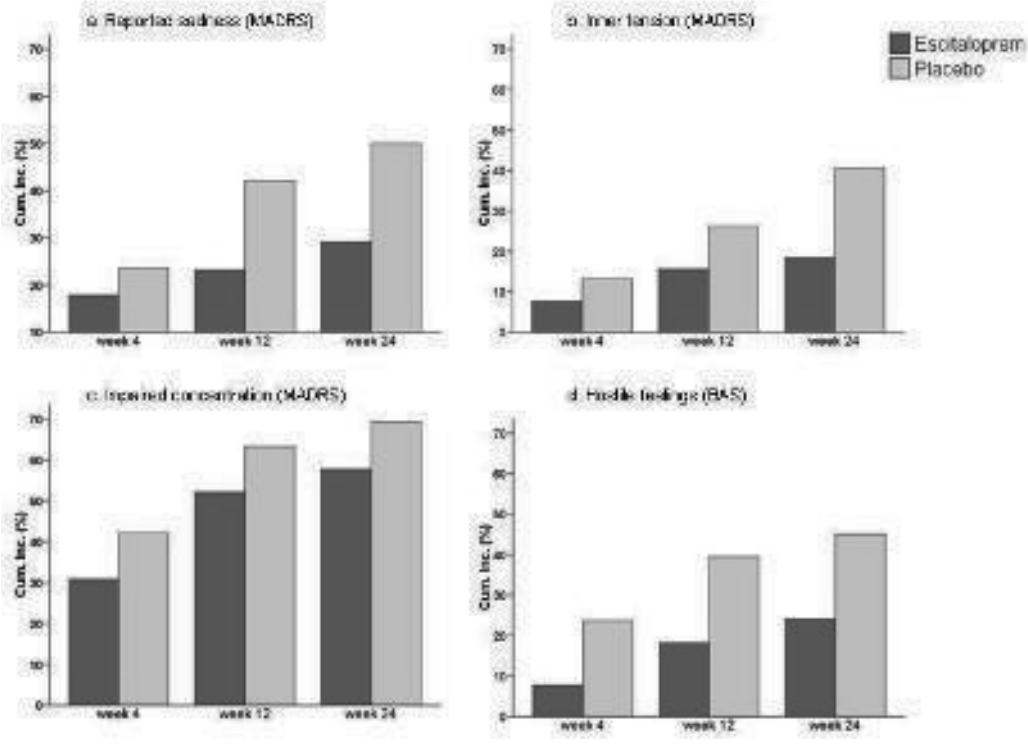


Figure 2 | Cumulative incidence (%) of primary outcomes: reported sadness, inner tension, impaired concentration (Montgomery –Asberg Depression Rating Scale (MADRS)) and Hostile feelings (Brief Anxiety Scale (BAS))



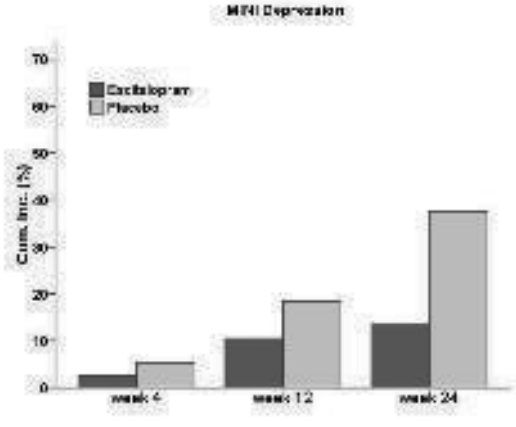
Baseline results of the scores of the different items and sum scores of the MADRS, BAS, BDI and SCL-90 were comparable between the escitalopram and placebo groups, with the exception of the item loss of interest of the MADRS. For a comparison: SCL-90 sumscores (mean±SD) at baseline were 130.64±35.9 in the escitalopram-group and 136.53±40.5 in the placebo-group; in the Dutch population mean sum scores are 108-115 in men and 117-129 in females. The flow of patients according to treatment allocation is summarized in Figure 1.

Outcome measures

The cumulative incidence of an increase of 2 points or more on the items reported sadness, inner tension of the MADRS and the item hostile feelings of the BAS and the incidence of a depressive episode according to the M.I.N.I. were all significantly more frequent ($p<0.05$) in the placebo group compared to the escitalopram group (Figure 2 and 3, and Table 2).

The use of one sum score of the four primary endpoints revealed an overall significant effect of escitalopram ($p=0.009$, Mann-Whitney U-test); the mean number of times a pa-

Figure 3|Cumulative incidence (%) of secondary outcome: MINI (Mini-International Neuropsychiatric Interview) depression according Diagnostic Statistical Manual-IV criteria



tient had an increase of 2 points or more on one of the four primary outcome measures (scoring range 0-4) was 1.23 in the escitalopram-group and 1.97 in the placebo-group. In addition, 15 patients in the escitalopram group did not have any increase (score 0) compared to 4 patients in the placebo-group. In 11 patients it was considered necessary to break the study code: in 4 patients in the escitalopram group and in 7 patients in the placebo group.

Some patient characteristics (table 1) were associated with the primary and secondary outcomes at baseline. The recent use of cannabis or hard drugs was associated with an impaired concentration (both p=0.02) at baseline, admission to an addiction facility was associated with reported sadness at baseline (p=0.01) and one or more depressive episodes prior to treatment was associated with the development of a depression during treatment ($p < 0.001$). However, after correction of these covariates with logistic regression modelling the treatment effects of escitalopram and placebo remained similar.

Additional analyses

The descriptive of the crude observed scores are given in Table 3. At week 4, 12 and 24 patients treated with escitalopram had significant lower total scores on the MADRS and lower scores on the item hostile feelings of the BAS. Specifically at week 4 and 24 the scores on the item inner tension of the MADRS were lower in the escitalopram group; there were also significant differences between escitalopram and placebo at the sleep item at week 4 and the appetite item at week 12 and 24, in favor of the escitalopram group (data not shown).

At week 4, 12 and 24 patients treated with escitalopram had lower scores on the BDI total score, BDI-somatic items, SCL total score, SCL-depression, SCL-anxiety, SCL-insufficiency in thinking and acting and SCL-hostility (Table 3). No differences at week 4 and 12 were observed between the two groups on the item SCL-somatic complaints.

Table 1 | Characteristics of all patients: general characteristics at baseline, route of transmission and genotype of hepatitis C virus, and psychiatric history, current treatment and substance abuse.

Characteristics	Escitalopram (n=40)	Placebo (n=39)	Total (n=79)
General			
Mean (SD) age, years	48.5 ± 9.7	44.6 ± 7.5	46.5 ± 8.8
Mean (SD) body weight, kg	79.1 ± 14.4	82.5 ± 14.3	80.8 ± 14.4
Mean (SD) BMI	26.1 ± 3.6	25.8 ± 4.3	26.0 ± 3.9
Sex (% males)	67.5	89.7	78.5
Route of transmission			
IVDU and/or tattoo	19	26	45
Iatrogenic	14	9	23
Sexual contact	1		1
Unknown	6	4	10
Genotype			
1 & 4	18	18	36
2 & 3	22	21	43
Psychiatric history, current treatment and substance abuse			
One or more depressive episodes	4	10	14
One or more depressive episodes including substance induced	8*	16*	24
Suicides attempts/overdose	5	6	11
Psychosis	14	15	29
Admission to psychiatric hospital	4	7	11
Admission to addiction facility	11	17	28
Current contact with Mental Health or Addiction Service	9	13	22
Daily use of: methadone or benzodiazepines	9 (6+3)	3 (2+1)	12 (8+4)
Daily us of: tobacco	25	29	54
Last week use of: alcohol	15	15	30
Last week use of: cannabis	6	10	16
Last month use of non-iv hard drugs	2	3	5

* Statistically significant difference between escitalopram and placebo group (Chi-square): p≤0.05.

Table 2 | The occurrence of psychiatric side-effects during antiviral therapy with peginterferon and ribavirin: the effects of prophylactic treatment with escitalopram or placebo.

		Escitalopram: Events (%) n=40	Placebo: Events (%) n=39	p-value (χ^2)
Increase of at least 2 points of:				
MADRS	Reported sadness	11 (27.5)	19 (48.7)	0.052
	Inner tension	7 (17.5)	15 (38.5)	0.038
	Impaired concentration	22 (55.0)	26 (66.7)	0.288
BAS	Hostile feelings	9 (22.5)	17 (43.6)	0.046
Occurrence of depression				
MINI	Depression	5 (12.5)	14 (35.9)	0.015

Table 3 | Mean scores with standard deviation of the different rating scales in the escitalopram and placebo group (at baseline, during antiviral therapy and during follow-up)

		Baseline		Week 4		Week 12
		Escitalopram	**Placebo**	**Escitalopram**	**Placebo**	**Escitalopram**
		N=40	**N=39**	**N=39**	**N=38**	**N=38**
MADRS	Sum score	4.58 ± 3.9	4.69 ± 4.7	6.69 ± 5.4	10.29 ± 7.3	8.26 ± 7.2
BAS	Hostile feelings	0.83 ± 1.0	0.95 ± 1.0	0.79 ± 1.2	1.24 ± 1.1	1.03 ± 1.4
		N=40	N=38	N=38	N=38	N=37
BDI	Sum score	9.05 ± 7.9	8.21 ± 7.7	8.87 ± 7.7	13.53 ± 10.1	10.76 ± 9.6
	Somatic items	3.25 ± 2.5	2.82 ± 2.8	4.08 ± 2.7	5.71 ± 3.7	4.57 ± 3.1
		N=39	N=38	N=37	N=38	N=37
SCL	Sum score	130.64 ± 35.9	136.53 ± 40.5	124.74 ± 29.2	156.37 ± 56.3	131.57 ± 40.9
	Depression	24.79 ± 9.3	25.74 ± 8.6	23.79 ± 8.0	29.24 ± 11.6	25.41 ± 10.4
	Anxiety	14.51 ± 5.1	14.82 ± 5.5	12.63 ± 3.4	16.66 ± 7.1	13.84 ± 4.0
	Insufficiency	14.92 ± 5.1	15.50 ± 5.0	14.24 ± 4.9	18.42 ± 7.0	15.59 ± 6.8
	Hostility	8.49 ± 3.6	8.08 ± 2.4	7.21 ± 2.0	9.63 ± 4.6	8.19 ± 2.9
	Somatic	18.33 ± 5.6	19.58 ± 6.7	20.92 ±6.5	25.32 ± 9.4	21.22 ± 6.9

All patients were asked to fill in the questionnaires, including those in whom the study code was broken. Incomplete questionnaires (administrative failure) were not taken into consideration.

Treatment effect over time was obtained using repeated measurement analysis using an unstructured covariance matrix of the time.

At follow up, escitalopram and placebo groups were compared using student t-test, * denotes significant differences ($p \leq 0.05$)

	Week 24		Treatment effect over time		Follow-up
Placebo	**Escitalopram**	**Placebo**		**Escitalopram**	**Placebo**
N=38	**N=33**	**N=34**		**N=28**	**N=34**
10.92 ± 6.6	7.39 ± 6.1	12.00 ± 6.0P=0.0024	5.04 ± 6.9	7.24 ± 7.0	
1.71 ± 1.0	0.91 ± 1.2	1.56 ± 1.3	P=0.0095	0.61 ± 1.0	0.94 ± 1.3
N=38	N=32	N=34		N=28	N=32
14.66 ± 9.2	8.09 ± 6.7	14.56 ± 8.8	P=0.0042	6.89 ± 8.6	9.59 ± 8.0
6.42 ± 3.5	3.91 ± 2.9	6.50 ± 3.4	P=0.0044	2.21 ± 2.1	4.34 ± 4.1
N=38	N=32	N=33		N=28	N=32
158,97 ± 50.0	125.59 ± 33.4	160.61 ± 50.1	P=0.0004	119.71 ± 34.8*	149.78 ± 55.6*
30,26 ± 1,2	23.94 ± 7.9	30.36 ±10.8	P=0.0043	23.11 ± 11.1	27.75 ±11.6
16,95 ± 6,5	13.00 ± 4.2	17.09 ± 6.7	P=0.0007	12.71 ± 3.4	16.38 ± 6.6
19,47 ± 7,0	15.16 ± 5.7	19.52 ± 6.9	P=0.0017	13.50 ± 5.5*	17.88 ± 7.4 *
9,76 ± 3,4	8.13 ± 3.2	9.85 ± 3.5	P=0.0028	7.46 ± 1.8	8.78 ± 3.8
24,76 ± 9,1	19.72 ± 8.1	25.21 ± 9.6	P=0.017	17.14 ± 4.6	21.75 ± 9.5

24 weeks post-antiviral treatment

At 24 weeks post-treatment, the MADRS-, BDI- and SCL-90-sum scores and BAS-hostile feelings score all returned to baseline in patients treated with escitalopram. In contrast, among the placebo-treated patients the BAS-hostile feelings score but not the MADRS-, BDI- and SCL-90-sumscores returned to baseline (Table 3).

Safety and tolerability

Three serious adverse events (SAE's) were observed during the study period. A 35-year old female developed a severe, persistent trombocytopenia; after cessation of all medication the trombocytopenia resolved. A 49-year old male, with as risk factor smoking, developed bilateral pulmonary embolism. A 53-year old female with recompensated liver cirrhosis had a new episode of decompensated liver disease with ascites. These SAE's occurred all in the escitalopram group, but none was considered to be related to the use of escitalopram. No dose reductions of PEGIFN or RBV were necessary due to psychopathology, also because of the possibility to break the study code and start open label treatment with antidepressants.

The use of escitalopram had no influence on either hematological or biochemical parameters (liver enzymes).

Response to antiviral treatment

The response to antiviral treatment in the escitalopram and in the placebo group was similar. The respective SVRs for escitalopram and placebo were, 46% and 50% for genotype 1, 83% and 80% for genotype 2 and respectively 73% and 86% for genotype 3. None of the 5 patients with genotype 4 reached SVR.

Concomitant use of benzodiazepines

The concomitant use of paracetamol and benzodiazepines was allowed. Before and after the start of antiviral therapy, the use of benzodiazepines was similar among both groups. At baseline 3 patients in the escitalopram-group and one patient in the placebo-group already used benzodiazepines. After antiviral therapy was started, 10 additional patients in the escitalopram-group and 13 in the placebo-group had benzodiazepines prescribed.

Patient withdrawal and patients lost to follow up during the study

Patient withdrawal was higher in the escitalopram-group (12 out of 40) than in the placebo- group (5 out of 39). The difference was not statistically significant (p=0.1 Fisher's Exact Test). There were several reasons for patient withdrawal: occurrence of side-effects (escitalopram three, placebo zero), viral nonresponse (escitalopram two, placebo two), and no obvious reason (escitalopram two, placebo zero). In two patients (one escitalopram, one placebo) data was not complete (administrative failure). Six patients were lost to follow up (four escitalopram, two placebo).

DISCUSSION

In this randomized double-blind placebo-controlled study, we demonstrated the efficacy of prophylactic escitalopram in the prevention of psychiatric side-effects during antiviral therapy for chronic hepatitis C. When started concomitantly with PEGIFN and RBV, escitalopram not only reduced the number of psychiatric events (increases to a clinically significant degree in depressed mood (reported sadness), anxiety (inner tension), concentration difficulties and in hostile feelings), but also the occurrence of a depressive syndrome. The study was designed such that the risk of premature discontinuation of antiviral treatment was minimized. When judged clinically necessary by the treating physician, the study code was broken and the patient was started on open label psychopharmacological treatment when indicated. Adherence to antiviral therapy was considered more important than to address the question whether prophylactic escitalopram-treatment itself would improve adherence and SVR. The concomitant use of escitalopram was safe and the SVR rates were similar in the two treatment groups. It is important to note that apart from observing a beneficial effect of escitalopram on three of the four pre-defined observer based outcome measures (symptom level), and on depression (syndromal level), these findings were corroborated with the use of self-administered questionnaires.

In agreement with the literature (7, 21-23), a substantial percentage of patients treated with placebo developed clinically relevant psychiatric side effects. Escitalopram had a beneficial effect on both the occurrence and the intensity of these side effects, such that the incidence of psychiatric side-effects was approximately halved compared to placebo. During follow up, no late adverse events related to escitalopram were observed; in contrary, although not being the aim of study, the beneficial effects of escitalopram seemed to be sustained 24 weeks after the end of antiviral therapy.

Results from three small open-label studies on the prophylactic treatment with antidepressants suggest that SSRIs are capable of preventing depression during PEGIFN-based treatment for HCV infection (24-26). In the first study, eight patients underwent successful antiviral re-treatment with concomitant SSRI prophylaxis with lower depression scores compared to those during the initial PEGIFN and RBV treatment (24). In the second study, 11 patients who were scheduled for IFN-based treatment and who were considered to have a clinical indication for antidepressant treatment, received SSRI prophylaxis. A comparison was made with 11 patients with a comparable psychiatric history and with 11 patients without such a history. In the prophylaxis group, less depression occurred (25). In the third study, 10 patients with a past history of but no current depression, were treated with escitalopram during antiviral treatment. Depression could be prevented in 9 out of 10 patients (26).

By now, five randomized controlled trials on prophylaxis with SSRIs have been performed. In oncology patients treated with high-dose IFN, paroxetine successfully attenuated depression (27). However, no beneficial effect could be demonstrated in the four trials in HCV patients. The first HCV-trial enrolled only 33 patients and different formulations of antiviral therapy were used (28). The second trial, performed by the same investigators but now using PEGIFN-based therapy only, did also not show a beneficial effect of paroxetine over placebo. However, this study had to be concluded prematurely because of recruitment difficulties (29). The third study was also closed prematurely due to a slow inclusion and had a very high drop-out rate 29 out of 61 patients) (30). The fourth study was the largest, studying the effects of 14 weeks escitalopram vs. placebo in 129 patients with hepatitis C treated with PEGIFN plus RBV (31). In comparison with other studies, the occurrence of depression was low. No beneficial effect of escitalopram could be demonstrated. With the appropriate sample size and power and 24-week treatment duration, we could demonstrate a significant beneficial effect of prophylactic treatment with escitalopram. A strong point of this study is the fact that those separate individual items considered to represent the most important types of IFN-induced psychopathology (reported sadness (i.e. mood lowering), inner tension (i.e. anxiety), concentration difficulties and aggression) were used as primary outcomes instead of only total composite scores or the occurrence of a full blown depression (32-35). The study-design was practically-oriented with the use of a fixed dose of escitalopram, the permitted use of psychotropic medication such as methadone and benzodiazepines, the concomitant start of escitalopram and

antiviral therapy, and with demographic characteristics of the participating HCV patients fitting well into every day practice of Western hospitals.

Although no official data is available, in Dutch clinical practice many HCV patients have a history of intravenous drug use and/or tattoo. For a comparison, US-veterans with HCV show an increased prevalence of psychiatric history and/or use of antidepressants (36), and a major part of these patients has been infected via intravenous drugs. But also HCV itself may induce psychopathological symptoms, suggested by a study showing increased presence of neuropsychiatric symptoms and impaired mood (depression and anxiety) in HCV patients without a background of drug abuse and with a stable social background (37). This might explain the high prevalence of a previous psychiatric history among our participating patients.

A weak point of the study might be the fact that patients with genotype 1 and 4 were treated for 48 weeks with peginterferon and ribavirin but received escitalopram or placebo for 26 weeks only. However, it is known that the occurrence of most psychiatric side-effects is within the first 24 weeks of antiviral therapy and is relatively rare thereafter (9, 38-39). One could also argue that as no lead in period with escitalopram was used, the SSRI might not have been fully active in the first weeks of antiviral therapy; consequently, the effect of prophylactic SSRI treatment might have been even greater. In addition, one might argue that despite an adequate randomization procedure, the placebo group displayed a greater vulnerability to the development of IFN-induced psychiatric disturbance, as shown by a tendency to have a history of more depressive episodes in the past and more admissions in psychiatric and addiction facilities. This might also explain why the scores of self-administered questionnaires did not return to baseline at 24 weeks post-treatment in the placebo-group. Finally, one could argue whether all or selected patients should receive prophylactic escitalopram because it cannot be excluded from this trial that patients with a psychiatric history benefit the more from escitalopram treatment; this warrants future study.

In conclusion, the substantial reduction of psychiatric side-effects in combination with the safety of escitalopram, suggests that prophylactic escitalopram treatment could be considered in hepatitis C patients treated with PEGIFN and ribavirin.

REFERENCES

1. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001;345:41-52.
2. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958-965.
3. 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. 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.
5. Russo S, Kema IP, Haagsma EB, Boon JC, Willemse PH, den Boer JA, de Vries EG, et al. Irritability rather than depression during interferon treatment is linked to increased tryptophan catabolism. Psychosom Med 2005;67:773-777.
6. Dusheiko G. Side effects of alpha interferon in chronic hepatitis C. Hepatology 1997;26:112S-121S.
7. Reichenberg A, Gorman JM, Dieterich DT. Interferon-induced depression and cognitive impairment in hepatitis C virus patients: a 72 week prospective study. Aids 2005;19 Suppl 3:S174-178.
8. Lieb K, Engelbrecht MA, Gut O, Fiebich BL, Bauer J, Janssen G, Schaefer M. Cognitive impairment in patients with chronic hepatitis treated with interferon alpha (IFNalpha): results from a prospective study. Eur Psychiatry 2006;21:204-210.
9. Bonaccorso S, Puzella A, Marino V, Pasquini M, Biondi M, Artini M, Almerighi C, et al. Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. Psychiatry Res 2001;105:45-55.
10. Pariante CM, Orru MG, Baita A, Farci MG, Carpiniello B. Treatment with interferon-alpha in patients with chronic hepatitis and mood or anxiety disorders. Lancet 1999;354:131-132.
11. 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.
12. Hauser P, Khosla J, Aurora H, Laurin J, Kling MA, Hill J, Gulati M, et al. A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. Mol Psychiatry 2002;7:942-947.
13. 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.
14. Diagnostic and Statistical Manual of mental Disorders 2000, DSM-IV TR. Arlington, USA: American Psychiatric Publishing; 2000.
15. World Health Organisation, ICD Version 2007. Available at: http://apps.who.int/classifications/apps/icd/icd10online. In.
16. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998;59 Suppl 20:22-33;quiz 34-57.
17. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry 1979;134:382-389.
18. Tyrer P, Owen RT, Cicchetti DV. The brief scale for anxiety: a subdivision of the comprehensive psychopathological rating scale. J Neurol Neurosurg Psychiatry 1984;47:970-975.
19. Beck AT, Steer RA, Carbin MG. Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. Clinical Psychology Review 1988;8:77-100.
20. Derogatis LR, Lipman RS, Covi L. SCL-90: an outpatient psychiatric rating scale--preliminary report. Psychopharmacol Bull 1973;9:13-28.

21. Asnis GM, De La Garza R, 2nd. Interferon-induced depression in chronic hepatitis C: a review of its prevalence, risk factors, biology, and treatment approaches. J Clin Gastroenterol 2006;40:322-335.
22. Leutscher PD, Lagging M, Buhl MR, Pedersen C, Norkrans G, Langeland N, Morch K, et al. Evaluation of depression as a risk factor for treatment failure in chronic hepatitis C. Hepatology 2010;52:430-435.
23. 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.
24. Kraus MR, Schafer A, Al-Taie O, Scheurlen M. Prophylactic SSRI during interferon alpha re-therapy in patients with chronic hepatitis C and a history of interferon-induced depression. J Viral Hepat 2005;12:96-100.
25. Schaefer M, Schwaiger M, Garkisch AS, Pich M, Hinzpeter A, Uebelhack R, Heinz A, et al. Prevention of interferon-alpha associated depression in psychiatric risk patients with chronic hepatitis C. J Hepatol 2005;42:793-798.
26. Gleason OC, Fucci JC, Yates WR, Philipsen MA. Preventing relapse of major depression during interferon-alpha therapy for hepatitis C--A pilot study. Dig Dis Sci 2007;52:2557-2563.
27. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, Greiner K, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med 2001;344:961-966.
28. 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.
29. Morasco BJ, Loftis JM, Indest DW, Ruimy S, Davison JW, Felker B, Hauser P. Prophylactic antidepressant treatment in patients with hepatitis C on antiviral therapy: a double-blind, placebo-controlled trial. Psychosomatics 2010;51:401-408.
30. 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.
31. 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.
32. Wade A, Friis Andersen H. The onset of effect for escitalopram and its relevance for the clinical management of depression. Curr Med Res Opin 2006;22:2101-2110.
33. Wade A, Michael Lemming O, Bang Hedegaard K. Escitalopram 10 mg/day is effective and well tolerated in a placebo-controlled study in depression in primary care. Int Clin Psychopharmacol 2002;17:95-102.
34. Lepola UM, Loft H, Reines EH. Escitalopram (10-20 mg/day) is effective and well tolerated in a placebo-controlled study in depression in primary care. Int Clin Psychopharmacol 2003;18:211-217.
35. Kennedy SH, Andersen HF, Lam RW. Efficacy of escitalopram in the treatment of major depressive disorder compared with conventional selective serotonin reuptake inhibitors and venlafaxine XR: a meta-analysis. J Psychiatry Neurosci 2006;31:122-131.
36. Rowan PJ, Al-Jurdi R, Tavakoli-Tabasi S, Kunik ME, Satrom SL, El-Serag HB. Physical and psychosocial contributors to quality of life in veterans with hepatitis C not on antiviral therapy. J Clin Gastroenterol 2005;39:731-736.
37. Lowry D, Coughlan B, McCarthy O, Crowe J. Investigating health-related quality of life, mood and neuropsychological test performance in a homogeneous cohort of Irish female hepatitis C patients. J Viral Hepat 2010;17:352-359.
38. Martin-Santos R, Diez-Quevedo C, Castellvi P, Navines R, Miquel M, Masnou H, Soler A, et al. De novo depression and anxiety disorders and influence on adherence during peginterferon-alpha-2a and ribavirin treatment in patients with hepatitis C. Aliment Pharmacol Ther 2008;27:257-265.
39. Dieperink E, Ho SB, Thuras P, Willenbring ML. A prospective study of neuropsychiatric symptoms associated with interferon-alpha-2b and ribavirin therapy for patients with chronic hepatitis C. Psychosomatics 2003;44:104-112.

Antidepressants during antiviral treatment for chronic hepatitis C infection:
a prognostic model to select patients for prophylaxis with SSRI therapy

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ABSTRACT

Objective Psychiatric side effects of interferon (IFN)-based therapy are the most important cause of early treatment discontinuation in chronic hepatitis C virus (HCV) infected patients. We aimed to identify those HCV patients who benefit most from prophylactic treatment with selective serotonin reuptake inhibitors (SSRIs) during antiviral therapy (AVT) and to quantify the effect of prophylactic treatment in these patients.

Method We analyzed risk factors for depression and studied the effect of prophylactic escitalopram on depressive symptoms among 78 HCV patients included in a prospective randomized controlled trial of escitalopram versus placebo during AVT with PEGIFN and ribavirin (RBV). At baseline, week 4, 12 and 24 of AVT, the Mini International Neuropsychiatric Interview and the Symptom Check List-90 (SCL-90) were conducted to diagnose depression and monitore depressive symptoms on treatment, respectively.

Results Depression occurred in 14 patients of the placebo-group and in 5 patients of the escitalopram-group (Pearson χ^2, p=0.01). The combination of a history of depression and previous intravenous drug use (IVDU) was independently associated with IFN-induced depression (odds ratio: 12.60; 95% confidence interval 2.47–64.34, p<0.01). Moreover, SSRI treatment was associated with a significant reduction in depressive symptoms (estimated mean depression score 35 versus 22 on the SCL-90 by week 4, for placebo versus SSRI respectively (Pearson χ^2 p=0.02).

Conclusion. HCV-infected patients with a history of depression and IVDU carry the highest risk to develop IFN-induced depression. In this subset of patients, prophylaxis with escitalopram in HCV-infected patients results in the most substantial decrease of IFN-induced depressive symptoms.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is a major health problem with 170 million people being infected worldwide. Antiviral therapy (AVT) with pegylated interferon (PEGIFN) and ribavirin (RBV) leads to sustained virologic response (SVR) in 41-84% of patients after 24 to 48 weeks of therapy. However, this AVT is associated with many side effects. In fact, psychiatric side effects of AVT have shown to be the most important cause of early treatment discontinuation. Irritability and depression are the most frequently reported psychiatric side effects with an incidence of 24% and 37% respectively (1-3). In our previously described retrospective cohort of HCV-infected patients treated with PEGIFN/RBV (4), we found a similarly high incidence of depression (30%) and association of previous intravenous drug use (IVDU) with new onset depression during AVT. Despite this high incidence of depression, depressive symptoms may be overlooked by routine clinical interviews, which are usually focused on physical rather than psychiatric complaints (3). Although new direct-acting antiviral regimens are being developed, these agents still require PEGIFN as a backbone of therapy and it can therefore be anticipated that interferon (IFN)-induced depression will remain a major challenge in the treatment of HCV.

Several baseline characteristics have been identified as predictors of depression of which higher depression scores at baseline and depression with previous IFN-based therapy are the most consistent ones in literature. A recent study suggested that antidepressant prophylaxis for each patient might prevent depressive symptoms and a major depressive episode (5), although other research groups could not demonstrate a significant advantage for SSRI prophylaxis in reducing the likelihood of developing major depression (6-7). A disadvantage of prophylactic SSRI treatment is that a significant amount of patients who would never have developed depressive symptoms would receive antidepressant treatment. Since IFN-induced depression affects only a subgroup of patients and has shown to be highly responsive to SSRI treatment, we hypothesized that the efficacy of SSRI prophylaxis in the prevention of IFN-induced depression would vary among HCV-infected patients. Therefore, we aimed to identify those HCV patients who would benefit the most from prophylactic SSRI treatment during PEGIFN/RBV and to quantify the effect of prophylactic treatment in these patients.

PATIENTS AND METHODS

We studied all patients included in a recent randomized, double-blind, placebo-controlled

trial that investigated the effect of prophylactic treatment with escitalopram versus placebo (5). We defined 'benefit' of SSRI prophylaxis as absence of a depressive syndrome according to current DSM-IV classification (8) and/or significant reduction in estimated mean depression score during AVT when dosed escitalopram instead of placebo. First, we studied baseline covariates in relation to the development of depression. After identification of baseline risk factors for the development of IFN-induced depression, we compared depression scores during AVT to identify which patients would benefit from antidepressant prophylaxis.

The original trial pursued to obtain a study population representative of the HCV-infected patient population in Western countries and therefore permitted use of psychotropic medication such as methadone and benzodiazepines. The existence of a depressive syndrome at screening was an exclusion criterion. Both treating clinician and psychiatrist were blinded with regard to type of study drug, which was initiated simultaneously with antiviral therapy (5). Psychiatric evaluation was performed at baseline, week 4, 12 and 24 of AVT and at 24 weeks of follow-up after cessation of AVT and included conduct of the relevant module of the Mini International Neuropsychiatric Interview (M.I.N.I) and the Symptom Check List-90 (SCL-90). The M.I.N.I. is a short structured diagnostic interview for DSM-IV-TR and ICD-10 psychiatric disorders and was used to diagnose the event 'depressive episode', or, briefly, 'depression'. The SCL-90 is a well-validated, multidimensional self-report symptom inventory, designed to assess various dimensions of psychopathology, including depressive symptoms. The SCL-90 was used to study the degree and course of depressive symptoms over time. The score of the depression scale is constructed with the results of 16 questions and interpreted according to its classification in one of the 7 groups (very low, low, below average, average, above average, high, very high). In the general Dutch population, the observed range is 16 – 76 with 16 – 19 corresponding to the classifications very low until below average, 20 – 23 to average, 24 to above average, 25 – 35 to high and ≥ 36 to very high (9). For our analysis, the following baseline covariates were collected: patient characteristics (gender, age, genotype 1 or other, race, living situation), data on psychiatric history (history of depression, history of psychosis, or previous suicide attempt; admission to a psychiatric hospital or admission to a center for addiction treatment; IVDU as route of transmission, former or current use of alcohol, tobacco, hard or soft drugs or psychotropic medication), routine laboratory examinations (sodium, albumin, bilirubin, prothrombin time, gammaglutaminyl transferase (GGT), alanine transaminase (ALT), thyroid function, creatinin) and two specific laboratory values hypothesized to underly psychiatric side ef-

fects of IFN-based AVT (serotonin, tryptophan) prior to initiation of AVT. Further, relation between success of AVT, SVR and risk factors for depression were investigated.

STATISTICS

Univariate logistic regression analysis was used to identify baseline factors associated with the development of depression in the placebo-group. In a multivariate logistic model, we investigated the same factors with the addition of the prophylactic therapy as to determine which factors were associated with depression in the total group with correction for study treatment (escitalopram/placebo). Determinants with statistical significance at the 0.20 level in the univariate analysis were included in the multivariate analysis with backward selection based on the likelihood ratio. Data were analyzed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). In general, strong co-linearity between two factors hampers the interpretation of the results of a multivariate logistic model. The baseline characteristic 'previous IVDU' almost fully covered patients who reported a past depressive episode. However, previous depression and psychiatric co-morbidity have been described in relation to IFN-induced depression. Therefore, we investigated these two covariates in the same multivariate model, and separately.

To study the development and severity of depressive symptoms over time, we investigated the scores on the SCL-90 subscale depression as continuous variable. The mean depression score of the SCL-90 was estimated using SAS mixed procedures with an autoregressive covariance structure taking the repeated measurement structure of the data into account. Covariates that had been identified as risk factors for depression with the above described logistic regression analysis were selected; these covariates (depression in history, previous IVDU) and the interaction with study treatment were studied in the mixed procedure as fixed effects.

RESULTS

Seventy-eight patients were included: 40 patients were randomized to escitalopram and 38 patients to placebo. Table 1a summarizes the baseline characteristics of these patients. In the escitalopram group, more female patients participated (p=0.04, Pearson Chi-square) and fewer patients had ever used hard drugs (p=0.04, Pearson Chi-square); otherwise baseline characteristics were comparable. Overall, 23 (29%) patients had a history positive for depression, 34 patients (44%) had been hospitalized in mental health centers or

Table 1a | Baseline characteristics of participants of the study on prevention of depression

		Escitalopram N=40	Placebo N=38	Total N=78
Male gender, n (%)		27 (68)*	33 (87)*	60 (77)
Age, years, mean (range)		48 (22-68)	45 (30-61)	47 (22-68)
Born in the Netherlands, n (%)		22 (55)	25 (66)	47 (60)
Living situation	with partner, n (%)	31 (78)	22 (58)	53 (68)
	other, n (%)	9 (23)	16 (42)	25 (32)
Paid job, n (%)		20 (50)	15 (39)	35 (45)
Route of transmission	intravenous drug use, n (%)	19 (48)	20 (53)	39 (50)
	other, n (%)	21 (53)	18 (47)	39 (50)
Race	Caucasian, n (%)	34 (85)	34 (89)	68 (87)
	other, n (%)	6 (15)	4 (11)	10 (8)
Genotype	1, n (%)	16 (40)	15 (39)	28 (36)
	2, n (%)	7 (18)	5 (13)	3 (4)
	3, n (%)	14 (35)	15 (39)	12 (15)
	4, n (%)	2 (3)	3 (8)	28 (36)
	Unknown, n (%)	1 (3)	0 (0)	1 (1)
Psychiatric medical history	One or more episodes of depression, n (%)	8 (20)	15 (39)	23 (29)
	Suicides attempts/overdose, n (%)	5 (13)	6 (16)	11 (14)
	Psychosis, n (%)	15 (38)	20 (53)	35 (45)
	Ever use of hard drugs, n (%)	19 (48)*	27 (71)*	46 (59)
	Intravenous use of hard drugs in past 3 months, n (%)	1 (3)	0 (0)	1 (2)
	Non-intravenous use of hard drugs in past 3 months, n (%)	2 (5)	3 (8)	5 (6)
	Admission to addiction facility, n (%)	11 (28)	17 (45)	28 (36)
	Current contact with Mental Health or Addiction Service, n (%)	9 (23)	13 (34)	22 (28)

* Statistically significant difference between escitalopram and placebo group (Pearson Chi-square, $p<0.05$)

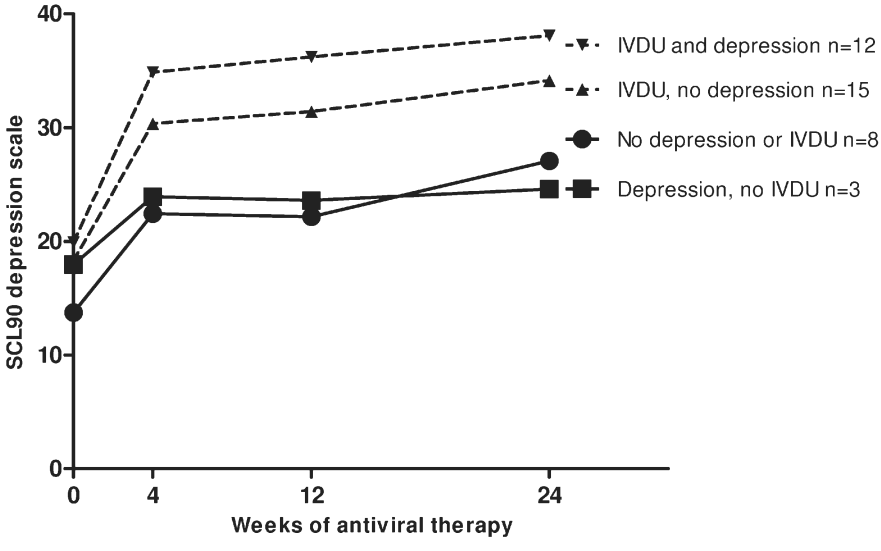
Table 1b | Results of multivariate logistic regression analyses on incidence of depression with antiviral therapy for chronic HCV

New onset depression with antiviral therapy	Placebo arm			All patients*		
Multivariate models	**OR**	**95% C.I.**	**p-value**	**OR**	**95% C.I.**	**p-value**
history of depression and IVDU * living without partner						
history of depression and IVDU	12.46	2.35 – 65.94	0.003	10.52	2.80 – 39.57	<0.001
living without partner	2.43	0.46 – 12.77	0.290	3.33	0.75 – 14.75	0.113
history of depression and IVDU * baseline serotonin level						
history of depression and IVDU	13.16	1.56 – 109.18	0.017	14.01	2.48 – 79.20	0.003
baseline serotonin level	0.93	0.84 – 1.04	0.203	0.91	0.84 – 0.99	0.105
history of depression and IVDU * gender						
history of depression and IVDU	13.53	2.55 – 71.75	0.002	10.45	2.90 – 37.70	<0.001
Gender	1.92	0.21 – 17.74	0.567	1.52	0.34 – 6.91	0.58
history of depression and IVDU * age						
history of depression and IVDU	12.48	2.37 – 65.66	0.003	10.23	2.86 – 36.59	<0.001
age	1.00	0.90 – 1.12	0.950	0.99	0.92 – 1.06	0.72

OR, odds ratio; 95% C.I., 95 percent confidence interval; BMI, body mass index; IVDU, intravenous drug use; AVT, antiviral therapy.

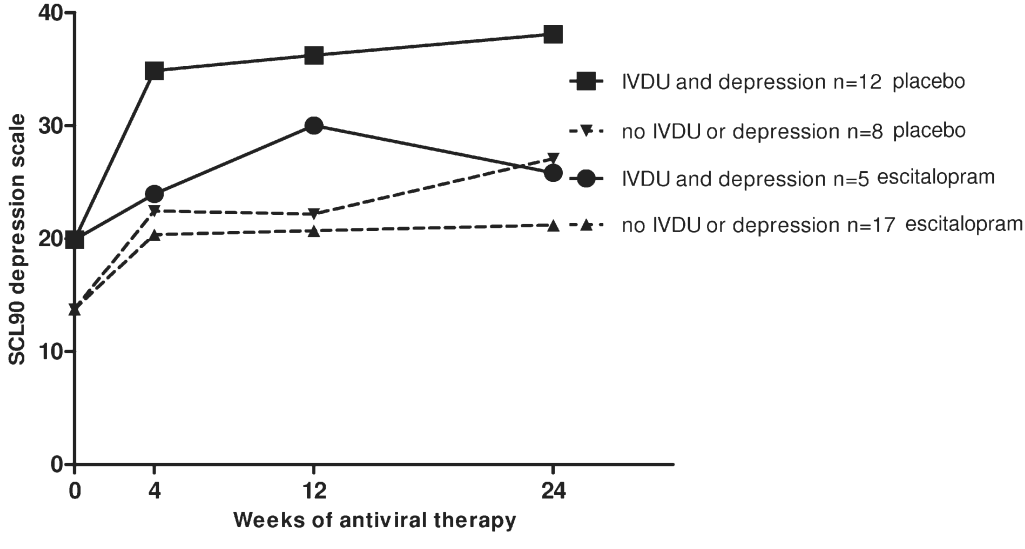
* After correction for randomization escitalopram or placebo.

Figure 1a. | Estimated mean depression scores during AVT in all placebo-dosed patients, according to the presence of depression in history or previous IVDU
AVT, antiviral therapy; IVDU, intravenous drug use



addiction centers and 22 patients (28%) were in contact with outpatient mental health service or addiction service at the time of intake. Forty-seven patients (60%) reported to have ever used hard drugs of which the majority (46 of 47 patients, 98%) reported IVDU; these 47 patients will be referred to as patients with previous IVDU from here. At screening, 6 of these 46 patients reported IVDU within the past 3 months. Baseline scores of SCL-90 were comparable between the escitalopram and placebo groups. SVR was achieved in 23 (35%) and 19 patients (29%) of the placebo and escitalopram arm, respectively (p=0.59). Depression occurred in 14 of 38 (37%) patients randomized to placebo and in 5 of 40 (13%) patients randomized to escitalopram (Pearson Chi-square, p=0.01). In the univariate logistic analysis in placebo-dosed patients, history of depression, past IVDU and IVDU as route of transmission were associated with the occurrence of depression during AVT. More importantly, the combination of history of depression and IVDU was strongly associated with the occurrence of depression. No association was found between the occurrence of depression and baseline scores of the psychiatric questionnaires or baseline laboratory results (table 1b).
Multivariate logistic regression analysis was hampered by co-linearity of history of depression and previous IVDU; 17 of the 23 patients (74%) with a history of depression had used intravenous drugs. Thirty-seven percent of patients who had ever injected drugs reported to have a history of depression. This co-linearity was even stronger with the

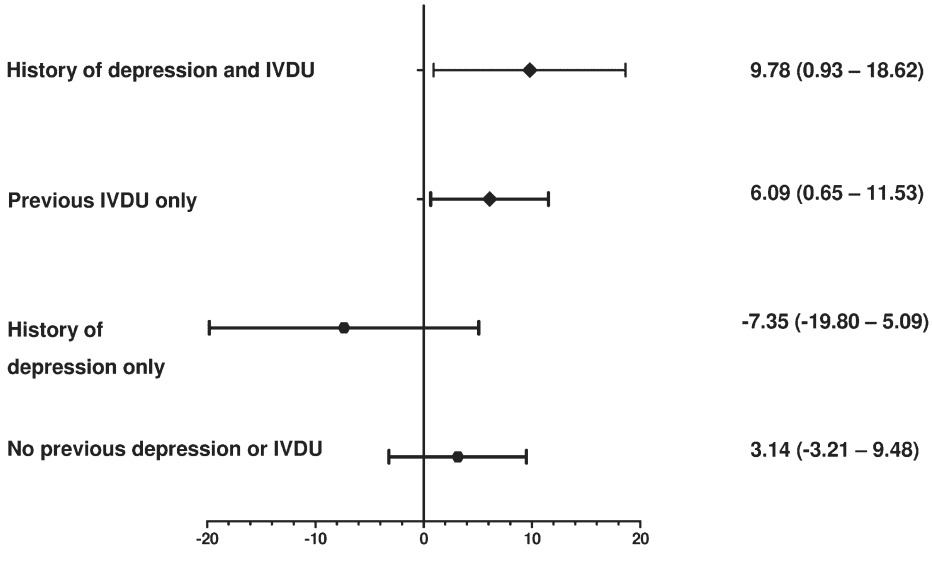
Figure 1b. | Estimated mean depression scores in patients on AVT with escitalopram or placebo, according to the presence of a history of depression and/or previous IVDU in this subgroup of patients AVT, antiviral therapy; IVDU, intravenous drug use With a history of depression and IVDU, escitalopram prevents the development of depressive symptoms (p-value 0.03).
Without a history of depression and/or IVDU, escitalopram prevents the development of depressive symptoms (p-value 0.04).



presence of the event depression; within this group 85% of patients with a history of depression reported to have ever injected drugs and 73% of patients who ever injected hard drugs had a history of depression. Multivariate analysis including the covariates "history of depression", "previous IVDU" and "gender", resulted in "history of depression" as covariate associated with the event depression: odds ratio (OR): 9.50, 95% confidence interval (C.I.) 2.08 – 43.50, p=0.004 in the placebo-arm and OR: 10.61, 95% C.I. 3.25 – 34.64, p<0.001 in the overall study group, corrected for study medication. A subsequent multivariate analysis was performed to overcome the described co-linearity, combining history of depression and previous IVDU as single covariate with other baseline covariates in a model. We included the following clinically relevant covariates in our multivariate analysis: female gender, younger age and living situation (alone or with partner) as a measure for lack of social support (table 1b) (10-13).

SVR rate was not significantly different in patients with (8, 57%) and without (34, 67%) a history of depression and IVDU (p=0.14).

Figure 2. | Estimated mean treatment effect of escitalopram on depression score during antiviral therapy, according to the presence of determinants of depression.
Mean treatment effect with 95% C.I.
IVDU, intravenous drug use; C.I., confidence interval.



Treatment effect of escitalopram.

Figure 1a shows the scores on the depression scale of the SCL-90 of placebo treated patients over time. One patient did not complete the SCL-90 questionnaire at week 0, therefore, this patients was not included in this analyses of depression score over time. In all patients, the development of depressive symptoms was most prominent within the first 4 weeks after start of AVT. Patients with a history of IVDU or depression showed significantly higher scores on the SCL-90 depression scale. Thus, patients with a history of depression and IVDU appear to be the most vulnerable subgroup for the development of depressive symptoms with AVT.

We selected patients with the presence of one of these two risk factors in both placebo arm and escitalopram arm. Subsequently, we compared the development and change in depressive symptoms over time (Figure 1b). Within this group escitalopram showed a significant decrease of 9.78 points on the depression score of patients with a history of depression and IVDU (95%C.I. 0.93 – 18.62, p=0.03) by preventing the development of depressive symptoms over time. Patients without both risk factors did not experience a

positive treatment effect against development of depressive symptoms; mean treatment effect of escitalopram was 3.14 points (95%C.I. -3.21 – 9.48, p=0.33) (figure 2).

DISCUSSION

Our study shows that chronic hepatitis C patients with a history of depression and IVDU are most vulnerable for the development of overt depression during treatment with PEGIFN and RBV. Furthermore, it shows that the development of depressive symptoms in these patients can be prevented with prophylactic treatment with the SSRI escitalopram.

Our study is important for clinical practice because it the first to identify patients at baseline who are prone for IFN-induced depression irrespective of prior IFN-treatment, and who are most likely to benefit from primary prevention with SSRI prophylaxis. Previously, several baseline covariates have been identified as risk factors for IFN-induced depression with elevation of depression scores just prior to initiation of AVT being the most consistent one (14-15). In our study we found no correlation between mood, anxiety and depression scores at baseline and depression, probably because patients with depression at baseline were excluded from the study and our study focussed on new onset IFN-induced depression. Other previously described risk factors for IFN-induced depression are depression with a previous IFN-based regimen for chronic HCV, younger age, lower social support, the personal trait “low self-directedness“ and reporting depressed feelings (10-11, 14, 16-17). We aimed to identify patients at risk for IFN-induced depression irrespective of prior treatment, as and not all patients received prior IFN-based AVT and depression is not always detected or documented. With respect to age, we could not demonstrate an association between age as continuous variable and the event depression in our study population. The other described parameters provide the possibility for identification of patients prone for depression at baseline. However, the identification of these parameters sometimes demands more than a standard medical interview. Hence, it can be difficult to apply the identification of these parameters in clinical practice. Genetic traits have also been investigated and even been identified as related to IFN-induced depression. These genetic predictors have not been confirmed in other studies yet, or have been confined to a very specific group of patients (12, 18).

Regarding prevention, previous research on prophylactic antidepressant treatment of the general HCV population has been inconsistent (5-6, 19-20). In high-dose IFN treatment for malignant melanoma, prophylactic treatment with paroxetine effectively prevented IFN-

induced depression. In HCV patients, Morasco et al (6) did demonstrate a reduction in depression of almost 50% with their double-blind placebo-controlled trial, but the study was underpowered to detect a clinically relevant significant difference. Hence, the authors concluded that prophylaxis was not beneficial. Diez-Quevedo et al (7) studied the effects of 14 weeks escitalopram vs. placebo on new onset depression in 129 HCV patients. Rates of depression were remarkably low (5.4%) and did not differ between placebo (3.2%) and escitalopram (7.6%). The results are difficult to translate to clinical practice as patients with baseline mental disorders and/or recent or concomitant drug use were excluded. This selection of patients might explain the low depression rate in the total group of patients. Schafer et al (19) demonstrated the beneficial effect of antidepressant prophylaxis in psychiatric HCV-infected patients on AVT, resulting in even lower rates of depression in HCV patients with psychiatric disorders than a control group of HCV patients without psychiatric risk factors. A recently conducted double-blind randomized placebo-controlled trial with escitalopram proved the efficacy of prophylactic treatment with a significant decrease in the incidence of depression in patients randomized to escitalopram (5). Taken these studies together, HCV-infected patients constitute a heterogenic group of patients with varying degrees of psychiatric co-morbidity and results of such trials might be difficult to compare. In combination with the fact that about 70% of HCV-infected patients will not suffer depression with IFN-based AVT, this asks for refinement of the indication for prophylactic SSRI treatment. The results of the current study suggest that it is possible to select these patients who benefit the most from SSRI prophylaxis.

An alternative strategy to prophylactic SSRI treatment is to detect and treat psychiatric symptoms as soon as they develop during AVT. Kraus et al (21) have demonstrated the high responsiveness of IFN-induced depression for serotonergic antidepressant medication, when initiated after early detection with a psychometric instrument (21). Nevertheless, we would favour pre-emptive treatment of patients at high risk for depression, rather than to wait for these symptoms to occur, especially since these symptoms may be underreported and are known to develop early in the course of AVT. Furthermore, it may be difficult to implement the use of psychological instruments in the routine clinic of somatically oriented hepatologists and infectious disease specialists. Also, inherent to the disorder, depressed patients might not be compliant to all medical appointments including the psychometric assessments. We offer a practical strategy to prevent depressive symptoms in vulnerable patients with a minimal risk of excessive treatment. Although we could not demonstrate a difference in SVR with

SSRI prophylaxis in patients who would experience less depressive symptoms during AVT, Leutscher et al (3) have shown the presence of depression to be associated with a poorer rate of SVR. Therefore, prevention of IFN-induced side effects would mean an important step forward in the treatment of chronic hepatitis C. Further, our analysis is based on a randomized-controlled trial. Both clinician and patient could be expected to be at least or even more motivated to avoid early drop-out. Therefore, it is unclear whether SSRI prophylaxis in patients prone for the development of depression and depressive symptoms would positively affect drop-out rate in clinical practice and, as a consequence, increase rate of SVR.

It should be noted that the size of our study population was limited. Therefore, less strong associations between baseline characteristics and depression may have remained undetected in our analysis. Nevertheless, the study population contained sufficient power to detect a strong association between history of depression and IVDU with PEGIFN/RBV therapy in the placebo-dosed patients and the total group of participants, when corrected for the use of SSRI. A second remark that should be made is that, for the analysis of depressive symptoms over time, we used the results of a single screening tool, i.e. the depression scale of the SCL-90. Some studies use other or multiple screening tools to describe depressive symptoms. However, the SCL-90 is a validated and frequently used screening tool for psychiatric symptoms. We diagnosed the event depression according to DSM-IV criteria and investigated predictors for depression in relation to the actual event. The SCL-90 was therefore not used as a screening tool for depression, but as a measure of degree of depressive symptoms over time.

In conclusion, prophylactic treatment with escitalopram reduces depressive symptoms significantly in HCV-infected patients with a history of both depression and IVDU during AVT and should therefore be considered in this subset of patients.

REFERENCES

1. Everson GT, Hoefs JC, Seeff LB, Bonkovsky HL, Naishadham D, Shiffman ML, Kahn JA, et al. Impact of disease severity on outcome of antiviral therapy for chronic hepatitis C: Lessons from the HALT-C trial. Hepatology 2006;44:1675-1684.
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. Leutscher PD, Lagging M, Buhl MR, Pedersen C, Norkrans G, Langeland N, Morch K, et al. Evaluation of depression as a risk factor for treatment failure in chronic hepatitis C. Hepatology 2010;52:430-435.
4. 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.
5. de Knegt RJ, Bezemer G, Van Gool AR, Drenth JP, Hansen BE, Droogleever Fortuyn HA, Weegink CJ, et al. Randomised clinical trial: escitalopram for the prevention of psychiatric adverse events during treatment with peginterferon-alfa-2a and ribavirin for chronic hepatitis C. Aliment Pharmacol Ther 2011;34:1306-1317.
6. Morasco BJ, Loftis JM, Indest DW, Ruimy S, Davison JW, Felker B, Hauser P. Prophylactic antidepressant treatment in patients with hepatitis C on antiviral therapy: a double-blind, placebo-controlled trial. Psychosomatics 2010;51:401-408.
7. 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.
8. Association AP. Diagnostic and statistical manual of mental disorders. 2000;4th, revised edition.
9. Derogatis LR, Lipman RS, Covi L. SCL-90: an outpatient psychiatric rating scale--preliminary report. Psychopharmacol Bull 1973;9:13-28.
10. Evon DM, Esserman DA, Ramcharran D, Bonner JE, Fried MW. Social support and clinical outcomes during antiviral therapy for chronic hepatitis C. J Psychosom Res 2011;71:349-356.
11. Evon DM, Ramcharran D, Belle SH, Terrault NA, Fontana RJ, Fried MW. Prospective analysis of depression during peginterferon and ribavirin therapy of chronic hepatitis C: results of the Virahep-C study. Am J Gastroenterol 2009;104:2949-2958.
12. Kraus MR, Al-Taie O, Schafer A, Pfersdorff M, Lesch KP, Scheurlen M. Serotonin-1A receptor gene HTR1A variation predicts interferon-induced depression in chronic hepatitis C. Gastroenterology 2007;132:1279-1286.
13. Schafer A, Scheurlen M, Seufert J, Keicher C, Weissbrich B, Rieger P, Kraus MR. Platelet serotonin (5-HT) levels in interferon-treated patients with hepatitis C and its possible association with interferon-induced depression. J Hepatol 2010;52:10-15.
14. 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.
15. Schaefer M, Hinzpeter A, Mohmand A, Janssen G, Pich M, Schwaiger M, Sarkar R, et al. Hepatitis C treatment in "difficult-to-treat" psychiatric patients with pegylated interferon-alpha and ribavirin: response and psychiatric side effects. Hepatology 2007;46:991-998.
16. Castellvi P, Navines R, Gutierrez F, Jimenez D, Marquez C, Subira S, Sola R, et al. Pegylated interferon and ribavirin-induced depression in chronic hepatitis C: role of personality. J Clin Psychiatry 2009;70:817-828.
17. Asnis GM, De La Garza R, 2nd. Interferon-induced depression in chronic hepatitis C: a review of its prevalence, risk factors, biology, and treatment approaches. J Clin Gastroenterol 2006;40:322-335.
18. Smith AK, Simon JS, Gustafson EL, Noviello S, Cubells JF, Epstein MP, Devlin DJ, et al. Association of a polymorphism in the indoleamine- 2,3-dioxygenase gene and interferon-alpha-induced depression in patients with chronic hepatitis C. Mol Psychiatry 2011.
19. Schaefer M, Schwaiger M, Garkisch AS, Pich M, Hinzpeter A, Uebelhack R, Heinz A, et al. Prevention of interferon-alpha associated depression in psychiatric risk patients with chronic hepatitis C. J Hepatol 2005;42:793-798.

20. Kraus MR, Schafer A, Al-Taie O, Scheurlen M. Prophylactic SSRI during interferon alpha re-therapy in patients with chronic hepatitis C and a history of interferon-induced depression. J Viral Hepat 2005;12:96-100.
21. 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.

Peginterferon-alpha induced changes in peripheral indicators of monoaminergic neurotransmission and their correlation with psychopathology

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ABSTRACT

Introduction Treatment of hepatitis C with pegylated interferon-alpha (PEGIFN) frequently leads to psychopathology such as depression, anxiety and anger. Simultaneously PEGIFN induces disturbances in peripheral indicators of brain metabolism, which led to the hypothesis that these changes are correlated (as shown in some earlier studies) thus providing information on changes in brain functioning. This study aimed to explore these changes in psychopathology and peripheral parameters of the brain metabolism and their relationship.

Methods 79 patients receiving PEGIFN and ribavirin were evaluated - at baseline, 4, 12 and 24 weeks after starting treatment, and 24 weeks after stopping treatment - with different psychometric questionnaires during treatment with either escitalopram (a selective serotonin reuptake inhibitor) or placebo in an randomized controlled trial into the prophylactic potential of escitalopram. At the same time points in 76 patients plasma levels were measured of serotonin (5-HT), its metabolite 5-hydroxyindolacetic acid (5-HIAA), its precursor tryptophan (TRP), several indicators of and co-factors of 5-HT, noradrenaline and dopamine synthesis (phenylalanine to tyrosine ratio (PHE/TYR-ratio), neopterin (NEOP), the ratio of TRP and tyrosine to other large amino acids, possibly toxic metabolites (kynurine (KYN)) and the ratio between citrulline (CITR) and arginine (ARG), an index of overall NO synthesis.

Results Decreases of TRP, CITR/ARG-ratio and 5-HT, and increases in PHE/TYR-ratio, NEOP and KYN were found. Changes in TRP levels and in the ratio of TRP to the other large neutral amino acids were not related to emerging psychopathology. Changes in PHE/TYR-ratio and TYR/LNAA-ratio were correlated with changes in mood, which indicates an important role for not only 5-HT but also noradrenaline and dopamine in the development of PEGIFN-induced psychopathology.

INTRODUCTION

A chronic infection with hepatitis C virus (HCV) can progress to severe liver damage, eventually leading to the development of end-stage liver cirrhosis and hepatocellular carcinoma. For this reason, a chronic hepatitis C-infection is the leading indication for liver transplantation in the U.S (1). The standard treatment consists of pegylated interferon-alpha (PEGIFN) and ribavirin (RBV) and successful treatment prevents the progression to liver cirrhosis. However, this treatment is associated with several, sometimes severe, side effects such as anemia, malaise and auto-immune phenomena, but also with psychopathology such as depression, aggression, anxiety and reduced concentration (2-6). Even cases of suicide attributed to treatment with PEGIFN are reported (7). SSRI's (Selective Serotonin Reuptake Inhibitors) are found to be effective in the treatment of PEGIFN-induced psychopathology (8-10).

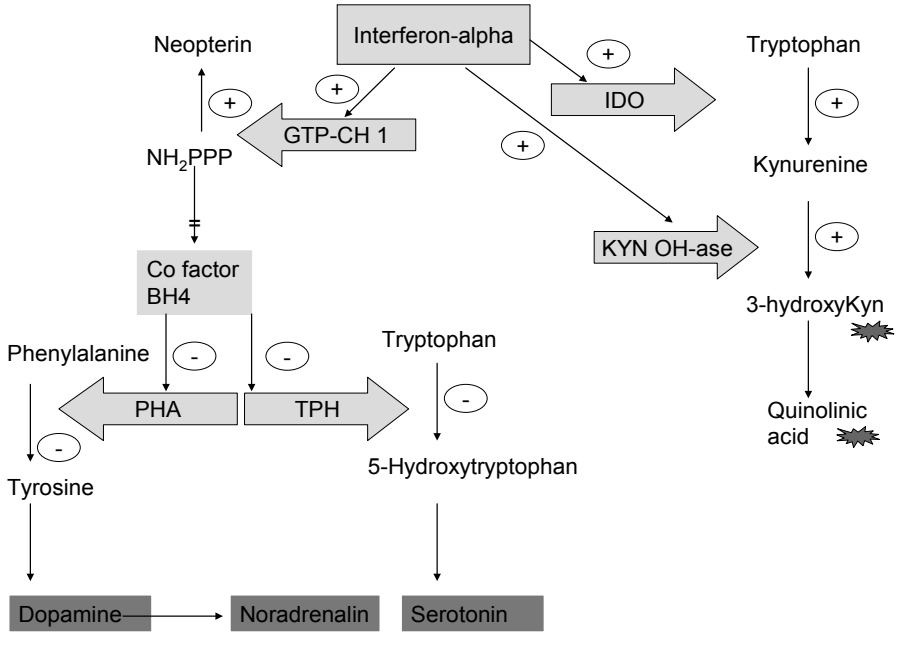
To investigate the efficacy of the SSRI escitalopram in the prevention of these psychiatric side effects, we performed a double-blind, placebo-controlled clinical trial. In the framework of this trial, we performed an extensive laboratory study into the underlying pathophysiology of PEGIFN-induced psychiatric side effects.

Changes in the cytokine network, the hypothalamic-pituitary-adrenal axis and brain monoaminergic system, including that of serotonin (5-HT) are hypothesized to cause PEGIFN-induced psychopathology (11). With regard to its influence on the central 5-HT-ergic system, firstly, (PEG)IFN induces the enzyme indoleamine 2,3-dioxygenase (IDO) catabolising the amino acid tryptophan (TRP), the precursor of 5-HT (12). At the blood-brain barrier, TRP competes with the other large neutral amino acids (LNAA: tyrosine (TYR), phenylalanine (PHE), valine, leucine and isoleucine) for gaining entrance into the central nervous system. The ratio of the concentration of TRP to those of the other LNAA (TRP/LNAA-ratio) reflects the central availability of TRP and is an index for the rate of central 5-HT synthesis. Indeed, in several studies treatment with (PEG)IFN was found to lower both TRP concentrations and the TRP/LNAA-ratio and in some (but not all) of these studies, relationships were observed between measures for depression and decrease in the TRP/LNAA-ratio (13-14). Next to depression, aggression and irritability have been found to be related to decreased concentrations of TRP (2).

With regard to the other mono-amines, tyrosine is the main precursor of dopamine and noradrenaline and its availability to the brain is reflected by the TYR/LNAA-ratio which, as TRP and TRP/LNAA-ratio, has also been found to be lowered in depressed patients (15).

Figure 1 | Hypothesis on effects of administration of interferon-alpha (IFN) on pathways in the brain. IFN stimulates a) the enzyme indoleamine-2,3-dioxygenase (IDO) that converts tryptophan into kynurenine, b) the enzyme kynurenine hydroxylase (KYN OH-ase) that hydroxylates kynurenine to the neurotoxic 3-hydroxykynurenine, c) the enzyme guanosine-triphosphate-cyclohydrolase-1 (GTP-CH1) that stimulates the formation of 7,8-dihydroneopterin triphosphate (NH2PPP) from GTP. In conditions of immune activation, the synthesis and release of neopterin by activated macrophages/microglia is initiated at the expense of tetrahydrobiopterin (BH4). BH4 is an important co-factor of phenylalanine-hydroxylase (PHA) that hydroxylates phenylalanine to tyrosine, the precursor of the bioactive dopamine and noradrenaline. BH4 is also co-factor of tryptophan-hydroxylase (TPH) which converts tryptophan to 5-hydroxytryptophan, the precursor of the bioactive serotonin. Shortage of BH4 will change levels op dopamine, noradrenaline and serotonin.

(Reprinted and adapted from 'Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms; Capuron L, Schroecksnadel S, Féart C, Aubert A, Higueret D, Barberger-Gateau P, Layé S, Fuchs D; Biol Psychiatry. 2011 Jul 15;70(2):175-82 with permission from Elsevier).



Secondly, (PEG)IFN is hypothesized to influence the metabolism of the pteridine tetrahydrobiopterin (BH4), an important cofactor in the biosynthesis of 5-HT (16), dopamine and noradrenaline: (PEG)IFN increases the concentration of neopterin (NEOP), a metabolite of one of the precursors of BH4, which might cause lower levels of BH4 resulting in a decrease in 5-HT synthesis. This may add to the reported reduction of BH4 levels by RBV (17). BH4 is also a cofactor for the enzyme (phenylalanine-hydroxylase) which hydroxyl-

ates PHE to TYR and the ratio of PHE to TYR (PHE/TYR-ratio) has been regarded as a measure of BH_4 activity (18-19) with an increased PHE/TYR-ratio suggesting reduced BH4 synthesis and resulting impaired synthesis of 5-HT, noradrenaline and dopamine.

Thirdly, the induction of IDO by (PEG)IFN increases the conversion of TRP to kynurenine (KYN) of which several metabolites are thought to have neurotoxic properties and thus may contribute to the development of depression (20-21). See figure 1.

Finally, possibly also by interfering with BH4 synthesis, PEGIFN influences the synthesis of Nitric Oxide (NO), the latter also modulating 5-HT-ergic function. The ratio between citrulline (CITR) and arginine (ARG) (CITR/ARG-ratio) is thought to reflect NO synthesis (22).

The aim of our study was to explore the influence of treatment with PEGIFN and RBV on these peripheral indices for central monoaminergic (5-HT, dopamine and noradrenaline) function, for NO-ergic function and for neurotoxic influences, and their correlation with psychological parameters.

MATERIAL & METHODS

Patients and treatment

80 patients, with a chronic hepatitis C-infection with genotype 1, 2, 3 or 4, participated in a double-blind randomized controlled trial comparing escitalopram versus placebo in the prevention of psychiatric symptoms during therapy with PEGIFNα-2a and RBV (23). During the (first) 26 weeks of treatment with PEGIFN and RBV patients received escitalopram or placebo.

Psychiatric evaluation

At baseline, week 4, 12, 24 and 24 weeks after stopping antiviral treatment (follow-up (FU)) symptoms of sadness, inner tension, impaired concentration and irritability were assessed by a trained physician using the Montgomery-Asberg Depression Rating Scale (MADRS) (24) and Brief Anxiety Scale (BAS) (25). The presence of a major depressive episode was assessed with the relevant module of the Mini-International Neuropsychiatric Interview (M.I.N.I.). The M.I.N.I. is a short structured diagnostic interview for DSM-IV TR and ICD-10 psychiatric disorders and was designed to perform a short but accurate structured psychiatric interview (26). At the same time patients completed the Beck Depression Inventory (BDI) (27) and the Symptom Check List-90 (SCL-90) as self-report questionnaires (28).

In case of a major psychopathology the study code was broken and, if indicated, open-label

psychopharmacological treatment was prescribed by a psychiatrist. This study was approved by the local Medical Ethics Committee and all patients gave written informed consent.
For further details of this study we refer to the publication by de Knegt et al. (23).

Biochemical analysis

At the same time points 5-HT, TRP, 5-HIAA (5-hydroxyindolacetic acid, the metabolite of 5-HT), LNAA, PHE, TYR, isoleucine, leucine and valine, CITR, ARG, NEOP and KYN were measured in plasma. EDTA blood was obtained by venipuncture. Almost all samples were taken in the morning, and patients were instructed to have a low-fat meal that morning. After immediate centrifugation (20 min at 2650 gmax) plasma was separated and frozen at -80°C. 5-HT and 5-HIAA were measured as described before (29). The amino acids PHE, TYR, TRP, CITR, ARG, isoleucine, leucine and valine were measured by means of high-performance liquid chromatography (30). The TRP/LNAA-ratio and TYR/LNAA-ratio were calculated as 100 times the concentration of respectively TRP and TYR divided by the summed concentrations of the other large neutral amino acids. NEOP was measured after acid oxidation as described earlier (19, 31). Concentrations of KYN were also determined by using reversed phase high performance liquid chromatography as described before (32).

Statistical analysis

All patients were included in the analysis when they still used PEGIFN, irrespective of use of (blinded) escitalopram, placebo or open-label antidepressants or other psychoactive medication, in the biochemical analysis. In case the PEGIFN was discontinued due to non-response to treatment or a serious adverse advent, data were censored from that time point for further analysis.

To explore the difference between the escitalopram and placebo group regarding the different biochemical and psychometric variables, only these patients were included in the analysis if they still used the blinded study medication. Only patients treated with (blinded) placebo were used to determine the correlation between the different biochemical variables and the observed psychopathology.

Data were stored and analysed using SPSS software, version 17.0 and SAS 9.2. Outcomes of the biochemical analysis at 4, 12, 24 and FU were compared to those at baseline applying a repeated measurements model and log transformation in case of non-normal distribution. Mean values at baseline and at FU were compared with reference values of healthy subjects (extracted from the database of the Laboratory of Psychiatry) using one-sample t-test. A bivariate model was used (with the variable t categorical and not-

significant) to estimate the correlation between changes in laboratory parameters and changes in psychiatric ratings. All reported P values are two-sided and a significance level of $\alpha \leq 0.05$ was used.

RESULTS

Patients

79 patients started treatment with PEGIFN and RBV. Characteristics at baseline are summarized in Table 1. In the escitalopram group, more females participated and less substance-induced depressive episodes were reported; otherwise baseline characteristics were comparable. As diagnosed with the M.I.N.I., 24 (30%) patients had a history of one or more depressive episodes.

Psychiatric evaluation

We observed significantly less psychiatric side effects for those treated with escitalopram as compared to placebo for all primary and secondary outcome measures, except for impaired concentration. Regarding the other psychometric measurements, patients treated with escitalopram scored significantly lower than placebo on sum scores and all subscales of the BDI and SCL-90 at all different evaluation points, except for SCL-90 somatic complaints at week 4 and 12 (Table 2). Changes in the different psychometric measurements and its differences between treatment with escitalopram and placebo were already observed from week 4 and persisted until week 24.

Biochemical analysis

One center did not participate in the biochemical analysis so 76 blood samples were obtained at baseline. At that time point no differences between patients treated with escitalopram or placebo were seen in the different laboratory parameters.

At baseline, mean values of our patients differed from mean values of the healthy subjects subjects (all $p< 0.01$) respectively 5-HIAA (33.76 ± 1.6 vs. 42 ± 11 nmol/l), TRP (43.71 ± 0.9 vs 46 ± 6.1), TYR/LNAA-ratio (14.47 ± 0.4.vs. 11.4 ± 2.17), PHE/TYR-ratio (0.82 ± 0.02 vs. 0.94 ± 0.15), NEOP (28.41 ± 1.5 vs. 16.7 ± 3.6) and 5-HT (28.47 ± 3.0 vs. 9.2 ± 5.5). The other parameters did not differ.

During antiviral treatment, at every time points and in both groups, a decrease of the concentration of TRP was observed, together with a direct and persistent increase in NEOP, KYN/TRP-ratio and PHE/TYR-ratio and decrease in CITR/ARG-ratio (additional table and Figure 2).

Table 1 | Baseline characteristics of patients

Demographic characteristics	Escitalopram (N=40)	Placebo (N=39)	Total (N = 79)
General			
Age – yr	48.5 ± 9.7	44.6 ± 7.5	46.5 ± 8.8
Body weight (kg)	79.1 ± 14.4	82.5 ± 14.3	80.8 ± 14.4
BMI	26.1 ± 3.6	25.8 ± 4.3	26.0 ± 3.9
Sex (% males)	67.5	89.7	78.5
Route of transmission			
IVDU and/or tattoo	19	26	45
Iatrogenic	14	9	23
Sexual contact	1		1
Unknown	6	4	10
Genotype			
1 & 4	18	18	36
2 & 3	22	21	43

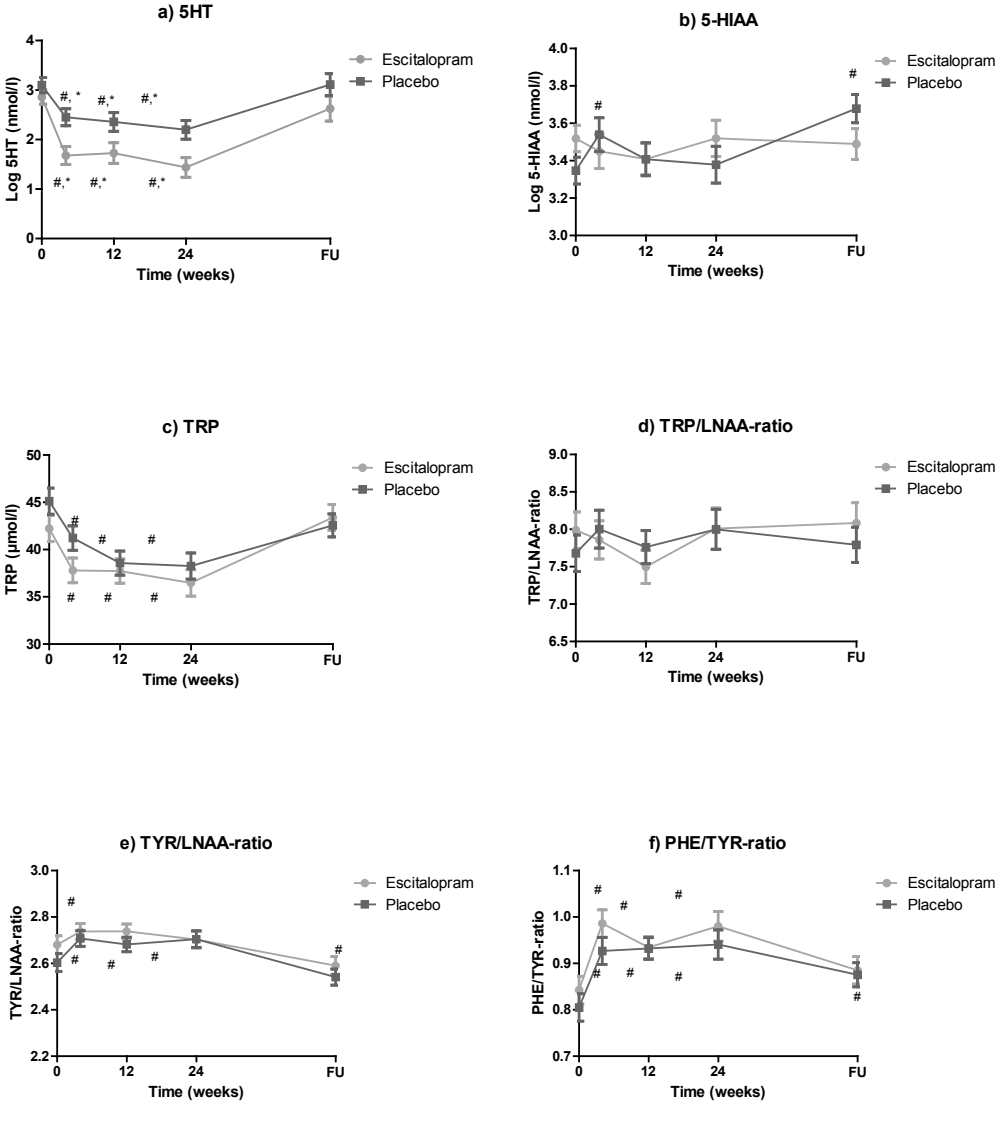
Table 2 | Mean scores with standard deviation of the different rating scales (Montgomery –Asberg Depression Rating Scale (MADRS), Brief Anxiety Scale (BAS), Beck Depression Inventory (BDI) and the Symptom Check List-90 (SCL-90)) in the escitalopram and placebo group. * = significant differences ($p < 0.05$) between escitalopram and placebo

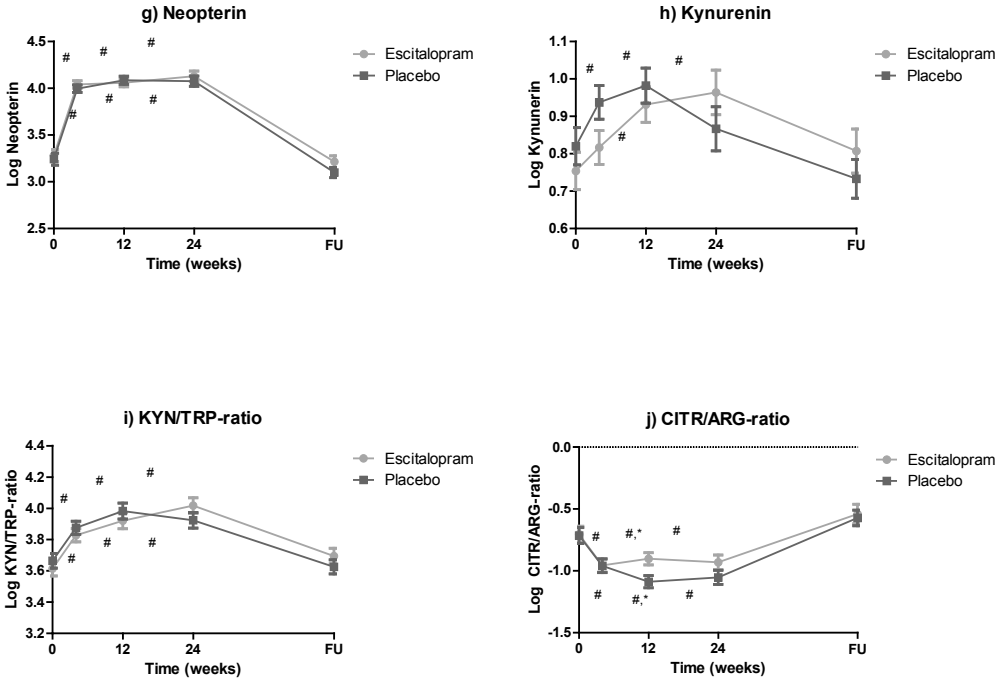
		Baseline	Week 4		Week 12		Week 24		Follow-up	
		Escitalopram	Escitalopram	Placebo	Escitalopram	Placebo	Escitalopram	Placebo	Escitalopram	Placebo
		N=40	N=39	N=38	N=40	N=38	N=33	N=34	N=28	N=34
MADRS	Sum score	4.58 ± 3.9	6.69 ± 5.4*	4.69 ± 4.7	8.26 ± 7.2*	10.29 ± 7.3*	7.39 ± 6.1*	12.00 ± 6.0*	5.04 ± 6.9	7.24 ± 7.0
BAS	Irritability	0.83 ± 1.0	0.79 ± 1.2*	0.95 ± 1.0	1.03 ± 1.4*	1.24 ± 1.1*	0.91 ± 1.2*	1.56 ± 1.3*	0.61 ± 1.0	0.94 ± 1.3
		N=40	N=38	N=38	N=37	N=38	N=32	N=34	N=28	N=32
BDI	Sum score	9.05 ± 7.9	8.87 ± 7.7*	8.21 ± 7.7	10.76 ± 9.6*	13.53 ± 10.1*	8.09 ± 6.7*	14.56 ± 8.8*	6.89 ± 8.6	9.59 ± 8.0
	Somatic items	3.25 ± 2.5	4.08 ± 2.7*	2.82 ± 2.8	4.57 ± 3.1*	5.71 ± 3.7*	3.91 ± 2.9*	6.50 ± 3.4*	2.21 ± 2.1	4.34 ± 4.1
		N=39	N=37	N=38	N=37	N=38	N=32	N=33	N=28	N=32
SCL	Sum score	130.64 ± 5.9	124.74 ± 29.2*	136.53 ± 40.5	131.57 ± 40.9*	156.37 ± 56.3 *	125.59 ± 33.4*	160.61 ± 50.1*	119.71 ± 34.8*	149.78 ± 55.6*
	Depression	24.79 ± 9.3	23.79 ± 8.0*	25.74 ± 8.6	25.41 ± 10.4*	29.24 ± 11.6*	23.94 ± 7.9*	30.36 ±10.8*	23.11 ± 11.1	27.75 ±11.6
	Anxiety	14.51 ± 5.1	12.63 ± 3.4*	14.82 ± 5.5	13.84 ± 4.0*	16.66 ± 7.1*	13.00 ± 4.2*	17.09 ± 6.7*	12.71 ± 3.4	16.38 ± 6.6
	Insufficiency	14.92 ± 5.1	14.24 ± 4.9*	15.50 ± 5.0	15.59 ± 6.8*	18.42 ± 7.0*	15.16 ± 5.7*	19.52 ± 6.9*	13.50 ± 5.5*	17.88 ± 7.4 *
	Hostility	8.49 ± 3.6	7.21 ± 2.0*	8.08 ± 2.4	8.19 ± 2.9*	9.63 ± 4.6*	8.13 ± 3.2*	9.85 ± 3.5*	7.46 ± 1.8	8.78 ± 3.8
	Somatic	18.33 ± 5.6	20.92 ±6.5	19.58 ± 6.7	21.22 ± 6.9	25.32 ± 9.4	19.72 ± 8.1*	25.21 ± 9.6*	17.14 ± 4.6	21.75 ± 9.5

Figure 2 | Mean values and standard error of the different biochemical parameters at baseline, week 4 and week 12, week 24 and at follow up (FU) with values of escitalopram and placebo users.

a) 5HT = serotonin, b) 5-HIAA = 5-hydroxyindolacetic acid, c) TRP = tryptophan, d) TRP/LNAA-ratio = tryptophan to large neutrophil amino acids ratio, e) TYR/LNAA-ratio = tyrosine to large neutrophil amino acids ratio, f) PHE/TYR-ratio = Phenanyl-Tyrosine ratio, g) neopterin, h) kynurenin, i) KYN/TRP-ratio = kynurenine to tryptphan ratio, j) CITR/ARG-ratio = Citruline-Arginine ratio

= Significant difference (P < 0.05) compared to baseline, * = significant difference (p<0.05) between escitalopram en placebo (with correction for non-normal distribution and multiple measurements)





KYN levels increased significantly at week 4 (mean = 2.52 µmol/l), week 12 (mean = 2.70 µmol/l) and week 24 (mean =2.61 µmol/l) compared to baseline (mean = 2.31 µmol/l) in the whole group, while 5-HT plasma levels decrease after the introduction of PEGIFN.

The pattern of PEFIFN-induced biochemical alterations is roughly similar when the escitalopram and placebo groups are analyzed separately (additional Table 3 and Figure 2) with the exception of the 5-HT levels in plasma showing a more profound decrease in the escitalopram group. When analyzing the biochemical data for a possible difference between the escitalopram and placebo groups, 5-HT levels differ at week 4,12 and 24 ($p < 0.03$) as different levels of CITR/ARG-ratio do at week 12 ($p = 0.01$).

24 weeks after cessation of antiviral therapy (FU) all different parameters have returned to their baseline levels except for 5-HIAA (increase from mean 33.76 nmol/l to 42.10 nmol/l), CITR/ARG-ratio (increase from mean 0.53 to 0.63) and TYR/LNAA-ratio (decrease from mean 14.47 to 13.23).

Mean values at FU of TRP, TYR/LNAA-ratio, PHE/TYR-ratio, NEOP and 5-HT still differed from mean values in the reference population (all $p<0.01$). CITR/ARG was higher at FU compared to the reference population (mean = 0.54, $p = 0.01$).

Correlation between biochemical variables and the observed psychopathology.

Changes compared to baseline in TYR/LNAA-ratio correlated negatively with changes compared to baseline of the BDI sum score (ρ = -0, 41). Changes compared to baseline of the PHE/TYR-ratio correlated with the different self-rating scores of the BDI sum score (ρ = 0,44) and SCL sum scores (ρ = 0,46) and different subscales of the SCL-90 (anxiety : ρ = 0,35 – hostility : ρ = 0,30 – depression : ρ = 0,47 – insufficiency : ρ = 0,35) and MADRS sum score (ρ = 0,46)

DISCUSSION

In this study we evaluated changes in peripheral biochemical parameters induced by the introduction of PEGIFN in patients with hepatitis C and correlated these with observed psychiatric disturbances in order to understand the pathophysiology underlying PEGIFN-induced psychiatric disturbance.

Firstly, interestingly almost all baseline values of our patients were different from those obtained from healthy subjects. Two earlier studies also reported reduced baseline levels of TRP in hepatitis C patients (33-34). Possible explanations of this phenomenon are the presence of changed IDO activity in macrophages of patients with HCV due to the chronic infection and neuroinvasion of the virus (35). However, also comparable changes were seen in cancer patients: additional analysis of a database of cancer patients treated with IFN showed also lower TRP, higher TYR/LNAA-ratio, higher PHE/TYR-ratio, higher NEOP levels, higher 5-HIAA levels and higher KYN levels at baseline. So changes in the monoaminergic system seem not only be induced by a chronic infection like HCV but also by malignancies. These changes may play a role in the complaints cocnceptualized as sickness behaviour, such as fatigue and concentration difficulties observed in these patients (36).

Secondly, and mostly in accordance with existing literature, we observed consistent changes in peripheral levels of several biochemical parameters: a decrease of the precursor of 5-HT, TRP (13-14, 16, 37-39), increase in the PHE/TYR-ratio (16, 37-38) and NEOP (16, 37-39) (index for co-factor function in mono-amine synthesis) and an increase of KYN (possibly giving rise to neurotoxic metabolites) and in KYN/LNAA-ratio (13, 38-40). Finally, we observed a decrease in the CITR/ARG-ratio (22), which may reflect impaired NO-synthesis, which may in turn influence 5-HT synthesis. However, in contrast with earlier studies (13), we did not observe changes in TRP/LNAA-ratio (14, 16, 37-38) (the marker

for the availability of TRP in the brain). Also in contrast with literature, rather consistent changes in TYR/LNAA-ratios were measured (14, 16, 37). In oncology patients treated with IFN (37-38) a decrease in 5-HIAA was seen, in contrast to our study. Summarized, one can hypothesize that the observed biochemical changes in the peripheral blood influence brain function more or less directly or may be seen as an index of processes in the brain.

Thirdly, not unexpected, only minor differences between patients treated with escitalopram and placebo in the different peripheral parameters were seen. As the prophylactic use of escitalopram had a protective effect on the development of psychiatric disturbances, this strongly suggests that if brain 5-HT-ergic neurotransmission is indeed affected by cytokine treatment and if the emerging psychopathology is mediated by decrease in 5-HT synthesis via the mechanisms investigated in this study, escitalopram has its effect downstream in the cascade of the brain/5HT-ergic neurotransmission in the prevention/ treatment of symptoms like depression, anger, anxiety and impaired concentration.
Fourthly, our data only suggest a correlation between the development of psychopathology and change in PHE/TYR-ratio and changes in the TYR/LNAA-ratio. This is in contrast to previous studies showing a correlation between levels of peripheral 5-HT, TRP and KYN and the development of psychiatric complaints in HCV patients as well in cancer patients (13-14, 39). However other studies could not establish a consistent relation in any of these parameters (38, 40). As mentioned before, the increased PHE/TYR-ratio possibly reflects a decreased BH4 activity, a co-factor in the biosynthesis of 5HT, noradrenaline and dopamine. Also changes in the TYR/LNAA-ratio may cause changes in levels of dopamine as TYR, the precursor of dopamine and the TYR/LNAA-ratio reflect the dopamine availability in the brain. Earlier studies showed increased levels of PHE/TYR-ratio in patients with chronic inflammation, for instance HIV (41-42) and in a recent study of Capuron et al.(43) correlation between neuropsychiatric symptoms and increased PHE/TYR-ratio levels was observed in elderly patients.
A strong point of our study is the broad evaluation of not only precursors of 5-HT but also of indicators of the biosynthesis of 5-HT, dopamine and noradrenaline and formation of toxic metabolites in a relatively large study population. The most prominent limitation of our study is the reliance on peripheral indicators of the brain metabolism, possibly bearing a more remote relationship with central processes than hypothesized.

Resuming, in the main study, PEGIFN induced different psychopathological symptoms which could partly be prevented by prophylactic use of escitalopram. PEGIFN induced changes in several biochemical parameters, which are thought to reflect the metabolism of monoaminergic neurotransmitters in the brain. However, only some of these changes were correlated with changes in mood, notably changes in PHE/TYR-ratio and TYR/LNAA-ratio, reflecting not only changes in 5-HT metabolism but also in that of noradrenaline and dopamine. Indicators of tryptophan metabolism did not appear to be of significance.

REFERENCES

1. Kim WR. The burden of hepatitis C in the United States. Hepatology. 2002 Nov;36(5 Suppl 1):S30-4.
2. Russo S, Kema IP, Haagsma EB, Boon JC, Willemse PH, den Boer JA, et al. Irritability rather than depression during interferon treatment is linked to increased tryptophan catabolism. Psychosom Med. 2005 Sep-Oct;67(5):773-7.
3. Reichenberg A, Gorman JM, Dieterich DT. Interferon-induced depression and cognitive impairment in hepatitis C virus patients: a 72 week prospective study. AIDS. 2005 Oct;19 Suppl 3:S174-8.
4. Lieb K, Engelbrecht MA, Gut O, Fiebich BL, Bauer J, Janssen G, et al. Cognitive impairment in patients with chronic hepatitis treated with interferon alpha (IFNalpha): results from a prospective study. Eur Psychiatry. 2006 Apr;21(3):204-10.
5. Bonaccorso S, Puzella A, Marino V, Pasquini M, Biondi M, Artini M, et al. Immunotherapy with interferon-alpha in patients affected by chronic hepatitis C induces an intercorrelated stimulation of the cytokine network and an increase in depressive and anxiety symptoms. Psychiatry Res. 2001 Dec 15;105(1-2):45-55.
6. Pariante CM, Orru MG, Baita A, Farci MG, Carpiniello B. Treatment with interferon-alpha in patients with chronic hepatitis and mood or anxiety disorders. Lancet. 1999 Jul 10;354(9173):131-2.
7. Janssen HL, Brouwer JT, van der Mast RC, Schalm SW. Suicide associated with alfa-interferon therapy for chronic viral hepatitis. J Hepatol. 1994 Aug;21(2):241-3.
8. Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS, et al. Paroxetine for the prevention of depression induced by high-dose interferon alfa. N Engl J Med. 2001 Mar 29;344(13):961-6.
9. Kraus MR, Schafer A, Scheurlen M. Paroxetine for the prevention of depression induced by interferon alfa. N Engl J Med. 2001 Aug 2;345(5):375-6.
10. Kraus MR, Schafer A, Schottker K, Keicher C, Weissbrich B, Hofbauer I, et al. Therapy of interferon-induced depression in chronic hepatitis C with citalopram: a randomised, double-blind, placebo-controlled study. Gut. 2008 Apr;57(4):531-6.
11. Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2005 Feb;29(2):201-17.
12. Brown RR, Ozaki Y, Datta SP, Borden EC, Sondel PM, Malone DG. Implications of interferon-induced tryptophan catabolism in cancer, auto-immune diseases and AIDS. Adv Exp Med Biol. 1991;294:425-35.
13. Bonaccorso S, Marino V, Puzella A, Pasquini M, Biondi M, Artini M, et al. Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. J Clin Psychopharmacol. 2002 Feb;22(1):86-90.
14. Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M, Dantzer R. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. Mol Psychiatry. 2002;7(5):468-73.
15. DeMyer MK, Shea PA, Hendrie HC, Yoshimura NN. Plasma tryptophan and five other amino acids in depressed and normal subjects. Arch Gen Psychiatry. 1981 Jun;38(6):642-6.
16. Van Gool AR, Fekkes D, Kruit WH, Mulder PG, Ten Hagen TL, Bannink M, et al. Serum amino acids, biopterin and neopterin during long-term immunotherapy with interferon-alpha in high-risk melanoma patients. Psychiatry Res. 2003 Jul 15;119(1-2):125-32.
17. Michaelis M, Michaelis R, Suhan T, Schmidt H, Mohamed A, Doerr HW, et al. Ribavirin inhibits angiogenesis by tetrahydrobiopterin depletion. Faseb J. 2007 Jan;21(1):81-7.
18. Anderson DN, Wilkinson AM, Abou-Saleh MT, Blair JA. Recovery from depression after electroconvulsive therapy is accompanied by evidence of increased tetrahydrobiopterin-dependent hydroxylation. Acta Psychiatr Scand. 1994 Jul;90(1):10-3.
19. Hoekstra R, van den Broek WW, Fekkes D, Bruijn JA, Mulder PG, Pepplinkhuizen L. Effect of electroconvulsive therapy on biopterin and large neutral amino acids in severe, medication-resistant depression. Psychiatry research. 2001 Sep 20;103(2-3):115-23.
20. Myint AM, Kim YK. Cytokine-serotonin interaction through IDO: a neurodegeneration hypothesis of depression. Medical hypotheses. 2003 Nov-Dec;61(5-6):519-25.

21. Wichers MC, Koek GH, Robaeys G, Verkerk R, Scharpe S, Maes M. IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. Molecular psychiatry. 2005 Jun;10(6):538-44.
22. Fekkes D, Bannink M, Kruit WH, Van Gool AR, Mulder PG, Sleijfer S, et al. Influence of pegylated interferon-alpha therapy on plasma levels of citrulline and arginine in melanoma patients. Amino Acids. 2007 Jan;32(1):121-6.
23. de Knegt RJ, Bezemer G, Van Gool AR, Drenth JP, Hansen BE, Droogleever Fortuyn HA, et al. Randomised clinical trial: escitalopram for the prevention of psychiatric adverse events during treatment with peginterferon-alfa-2a and ribavirin for chronic hepatitis C. Aliment Pharmacol Ther. 2011 Dec;34(11-12):1306-17.
24. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry. 1979 Apr;134:382-9.
25. Tyrer P, Owen RT, Cicchetti DV. The brief scale for anxiety: a subdivision of the comprehensive psychopathological rating scale. J Neurol Neurosurg Psychiatry. 1984 Sep;47(9):970-5.
26. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59 Suppl 20:22-33;quiz 4-57.
27. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961 Jun;4:561-71.
28. Derogatis LR, Lipman RS, Covi L. SCL-90: an outpatient psychiatric rating scale--preliminary report. Psychopharmacol Bull. 1973 Jan;9(1):13-28.
29. Borgdorff P, Fekkes D, Tangelder GJ. Hypotension caused by extracorporeal circulation: serotonin from pump-activated platelets triggers nitric oxide release. Circulation. 2002 Nov 12;106(20):2588-93.
30. Fekkes D, van Dalen A, Edelman M, Voskuilen A. Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated pre-column derivatization with o-phthaldialdehyde. Journal of chromatography. 1995 Jul 21;669(2):177-86.
31. Fukushima T, Nixon JC. Analysis of reduced forms of biopterin in biological tissues and fluids. Analytical biochemistry. 1980 Feb;102(1):176-88.
32. Myint AM, Kim YK, Verkerk R, Scharpe S, Steinbusch H, Leonard B. Kynurenine pathway in major depression: evidence of impaired neuroprotection. J Affect Disord. 2007 Feb;98(1-2):143-51.
33. Zignego AL, Cozzi A, Carpenedo R, Giannini C, Rosselli M, Biagioli T, et al. HCV patients, psychopathology and tryptophan metabolism: analysis of the effects of pegylated interferon plus ribavirin treatment. Dig Liver Dis. 2007 Sep;39 Suppl 1:S107-11.
34. Cozzi A, Zignego AL, Carpendo R, Biagiotti T, Aldinucci A, Monti M, et al. Low serum tryptophan levels, reduced macrophage IDO activity and high frequency of psychopathology in HCV patients. J Viral Hepat. 2006 Jun;13(6):402-8.
35. Laskus T, Radkowski M, Adair DM, Wilkinson J, Scheck AC, Rakela J. Emerging evidence of hepatitis C virus neuroinvasion. AIDS. 2005 Oct;19 Suppl 3:S140-4.
36. Dantzer R. Cytokine, sickness behavior, and depression. Immunol Allergy Clin North Am. 2009 May;29(2):247-64.
37. Van Gool AR, van Ojik HH, Kruit WH, Bannink M, Mulder PG, Eggermont AM, et al. Pegylated interferon-alpha2b treatment in melanoma patients: influence on amino acids, 5-hydroxyindolacetic acid and pteridine plasma concentrations. Anticancer Drugs. 2004 Jul;15(6):587-91.
38. Bannink M, Fekkes D, Van Gool AR, Kruit WH, Sleijfer S, van der Holt B, et al. Interferon-alpha influences tryptophan metabolism without inducing psychiatric side effects. Neuropsychobiology. 2007;55(3-4):225-31.
39. Capuron L, Neurauter G, Musselman DL, Lawson DH, Nemeroff CB, Fuchs D, et al. Interferon-alpha-induced changes in tryptophan metabolism. relationship to depression and paroxetine treatment. Biol Psychiatry. 2003 Nov 1;54(9):906-14.

40. Van Gool AR, Verkerk R, Fekkes D, Bannink M, Sleijfer S, Kruit WH, et al. Neurotoxic and neuroprotective metabolites of kynurenine in patients with renal cell carcinoma treated with interferon-alpha: course and relationship with psychiatric status. Psychiatry Clin Neurosci. 2008 Oct;62(5):597-602.
41. Neurauter G, Schrocksnadel K, Scholl-Burgi S, Sperner-Unterweger B, Schubert C, Ledochowski M, et al. Chronic immune stimulation correlates with reduced phenylalanine turnover. Curr Drug Metab. 2008 Sep;9(7):622-7.
42. Zangerle R, Kurz K, Neurauter G, Kitchen M, Sarcletti M, Fuchs D. Increased blood phenylalanine to tyrosine ratio in HIV-1 infection and correction following effective antiretroviral therapy. Brain Behav Immun. 2010 Mar;24(3):403-8.
43. Capuron L, Schroecksnadel S, Feart C, Aubert A, Higueret D, Barberger-Gateau P, et al. Chronic Low-Grade Inflammation in Elderly Persons Is Associated with Altered Tryptophan and Tyrosine Metabolism: Role in Neuropsychiatric Symptoms. Biol Psychiatry. 2011 Jan 28.

Additional Table | mean values and standard error of the different biochemical parameters at baseline, week 4 and week 12, week 24 and at follow up (FU) with values of escitalopram and placebo users. 5HT = serotonin, 5-HIAA = 5-hydroxyindolacetic acid, TRP = tryptophan, TRP/LNAA- ratio = tryptophan to large neutrophil amino acids ratio, TYR/LNAA-ratio = tyrosine to large neutrophil amino acids ratio, PHE/TYR-ratio = Phenanyl-Tyrosine ratio, NEOP = neopterine, KYN = kynurenine, KYN/TRP-ratio = kynurenine to tryptophan ratio, CITR/ARG-ratio = Citruline-Arginine ratio,

* = Significant difference (P < 0.05) compared to baseline, # = significant difference (p<0.05) between escitalopram en placebo (with correction for non-normal distribution and multiple measurements)

Week	Total	Escitalopram	Placebo
5HT (nmol/l) **0**	28.47 ± 3.0 (n=71)	24.09 ± 3.2 (n=37)	33.22 ± 5.1 (n=34)
4	17.00* ± 6.2 (n=59)	9.42*# ± 2.4 (n=28)	23.86*# ± 11.6 (n=31)
12	10.46* ± 1.7 (n=57)	6.56*# ± 1.6 (n=22)	14.54*# ± 3.0 (n=27)
24	10.06* ± 1.5 (n=45)	6.59*# ± 1.6 (n=19)	12.84*# ± 2.6 (n=21)
FU	33.26 ± 5.6 (n=45)	24.84 ± 5.5 (n=20)	39.76 ± 9.0 (n=25)
5-HIAA (nmol/l) **0**	33.76 ± 1.6 (n=75)	36.58 ± 2.7 (n=38)	30.87± 1.6 (n=37)
4	40.20 ± 4.4 (n=73)	37.14 ± 5.8 (n=36)	43.18 ± 6.9 (n=37)
12	37.98 ± 5.6 (n=66)	34.09 ± 4.0 (n=32)	32.95 ± 3.3 (n=28)
24	37.30 ± 4.8 (n=65)	44.49 ± 10.1 (n=29)	32.17 ± 3.4 (n=28)
FU	42.10* ± 4.2 (n=62)	35.11 ± 2.7 (n=28)	47.85* ± 7.2 (n=34)
TRP (µmol/l) **0**	43.71 ± 0.9 (n=76)	42.23 ± 1.4 (n=38)	45.20 ± 1.4 (n=38)
4	39.57*± 0.9 (n=74)	37.80*± 1.3 (n=37)	41.21*± 1.3 (n=37)
12	38.44* ± 1.0 (n=70)	37.73* ± 1.3 (n=32)	38.57* ± 1.3 (n=32)
24	37.06*± 1.0 (n=67)	36.47*± 1.4 (n=29)	38.26*± 1.4 (n=29)
FU	43.40 ± 1.0 (n=60)	43.39 ± 1.4 (n=26)	42.56 ± 1.2 (n=34)
TRP/LNAA-ratio 0	7.84 ± 0.4 (n=76)	7.99 ± 0.3 (n=38)	7.68 ± 0.2 (n=38)
4	7.92 ± 0.2 (n=74)	7.88 ± 0.3 (n=37)	7.95 ± 0.2 (n=37)
12	7.67 ± 0.2 (n=70)	7.51 ± 0.2 (n=32)	7.56 ± 0.3 (n=32)
24	7.98 ± 0.2 (n=67)	8.00 ± 0.3 (n=29)	7.83 ± 0.3 (n=29)
FU	8.05 ± 0.2 (n=60)	8.24 ± 0.3 (n=26)	7.90 ± 0.2 (n=34)
TYR/LNAA-ratio 0	14.47 ± 0.4 (n=76)	15.03 ± 0.6 (n=38)	13.92 ± 0.5 (n=38)
4	15.69* ± 0.4 (n=74)	15.97* ± 0.7 (n=37)	15.40* ± 0.5 (n=37)
12	15.44* ± 0.4 (n=70)	16.09 ± 0.8 (n=32)	14.56* ± 0.4 (n=32)
24	14.92* ± 0.4 (n=67)	14.91 ± 0.7 (n=29)	14.81* ± 0.5 (n=29)
FU	13.23* ± 0.3 (n=60)	13.64* ± 0.6 (n=26)	12.92 ± 0.4 (n=34)

PHE/TYR-ratio 0	0.82 ± 0.02 (n=76)	0.84 ± 0.03 (n=38)	0.80 ± 0.03 (n=38)
4	0.95* ± 0.02 (n=74)	0.99* ± 0.03 (n=37)	0.92* ± 0.02 (n=37)
12	0.93* ± 0.02 (n=70)	0.93* ± 0.03 (n=32)	0.93* ± 0.02 (n=32)
24	0.97* ± 0.02 (n=67)	0.99* ± 0.04 (n=29)	0.96* ± 0.03 (n=29)
FU	0.87* ± 0.2 (n=60)	0.86 ± 0.03 (n=26)	0.88* ± 0.02 (n=34)
NEOP (nmol/l) **0**	28.41 ± 1.5 (n=76)	30.03 ± 2.5 (n=37)	27.14 ± 1.6 (n=38)
4	57.78* ± 1.6 (n= 74)	58.44* ± 2.6 (n=37)	56.22* ± 2.0 (n= 37)
12	60.27* ± 1.6 (n=74)	60.06* ± 2.7 (n=34)	60.77* ± 2.1 (n=33)
24	62.21* ± 2.3 (n=67)	64.78* ± 4.3 (n=29)	59.15* ± 2.9 (n=29)
FU	23.27 ± 1.0 (n=60)	24.19 ± 1.8 (n=26)	22.56 ± 1.0 (n=34)
KYN (µmol/l) **0**	2.31 ± 0.09 (n=76)	2.28 ± 0.1 (n=37)	2.35 ± 0.1 (n=38)
4	2.52* ± 0.09 (n=74)	2.40 ± 0.1 (n=37)	2.64* ± 0.1 (n=37)
12	2.70* ± 0.09 (n=70)	2.67* ± 0.1 (n=32)	2.73* ± 0.1 (n=32)
24	2.61* ± 0.09 (n=67)	2.68* ± 0.1 (n=24)	2.54 ± 0.1 (n=24)
FU	2.22 ± 0.08 (n=60)	2.33 ± 0.1 (n=26)	2.17 ± 0.1 (n=34)
KYN/TRP-ratio 0	39,86 ± 1.5 (n=76)	39,61 ± 2.3 (n=38)	40.53 ± 1.9 (n=38)
4	49.03* ± 1.5 (n=74)	48.16* ± 2.5 (n=37)	49.73* ± 1.8 (n=37)
12	54.23* ± 2.0 (n=70)	53.28* ± 2.6 (n=32)	56.47* ± 3.4 (n=32)
24	54.69* ± 1.6 (n=67)	56.93* ± 2.8 (n=29)	52.83* ± 2.6 (n=29)
FU	39.13 ± 1.2 (n=60)	40.15 ± 1.8 (n=26)	38.35 ± 1.7 (n=34)
CITR/ARG-ratio 0	0.53 ± 0.03 (n =76)	0.56 ± 0.05 (n=38)	0.52 ± 0.03 (n =38)

Psychiatric side effects and fluctuations in serotonergic parameters in the treatment of chronic hepatitis C infection

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ABSTRACT

Introduction Treatment of hepatitis C with peginterferon induces psychiatric side effects. This might include changes in serotonergic function.

Methods Twenty-two hepatitis C patients were treated with peginterferon. At different time-points, psychometric assessment was performed using the Profile of Mood States (POMS). Plasma samples were taken to study serotonergic parameters.

Results Anger and depression increased compared to baseline, starting with anger (>week 3), followed by depression (>week 7). Other scores did not show consistent changes. No consistent changes were observed in tryptophan, tryptophan/large neutral amino acids-ratio, biopterin and 5-hydroxyindoleacetic acid. The tyrosine/large neutral amino acids-ratio, neopterin, phenylalanine/tyrosine-ratio, and prolactin concentrations increased compared to baseline. Prolactin levels were associated with the occurrence of depression and anger.

Discussion Particularly anger and depression increased during treatment. Neither a decrease in tryptophan and tryptophan availability was seen, nor a relationship between these parameters and the development of psychopathology. Therefore, other mechanisms in the induction of psychopathology should be considered. The observed increases in neopterin and phenylalanine/tyrosine-ratio are indicative for changes in tetrahydrobiopterin, which is involved in the metabolism of serotonin, noradrenaline and dopamine, and possibly mediating the increase in prolactin. The increase in prolactin levels and its relationship with depression and anger needs further exploration.

INTRODUCTION

About 170 million people are chronically infected with hepatitis C virus (HCV), of whom about 20 percent develop end stage liver disease (1). As a result, hepatitis C is now the leading indication for liver transplantation in developed nations. Successful treatment with a combination of (peg)interferon-alpha ((PEG)IFN) and ribavirin (RBV) leads to a permanent eradication of the virus which prevents progression to end stage liver disease and reduces mortality caused by HCV (2-3). Besides somatic side effects such as headache, thyroid dysfunction and anemia, this treatment is associated with sometimes severe psychiatric side effects. These psychiatric side effects comprise a wide variety of symptoms and syndromes: depression, aggression, anxiety and cognitive symptoms such as reduced concentration and memory (4). The exact pathophysiology of these side effects is still unknown but is thought to be mediated by IFN-induced changes in the cytokine network, the hypothalamic-pituitary-adrenal axis, the hypothalamic-pituitary-thyroid axis and in the brain serotonin (5-HT) function (5).

With regard to its influence on the central 5-HT-ergic system, firstly, (PEG)IFN induces the enzyme indoleamine 2,3-dioxygenase (IDO) catabolising the amino acid tryptophan (TRP), the precursor of 5-HT. At the blood-brain barrier, TRP competes with the other large neutral amino acids (LNAA: tyrosine, phenylalanine, valine, leucine and isoleucine) for gaining entrance into the central nervous system (CNS). The ratio of the concentration of TRP to those of the other LNAA (TRP/LNAA-ratio) reflects the central availability of TRP and is an index for the rate of central 5-HT synthesis. Indeed, in several studies treatment with (PEG)IFN was found to lower both TRP concentrations and the TRP/LNAA-ratio and in some of these studies, relationships were observed between measures for depression and decrease in the TRP/LNAA-ratio (6-8). Next to depression, aggression and irritability have been found to be related to decreased concentrations of TRP (9).

Secondly, (PEG)IFN is hypothesized to influence the metabolism of the pteridine tetrahydrobiopterin (BH4), an important co-factor in the biosynthesis of 5-HT (8). (PEG)IFN increases the concentration of neopterin, a metabolite of one of the precursors of BH4, which might cause lower levels of BH4 resulting in a decrease in 5-HT synthesis. This may add to the recently reported reduction of BH4 levels by RBV (10). BH4 is also a cofactor for the enzyme which hydroxylates phenylalanine (PHE) to tyrosine (TYR) and the ratio of PHE to TYR (PHE/TYR-ratio) has been regarded as a measure of BH4 activity (11-12). An increased PHE/TYR ratio suggests reduced BH4 synthesis. In addition, BH4 is involved in

the biosynthesis of noradrenaline (precursor: PHE) and dopamine (precursor: TYR). Thirdly, the induction of IDO by (PEG)IFN increases the conversion of TRP to kynurenine of which several metabolites are thought to have neurotoxic effects and thus may contribute to the development of depression (13-14).

In this study of HCV-infected patients treated with PEGIFN, the course of several peripheral indicators for CNS 5-HT-ergic function were explored as well as the correlation between these 5-HT-ergic indices and psychological parameters, most notably depression and aggression. We hypothesized that TRP/LNAA, 5-HIAA and prolactin (PRL) levels would decrease during PEGIFN treatment. As far as we know this study is the first one that provides insight into the very early changes in different changes in the 5-HT-ergic system with assessments already in the first days and weeks after the introduction of PEGIFN.

MATERIAL AND METHODS

Dutch patients, participating in the international DITTO-Study (15), received antiviral treatment with a standard dose of peginterferon-alfa 2a (Pegasys, Hoffmann-LaRoche, Basel, Switzerland) 180 microgram once weekly and 1000-1200 mg RBV daily (Copegus, Hoffmann-LaRoche) during the first six weeks of treatment. Thereafter all patients continued their treatment with PEGIFN, some with RBV some without RBV and some with additional histamine, according to their virological response and randomization according to the study protocol. At day 0, 1, 4, 7 and 8 and at week 2, 3, 4, 6, 7, 8, 10 and 12, plasma samples were obtained for biochemical analysis. At the same time points patients completed the (Dutch shortened version of the) Profile of Mood States (POMS), a self-rating questionnaire assessing five domains of psychic functioning: depression-dejection (range: 0-32), anger-hostility (range: 0-28), fatigue-inertia (range: 0-24), tension-anxiety (range: 0-24) and vigor-activity (range: 0-20). These domains cover the most frequently occurring psychiatric side effects of IFN-based treatment. The POMS has been extensively used and is particularly suitable for frequently repeated assessments of psychological functioning. With the exception of vigor, higher scores indicate more complaints. Data on patient characteristics (mode of transmission and concomitant psycho-active medication) and disease-related characteristics (genotype, HCV-RNA) were also collected. Use of benzodiazepines was permitted whereas use of antidepressants and antipsychotic drugs was a reason for exclusion. Laboratory and psychometric data from patients after the time

point when prohibited medication was prescribed were excluded from the analysis, as were data after cessation of PEGIFN. This study was approved by the local Medical Ethics Committee and all patients have given written informed consent.

Biochemical analysis

TRP and the TRP/LNAA-ratio were assessed, as indices for the availability of TRP to the CNS. The BH4 activity was estimated by the determination of the PHE/TYR-ratio. In addition, the levels of the pteridins neopterin and biopterin were measured. The ratio of tyrosine to the LNAA (TYR/LNAA-ratio) was used as an index for central dopamine synthesis. Lastly, peripheral 5-hydroxyindoleacetic acid (5-HIAA) and PRL were assessed. 5-HIAA is the main metabolite of 5-HT and reflects total 5-HT turnover. As the secretion of PRL is at least partially controlled by 5-HT, PRL levels were considered indicative of the central activity of 5-HT. EDTA blood was obtained by venipuncture. Samples were not taken under fasting conditions. However, almost all samples were taken early in the morning. After immediate centrifugation (20 min at 2650 g_{max}) plasma was separated and frozen at -80°C. The amino acids PHE, TYR, TRP, isoleucine, leucine and valine were measured with high-performance liquid chromatography (16). The TRP/LNAA-ratio was calculated as 100 times the concentration of TRP divided by the sum of concentrations of the other large neutral amino acids. The TYR/LNAA-ratio was calculated in the same manner, substituting TYR for TRP. The pteridines were measured after acid oxidation as described earlier (12, 17). The concentrations of 5-HIAA were measured twice by high performance liquid chromatography (electrochemical detection with limit in plasma: 1 nmol/L) (18). The mean recovery (± SD) of 5-HIAA added to plasma was 72±8%. PRL levels were measured in serum by Immunolite 2000 using two-site chemiluminescent immunometric assay.

Statistical analysis

Scores of the different items of the POMS and the concentrations of the biochemical parameters at the different time points were compared with their respective baseline values with repeated measurements analysis using a linear mixed regression with random intercept and slope over time. In addition, scores of the different items of the POMS were compared to baseline with the Mann-Whitney-U-test. Data on TRP at baseline were compared to healthy controls from our neuropsychiatric laboratory (corrected for gender and age), using the t-test. Associations between (changes in) psychometric parameters and (changes in) biochemical parameters were tested with linear mixed analysis using SAS 9.2. Scores on the items anger and depression were divided in two groups: patients who

Table 1 | Scores on the subscales of the Profile of Mood States (POMS) at baseline and during therapy with PEGIFN

Time		d0	d1	d4	d7	d8	W2	W3	W4	W6	W7	W8	W10	w12
Depression	N	18	20	20	17	20	19	18	18	16	14	13	8	9
Median		3.0	3.0	4.0	5.5	4.0	7.0	4.0	8.0	2.5	9.0*	6.5*	5.0*	4.0*
Range		28	26	25	27	24	28	27	24	30	29	28	30	28
Anger	N	18	20	19	17	20	19	18	18	16	14	13	8	9
Median		2.5	3.5	4.0	8.0	6.0	9.0	7.5*	8.0*	6.5*	11.5*	9.0*	14.0	13.0
Range		27	25	28	24	23	25	28	22	28	24	26	25	24
Fatigue	N	16	19	18	15	18	18	17	17	15	14	13	8	9
Median		1.5	11.0	10.5	12.0	7.5	13.0	8.0	13.0*	10.0*	12.5*	11.0	6.5*	7.0
Range		23	24	24	22	24	23	24	22	24	24	23	23	23
Tension	N	18	20	20	17	20	19	18	18	16	14	13	8	9
Median		5.0	5.5	6.0	10.0	6.0	7.0	3.0	6.5	2.5	6.5	3.0	3.5	5.0
Range		17	19	18	18	19	19	22	18	23	19	18	19	18
Vigor	N	18	20	20	17	19	19	18	18	16	14	13	8	9
Median		7.0	6.0	6.5	6.0	6.0	5.0*	7.0	6.5	5.5	5.5*	5.0	6.5	6.0
Range		19	19	19	10	18	19	18	19	19	19	19	19	18

* Significant difference ($p < 0.05$) compared to baseline (Mann-Whitney test)

Table 2 |5-HTergic parameters at baseline and during treatment with PEGIFN

Time point of treatment		d0	d1	d4	d7	d8	W2	W3	W4	W6	W7	W8	W10	W12
TRP (mmol/l)	N	21	22	21	18	22	21	22	18	15	13	10	7	8
Mean		40.36	38.77	40.38	43.34	40.55	39.45	36.95*	36.96*	37.02*	34.71*	36.25	36.14	38.27
SD		7.95	7.85	9.64	8.02	8.56	7.67	7.07	6.06	7.57	5.82	6.67	5.54	6.04
TRP/LNAA-ratio	N	21	22	21	18	22	21	22	18	15	13	10	7	8
Mean		7.51	7.22	7.01*	7.20	7.15	7.36	7.31	7.55	7.49	7.14	7.30	7.41	7.21
SD		0.98	0.91	0.82	1.14	0.68	0.82	0.85	1.11	0.87	0.84	0.43	1.10	0.72
TYR/LNAA-ratio	N	21	22	21	18	22	21	22	18	15	13	10	6	8
Mean		13.05	14.06*	13.52	15.08	12.86	14.07	14.00*	13.65*	13.44*	13.73*	12.24*	14.22*	12.60
SD		5.37	5.64	5.26	3.61	4.94	6.02	5.55	5.87	6.30	5.19	4.51	1.98	5.31
Neopterin (nmol/l)	N	21	22	21	18	22	21	22	20	17	14	12	7	6
Mean		30.14	99.44*	87.86*	68.37*	63.45*	55,47*	55.00*	55.63*	59.16*	55.96*	54.07*	61.58*	49.47*
SD		13.22	29.66	25.88	17.59	19.19	16.39	15.69	15.91	15.28	12.55	16.31	14.27	14.39
Biopterin (nmol/l)	N	21	22	21	18	22	21	22	20	17	14	12	7	6
Median		7.36	9.31*	7.90*	8.00	7.68	6.92	6,61*	6,40*	6.16*	6.01*	6.52	6.94	7.79
SD		1.84	2.12	2.48	2.44	1.92	1.48	1,55	1,81	1.58	2.31	1.97	1.21	1.75
PHE/TYR-ratio	N	21	22	21	18	22	21	22	18	15	13	10	7	8
Mean		0.80	0.96*	1.00*	0.94*	0.99*	0.93*	0.94*	0.95*	0.94	0.94	1.05	1.05	0.96
SD		0.20	0.22	0.20	0.19	0.18	0.21	0.22	0.23	0.23	0.17	0.19	0.15	0.24
5-HIAA (mmol/l)	N	17	18	17	14	18	17	17	17	14	121	10	7	9
Mean		49.87	39.76	46.49	52.99	41.17	32.95*	29.91*	50.94*	40.03	29.17	30.47	51.67	62.39
SD		39.56	20.54	31.26	45.45	31.57	9.52	8.51	73.09	18.11	7.55	8.26	51.78	87.94
Prolactin (µg/l)	N	22	22	21	18	21	21	22	21	18	16	14	8	10
Mean		5.6	6.8	7.2*	7.4	8.5*	7.6*	8.1*	8.3*	9.5*	9.6*	8.8*	8.4*	11.8*
SD		2.4	3.7	3.7	3.4	6.0	3.2	4.0	3.8	4.4	5.7	4.1	3.8	6.3

SD: standard deviation; * Significant difference ($p < 0.05$) compared to baseline with linear mixed regression corrected for repeated measurements

Figure 1| Relation between baseline levels of prolactin (µg/l) and change (0-1) on increase of anger-scale of the POMS compared to baseline during treatment with PEGIFN

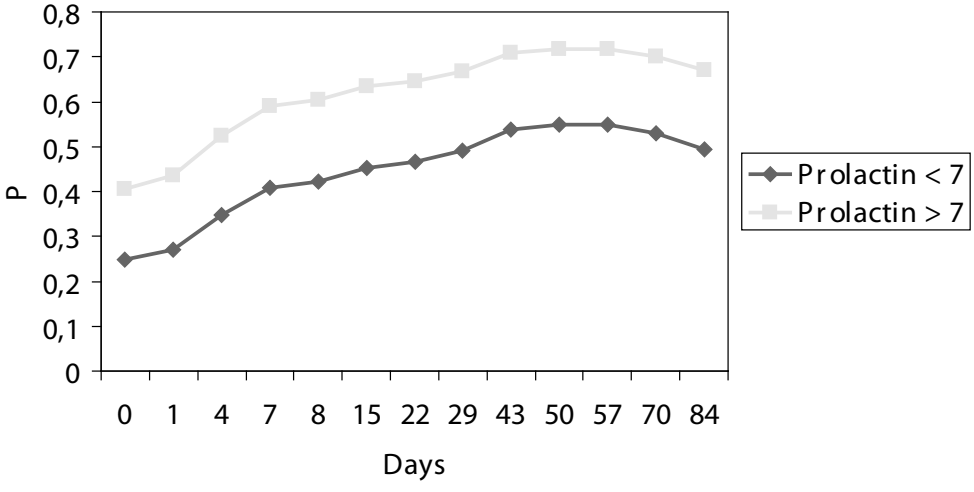
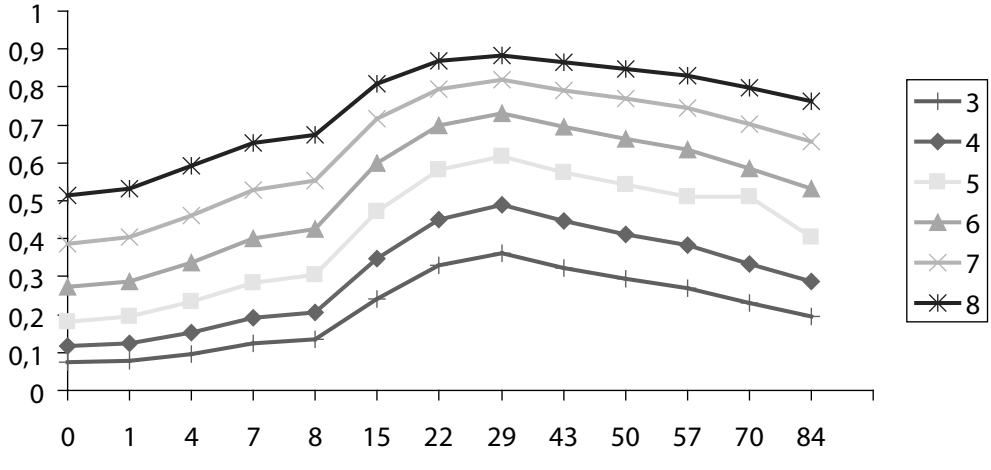


Figure 2| Relationship between levels of prolactin (µg/l) during treatment wih PEGIFN and the chance (0-1) on an increase on depression-scale of the POMS compared to baseline



did show an increase compared to baseline and those who did not. To relate the different 5-HT-ergic values with the items anger and depression, three different approaches were used: changes compared to baseline, changes compared to previous visit and baseline values of these different 5-HT-ergic values. All reported p-values are 2-sided, and a significance level of $\alpha = 0.05$ was used.

RESULTS

Twenty-two patients, 16 men and 6 women, participated in the study. Mean age was 44.2 years (range: 31-58). The mode of transmission for 8 of the patients was unknown, for twelve of them it was through intravenous drug use and/or tattooing and for the remaining 2 it was iatrogenic. Thirteen patients were infected with HCV genotype 1, 2 patients with genotype 2, 5 patients with genotype 3 and 2 patients with genotype 4. During treatment with PEGIFN, 10 patients received benzodiazepines and 6 patients were clinically diagnosed with PEGIFN-induced depression and were prescribed antidepressants. Diagnosis of depression and prescription of antidepressant took place in all cases after day 29. As mentioned, after initiation of antidepressants, psychometric and laboratory data were excluded from analysis. No antipsychotic drugs were used. In the course of time, data went missing due to administrative failure or discontinuation of antiviral therapy (Table 1 and 2). With non-parametric testing, mean scores on the item fatigue, tension and vigor did not show any consistent changes compared to baseline during the first 12 weeks of treatment. However, scores on the item anger increased significantly compared to baseline from week 3 until week 8. Also scores on the items depression increased, though only from week 7 (Table 1). With repeated measures analysis, statistically significant trends were found for increase in depression ($p=0.036$), anger ($p=0.001$) and vigor ($p=0.002$), all signifying an increase in complaints.

Baseline levels of TRP were significantly lower ($p<0.01$) and neopterin levels were significantly higher ($p<0.01$) compared to the healthy controls.

Laboratory values in the first week as tested with repeated measurements analysis yielded statistically significant increases for neopterin and the PHE/TYR-ratio at all four time points in the first week. Biopterin and PRL were found to be increased at two time points, whereas the TRP/LNAA-ratio and the TYR/LNAA-ratio were only decreased once (Table 2). After the first week, neopterin and PRL were consistently increased at all time points. Levels of TRP were decreased at weeks 3, 4, 6 and 7, whereas the TRP/LNAA-ratio was not found to be changed, and the TYR/LNAA-ratio was increased at weeks 3 to 10. The PHE/TYR-ratio was increased at weeks 1 to 4, biopterin was decreased (after the aforementioned increase at day 1 and 4) at weeks 3 to 7, and 5-HIAA was found to be decreased at week 2 to 4 (Table 2). In the multivariate analysis changes in TRP, TYR/LNAA-ratio, neopterin, biopterin, PHE/TYR-ratio and 5-HIAA were not associated with changes in the scores on the items depression and anger. Contrary to expectation, change in the TRP/LNAA-ratio was positively as-

sociated with increased anger (p=0.03). No relationship was seen between change in the TRP/LNAA-ratio and change in depression.

Patients with higher concentrations of PRL at baseline were associated to have an increase in anger: the chances on an increase on the anger subscale, compared to baseline were consistently higher at all time points during treatment with PEGIFN in patients with PRL levels at baseline higher than 7 µg/l compared to patients with PRL levels at baseline lower than 7 µg/l (p<0.01; Figure 1). At all time points during treatment, higher PRL levels were associated with an increase on the item depression compared to baseline at the same time point (p=0.03; Figure 2).

Furthermore, we compared the laboratory values of the patients who developed clinical depression in the course of treatment with the remaining patients who did not. No differences were observed between the groups at baseline and at day 29 regarding the concentrations of TRP, TYR, neopterin and PRL, and with regard to the TRP/LNAA-ratio and PHE/TYR-ratio, with the exception of the TRP/LNAA-ratio being higher (8.61 + 1.2 vs. 7.03 + 0.6) and the PHE/TYR-ratio being lower (0.79 + 0.2 vs. 1.03 + 0.2) in the 6 patients that went on to develop depression.

Finally, to investigate if changes in laboratory values were more predictive than the absolute concentrations or ratios on a specific point in time, we analyzed the changes in the concentrations of TRP, TYR, neopterin and PRL, and in the TRP/LNAA-ratio and PHE/TYR-ratio occurring over the first week compared to baseline and over the first 4 weeks compared to baseline, again in the patients who developed clinical depression in the course of treatment and who did not. No differences were observed between the two groups.

DISCUSSION

In this study on 22 HCV patients treated with PEGIFN, we assessed psychopathology in combination with an exploration of a set of laboratory parameters considered indicative of the hypothetic 5-HT-ergic dysfunction underlying PEGIFN-induced psychiatric side effects. During treatment, scores on the items anger and depression (and vigor to a lesser degree) tended to increase. The increase in irritability is in accordance with the data reported by Russo et al 9, who described aggression as the main psychiatric side effect of IFN.

Regarding the exploration of 5-HT-ergic parameters, baseline levels of TRP were significantly lower compared to the general population, in accordance with earlier publications (19). With regard to levels of TRP and TRP/LNAA-ratio, indices for the availability of TRP

for the CNS 5-HT synthesis, only for TRP a decrease was observed in the at some time points. No consistent changes in 5-HIAA were observed. Secondly, concerning indicators for the BH4 activity, the PHE/TYR-ratio increased in the first four weeks, in part in line with data from studies on cancer patients treated with (PEG)IFN, suggestive for a lower BH4 activity, potentially resulting in an impaired synthesis of 5-HT, dopamine and noradrenaline (8, 20-21). Due to the observed consistent increase in neopterin levels one could have expected a decrease in biopterin. However, this was not the case.

Contrary to our expectations, levels of PRL, a hormone under the control of 5-HT and dopamine, increased soon after the start of PEGIFN and remained elevated during treatment. These findings are in accordance with two recent studies (22-23), but in contrast with another (24). In case of decreased 5-HT-ergic neurotransmission, one might expect a decrease in PRL. Perhaps the observed augmented secretion is due to a decreased dopaminergic inhibition of PRL secretion caused by a PEGIFN-induced decreased dopamine synthesis. Decreased dopamine synthesis in turn, might be due to reduced BH4 synthesis, reflected in our study by an increase in the PHE/TYR-ratio. Also augmented stress due to the side effects caused by PEGIFN treatment could cause higher levels of prolactin (25).

A substantial proportion of patients were clinically diagnosed with depression, in all cases after the 6th week of antiviral treatment. We did not succeed to identify these patients with the use of laboratory parameters, as these were mostly not different between patients who did develop depression and who did not. The higher TRP/LNAA-ratio and the lower PHE/TYR-ratio at day 29 in patients who developed depression later are counterintuitive, as one would expect that lower TRP availability to the CNS and less BH4 activity would induce depression. Furthermore, changes in laboratory parameters compared to baseline did not seem to matter.

No consistent correlations could be found between scores on the items anger and depression and different 5-HT-ergic parameters. Some but not all studies showed a correlation between 5-HT-ergic parameters and depressive symptoms. Neither Bannink (20) nor Wichers (14) could find a relationship between changes in TRP and the TRP/LNAA-ratio and depressive symptoms. On the whole, these findings suggest a more broad exploration of the metabolism of TRP and 5-HT, e.g. by studying kynurenine and PRL. Whereas in humans TRP can only be measured in the peripheral blood, PRL might reflect more directly the central monoaminergic activity. In this study we observed an interesting relationship between baseline levels PRL and an increase in anger and between PRL and depression.

Limitations of our study were the small patient numbers, the wide intra-subject and inter-subject variability of the different parameters and the number of missing data due to treatment cessation or administrative failure.

The data should be interpreted against the background of the high rates of psychopathology found in HCV patients, comprising a broad spectrum of psychiatric and addiction disorders (26, 27). Currently, the differing theories on the pathophysiology underlying the psychiatric disturbance in HCV patients presume biological (e.g. the possibility of a CNS infection by HCV), psychiatric and sociological factors (27). In view of this, the extrapolation from data from IFN-induced depression to depression in general should be undertaken with care.

In summary, we observed both anger and depression as psychiatric side effects of antiviral therapy, although no consistent changes were seen in laboratory parameters reflecting TRP availability to the brain, whereas changes were seen in indices of BH4 activity, as well as in PRL levels. Only PRL concentrations at baseline and during treatment were found to be related to emerging psychiatric side effects. This warrants further research.

REFERENCES

1. Lauer GM, Walker BD. Hepatitis C virus infection. The New England journal of medicine. 2001 Jul 5;345(1):41-52.
2. Veldt BJ, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, et al. Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. Ann Intern Med. 2007 Nov 20;147(10):677-84.
3. Imazeki F, Yokosuka O, Fukai K, Saisho H. Favorable prognosis of chronic hepatitis C after interferon therapy by long-term cohort study. Hepatology. 2003 Aug;38(2):493-502.
4. Van Gool AR, Kruit WH, Engels FK, Stoter G, Bannink M, Eggermont AM. Neuropsychiatric side effects of interferon-alfa therapy. Pharm World Sci. 2003 Feb;25(1):11-20.
5. Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2005 Feb;29(2):201-17.
6. Bonaccorso S, Marino V, Puzella A, Pasquini M, Biondi M, Artini M, et al. Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. J Clin Psychopharmacol. 2002 Feb;22(1):86-90.
7. Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M, Dantzer R. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. Mol Psychiatry. 2002;7(5):468-73.
8. Van Gool AR, Fekkes D, Kruit WH, Mulder PG, Ten Hagen TL, Bannink M, et al. Serum amino acids, biopterin and neopterin during long-term immunotherapy with interferon-alpha in high-risk melanoma patients. Psychiatry Res. 2003 Jul 15;119(1-2):125-32.
9. Russo S, Kema IP, Haagsma EB, Boon JC, Willemse PH, den Boer JA, et al. Irritability rather than depression during interferon treatment is linked to increased tryptophan catabolism. Psychosomatic medicine. 2005 Sep-Oct;67(5):773-7.
10. Michaelis M, Michaelis R, Suhan T, Schmidt H, Mohamed A, Doerr HW, et al. Ribavirin inhibits angiogenesis by tetrahydrobiopterin depletion. Faseb J. 2007 Jan;21(1):81-7.
11. Anderson DN, Wilkinson AM, Abou-Saleh MT, Blair JA. Recovery from depression after electroconvulsive therapy is accompanied by evidence of increased tetrahydrobiopterin-dependent hydroxylation. Acta Psychiatr Scand. 1994 Jul;90(1):10-3.
12. Hoekstra R, van den Broek WW, Fekkes D, Bruijn JA, Mulder PG, Pepplinkhuizen L. Effect of electroconvulsive therapy on biopterin and large neutral amino acids in severe, medication-resistant depression. Psychiatry research. 2001 Sep 20;103(2-3):115-23.
13. Myint AM, Kim YK. Cytokine-serotonin interaction through IDO: a neurodegeneration hypothesis of depression. Medical hypotheses. 2003 Nov-Dec;61(5-6):519-25.
14. Wichers MC, Koek GH, Robaeys G, Verkerk R, Scharpe S, Maes M. IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. Molecular psychiatry. 2005 Jun;10(6):538-44.
15. Zeuzem S, Pawlotsky JM, Lukasiewicz E, von Wagner M, Goulis I, Lurie Y, et al. International, multicenter, randomized, controlled study comparing dynamically individualized versus standard treatment in patients with chronic hepatitis C. J Hepatol. 2005 Aug;43(2):250-7.
16. Fekkes D, van Dalen A, Edelman M, Voskuilen A. Validation of the determination of amino acids in plasma by high-performance liquid chromatography using automated pre-column derivatization with o-phthaldialdehyde. Journal of chromatography. 1995 Jul 21;669(2):177-86.
17. Fukushima T, Nixon JC. Analysis of reduced forms of biopterin in biological tissues and fluids. Analytical biochemistry. 1980 Feb;102(1):176-88.
18. Borgdorff P, Fekkes D, Tangelder GJ. Hypotension caused by extracorporeal circulation: serotonin from pump-activated platelets triggers nitric oxide release. Circulation. 2002 Nov 12;106(20):2588-93.
19. Cozzi A, Zignego AL, Carpendo R, Biagiotti T, Aldinucci A, Monti M, et al. Low serum tryptophan levels, reduced macrophage IDO activity and high frequency of psychopathology in HCV patients. J Viral Hepat. 2006 Jun;13(6):402-8.

20. Bannink M, Fekkes D, Van Gool AR, Kruit WH, Sleijfer S, van der Holt B, et al. Interferon-alpha influences tryptophan metabolism without inducing psychiatric side effects. Neuropsychobiology. 2007;55(3-4):225-31.
21. Van Gool AR, van Ojik HH, Kruit WH, Bannink M, Mulder PG, Eggermont AM, et al. Pegylated interferon-alpha2b treatment in melanoma patients: influence on amino acids, 5-hydroxyindolacetic acid and pteridine plasma concentrations. Anticancer Drugs. 2004 Jul;15(6):587-91.
22. Plockinger U, Kruger D, Bergk A, Weich V, Wiedenmann B, Berg T. Hepatitis-C patients have reduced growth hormone (GH) secretion which improves during long-term therapy with pegylated interferon-alpha. Am J Gastroenterol. 2007 Dec;102(12):2724-31.
23. Kraus MR, Schafer A, Bentink T, Scheurlen M, Weissbrich B, Al-Taie O, et al. Sexual dysfunction in males with chronic hepatitis C and antiviral therapy: interferon-induced functional androgen deficiency or depression? J Endocrinol. 2005 May;185(2):345-52.
24. Durazzo M, Premoli A, Di Bisceglie C, Bertagna A, Faga E, Biroli G, et al. Alterations of seminal and hormonal parameters: An extrahepatic manifestation of HCV infection? World J Gastroenterol. 2006 May 21;12(19):3073-6.

Sperm dna integrity is not affected by treatment with peginterferon alfa and ribavirin for chronic hepatitis c

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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 <15x106/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 x 106 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 ≥ 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 comedication included selective serotonin reuptake inhibitors, benzodiazepines and antipsychotics which were used in 9 patients. All patients were naïve to antiviral treatment and were treated with peginterferon 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.

Table 1 | Baseline characteristics

Number of patients	**23**
Age (mean, range in years)	43 (22-62)
BMI (mean, range in kg/m2)	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%)

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 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 x 106/ml (range 3-344 x 106/ml). Median sperm concentrations at week 12, 24, 48 and FU24 were 38, 27, 37 and 54 x 106/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

Figure 1|Seminal volume (A), semen concentration (B), progressive motility (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

Figure 1a

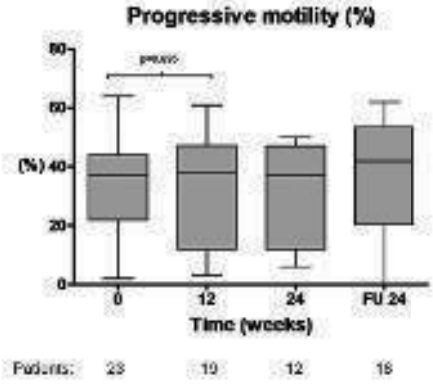


Figure 1b

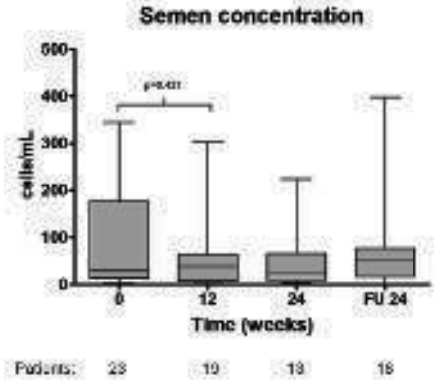


Figure 1c

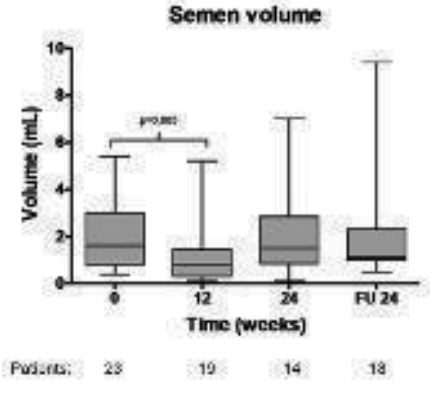
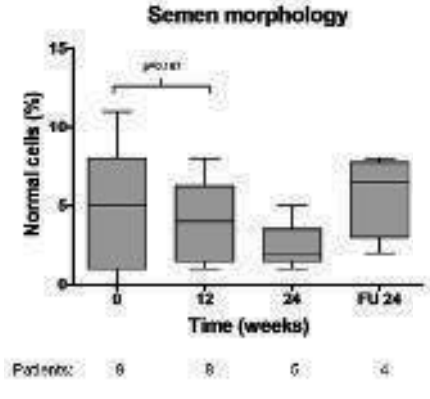


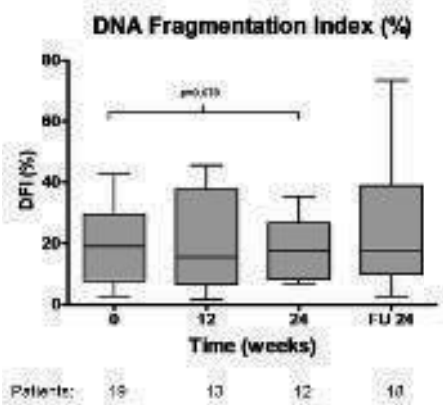
Figure 1d



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).

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 (fig·

Figure 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



ure 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).

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 progessive 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.

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 x 106	51.0 x 106	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 x 106	59.0 x 106	0.31
	Motility	22.5%	36.0%	0.18
	Sperm DNA integrity	21.9%	17.2%	0.35

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

An 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)

Figure 3a

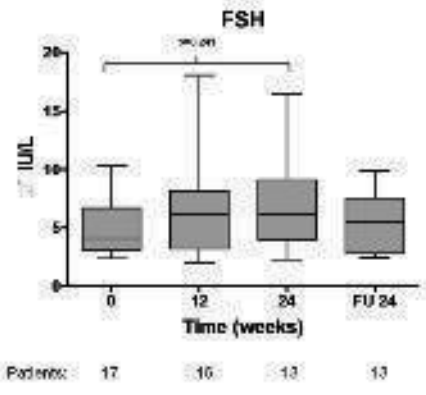


Figure 3b

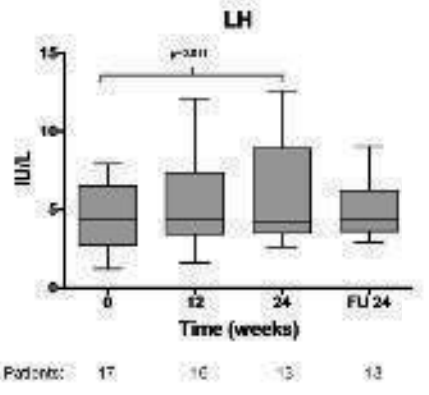


Figure 3c

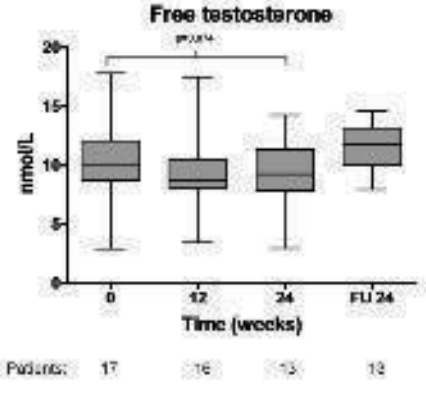


Figure 3d

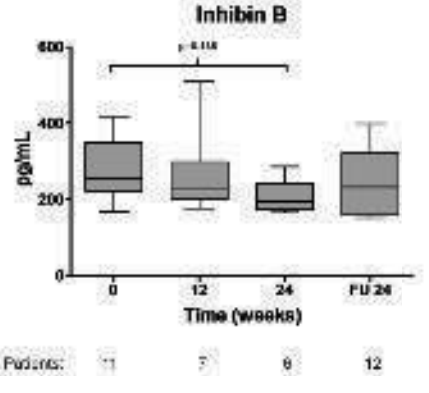


Figure 4 | DFI in patients using methadone (A) antipsychotics (B) (median, range)
DFI was significantly increased in patients using methadone and antipsychotics

Figure 4a

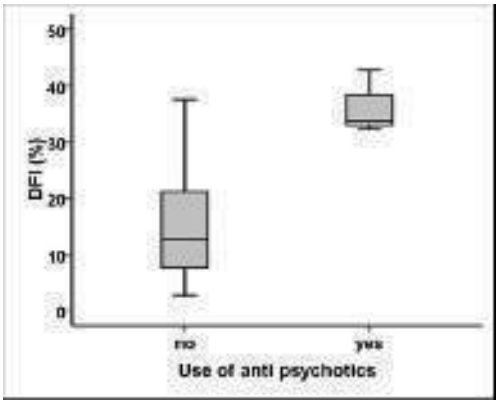
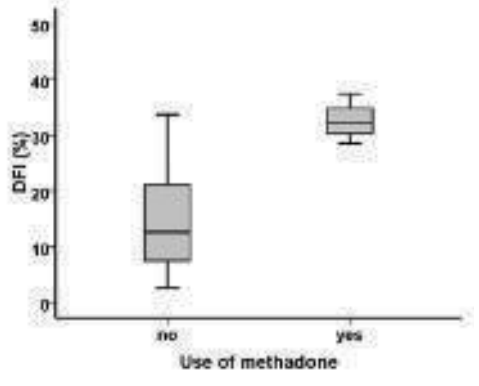


Figure 4b



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 disagree-

ment 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 teratogenity, 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. Trigonocephaly and valproate: a case report and review of literature. Prenat Diagn 2008;28:259-261.
12. Bianca S, Ettore G. Male periconceptional ribavir-ininterferon 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, Staufer 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_ntenbijsluiter_Rebetol_tcm_169-189839.pdf.
18. 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.
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 Roijen 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.

Long-term effects of treatment and response in patients with chronic hepatitis C on quality of life. An international, multicenter, randomized, controlled study.

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ABSTRACT

Background Hepatitis C decreases health related quality of life (HRQL) which is further diminished by antiviral therapy. HRQL improves after successful treatment. This trial explores the course of and factors associated with HRQL in patients given individualized or standard treatment based on early treatment response (Ditto-study).

Methods The Short Form (SF)-36 Health Survey was administered at baseline (n= 192) and 24 weeks after the end of therapy (n= 128).

Results At baseline HRQL was influenced by age, participating center, severity of liver disease and income. Exploring the course of HRQL (scores at follow up minus baseline), only the dimension general health increased. In this dimension patients with a relapse or sustained response differed from non-responders. Men and women differed in the dimension bodily pain. Treatment schedule did not influence the course of HRQL.

Conclusions Main determinants of HRQL were severity of liver disease, age, gender, participating center and response to treatment. Our results do not exclude a more profound negative impact of individualized treatment compared to standard, possibly caused by higher doses and extended treatment duration in the individualized group. Antiviral therapy might have a more intense and more prolonged negative impact on females.

BACKGROUND

Patients chronically infected with hepatitis C virus (HCV) have a decreased health related quality of life (HRQL) compared to the general population (1,2). The impact of the disorder is comparable with other stressful life events and chronic diseases, like diabetes (3). In part, the reduction in HRQL is due to the mental components of HRQL. With regard to these mental components, patients aware of their diagnosis have a more reduced HRQL than those who are unaware (4). Furthermore, many HCV patients have a previous or ongoing addiction and/or psychiatric problems, reflected in lower HRQL. In addition, patients with hepatitis C are stigmatized in society and the majority of the population of hepatitis C patients has a lower social economic status compared to the general population (5).

The reduction of HRQL is probably also due to physical and psychiatric symptoms as a direct consequence of this chronic infection and its sequelae (such as cirrhosis). The chronic inflammation is believed to signal the brain and to give rise to neurovegetative symptoms (e.g. malaise and fatigue) and to amongst others depression and concentration difficulties (6). Possibly, also the brain itself is infected by HCV (7).

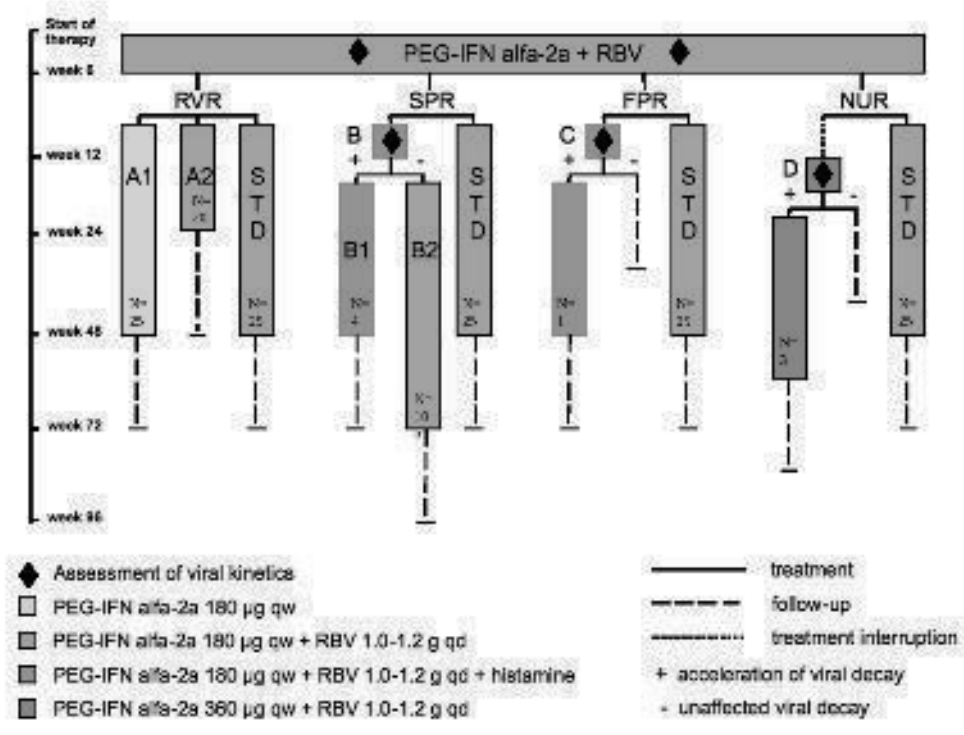
Finally, treatment of chronic HCV with (peg)interferon-alpha ((PEG)IFN) and ribavirin (RBV) further diminishes HRQL due to its side effects. The introduction of PEGIFN provided a significant improvement over standard IFN, with the result that the decrease of HRQL during treatment with PEGIFN is less than with standard IFN (8,9,10,11). In case of successful treatment (obtaining a sustained virological response (SVR)) an improvement of HRQL-scores is observed (2,8,9,10).

In the Ditto-study (12) a dynamically individualized treatment schedule depending on the on-treatment response was compared to a standard combination therapy with PEGIFN alfa-2a (180 µg qw) plus RBV (1000-1200 mg qd) for 48 weeks (Figure 1). The primary aim of the Ditto study was to improve the SVR rate by individualizing the treatment schedule, but this could not be established.

This large scale, multi-centre, international trial, firstly, provides the opportunity to explore a variety of factors associated with HRQL in HCV patients at baseline (treatment naïve; t=0). Secondly, although type of treatment, treatment intensity and treatment duration were heterogeneous this study enabled us to investigate the course of HRQL during PEGIFN-based treatment and to add to the existing knowledge on the impact of cytokine-based antiviral treatment on HRQL. (E.g. do patients recover 24 weeks after completion of therapy (follow up) and which factors are associated with the course of HRQL?)

Figure 1 | Treatment allocation of patients with chronic hepatitis C according to initial virologic response pattern in the Ditto study

Abbreviations: Ribavirin (RBV), Rapid viral response (RVR), Slow partial response (SPR), Flat partial response (FPR), null response (NUR), Standard combination therapy (STD)



METHODS

Patients

273 patients were included in the study, after informed consent was obtained, and 270 patients were randomized after 6 weeks of standard treatment. The ethical review boards of the participating centers approved the study. After randomization, 134 patients received standard treatment and 136 patients were treated according to an individualized treatment schedule depending on the response at week 4 and 12 of treatment. 216 patients completed the treatment per protocol and 249 patients completed the end of follow up. At t=0 HRQL was measured in 192 patients (not every center participated in the HRQL-analysis) and at follow up (24 weeks after completion of therapy) in 128 patients. In 120 patients, HRQL could be assessed at both time points.

Table 1 | Determinants of Health Related Quality of Life on t=0 and course of Health Related Quality of Life

	Subscale	t=0		P-value	Course (follow up – t=0)		P-value
Age		Age category (20-29, 30-39, 40-9, 50-59)			Age category (20-29, 30-39, 40-49,50-59)		
	Physical functioning	90.6, 93.1, 86.1, 78.1		0.01			
	Subscale	**t=0**		**P-value**	**Course (follow up – t=0)**		**P-value**
Cirrhosis No/Yes		No	Yes		No	Yes	
	Mental component summary scale	44.7	53.7	0.001			
	General health	61.3	73.5	0.003			
	Bodily pain	74.2	88.4	0.04			
	Role limitation because of emotional problems	68.9	88.1	0.05			
	Mental health	66.4	80.4	0.0004			
	Subscale	**t=0**		**P-value**	**Course (follow up – t=0)**		**P-value**
Income in Euros	Subscale SF-36	Correlation coefficient		P-value	Correlation coefficient		P-value
	Physical component summary scale	0.30		0.003			
	Role limitation because of physical health	0.23		0.002			
	Vitality				.30		0.02
	Bodily pain	0.18		0.07			
	Mental health				.21		0.03
	Subscale	**t=0**		**P-value**	**Course (Follow up – t=0)**		**P-value**
Gender (M/F)		M	F		M	F	
	Bodily pain				+ 4.1	- 7.2	0.02
	Social functioning				+ 6.3	- 5.8	0.008
	Vitality				+ 7.2	- 2.7	0.03
	Role limitation because of emotional problems				+ 9.3	- 8.3	0.05

Results of statistical analysis of the influence of age (ANOVA), presence of cirrhosis (t-test), gender (t-test) and income in Euros (correlation analysis: Spearman) on Health Related Quality of Life at start of treatment (t=0) and the course of Health Related Quality of Life (scores at 24 weeks after treatment (Follow up) minus scores at baseline). Data were shown if relationships were statistically significant.

Study Design

This phase III, open-label, randomized, multicenter trial was conducted by the DITTO-HCV study group between February 2001 and November 2003 at 9 centers in France, Germany, Greece, Israel, Italy, the Netherlands, Spain, Sweden, and Switzerland.

Assessments

The Short Form (SF)-36 Health Survey was used to measure HRQL. The SF-36 generates a profile of HRQL outcomes by measuring health across eight different dimensions: physical functioning, role limitation because of physical health, social functioning, vitality, bodily pain, mental health, role limitation because of emotional problems and general health. Responses to each question within a dimension are combined to generate a score from 0 to 100, where 100 indicates "good health". The eight multi-item subscales were also converted into a physical component summary scale and a mental component summary scale [13]. Demographic (such as age, gender, and income in Euros) and disease related factors were assessed before start of treatment. At the moment the SF-36 was completed at follow up both patients and the treating physician were unaware of the response to the treatment because HCV-RNA testing results were not known yet, however non-responders were aware of their (positive) HCV-RNA status at relevant time points during treatment.

DATA ANALYSIS

Power calculation

The HRQL-analysis was part of the main study which was powered on significant differences in SVR-rates between standard and individualized treatment. Therefore no separate power analysis was performed for this analysis.

Determinants of HRQL before treatment

To investigate the influence of different characteristics such as age, grade of fibrosis, and income in Euros, t-test analysis for dichotomous categorical, univariate analysis for multicategorical and correlation analysis (Spearman) for continuous data were performed (n=192).

Course of HRQL

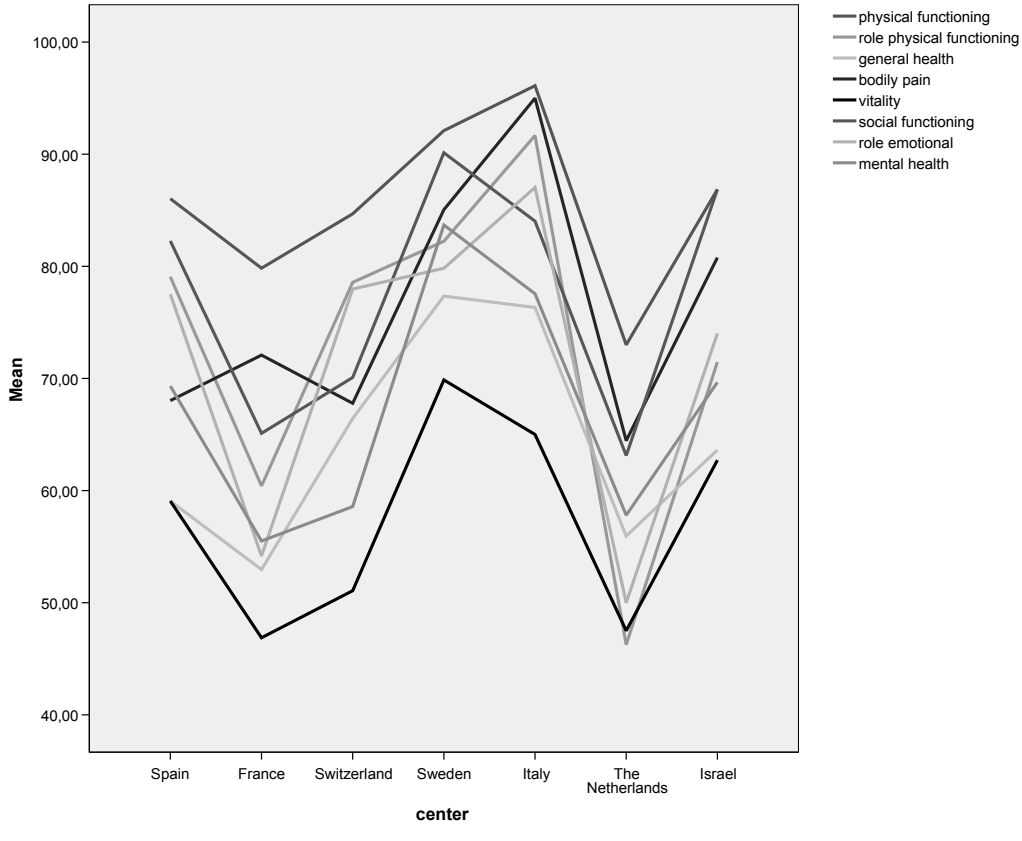
The differences between HRQL at t=0 and follow up were investigated with analysis of variance (ANOVA) and t-tests for Equality of Means (n=120).

Explanatory variables on the course of HRQL

a) Effects of response to treatment on the course of HRQL

The course of HRQL was analyzed separately for patients with SVR, relapse or non-re-

Figure 2 | Mean scores of the different SF36 dimensions at t = 0 from the participating centers
Barcelona; Spain, Paris; France, Geneva; Switzerland, Gothenburg; Sweden, Parma; Italy, Rotterdam; The Netherlands, Rehovot; Israel



sponse with t-tests. Comparison between patients with SVR, with relapse and non-response was assessed by means of ANOVA.

b) Effects of treatment schedule

The mean differences in quality of life between t=0 and follow up were compared between patients treated with standard and individualized treatment using ANOVA and t-tests for Equality of Means. Also the mean differences were compared between the groups with RVR (A1, A2 and standard treatment with RVR) and the groups B2, D and A2 (extended versus shortened therapy, see Figure 1).

c) Effects of patient characteristics on the course of HRQL

With t-test analysis for dichotomous categorical, univariate analysis for multicategorical and correlation analysis (Spearman) for continuous data, the influence on the differences between HRQL at t= 0 and follow up was determined for the factors age (<20, 20-29, 30-39,

Figure 3 | Mean scores with 95 % confidence intervals of the different dimensions of the SF-36 in men at baseline and at 24 weeks after completion of treatment (follow up)

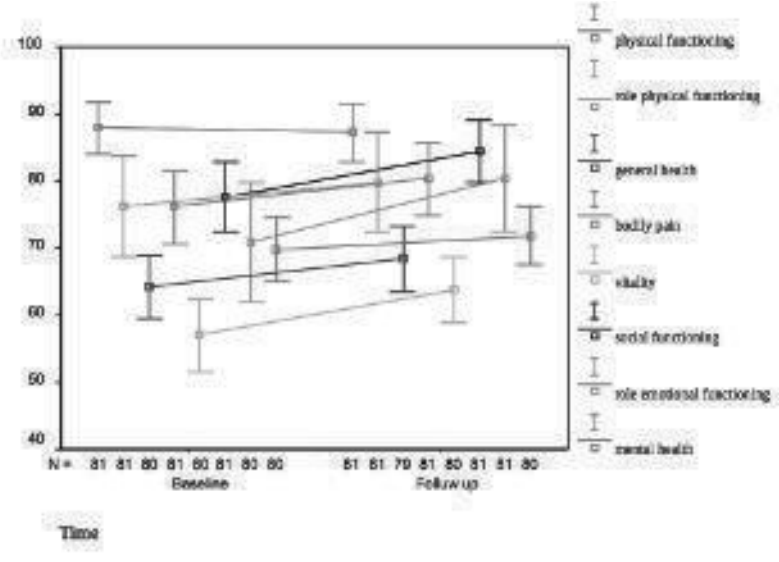
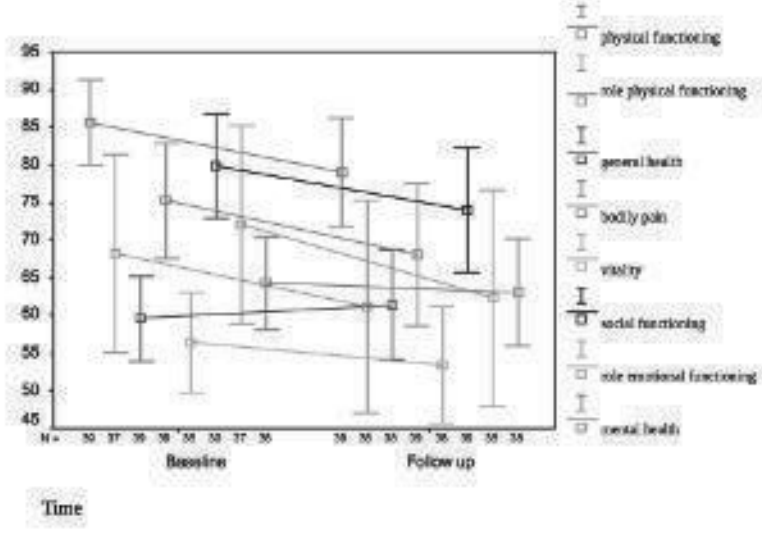


Figure 4 | Mean scores with 95 % confidence intervals of the different dimensions of the SF-36 in females at baseline and at 24 weeks after completion of treatment (follow up)



40-49, 50-59, 60-69 years), gender, participating center, presence of cirrhosis, genotype (1, 4, and 5 versus 2 and 3) and income in Euros.

d) Multivariate analysis

For further exploration of the influence of the different factors mentioned above on the

course of HRQL, a multivariate analysis was performed with a correction for age and baseline values of the SF-36.

RESULTS

Baseline host and virus-related variables were similar in the standard and the individualized treatment groups (data not shown).

Determinants of HRQL before treatment

HRQL varied with age, participating center, severity of liver disease and income in Euros (Table 1). As might be expected, younger patients had a better performance compared to older patients, especially on the physical components of HRQL. Surprisingly, cirrhotic patients had a better performance than non-cirrhotic patients, having significantly higher scores on several dimensions of the SF-36, mostly on mental components. Higher income in Euros had a weak positive effect on the physical component summary scale and role limitation because of physical health scale of HRQL, with a correlation coefficient of 0.30 (p=.003) and 0.23 (p=.002) respectively. Finally, HRQL differed among the different participating centers: the mental component summary scale and the dimensions physical functioning, role limitation because of physical health, general health, bodily pain, vitality, social functioning and mental health were all significantly different between the participating centers (Figure 2).

Course of HRQL

General health improved significantly at follow up compared to general health at start of treatment (mean improvement 3.5, p =.04). The subscale physical functioning tended to worsen with a mean decrease of – 3.4 (p =.06) compared to t=0. Other dimensions did not change.

Explanatory variables on the differences between HRQL at baseline and at follow up

a) Effect of response to treatment on course of HRQL

In patients with SVR, as well as with a relapse or non-response, HRQL did not change compared to t=0 after treatment with PEGIFN antiviral therapy, except for an increase in social functioning in sustained viral responders (78.8 at baseline, 83.3 at follow up, p =.04). Among the different groups changes compared to baseline in the dimensions general health differed significantly (p =.02) between patients with a relapse (+ 5.3), SVR (+ 6.1) and non-response (- 6.3) to treatment.

Table 2 | Multivariate analysis

	Physical component summary scale	Mental component summary scale	Physical functioning	Role limitation because of physical health	General health	Bodily pain	Vitality	Social functioning	Role limitation because of emotional problems	Mental health
Gender		.043	.050	.030		.006	.009	.001	.024	.023
SVR					.047					
Standard vs Individualized treatment	.031					.019	.027	.031		.075

Multivariate analysis (corrected for baseline value and age) of the influence of gender, response to treatment and kind of therapy on the mean difference between baseline and follow up (course of Health Related Quality of Life) of the different dimensions of the SF-36.

Only significant p-values are shown in this table.

Abbreviations: Sustained Virological Response (SVR).

b) Effect of treatment schedule on course of HRQL

No significant differences were observed in changes in the different dimensions of the SF-36 between standard and individualized treatment. A sub-analysis comparing responders and non-responders separately in standard and individualized treatment showed no difference, either. Patients treated for 24 weeks and patients treated with double dose PEGIFN showed a decline on the dimensions mental health whereas patients treated for 72 weeks had an increase on this dimension: respectively – 7.9, - 2.0 and + 18.5.

c) Effects of patient characteristics on course of HRQL

Neither age, genotype nor grade of fibrosis had an influence on changes in the different dimensions of HRQL. A significant difference was seen between men and women in the dimensions bodily pain (males: + 4.1, females: - 7.2, p =.02), social functioning (males: + 6.3, females: - 5.8, p =.008), vitality (males: + 7.2, females: - 2.7, p =.03) and role limitations due to emotional problems (males: + 9.3, females: - 8.3, p=.05). There was a weak significant positive correlation between income in Euros and increase in vitality and mental health between baseline and follow up: correlation coefficient .30 (p =.02) for vitality and .21 (p =.03) for mental health. See figure 3 and figure 4.

d) Multivariate analysis

Also in a multivariate analysis gender was a significant factor on the course of HRQL. All differ-

ent dimensions, except for physical component summary scale and general health, were significantly different between men and women (Table 2). Response to treatment was again significantly influencing general health.

In the multivariate analysis, individualized treatment had a significantly negative influence on the course of HRQL on the dimensions physical component summary scale, bodily pain, vitality and social functioning, compared to patients treated with standard treatment (Table 2). The presence or absence of cirrhosis and grade of fibrosis did not play a role in the course of HRQL in this analysis. Also different kind of genotypes (1, 4 and 5 versus 2 and 3) did not influence changes in HRQL.

DISCUSSION

In this study we explored the influence of PEGIFN-based antiviral treatment with various regimens and of patients characteristics on HRQL before as well as at 24 weeks after completion of treatment. Main determinants of HRQL in this study were the severity of liver disease (non-cirrhosis vs. cirrhosis), age, gender and participating center and – to some extent - response to treatment. With regard to the severity of the liver disease, patients with cirrhosis did better compared to those without cirrhosis. We are unable to explain this difference.

In our study almost all dimensions of the SF-36 did not change regardless of response, indicative of a satisfying recovery of patients after cytokine-based therapy. However, a significant increase was found on the subscale general health despite a therapy-induced worsening of the subscale physical functioning. This improvement was seen in patients with both SVR and relapse, whereas non-responders showed a decrease in general health. In our study, interestingly the relapsers who were ignorant of treatment effect when filling in the questionnaire, had a far more higher score on general health compared to the non-responders who were aware of their previous non-response to therapy. This underlines the mental aspects of the impact on HRQL in this disease (14). The observed recovery in HRQL is in line with previous studies reporting an improvement on (almost) all different dimensions of the SF-36 after a successful treatment with PEGIFN and RBV compared to baseline scores (2,8,9,10).

The presence of cirrhosis did not influence the course of HRQL during treatment, but patients with a cirrhosis had higher scores on some, mostly mental, dimensions of the SF-36 at t=0 and at follow up, which is in contrast with earlier studies (8,15). Patients with decompensated cirrhosis were excluded to participate in the study, but still we cannot

explain this difference. Higher age was associated with a decrease in HRQL, according to literature data (16).

Men and women differed in the course of HRQL with an increase on scores for men on several dimensions and women experiencing a decrease. These differences between men and women were also significant in a multivariate analysis for all dimensions except for general health. In the general population, men report a higher HRQL (16,17,18), a finding possibly of relevance for our findings. But also, therapy with PEGIFN and RBV might have a more intense and more prolonged impact on females. Studies show more severe anemia in women during treatment with PEGIFN and RBV (19). As anemia plays an important role in HRQL during antiviral treatment this could be one of the explanations for the observed difference (20). With the fixed doses of PEGIFN women may be treated with higher doses of PEGIFN per kg, which may contribute to more side-effects and a lower HRQL during treatment and at 24-weeks follow-up. To our knowledge, only one study found sex differences in recovery, with males doing slightly worse (21).

HRQL at baseline was found to be differing between the participating centers (in this case different countries/cultural regions). In the study by Ware et al (9) a similar observation was made. Although much attention has been devoted to assure the comparability between different cultures and languages, this observation suggests that studies from different countries concerning side effects (for instance decrease of HRQL, but also psychopathology) should be interpreted with caution (22,23).

An important limitation of this study is the large number of variables in relation to the number of participants and the many different treatment schedules. Also the relative high number of drop-outs with data of 192 patients at baseline and of 128 patients at follow up could be of importance, however non-responders or relapsers were not significantly more non-compliant compared to responders (data not shown). In retrospect an additional time point at the end of treatment would have given more insight.

In conclusion: our findings support the observation, that in general patients exposed to PEGIFN-based treatment do recover to their pre-treatment level of baseline, six months after completion of antiviral therapy or even surpass that level. This underlines again the safe profile of this intensive treatment, irrespective of the used dosage and/or duration in this study. Before treatment and after treatment with PEGIFN in patients chronically in-

fected with HCV health related quality of life is mostly influenced by presence of cirrhosis, age, gender, participating center (or country) and response to treatment. Also, awareness of response status to therapy seems important.

REFERENCES

1. Foster GR, Goldin RD, Thomas HC. Chronic hepatitis C virus infection causes a significant reduction in quality of life in the absence of cirrhosis. Hepatology 1998;27:209-212.
2. Spiegel BM, Younossi ZM, Hays RD, Revicki D, Robbins S, Kanwal F. Impact of hepatitis C on health related quality of life: a systematic review and quantitative assessment. Hepatology 2005;41:790-800.
3. Castera L, Constant A, Bernard PH, de Ledinghen V, Couzigou P. Psychological impact of chronic hepatitis C: comparison with other stressful life events and chronic diseases. World J Gastroenterol 2006;12:1545-1550.
4. Rodger AJ, Jolley D, Thompson SC, Lanigan A, Crofts N. The impact of diagnosis of hepatitis C virus on quality of life. Hepatology 1999;30:1299-1301.
5. Niederau C, Bemba G, Kautz A. [Socioeconomic characteristics, quality of life, and state of knowledge of patients with hepatitis C viral infection in Germany--socioeconomic aspects in hepatitis C]. Z Gastroenterol 2006;44:305-317.
6. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008;9:46-56.
7. Weissenborn K, Krause J, Bokemeyer M, Hecker H, Schuler A, Ennen JC, Ahl B, Manns MP, Boker KW. Hepatitis C virus infection affects the brain-evidence from psychometric studies and magnetic resonance spectroscopy. J Hepatol 2004;41:845-851.
8. Bonkovsky HL, Woolley JM. Reduction of health-related quality of life in chronic hepatitis C and improvement with interferon therapy.The Consensus Interferon Study Group. Hepatology 1999;29:264-270.
9. Ware JE, Jr., Bayliss MS, Mannocchia M, Davis GL. Health-related quality of life in chronic hepatitis C: impact of disease and treatment response. The Interventional Therapy Group. Hepatology 1999;30:550-555.
10. McHutchison JG, Ware JE, Jr., Bayliss MS, Pianko S, Albrecht JK, Cort S, Yang I, Neary MP, Hepatitis Interventional Therapy Group. The effects of interferon alpha-2b in combination with ribavirin on health related quality of life and work productivity. J Hepatol 2001;34:140-147.
11. Hassanein T, Cooksley G, Sulkowski M, Smith C, Marinos G, Lai MY, Pastore G, trejo-Estrada R, Horta E, Vale A, Wintfield N, green J. The impact of peginterferon alfa-2a plus ribavirin combination therapy on health-related quality of life in chronic hepatitis C. J Hepatol 2004;40:675-681.
12. Zeuzem S, Pawlotsky JM, Lukasiewicz E, von Wagner M, Goulis I, Lurie Y, Gianfranco E, Vrolijk JM, Esteban JI, Hezode C, Lagging M, Negro F, Soulier A, Verheij-Hart E, Hansen B, Tal R, Ferrari C, Schalm SW, Neumann AU, DITTO-HCV Study Group. International, multicenter, randomized, controlled study comparing dynamically individualized versus standard treatment in patients with chronic hepatitis C. J Hepatol 2005;43:250-257.
13. Ware JE, Jr., Kosinski M, Bayliss MS, McHorney CA, Rogers WH, Raczek A. Comparison of methods for the scoring and statistical analysis of SF-36 health profile and summary measures: summary of results from the Medical Outcomes Study. Med Care 1995;33(Suppl):AS264-79.
14. Wessely S, Pariante C. Fatigue, depression and chronic hepatitis C infection. Psychol Med 2002;32:1-10.
15. Kallman J, O'Neil MM, Larive B, Boparai N, Calabrese L, Younossi ZM. Fatigue and health-related quality of life (HRQL) in chronic hepatitis C virus infection. Dig Dis Sci 2007;52:2531-2539.
16. Ware JE SK, Kosinski M, Gandek B. SF-36 Health Survey Manual and Interpretation Guide. Boston, MA: New England Medical Center, The Health Institute. 1993.
17. Aaronson NK, Muller M, Cohen PD, Essink-Bot ML, Fekkes M, Sanderman R, Sprangers MA, te Velde A, Verrips E. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. J Clin Epidemiol 1998;51:1055-1068.
18. Lewin-Epstein N, Sagiv-Schifter T, Shabtai EL, Shmueli A. Validation of the 36-item short-form Health Survey (Hebrew version) in the adult population of Israel. Med Care 1998;36:1361-1370.
19. Sulkowski MS, Wasserman R, Brooks L, Ball L, Gish R. Changes in haemoglobin during interferon alpha-2b plus ribavirin combination therapy for chronic hepatitis C virus infection. J Viral Hepat 2004;11:243-250.

20. Pockros PJ, Shiffman ML, Schiff ER, Sulkowski MS, Younossi Z, Dieterich DT, Wright TL, Mody SH, Tang KL, Goon BL, Bowers PJ, Leitz G, Afdhal NH, PROACTIVE Study Group. Epoetin alfa improves quality of life in anemic HCV-infected patients receiving combination therapy. Hepatology 2004;40:1450-1458.
21. Bonkovsky HL, Snow KK, Malet PF, Back-Madruga C, Fontana RJ, Sterling RK, Kulig CC, Di Bisceglie AM, Morgan TR, Dienstag JL, Ghany MG, Gretch DR, HALT-C Trial Group. Health-related quality of life in patients with chronic hepatitis C and advanced fibrosis. J Hepatol 2007;46:420-431.
22. Wagner AK, Gandek B, Aaronson NK, Acquadro C, Alonso J, Apolone G, Bullinger M, Bjorner J, Fukuhara S, Kaasa S, Leplege A, Sullivan M, Waud-Dauphinee S, Ware JE Jr. Cross-cultural comparisons of the content of SF-36 translations across 10 countries: results from the IQOLA Project. International Quality of Life Assessment. J Clin Epidemiol 1998;51:925-932.
23. Bullinger M, Alonso J, Apolone G, Leplege A, Sullivan M, Wood-Dauphinee S, Gandek B, Wagner A, Aaronson N, Bech P, Fukuhara S, Kasa S, Ware JE Jr. Translating health status questionnaires and evaluating their quality: the IQOLA Project approach. International Quality of Life Assessment. J Clin Epidemiol 1998;51:913-923.

Summary and discussion

samenvatting en discussie

Optimal adherence to antiviral treatment for a chronic Hepatitis C virus (HCV) infection is crucial for complete eradication of the virus (as indicated by the attainment of a sustained virological response). However the current therapy with peginterferon (PEGIFN) and ribavirin (RBV) is hindered by many side effects. One of the most frequently observed, severe side effects is psychiatric disturbance such as depression, anger, anxiety and cognitive impairment. This thesis focuses on the manifestation, the underlying pathophysiological mechanisms, the prevention, detection and treatment of psychiatric disturbances due to cytokine-based treatment (as PEGIFN is). Cytokine-based treatment influences a variety of other organ systems apart from the central nervous system. A separate chapter is included on the effects of PEGIFN on semen quality.

Antiviral treatment and psychopathology - Review –

Chapter 1 reviews both the psychopathology in the natural history of HCV patients and in patients during therapy with PEGIFN, and theories about the pathophysiology underlying these psychiatric disorders. Psychopathology appears to be a common phenomenon in HCV, not only because of the social background and associated problems of these patients but probably also due to the chronic infection itself. This psychopathology complicates the start and completion of antiviral treatment with PEGIFN, as these symptoms frequently exacerbate during the treatment. On top of this, cytokine-induced psychiatric disturbance is one of the main reasons to stop the antiviral therapy prematurely with the result that chances of clearing the virus decline considerably.

Cytokine induced psychiatric side effects not only include depressive syndromes according to current psychiatric standards such as the DSM-IV, but a wider range of individual symptoms. For instance, increased irritability has been described as occurring frequently without a full depressive episode being present.

Several biochemical pathways are proposed by which PEGIFN might signal the brain to induce these psychiatric symptoms such as signaling at the blood-brain barrier, via the vagal nerve and by lowering of the availability of tryptophan (TRP) to the central nervous system. This leads to central changes, such as changes in the cytokine network in the brain, in brain mono-aminergic activity and the hypopituitary-hypophysis-adrenal axis (which regulate adaptation to stress). Selective serotonin reuptake inhibitors (SSRI's) play a key role in the treatment of these psychiatric disturbances and are widely advocated in

this setting. This is perhaps surprising, in view of the doubts that have been raised in the last decade about the efficacy of modern antidepressants.

Antiviral treatment and psychopathology - Clinical studies –

Prophylactic treatment with SSRIs in patients treated with high doses of interferon was effective in an earlier study with oncology patients. Also some studies in selected HCV patients suggested a beneficial effect of prophylactic treatment with SSRIs in patients who had (PEGIFN-induced) depressive syndromes during previous antiviral treatment. Nevertheless, the efficacy and beneficial effect of prophylactic SSRI use in unselected and treatment naive HCV patients during therapy with PEGIFN is still to be determined. **Chapter 2** reports on a double-blind placebo controlled randomized clinical trial to explore this. The incidence of a clinically relevant PEGIFN/ribavirin induced increase of individual symptoms (of lowered mood, hostile feelings and inner tension), but also of the (syndromal) major depression was lower in patients who received prophylactic treatment with the SSRI escitalopram compared to those who received placebo. One other large study confirms the beneficial effect of prophylactic use of an SSRI; however, several smaller studies could not confirm this. Identification of patients who are most at risk to develop psychopathology due to the treatment with PEGIFN gives us the opportunity to select patients who benefit the most from prophylaxis with an SSRI. In **Chapter 3** an analysis of the trial mentioned before demonstrated that patients with a previous history of intravenous drug use and depression were most at risk to develop a depression due to PEGIFN. This is in accordance with previous studies. More important, in this trial patients at risk were partly protected from developing (more) depressive symptoms due to PEGIFN by the use of escitalopram. Concluding, SSRIs do not only play an important role in the treatment of PEGIFN-induced psychopathology, but probably also in the prevention of these side effects if given prophylactically as our results strongly suggested. This study closely resembled daily clinical practice of unselected HCV patients and we consider the observed decrease in psychiatric complaints as clinically relevant. The prevention of a major depression (syndromal depression, our secondary endpoint) by prophylaxis with escitalopram was most in line with existing data and is clinically relevant anyway. This is less clear for our primary endpoint as there is no equivalent of it in the literature on PEGIFN-induced psychiatric disturbance. However, at the outset, we were particularly interested to gain insight into the prevalence

and prevention of psychiatric symptomatology defined broader then only major depression. In our view, this could be clinically relevant most notably in view of the long duration of antiviral treatment. For instance, we assumed that increased irritability during many months of treatment resulting in marital conflicts or severe concentration difficulties resulting in underperformance at work, again over a period of many months, need not to go along with the presence of a full-blown depressive syndrome but yet might well compromise quality of life of patients and add to the burden of treatment. To our knowledge, no other studies took this standpoint. At the end, we feel justified in taking this approach as the cumulative incidence of clinically relevant increase of the selected individual target symptoms (lowered mood, concentration difficulties, hostile feelings and increased tension) clearly exceeded the incidence of major depression.

Form a standpoint of ethics, one might argue that a group of patients – and perhaps even a considerable percentage of it - in the study was treated with an SSRI although they would not have developed psychopathology anyway. This is true, but the side effects of this treatment seemed not to outweigh the side effects PEGIFN and RBV cause. No patient stopped because of side effects of the SSRI. However this study was not designed to monitor these side effects properly. A much larger study is needed to do this before standard prophylaxis with an SSRI is to be introduced. However, for the selected patients at risk (the patients with a previous history of intravenous drug use and depression) the introduction of prophylactic treatment seems to be very reasonable.

Antiviral treatment and psychopathology – Biochemical background –

The high incidence of psychiatric symptoms during antiviral immunotherapy and the effectiveness of SSRIs at least suggest an influence of PEGIFN on the brain function: peripherally administrated cytokine-based treatment leads to central changes. For this reason PEGIFN-induced psychopathology has been used as a model to study the interaction/communication of the immune system with the brain. Several theories have been introduced on the effector mechanisms underlying this interaction: alterations in brain monoaminergic activity, alterations in brain cytokines, accumulation of neurotoxic metabolites and changes in the hypothalamic-pituitary-adrenal (HPA) axis.

Several previous studies explored peripheral changes reflecting the above mentioned effector mechanisms in patients treated with PEGIFN. We focussed on different peripheral

parameters reflecting brain metabolism in relation to tryptophan and monoaminergic neurotransmission in a broader sense. In **Chapter 4 and 5** we describe the biochemical changes in two different patient groups treated with PEGIFN and RBV. As observed before, PEGIFN decreases peripheral levels of TRP. TRP is the precursor of the monoamine serotonin (5-hydroxytryptamine; 5-HT), and as a consequence PEGIFN could inhibit peripheral and central 5-HT synthesis. The Phenylalanine/Tyrosine (PHE/TYR) ratio was used as a marker of the availability of tetrahydrobiopterin, an important co-factor of the synthesis of 5-HT but also of the monoamines dopamine and noradrenaline. A consistent rise in PHE/TYR-ratio was seen, correlating with development of psychopathology. Strikingly, this rise in the PHE/TYR-ratio was consistently seen in other studies done in oncology patients treated with (PEG)IFN. Also levels of the neurotoxic metabolite kynurenin increased during PEGIFN treatment. The level of the hormone prolactine is influenced by dopamine synthesis. The observed rise in prolactin levels correlated with emerging symptoms of anger and depression. At last the decreased ratio between citruline and arginine is thought to reflect an impaired nitric oxide synthesis, which in turn influences the 5-HT release. Concluding PEGIFN might not only influence 5-HT synthesis (via decreased levels of TRP), but could also induce psychopathology (or increase the vulnerability to develop psychiatric symptoms) by influencing the synthesis of dopamine and noradrenaline and by formation of neurotoxic metabolites. However, in our research, few relationships were observed between biochemical alterations and the emergence of psychic complaints

Notably only minor differences were seen in these biochemical parameters between patients treated with the SSRI escitalopram or placebo. However, in the escitalopram group significantly less psychopathology was observed. This strongly suggests that the effect of the SSRI is downstream in the cascade of the switched systems of the brain.

Earlier studies on the pathophysiology of (PEGIFN-induced) depressive syndromes focussed mainly on TRP depletion. More recent studies, like ours, show that the effector mechanism is far more complex. PHE/TYR ratio was the most consistent changed parameter with the most consistent relation to clinical changes. Therefore this parameter needs to be more studied in PEGIFN treated patients, leading to more focus on changes in dopamine and adrenaline synthesis. However correlation with clinical observed psychopathology was not only small but also studied in peripheral parameters reflecting brain metabolism. Still further research in PEGIFN induced psychopathology remains interest-

ing as it gives the opportunity to study the role of (peripheral) cytokines (as part of the immune system) in the pathogenesis of psychiatric symptoms in a rather fixed setting. Functional brain imaging could be of help to clarify this pathogenesis.

Antiviral treatment and semen quality

Because of the unknown effects of PEGIFN on semen double contraception during and until six months after treatment with PEGIFN is required. **Chapter 6** is a descriptive study on semen quality performed in 20 HCV patients. Semen abnormalities were frequently seen in men prior to treatment with PEGIFN but did not increase during treatment. Also decreased sperm DNA integrity, which is associated with poor reproductive outcome and with a higher miscarriage risk, was not observed due tovan treatment with PEGIFN and RBV. These results raise doubts about the standard contraception that is advised. Larger studies are needed to get more details on this subject.

Antiviral treatment and quality of life

Hepatitis C decreases health related quality of life (HRQL) which is further diminished by antiviral therapy with PEGIFN due to the side effects. However earlier studies also show an improvement of HRQL after successful treatment. **Chapter 7** explores the course of and factors associated with HRQL in patients given individualized or standard treatment based on early treatment response (Ditto-study). Main determinants of HRQL were severity of liver disease, age, gender, participating center and response to treatment. Antiviral therapy might have a more intense and more prolonged negative impact on female patients because of the fixed and sometimes high doses of PEGIFN used in the trial.

SAMENVATTING EN DISCUSSIE

Bij de antivirale behandeling van een chronische hepatitis C virus (HCV) infectie is optimale therapietrouw van cruciaal belang voor de complete eradicatie van het virus (aangeduid als een blijvende virologische repsons). Bij de huidige behandeling met peginterferon (PEGIFN) en ribavirine (RBV) treden echter frequent bijwerkingen op die het niet gemakkelijk maken de behandeling te volgen. De meest frequent optredende groep bijwerkingen is psychiatrisch van aard: depressie, agressie, angst en cognitieve veranderingen. Dit proefschrift richt zich op de manifestatie, het onderliggende pathofysiologische mechanisme, de preventie, detectie en behandeling van deze psychiatrische ontregeling ten gevolge van behandeling met het cytokine PEGIFN. Therapieën met cytokines hebben naast invloed op het centraal zenuwstelsel ook invloed op verscheidene andere organen. Een apart hoofdstuk is daarom gewijd aan het effect van PEGIFN op de kwaliteit van sperma.

Antivirale behandeling en psychopathologie – bespreking-

Hoofdstuk 1 bespreekt zowel de psychopathologie zoals die beschreven is in het natuurlijk beloop van patiënten met een HCV-infectie (dus zonder behandeling) en bij patiënten die behandeld worden met PEGIFN, alsook theorieën over de onderliggende pathofysiologie van deze psychiatrische stoornissen. Psychopathologie is een veel voorkomend probleem onder HCV-patiënten. Dat heeft niet alleen te maken met de sociale achtergrond met bijhorende problematiek van deze patiënten (velen liepen hun infectie op door intraveneus drugsgebruik), maar waarschijnlijk ook met de gevolgen van een chronische infectie zelf. Deze psychopathologie compliceert het volbrengen van de antivirale behandeling met PEGIFN, te meer omdat deze voor de behandeling aanwezige psychiatrische symptomen gedurende de antivirale behandeling vaak verergeren. Deze cytokine-geïnduceerde psychiatrische klachten zijn dan ook één van de voornaamste redenen is om de behandeling voortijdig te stoppen, en voortijdig stoppen resulteert weer in een sterk verminderde kans om het virus definitief te klaren.

Cytokine-geïnduceerde psychiatrische klachten behelzen niet alleen depressieve syndromen (bijvoorbeeld de depressieve episode zoals die beschreven is volgens de huidige DSM-IV criteria), maar ook een scala aan individuele psychische symptomen. Toegenomen geïrriteerdheid bijvoorbeeld, zonder dat er verder een depressieve episode aanwezig is, komt bijvoorbeeld frequent voor.

Er zijn verschillende voorgestelde routes beschreven waarlangs PEGIFN het brein kan bereiken en de hersenfunctie kan beïnvloeden om deze psychiatrische symptomen te veroorzaken. Bijvoorbeeld via de bloed-hersenbarrière (waar cytokines in het bloed boodschappen kunnen afgeven die worden doorgeleid naar de hersenen zelf), via de nervus vagus of door verminderde beschikbaarheid van tryptofaan aan het centrale zenuwstelsel. Dit leidt waarschijnlijk weer tot veranderingen in de hersenen zoals activatie van het cytokine netwerk in het brein, veranderingen in de mono-aminerge activiteit (een aantal van de prikkeloverdrachtstoffen die de zenuwcellen gebruiken om boodschappen over te brengen) of in de hypothalamus-hypofyse-bijnierschors as (die de adaptatie aan stress reguleert). Specifieke serotonineheropnameremmers (SSRI's) spelen een hoofdrol in de behandeling van deze psychiatrische klachten en worden daarom voor deze indicatie aanbevolen. Dit is misschien verrassend gezien de twijfels die er de laatste decennium gerezen zijn over de effectiviteit van de moderne antidepressiva.

Antivirale therapie en psychopathologie – klinische studies-

Profylactische behandeling met een SSRI was effectief in eerdere studies met oncologische patiënten die behandeld werden met hoge dosis interferon. Ook enkele studies met geselecteerde HCV patiënten suggereren een gunstig effect van profylactische behandeling met een SSRI bij patiënten die een (PEGIFN-geïnduceerde) depressie hadden ten tijde van eerdere antivirale therapie.

Desondanks is de effectiviteit en het gunstige effect van profylactisch gebruik van SSRI's in niet-geselecteerde, niet-eerder behandelde HCV patiënten nog onbekend. **Hoofdstuk 2** beschrijft een dubbelblinde, gerandomiseerde en met placebo gecontroleerde studie die dit onderzoekt. De incidentie van een klinisch relevante, door PEGIFN-geïnduceerde, toename van individuele symptomen (zoals verminderde stemming, vijandige gevoelens en innerlijke spanning) evenals de incidentie van een depressieve episode (depressief syndroom) was lager in patiënten die profylactisch behandeld werden met de SSRI escitalopram vergeleken met patiënten die placebo kregen.

Een andere, grotere studie bevestigt het gunstig effect van profylactisch gebruik van een SSRI; andere studies tonen dit echter niet aan. Het identificeren van patiënten die het grootste risico hebben om depressieve klachten te ontwikkelen ten gevolge van behandeling met PEGIFN stelt ons in staat patiënten te selecteren die het meest baat zullen hebben van profylactische behandeling met een SSRI. In **Hoofdstuk 3** laat een aanvullende

analyse van de hiervoor genoemde studie zien dat patiënten die eerder intraveneus drugs gebruikten en eerder in hun leven een depressie hadden, het grootste risico lopen op het ontwikkelen van een depressief syndroom ten gevolge van PEGIFN. Dit komt overeen met eerdere studies. Nog belangrijker, in deze studie waren deze 'at risk' patiënten gedeeltelijk beschermd door het gebruik van escitalopram tegen het ontwikkelen van (meer) depressieve klachten ten gevolge van PEGIFN.

Concluderend spelen SSRI's niet alleen een belangrijke rol in de behandeling van PEGIFN-geïnduceerde psychopathologie, maar waarschijnlijk ook, zo is de sterke indruk op grond van onze resultaten, in de preventie van deze bijwerkingen als deze SSRI's profylactisch worden gegeven. De patiënten die aan deze studie deelnamen kwamen sterk overeen met de niet-geselecteerde HCV patiënten die men in de dagelijkse klinische praktijk ziet. Ook beschouwen we de geobserveerde afname in psychiatrische klachten zoals gedaalde stemming en prikkelbaarheid als klinisch relevant. De preventie van een klinische depressie (depressieve episode volgens DSM-IV-criteria, ons secundaire eindpunt) door profylaxe met escitalopram was het meest in overeenkomst met al eerder beschreven data en is in ieder geval klinisch relevant. Dit is misschien minder duidelijk voor ons primaire eindpunt, omdat hiervan geen equivalent te vinden is in de literatuur over PEGIFN-geïnduceerde psychopathologie. Echter, bij de opzet van de studie, waren we vooral geïnteresseerd om meer inzicht te krijgen in de prevalentie en preventie van psychiatrische symptomen die een breder gebied beslaan dan alleen een depressief syndroom. Vanuit ons standpunt is de studie van individuele symptomen klinisch relevant gezien de lange behandelduur van de antivirale therapie. Onze veronderstelling was bijvoorbeeld dat de ernst en het ermee gepaard gaande lijden van bijvoorbeeld toegenomen geïrriteerdheid gedurende vele maanden resulterend in huwelijksproblemen, of van ernstige concentratieproblemen resulterend in onderprestaties op werk (ook voor vele maanden) misschien niet overeenkomen met de ernst en het lijden van een klinische depressie. We veronderstelden dat deze individuele symptomen wel degelijk van grote invloed zouden zijn op de kwaliteit van leven van patiënten en de zwaarte van de behandeling en ook bij méér patiënten zullen voorkomen dan een volledig depressief syndroom. Voor zover wij weten zijn er geen andere studies van dit standpunt uitgegaan. Uiteindelijk voelen wij ons gesteund in onze benadering gezien het feit dat de cumulatieve incidentie van de gekozen symptomen (stemmingsdaling, concentratieproblemen, agressieve gevoelens en toegenomen angst en spanning) duidelijk

de incidentie van een klinische depressie overschrijdt.

Vanuit ethisch oogpunt kan men tegenwerpen dat een groep patiënten – en misschien zelfs een aanzienlijk percentage – in de studie profylactisch behandeld is met SSRI's terwijl zij hoe dan ook geen psychopathologie ontwikkeld hadden. Dit is zeker waar, maar het alternatief zou geweest zijn dat de patiënten eerst een zekere mate van depressieve of andere psychiatrische symptomen ontwikkeld zouden moeten hebben voordat we ze behandeld zouden hebben met een antidepressivum. En zoals eerder gesteld, psychiatrische bijwerkingen zijn een voorname reden om de antivirale behandeling te stoppen, wat ook ongunstig zou zijn voor de patiënt. Verder houdt een depressie ook een zeker risico in, bijvoorbeeld op suïcide. Gelukkig bleken de bijwerkingen van deze interventie de bijwerkingen ten gevolge van PEGIFN en RBV niet te overstijgen. Geen van de patiënten stopte de behandeling ten gevolge van de bijwerkingen van de SSRI. Aan de andere kant was deze studie niet ontworpen om alle mogelijke bijwerkingen gericht te inventariseren. Het geheel overziend zijn meer en veel grotere studies nodig alvorens profylactische behandeling met een SSRI ingevoerd kan worden. Daarentegen lijkt voor geselecteerde patiënten (de patiënten met een voorgeschiedenis van intraveneus drugsgebruik en depressie) de introductie van profylactische behandeling op grond van onze resultaten wel plausibel.

Antivirale behandeling en psychopathologie- Biochemische achtergrond-

De toegenomen incidentie van psychiatrische symptomen ten tijde van antivirale immunotherapie en de effectiviteit van SSRI's suggereert tenminste invloed van PEGIFN op het functioneren van het brein: perifeer toegediende cytokines leiden tot centrale veranderingen. Om deze reden werd PEGIFN-geïnduceerde psychopathologie gebruikt als een model om de interactie/communicatie tussen het immuunsysteem en het brein te onderzoeken. Verschillende theorieën zijn geïntroduceerd over de effector-systemen die ten grondslag liggen aan deze interactie: vooral wordt gedacht aan activatie van immuunactiviteit in de hersenen zelf, aan veranderingen in monoaminerge activiteit in het brein, opstapeling van neurotoxische metabolieten en veranderingen in de hypothalamus-hypofyse-bijnierschors as.

Verschillende eerdere studies verkenden perifere parameters (laboratorium onderzoek in perifeer bloed) in patiënten die met PEGIFN werden behandeld, waarvan men aanneemt dat die parallel lopen aan deze hierboven vermelde veranderingen in de effectorsystemen in de hersenen. Wij richtten ons vooral op de verschillende perifere parameters die het metabolisme van het brein weerspiegelen in relatie tot tryptofaan en monoaminerge

neurotransmissie in bredere zin. In **Hoofdstuk 4 en 5** beschrijven we de biochemische veranderingen die optraden in twee verschillende groepen van patiënten die behandeld werden met PEGIFN en RBV. Zoals al eerder werd beschreven, verlaagde PEGIFN de perifere spiegels van tryptofaan. Tryptofaan is de voorloper van de monoamine serotonine (5-hydroxytryptamine; 5-HT), en dientengevolge zou PEGIFN de perifere en centrale synthese van 5-HT remmen met als mogelijk gevolg psychiatrische verschijnselen zoals depressie. De phenylalanine/tyrosine (PHE/TYR) ratio werd gebruikt als een marker voor de beschikbaarheid van tetrahydrobiopterine, een belangrijke cofactor in de synthese van 5-HT, maar ook de aanmaak van de monoamines dopamine en noradrenaline. Een consistente toename in de PHE/TYR ratio werd gemeten, correlerend met de ontwikkeling van sommige aspecten van de opgetreden psychopathologie. Opvallend genoeg werd deze toename in de PHE/TYR ratio ook bij herhaling gezien in studies met oncologiepatiënten die met (PEG) IFN werden behandeld. Dus mogelijk beïnvloedt therapie met cytokines de activiteit van tetrahydrobiopterine en via tetrahydrobiopterine ook de neurotransmissie met stoffen als 5-HT en dopamine. De spiegel van perifeer dopamine staat onder invloed van prolactine. De gemeten toename van de spiegel van prolactine correleerde met toenemende klachten van boosheid en depressie. Ook spiegels van de neurotoxische metaboliet kynurenine stegen gedurende de behandeling met PEGIFN. Tenslotte werd de waargenomen afname in de ratio tussen citrulline en arginine verondersteld de aangedane nitride oxide-synthese te weerspiegelen. Stikstof beïnvloedt weer de 5-HT afgifte.
Samenvattend – op basis van deze gevonden veranderingen gemeten in perifeer bloed – zou PEGIFN in de hersenen niet alleen de 5-HT synthese (via bijvoorbeeld verminderde spiegels van tryptofaan) kunnen beïnvloeden, maar zou PEGIFN ook psychopathologie kunnen induceren (of de kwetsbaarheid om psychiatrische symptomen te ontwikkelen verhogen) door de synthese van dopamine en noradrenaline te beïnvloeden en door de vorming van neurotoxische metabolieten. Echter werden er maar weinig consistente correlaties gezien tussen biochemische perifere veranderingen en de toename in psychiatrische klachten.

Opmerkelijk genoeg werden er maar weinig verschillen in de biochemische parameters gezien tussen de patiënten die met escitalopram of placebo werden behandeld, terwijl er in de placebogroep meer psychopathologie werd gezien. Dit wekt sterk de indruk dat de SSRI weliswaar werkt op de serotonerge neurotransmissie in de hersenen maar zijn

uiteindelijke therapeutische effect verderop in een cascade van geschakelde systemen in het brein heeft.

Eerder verrichte studies naar de pathofysiologie van (PEGIFN-geïnduceerde) depressieve symptomen richtten zich vooral op de depletie van tryptofaan. Daar konden we in onze studies geen aanwijzingen voor vinden. Mogelijk illustreren studies als de onze dat het effector mechanisme veel complexer is en dat er diverse effector mechanismes tegelijkertijd bij betrokken zijn, zoals misschien een effector mechanisme waarvan tetrahydriobiopterine deel uitmaakt. PHE/TYR ratio was namelijk de meeste consistente parameter die veranderde met ook hierbij de meest consistente correlatie met klinische veranderingen. Daarom zou deze parameter verder onderzocht dienen te worden in patiënten die met PEGIFN behandeld worden, zodat er meer aandacht komt voor de veranderingen in de synthese van dopamine en noradrenaline. Desondanks blijft de studie naar de PEGIFN-geïnduceerde psychopathologie interessant omdat het de mogelijkheid biedt om ten tijde van toediening van (perifere) cytokines met seriële metingen de pathogenese van door die cytokines uitgelokte psychiatrische symptomen te onderzoeken. Functionele beeldvorming van de hersenen zou hierbij een rol kunnen spelen om deze psychopathologie verder te verklaren.

Antivirale behandeling en de kwaliteit van sperma

Vanwege de onbekende effecten van PEGIFN op sperma is dubbele anticonceptie benodigd gedurende de behandeling met PEGIFN en tot zes maanden nadien. **Hoofdstuk 6** is een beschrijvende studie van twintig HCV patiënten over kwaliteit van sperma. Afwijkingen in sperma werden frequent gezien in mannen met een HCV infectie voor behandeling met PEGIFN, dit nam echter niet toe ten tijde van de behandeling. Ook een afname in DNA integriteit van sperma, dat geassocieerd is met verminderde spontane zwangerschappen en een toename van miskramen, werd niet gezien ten gevolge van behandeling met PEGIFN en RBV. Deze resultaten doen twijfelen aan het nu standaard advies tot dubbele anticonceptie tijdens en na de behandeling met PEGIFN.

Antivirale behandeling en kwaliteit van leven

Een chronische HCV infectie vermindert de kwaliteit van leven. Vanwege de bijwerkingen van PEGIFN vermindert deze kwaliteit nog verder ten tijde van de behandeling. Echter, na

een succesvolle behandeling neemt deze kwaliteit van leven juist weer toe. **Hoofdstuk 7** verkent het beloop van en de factoren die een rol spelen op de kwaliteit van leven in patiënten die dan wel standaard dan wel geïndividualiseerde behandeling kregen gebaseerd op vroege virologische respons (Ditto-studie). De belangrijkste determinanten voor kwaliteit van leven waren ernst van de leverziekte, leeftijd, participerend centrum en respons op behandeling. Antivirale behandeling heeft mogelijk een meer intens en meer aanhoudend negatief effect op vrouwen mogelijk vanwege de vaste doseringen (vrouwen zijn gemiddeld lichter) van PEGIFN die gebruikt werden in deze studie.

BIBLIOGRAFIE

1. Tanis W, Mancham S, Binda R, Janssen HL, Bezemer G, IJzermans JN, Tilanus HW, Laman JD, de Wit H, Drexhage HA, Schalm SW, Kwekkeboom J. Human hepatic lymph nodes contain normal numbers of mature myeloid dendritic cells but few plasmacytoid dendritic cells. Clin Immunol. 2004 Jan;110(1):81-8.
2. Bezemer G, Schalm SW, van Gool AR, de Knegt RJ. Wijzigingen in het beleid bij patiënten met bijwerkingen van de behandeling van hepatitis C Ned Tijdschr Geneeskd. 2007 Mar 3;151(9):525-30
3. de Knegt RJ, Bezemer G, Van Gool AR, Drenth JP, Hansen BE, Droogleever Fortuyn HA, Weegink CJ, Hengeveld MW, Janssen HLA Randomised clinical trial: escitalopram for the prevention of psychiatric adverse events during treatment with peginterferon-alfa-2a and ribavirin for chronic hepatitis C. Aliment Pharmacol Ther. 2011 Dec;34(11-12):1306-17
4. Bezemer G, Van Gool AR, Verheij-Hart E, Hansen BE, Lurie Y, Esteban JI, Lagging M, Negro F, Zeuzem S, Ferrari C, Pawlotsky JM, Neumann AU, Schalm SW, de Knegt RJ; DITTO-HCV Study Group. Long-term effects of treatment and response in patients with chronic hepatitis C on quality of life. An international, multicenter, randomized, controlled study. BMC Gastroenterol. 2012 Jan 31;12:11
5. Bezemer G, Van Gool AR, Fekkes D, Vrolijk JM, Hansen BE, Janssen HL, de Knegt RJ. Psychiatric side effects and fluctuations in serotonergic parameters in the treatment of chronic hepatitis C infection. Neuropsychobiology. 2012;65(3):126-32.
6. Hotho DM, Bezemer G., Hansen BE, Van Gool AR, de Knegt RJ, Veldt BJ, Janssen HLA, Antidepressants during antiviral treatment for chronic hepatitis C infection: a prognostic model to select patients for prophylaxis with SSRI therapy, Submitted
7. Roomer R, Bezemer G, van Brakel J, Romijn JC, Hansen BE, de Jong FH, Dohle GR, Janssen HLA and de Knegt RJ, Sperm DNA integrity is not affected by treatment with peginterferon alfa and ribavirin for chronic hepatitis C, Submitted
8. Bezemer G, de Knegt RJ, Janssen HLA, Van Gool AR, Psychopathology in hepatitis C: its clinical features and underlying biochemical mechanism, Submitted

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CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 2 oktober 1978 te Hendrik-Ido-Ambacht. Na het behalen van zijn eindexamen VWO aan het Wartburg College te Rotterdam, startte hij in 1996 met de studie Geneeskunde aan de Medische Faculteit van de Universiteit Antwerpen. Na twee kandidaturen en na uiteindelijk ingeloot te zijn, vervolgde hij zijn studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. In 2001 behaalde hij zijn doctoraal examen en volgden twee jaar co-schappen. Na het artsexamen in 2003 was hij drie maanden arts-assistent psychiatrie in de Delta Bouman Kliniek. Aansluitend was hij werkzaam als arts-assistent Inwendige Geneeskunde in het Ikazia ziekenhuis te Rotterdam tot december 2004. Nadien startte hij zijn promotieonderzoek op de afdeling Maag-, Darm- en leverziekten onder leiding van Prof. dr. H.L.A. Janssen, Dr. R.J. de Knegt en (vanuit de afdeling psychiatrie) Dr. A.R. Van Gool naar de bijwerkingen van de behandeling van Hepatitis C. In 2008 startte hij de (voor)opleiding Inwendige Geneeskunde in het Erasmus Medisch Centrum te Rotterdam (opleider: Prof. J.L.C.M. van Saase). Vanaf januari 2010 tot nu vervolgt hij zijn opleiding tot Maag-, Darm- en Leverarts in het Erasmus Medisch Centrum te Rotterdam (opleider Dr. R.A. de Man).

SUMMARY OF PHD TRAINING AND TEACHING

Name PhD student: Geert Bezemer

Erasmus MC Department: Gastroenterology and Hepatology

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

PhD period: 2005-2008

Summary of PhD training activities

(Inter)national conferences

2005 40th Annual Meeting of the European Association for the Study of the Liver; *April 13-17, 2005 - Paris, France*

20th Erasmus Liver Day; *June 10, Rotterdam, the Netherlands*

Annual meeting of the Dutch Society of Gastroenterology and Hepatology; *October 6-7, Veldhoven, the Netherlands*

2006 41st Annual Meeting of the European Association for the Study of the Liver; *April 26-30, 2006 - Vienna, Austria*

21st Erasmus Liver Day; *November 23, Rotterdam, the Netherlands*

Annual meeting of the Dutch Society of Gastroenterology and Hepatology; *October 5-6, Veldhoven, the Netherlands*

2007 Annual meeting of the Dutch Society of Gastroenterology and Hepatology; *October 4-5, Veldhoven, the Netherlands.*

22nd Erasmus Liver Day; *December 6, Rotterdam, the Netherlands*

2008 59th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD); *October 31-November 4, San Fransisco, CA, United States of America*

Annual meeting of the Dutch Society of Gastroenterology and Hepatology; *October 2-3, Veldhoven, the Netherlands.*

23rd Erasmus Liver Day; November 27, Rotterdam, the Netherlands

2009 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD); October 30-November 3, 2009, Boston, Massachussets, USA

Oral and poster presentatations

2005 Use of antidepressants during treatment of Hepatitis C, *National Hepatitis Day, National Hepatitis Center, Amersfoort, the Netherlands (oral)*

2006 Health Related Quality of Life of hepatitis C patients in the Ditto-study, *NVGE, Veldhoven, the Netherlands (oral)*

2007 Psychiatric side-effects and the fluctuations in related amino acids in the treatment of chronic hepatitis C infection, *NVGE, Veldhoven, the Netherlands (oral)*

2008 A double blind, placebo-controlled trial with escitalopram to prevent psychiatric adverse events during treatment with pegylated interferon-alpha and ribavirin for chronic hepatitis C: The "Prevention Of Psychiatric Side effects (POPS)-study" *NVGE, Veldhoven, the Netherlands (oral) & AASLD, San Fransisco, United States of America (poster)*

2009 Escitalopram prevents psychiatric side effects of interferon-alfa in Hepatitis C, *Annual meeting of the Dutch Society of Psychiatry, Groningen, the Netherlands, poster*
Psychiatric side-effects and the fluctuation in serotonergic parameters during treatment of chronic hepatitis C-infection with peginterferon and ribavirin, *NVGE, Veldhoven, the Netherlands (oral) & AASLD, Boston, United States of America (poster)*

Courses

2005 Netherlands Insitute for Health Sciences (NIHES), Erasmus Summer Program, Statistics

Memberships

2005 Dutch Society of Gastroenterology (NVGE)
Dutch Society of Hepatology (NVH)