Clinical and therapeutic aspects of viral hepatitis: exploring the role of ribavirin

Ludi Koning

ABBREVIATIONS

AE	Adverse Event
AUC	Area Under the Curve
AZA	Azathioprine
BMI	Body Mass Index
BOC	Boceprevir
BPAR	Biopsy-Proven Acute Rejection
CHF	Congestive Heart Failure
CI	Confidence Interval
CNI	Calcineurin inhibitor
Ct	Cycle threshold
CsA	Cyclosporine
C0(s)	Through level(s)
DAA(s)	Direct Acting Antiviral(s)
DFI	DNA fragmentation index
(c/p)EVR	(complete/partial) Early Virological Response
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
FU	Follow-Up
GFR	Glomerular Filtration Rate
HAI	Histology Activity Index
Hb	H(a)emoglobin
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HDR	High-Dose Ribavirin
HEV	Hepatitis E virus
HIV	Human Immunodeficiency Virus
HTX	Heart Transplant
IDP	Intended Dose/Duration Population
(s)IFN	(standard) Interferon
IL-28B	Interleukin-28B
ITP	Idiopathic Thrombocytopenic Purpura
ITT	Intention To Treat
LH	Luteinising Hormone
LLOD	Lower Limit Of Detection
LLOQ	Lower Limit Of Quantification
LT	Liver Transplant
MITT	Modified Intention To Treat
MMF	Mycophenolate Mofetil
NTX	Kidney Transplant
ORF	Open Reading Frame
PC	Platelet Count
PEG(-IFN)	Pegylated Interferon
RBV	Ribavirin
RVR	Rapid Virological Response
SAE	Serious Adverse Event
SBP	Spontaneous Bacterial Peritonitis
SD	Standard Deviation
SDR	Standard-Dose Ribavirin
SOT	Solid Organ Transplant
SUSAR	Suspected Unexpected Serious Adverse Reaction
SVR	Sustained Virological Response
TAC	Tacrolimus
TIW	Three Times per Week
TVR	Telaprevir
ULN	Upper Limit of Normal
WBC	White Blood Cell Count

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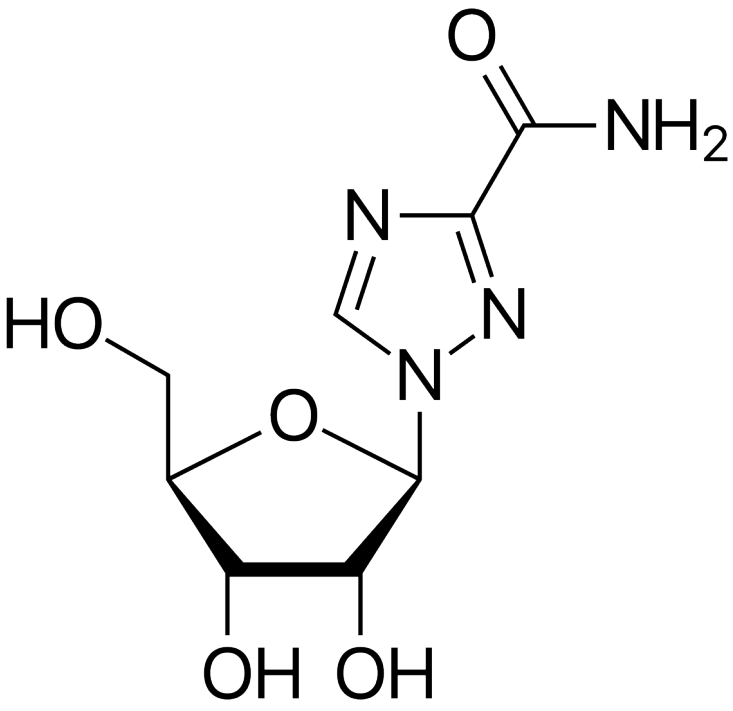

(Formule ribavirine)

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Clinical and therapeutic aspects of viral hepatitis: exploring the role of ribavirin

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de rol van ribavirine uitgediept

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CHAPTER 1

General introduction

Ribavirin is a synthetic nucleoside (guanosine) analogue, first discovered in 1972 to have broad-spectrum antiviral activity [1]. Since its discovery, ribavirin has played an important role in optimizing therapy for liver disease caused by viral hepatitis infections. Though the effect of ribavirin for the treatment of chronic hepatitis B virus (HBV) infection has been disappointing [2], optimizing the treatment of chronic hepatitis C virus and hepatitis E virus infections through the addition of ribavirin is an ongoing topic of research. Up to now, ribavirin has only been registered for the treatment of chronic HCV infection, in combination with other antiviral medications, including pegylated interferon (peginterferon) and direct acting antivirals (DAAs).

Epidemiology of Hepatitis C

HCV was referred to as non-A, non-B hepatitis, until its genetic determination in 1989 [3, 4]. HCV is an enveloped, positive-sense, single stranded RNA virus, distinguished into six major genotypes (1-6) [5].

HCV is transmitted through blood-blood contact, such as sharing of needles for intravenous drug use and unsafe (medical) procedures including blood transfusions, tattooing and piercing. After infection, HCV becomes chronic in approximately 55-85% of patients with an estimated 130-150 million people worldwide infected, according to the World Health Organization (www.who.int). At least 20% of chronically infected HCV patients develop liver cirrhosis within 20 years [6]. Liver cirrhosis may lead to liver failure or the development of hepatocellular carcinoma. Both conditions ultimately require liver transplantation. In fact, HCV infection is still the leading indication for liver transplantation in the western world. However, as the obesity and metabolic syndrome epidemic has reigned for many years, non-alcoholic steatohepatitis (NASH) as the second most common indication for liver transplantation among adults is soon expected to beat HCV [7].

After liver transplantation, HCV infection recurs in virtually all patients with up to 30% developing liver cirrhosis within 5 years [8]. Adequate treatment of HCV infection is essential to reduce the current, immense global burden.

Ribavirin for the treatment of hepatitis C virus

The first effective therapy for HCV infection was monotherapy with interferon alfa, which had a very modest sustained virological response (SVR; HCV RNA negativity 24 weeks after discontinuation of treatment) of 5-20%. When ribavirin was added to interferon, SVR rates improved to 40-50%. Eventually, a pegylated version of interferon (peginterferon), became available. Pegylation decreases the elimination of interferon from the body, therefore reducing the necessary frequency of injection from three times to once a week. Weekly peginterferon injection in combination with daily ribavirin tablets or capsules has been the backbone of HCV therapy for years.

The efficacy of this combination therapy is moderate, with SVR rates around 54-63% [9] and at the cost of serious side effects. SVR rates vary greatly depending on individual patient characteristics; especially HCV genotype, baseline viral load, IL28B genotype and advanced liver disease are known to influence SVR rates in patients treated with peginterferon and ribavirin [10-16].

As stated earlier, peginterferon and ribavirin have many side effects, among others; bone marrow suppression resulting in thrombocytopenia, leukopenia and anemia (ribavirin induces haemolytic anemia), influenza like symptoms, depression and gastrointestinal symptoms [17]. On top of that, guidelines advise double contraception during and until 7 months after treatment for male patients and their female partners, since ribavirin may influence reproductive systems and even be teratogenic, though this has never been established in human studies [18-23]. Other guideline rules have been questioned previously, such as the advice to reduce the dose of peginterferon when (severe) cytopenias occur on treatment. Several studies have shown that leukopenia and trombocytopenia are not associated with respectively infection and bleeding [24-35]. It is important to minimize dose reductions in HCV treatment, since adherence leads to better response rates [36]. Therefore, strategies to minimize the need for dose reduction are an important research topic.

Considering the long treatment duration with peginterferon and ribavirin of up to 48 weeks, the major side effects and moderate SVR rates, especially in patients with known difficult to treat characteristics, the arrival of new treatment regimens with DAAs (direct acting antivirals), starting with the protease inhibitors boceprevir and telaprevir in 2011, has greatly improved the treatment landscape of hepatitis C. The addition of the first generation of DAAs to HCV therapy led to more side effects in all patients and great treatment challenges in transplant recipients due to drug-drug interactions with immunosuppressive medication[37, 38]. Fortunately, the newest treatment regimens with DAAs have excellent SVR profiles and are much better tolerated than classic peginterferon and ribavirin therapy [39, 40]. Guidelines for HCV treatment are changing quicker than ever due to the accumulation of HCV trials addressing different patient groups. Peginterferon-free treatments are now the standard of care in most developed countries, but ribavirin is expected to remain part of treatment with DAAs for the foreseeable future [41]. The addition of ribavirin to DAA treatments can be beneficial depending on genotype and treatment-experience, with duration of several treatment regimens cut in half, since SVR rates can be similar for 24 weeks duo therapy with DAAs or 12 weeks triple therapy through the addition of ribavirin [42]. Nonetheless, due to high costs and lack of availability of DAAs, peginterferon and ribavirin are currently still the standard of care in certain parts in the world [43-46]. Optimizing peginterferon and ribavirin duo therapy regimens to achieve better SVR rates in patients who cannot be treated with DAAs is therefore still important. Especially

the fine-tuning of optimal ribavirin dosing may be an important means through which better SVR rates can be achieved [47-52].

The exact mechanism of action of ribavirin for the treatment of HCV infection has been poorly understood, despite years of extensive research efforts [53]. Ribavirin is a weak in vitro inhibitor of HCV replication, but monotherapy of ribavirin in HCV infected patients has no effect on HCV viral loads and liver-related morbidity and mortality and therefore ribavirin should always be used in combination therapy [54]. Several modes of action have been proposed including ribavirin having a direct inhibitory effect on viral RNA-dependent RNA-polymerases (though plasma levels needed to induce such an effect are practically unachievable); lethal mutagenesis of viral nucleic acids; depletion of the cell guanosine triphosphate pool by inhibiting the enzymatic activity of the inosine monophosphate dehydrogenase; modulation of the Th1/Th2 T-lymphocyte balance; impairment of the translation via eIF4E inhibition; and the ability to potentiate interferon alfa signaling [55, 56]. Nonetheless, none of these possible pathways are conclusive for the mode of action of ribavirin and finding the true mechanism remains a challenge.

Epidemiology of Hepatitis E

Hepatitis E virus (HEV), first isolated in 1990, is a non-enveloped virus with a single-stranded, positive sense RNA genome of approximately 7,500 base pairs and three partially overlapping open reading frames (ORF 1-3) [57, 58]. Up to date, four genotypes are known to infect humans. Genotype 1 and 2 are endemic in developing countries and mainly transmitted through contaminated water, while genotype 3 and 4 are sporadically seen in industrialized countries and thought to be zoonotic of origin [59, 60]. Possible zoonotic reservoirs for HEV genotype 3 or 4 able to infect humans are: pigs, wild boar and deer, and recently, also rabbits and camels [61]. Consumption of raw or undercooked meat or products of these animals may lead to HEV infection in humans.

An estimated 20 million people are yearly infected with HEV, according to the World Health Organization (www.who.int). Though HEV infection usually is self-limiting within 2-6 weeks, genotype 1 or 2 infection can have a notoriously grave clinical course in pregnant women, especially in the third trimester of pregnancy, leading to an estimated mortality rate of 20% [62, 63]. Until quite recently, hepatitis E genotype 3 or 4 infections were thought to be rare in industrialized countries and cause exclusively acute infections, sometimes leading to severe hepatitis with sporadic cases of acute liver failure (e.g., [64-67]). In 2008, several reports emerged on immunocompromised patients, especially solid organ transplant recipients, developing chronic HEV [68-71]. Instead of clearing HEV within a couple of weeks, (the normal course of HEV infection), these patients have a positive HEV RNA PCR for more than six months.

Importantly, HEV seroconversion in these immunocompromised patients often has aberrant patterns, or is even completely absent, and great variability in detection rate is observed between serologic assays [72, 73]. Therefore, to confirm the diagnosis in immunocompromised patients, an HEV RNA PCR test in serum should be performed. HEV RNA can also be detected in stool samples, though fecal shedding of HEV may be prolonged, increasing the risk of false positive results in patients who have already cleared HEV [74].

Limited availability of HEV assays, even in industrialized countries, is a major culprit in the lack of data on the prevalence of chronic HEV worldwide. For example, the Food and Drug Administration (FDA) of the United States has not yet approved any HEV tests. Insight into the burden of chronic HEV infection is all the more important since chronic HEV infection in immunocompromised patients may lead to rapid fibrosis and results in cirrhosis in around 14% of solid organ transplant recipients, warranting treatment to prevent end-stage liver disease [69, 70, 75]. Of note, infection with HEV is also associated with extrahepatic manifestations, in particular involving the nervous system, but also hematological disorders and even acute pancreatitis and glomerulonephritis have been reported [76].

Ribavirin for the treatment of hepatitis E virus

The first step in the management of chronic HEV infection is reduction of immunosuppressive medication. However, dose reduction is not always possible, nor does it always lead to clearance of HEV. Interestingly, the two medications that have been the backbone of HCV therapy for years, peginterferon and ribavirin, have played an important role in emerging treatment strategies for chronic HEV infection [76].

Due to its broad-spectrum and strong antiviral properties, peginterferon was first tried, with some success, as a treatment for chronic HEV [77-80]. Since not all patients are eligible for peginterferon treatment, soon reports on chronic HEV infected patients treated with ribavirin emerged [81-83]. Currently, optimal treatment strategies for chronic HEV infection remain an important research topic.

Outline of this thesis

In this thesis, the evolving role of ribavirin in the treatment of viral hepatitis is assessed. The thesis has been sub-divided in two sections: the first part concerning HCV infection and the second part exploring HEV infection.

PART 1 – RIBAVIRIN IN HCV INFECTED PATIENTS

In *chapter 2* the results of the Dutch VIRID-study, a randomized-controlled, multicenter trial assessing the effect of high-dose ribavirin on SVR in difficult to treat patients

with HCV genotype 1 or 4 and a high baseline viral load, are reported. To minimize the need for ribavirin dose reductions, epoetin was added when anemia occurred. This study was designed to test the hypothesis, based on a pilot study [84], that increasing the dose of ribavirin in peginterferon duo therapy leads to higher SVR rates.

In *chapter 3* optimizing adequate ribavirin exposure in patients is assessed through measuring the area under the curve and, if necessary, adjusting the ribavirin dose after a test dose of ribavirin in patients previously treated for HCV.

In *chapter 4* the hypothesis that ribavirin affects sperm quality is tested through measuring sperm DNA integrity before, during and after HCV therapy. Semen analysis and data on endocrinological parameters are also discussed.

In *chapter 5* the hypothesis that cytopenia's are not associated with infection and bleeding on treatment is tested in HCV infected liver transplant patients

In *chapter 6* the real life experience of the addition of first generation protease inhibitors telaprevir and boceprevir to the treatment of peginterferon and ribavirin in HCV infected liver transplant patients is reported. The efficacy of this treatment and the interaction between DAAs and immunosuppressive medication is assessed for the first time in liver transplant patients in this study.

Chapter 7 provides an in-depth analysis of anemia and renal insufficiency related to ribavirin troughs and drug-drug interactions in the setting of triple therapy with boceprevir in liver transplant recipients.

PART 2 – RIBAVIRIN IN HEV INFECTED PATIENTS

In *chapter 8* the prevalence of HEV infection is studied in patients who underwent liver transplantation for chronic HCV in the United States. Particular emphasis is put on the influence of treatment with interferon and/or ribavirin on the prevalence and incidence of HEV in this population.

In *chapter 9* the clinical course of hepatitis E infection in a group of heart transplant recipients is described. The effect of treatment with ribavirin in patients who did not clear the virus after dose reduction of immunosuppressive medication is reported as well.

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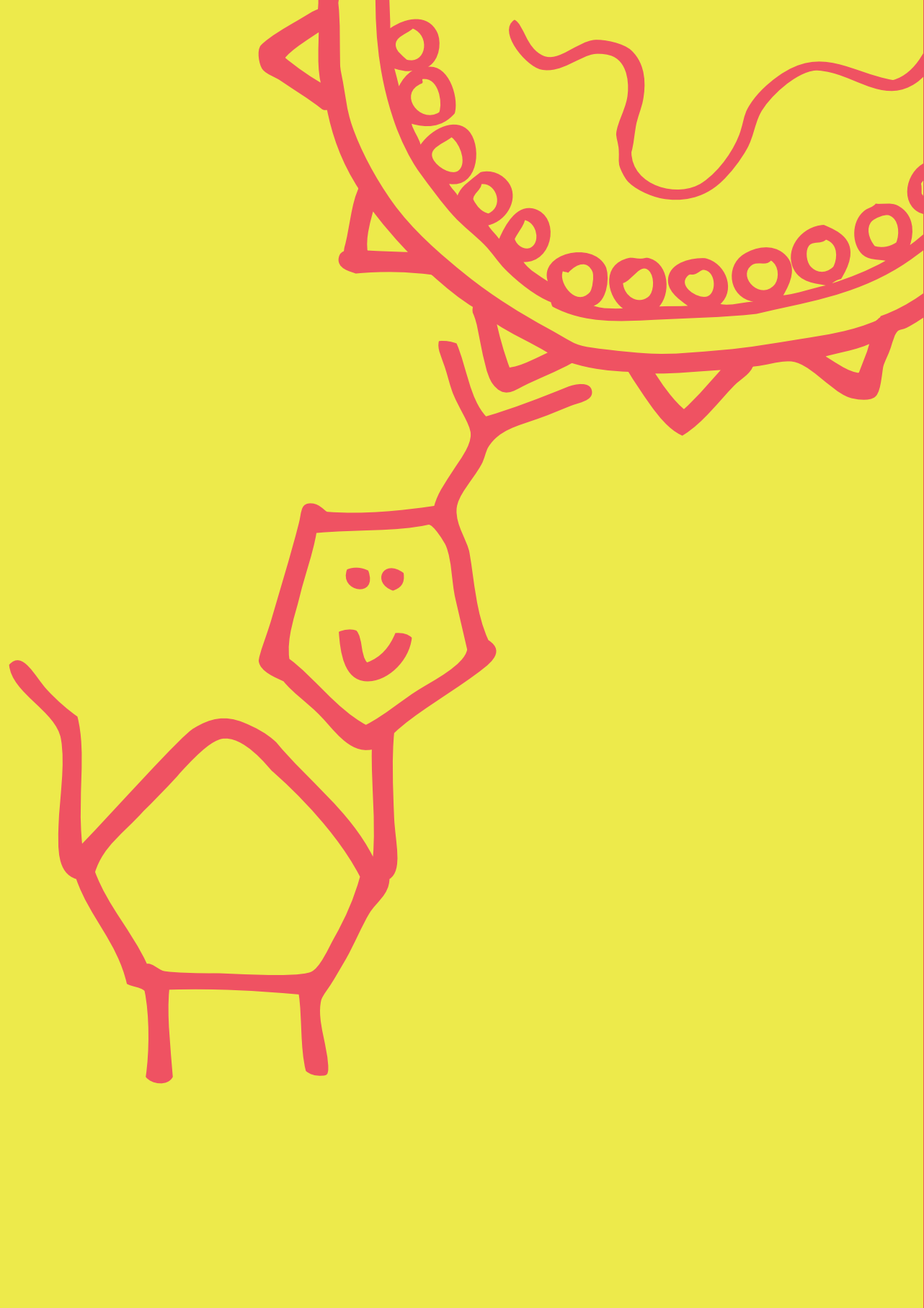

PART 1

RIBAVIRIN IN HCV INFECTED PATIENTS

CHAPTER 2

A randomized controlled trial to compare high-dose versus standard-dose ribavirin for the treatment of chronic hepatitis C genotype 1 or 4 (VIRID)

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SUBMITTED

ABSTRACT

OBJECTIVES*:*
Optimal ribavirin doses are essential to achieve sustained virological response (SVR) in hepatitis C virus (HCV) infected patients on peginterferon therapy. We investigated if high-dose ribavirin (HDR) improves SVR rates.

MATERIALS AND METHODS
N=110 patients with a chronic HCV genotype 1 or 4 infection and a high baseline viral load (HCV RNA >400,000 IU/mL) were randomly assigned to peginterferon alfa-2a 180µg with either HDR (25-29 mg/kg/day) or SDR (12-15 mg/kg/day) for a treatment period of 48 weeks (Clinicaltrials.gov: NCT00662220).

RESULTS
A total of 28/52 (56.0%) patients in the HDR group and 26/58 (45.6%) patients in the SDR group achieved an SVR (intention to treat: p=0.28). In multivariate analysis, corrected for IL28B genotype and ethnicity, HDR showed a trend towards positive association with SVR (OR 2.4; 95%CI 1.0-6.0). In patients who adhered to treatment (continued prescription of at least 80% of peginterferon and ribavirin for at least 80% of the planned treatment duration) SVR was achieved in 65.8% of the HDR group and 50.0% of the SDR group (p=0.14). In multivariate analysis among adherent patients, again corrected for IL28B genotype and ethnicity, HDR was associated with SVR (OR 3.9; 95%CI 1.3-11.2). Anemia prevention with epoetin beta and blood transfusions led to HDR being relatively well tolerated with only 1 patient in the HDR group developing grade 4 anemia.

CONCLUSIONS
HDR is relatively well tolerated, and seems to improve SVR rates in patients who adhere to treatment, though no significant effect on SVR was seen in the intention to treat group.

INTRODUCTION

For years, peginterferon and ribavirin have been the backbone of hepatitis C virus (HCV) treatment. Sustained virological response (SVR) rates with this duo therapy have been notoriously low in genotype 1 and 4 HCV infected patients with a high baseline viral load, leaving around half of patients uncured [1-3]. With the introduction of direct-acting antivirals, SVR rates have increased considerably. However, worldwide access to these new medications is still limited due to high costs and/or availability [4, 5].

The addition of ribavirin to peginterferon therapy increases SVR rates significantly [6]. Nonetheless, the optimal dosing regimen for ribavirin remains an important research topic. SVR rates in genotype 1 and 4 patients can be augmented when forgoing flat-dose ribavirin for weight-based ribavirin [7-9]. Weight-based ribavirin is associated with lower relapse rates compared to flat-dose ribavirin [10]. Indeed, relapse rates were significantly lower in patients receiving ribavirin doses ≥13.2 mg/kg compared to patients who received <13.2 mg/kg [11]. Moreover, a prediction model based on treatment data in 1732 genotype 1 patients, suggests that every 12-16 mg/kg dose increase of ribavirin increases the probability of SVR with 40-50% [12]. This illustrates that optimal ribavirin doses in HCV genotype 1 and 4 patients are essential to achieve SVR. However, no prospective studies have formally investigated the effect of higher ribavirin doses on SVR in this difficult to treat group. In a pilot study, 9 out of 10 HCV patients with genotype 1 achieved SVR after treatment with peginterferon and high-dose ribavirin (1600 mg-3600 mg/day) [13]. These study results are promising, but require confirmation through a larger controlled study.

We therefore conducted this multicenter, open label, randomized controlled study to compare SVR in previously untreated patients with chronic HCV genotype 1 or 4 and a high viral baseline load (>400.000 IU/ml) after 48 weeks of treatment with peginterferon alfa-2a and either standard-dose weight-based ribavirin (12-15 mg/kg) or high-dose weight-based ribavirin (25-29 mg/kg).

METHODS

The VIRID study is registered under Identifier NCT00662220 at http://clinicaltrials.gov. The summarized study protocol can be found at http://clinicaltrials.gov and the full protocol can be provided upon request.

STUDY PATIENTS

From April 2008 to April 2012 N=144 ‘difficult-to-treat’ treatment naïve patients with chronic HCV genotype 1 or 4 infection were screened at 18 sites in The Netherlands, divided over 14 treatment regions.

Eligibility criteria included a high viral load (>400.000 IU/mL) and age 18-70 years. Patients were excluded in case of evidence of hepatocellular carcinoma or decompensated cirrhosis and other liver disease. Complete in- and exclusion criteria can be found at http://clinicaltrials.gov (study Identifier NCT00662220).

STUDY DESIGN

Study design and rationale of the study have been published before [14]. The primary objective was to study whether 48 weeks of daily high-dose ribavirin (HDR) in combination with peginterferon alfa-2a will lead to a higher SVR rate (HCV RNA negativity 24 weeks after end of treatment) compared with standard-dose ribavirin (SDR).

Secondary endpoints were HCV RNA negativity at weeks 4 (Rapid Virological Response; RVR), 12 (complete Early Virological Response; cEVR), 24 and 48 and a >2log decline in HCV RNA from baseline at week 12 (partial Early Virological Response; pEVR). Failure to achieve pEVR or HCV RNA negativity at week 24 were, per international guidelines, considered stopping rules. Safety-related secondary outcomes were the occurrence of anemia and serious adverse events and the percentage of people completing treatment on full or >80% of total intended dose and duration.

This study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. The study protocol was reviewed and approved by the institutional review boards of all participating centers and the regulatory agencies. Written informed consent was obtained from all study subjects before screening.

Patients were randomized 1:1 in blocks of 4 per participating region to receive once weekly 180 µg peginterferon alfa-2a (Pegasys, Roche) in combination with either 25-29 (mean 26.2) mg/kg/day ribavirin (Copegus, Roche) or 12-15 (mean 13.3) mg/kg/day for a total treatment period of 48 weeks. At the start of the study, the statistician (BEH) generated the allocation numbers. The sealed, opaque allocation envelopes were stored in sequentially numbered containers, classified by region, at the Erasmus MC Medical Center. After the principal investigator (RJK or JPHD) had determined that a patient could be included, a staff member not involved in the screening or inclusion of patients, opened the subsequent allocation envelope in the sequence to randomize the patient into one of the treatment groups.

After the 48 week treatment period was completed, patients subsequently underwent a follow-up period of 24 weeks. Stopping rules were maintained according to international guidelines: <2log decrease in HCV RNA load at week 12 or HCV RNA positive at week 24 of treatment.

Samples were collected for central HCV RNA testing with the Siemens Versant HCV RNA 3.0 Assay (bDNA) (LLOQ=308 IU/mL). HCV RNA negativity was con-

firmed with the Siemens Versant HCV RNA Qualitative Assay (TMA) (LLOD=≤5.3 IU/mL). Missing SVR data (central HCV RNA) was, per data analysis plan, replaced with local SVR data, when available. Il28B (rs12979860) genotype was determined by real-time PCR.

SAFETY

To avoid the development of (severe) anemia, all participants with a haemoglobin (Hb)<6.8 mmol/l were prescribed a weekly fixed dose of epoetin beta 30.000 IU (NeoRecormon, Roche). Epoetin beta had to be discontinued in case Hb rose above 7.5 mmol/l. A Hb drop below 5.0 mmol/l, led to the reduction of ribavirin with 400 mg/day (HDR group) or 200 mg/day (SDR group). The previous ribavirin dose was resumed if Hb rose above 5.0 mmol/l. Ribavirin was to be discontinued if Hb dropped below 4.0 mmol/l. Ribavirin could be restarted if Hb level reached at least 5.0 mmol/l within 14 days. Patients on HDR were to restart with a dose reduction of 400 mg/day. When Hb level did not rise above 5.0 mmol/l within 14 days, ribavirin had to be discontinued permanently. When Hb dropped below 4.0 mmol/l for the second time, ribavirin had also to be discontinued permanently.

Peginterferon doses were per protocol adjusted according to current treatment standards in case of neutropenia and/or thrombocytopenia.

STATISTICAL ANALYSIS

The power analysis was based on the primary endpoint (SVR) assuming 67.5% SVR with HDR and peginterferon alfa-2a (based on a cautious estimation of 20-25% improvement compared to standard treatment) and 45% SVR with SDR and peginterferon alfa-2a (based on literature). With a two-sided 5% significance test, a power of 80%, a 1:1 randomization and a 10% drop-out rate, a minimum of 85 patients in both arms was needed. However, due to the arrival of the direct-acting antiviral agents (boceprevir and telaprevir), replacing standard of care for genotype 1 infected patients per April 2011 in The Netherlands, it was decided to terminate the study and subsequently the preplanned inclusion number could not be met.

Analysis of the primary endpoint was carried out for the Modified Intention To Treat (MITT) population (all patients receiving at least one dose of study medication) and repeated in the Intended Dose/Duration Population (IDP or 80/80 rule; adherence to therapy was defined by continued prescription of at least 80% of peginterferon and ribavirin for at least 80% of the planned treatment duration). Chi-Square tests were used to compare viral response between treatment arms. To gain further insight in factors predicting the efficacy outcome, analysis of the effects of (essential) baseline covariates (and potential interactions) were performed with logistic regression analysis. All analysis were performed in SPSS version 22.0 (SPSS Inc., Chicago, IL, USA)

and the SAS 9.3 program (SAS Institute Inc., Cary, NC, USA).

RESULTS

From April 2008 to April 2012 a total of 110 patients were randomized to high-dose ribavirin (HDR; N=52) or standard-dose ribavirin (SDR; N=58). Last follow up took place in June 2013. The inclusion flowchart is given in figure 1.

All randomized patients (intention to treat; ITT) received at least one dose of study medication (modified intention to treat; MITT). Six patients in the HDR group and 5 in the SDR group discontinued treatment prematurely. Baseline characteristics of both treatment groups are given in table 1.

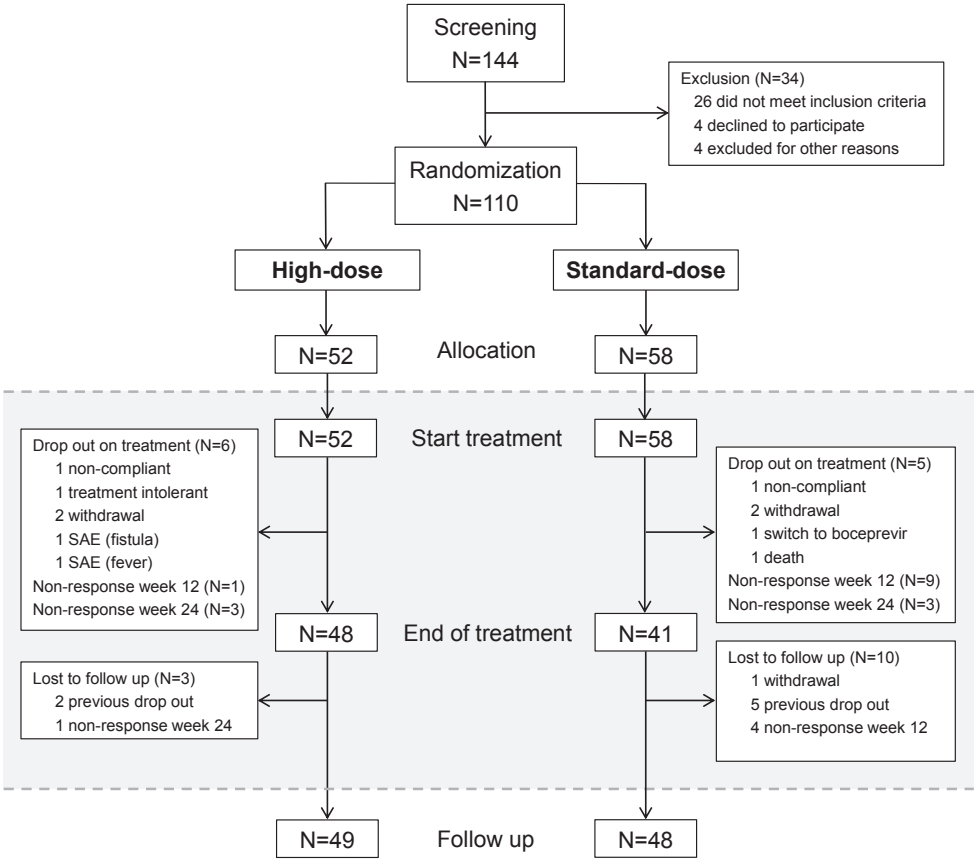

Figure 1. Inclusion flowchart

Table 1. Baseline characteristics

	High-dose ribavirin (N=52)	Standard-dose ribavirin (N=58)
Mean age in years (SD)	47.4 (+/- 9.8)	43.4 (+/- 9.5)
Sex		
Male	40 (76.9%)	45 (77.6%)
Female	12 (23.1%)	13 (22.4%)
BMI kg/m^2 (SD)	25.7 (+/- 3.8)	26.0 (+/- 4.2)
Fibrosis (Metavir)		
F0-F1	20 (38.5%)	30 (51.8%)
F2	15 (28.8%)	14 (24.1%)
F3	14 (26.9%)	8 (13.8%)
F4	3 (5.8%)	6 (10.3%)
HCV genotype		
1	48 (92.3%)	51 (87.9%)
4	4 (7.7%)	7 (12.1%)
Baseline HCV RNA load (log IU/mL)	6.00 (+/-0.52)	5.94 (+/-0.56)
Race		
Caucasian	44 (84.6%)	42 (72.4%)
Asian*	2 (3.8%)	6 (10.3%)
African	4 (7.7%)	6 (10.3%)
Other/unknown	2 (3.8%)	4 (6.9%)
Il28B genotype (rs12979860)		
CC	13 (25.0%)	21 (36.2%)
CT	30 (57.7%)	26 (44.8%)
TT	7 (13.5%)	10 (17.2%)
Unknown	2 (3.8%)	1 (1.7%)

*Including mixed Caucasian

VIROLOGY

A total of 28/52 (56.0%) patients in the HDR group and 26/58 (45.6%) patients in the SDR group achieved SVR (p=0.28). Virology results at different time points are given in figure 2. Patients in the SDR group were more likely to meet the stopping rules at week 12 (<2log decline of HCV RNA; p=0.001) and 24 (HCV RNA positivity; p=0.009). No difference was seen in HCV negativity at week 4, 12 or 48 between treatment groups. However, when grouped according to IL28B genotype, non-CC patients from the HDR group were more likely to obtain HCV RNA negativity at week 12 (p=0.004) and 48 (p=0.041). This led to a trend towards higher SVR rates (p=0.067; figure 3).

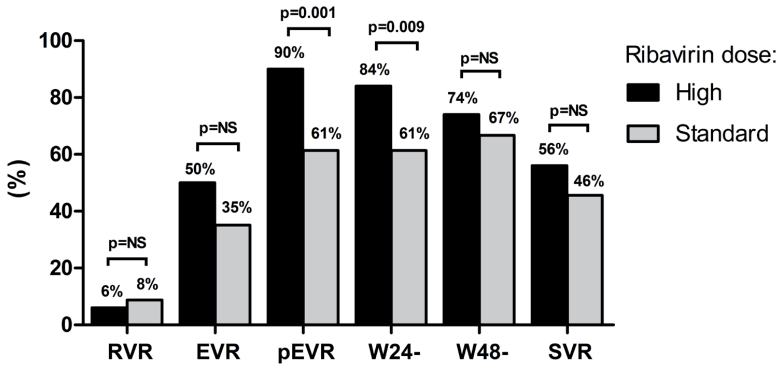

Figure 2. Virological results - modified intention to treat
RVR=rapid virological response; EVR=early virological response; pEVR=partial early virological response; W24-= HCV RNA negative at week 24 of treatment; W48-= HCV RNA negative at week 48 of treatment; SVR= Sustained virological response

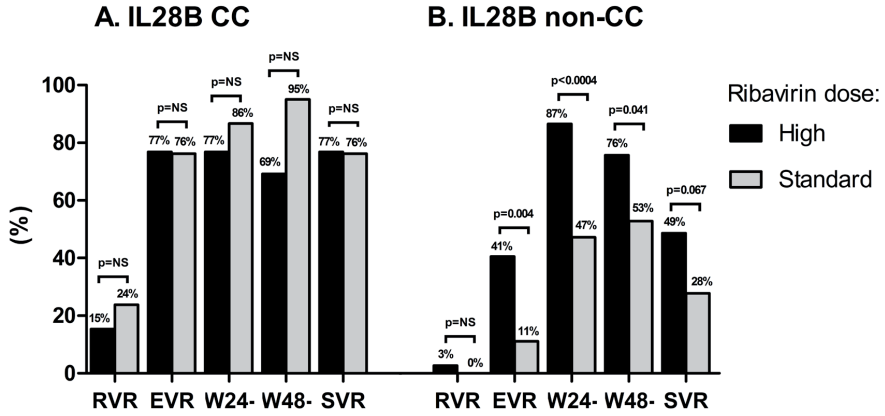

Figure 3. Virological results by IL28B A. CC and B. non-CC genotype - modified intention to treat
RVR=rapid virological response; EVR=early virological response; W24-= HCV RNA negative at week 24 of treatment; W48-= HCV RNA negative at week 48 of treatment; SVR= Sustained virological response

In multivariate logistic regression, Caucasian race was inversely associated with SVR (OR 0.25; 95%CI 0.084-0.76) while Il28B CC-genotype was positively associated with SVR (OR 6.9; 95%CI 2.5-18.9). HDR showed a trend towards positive association with SVR (OR 2.4; 95%CI 1.0-6.0, p=0.051) (table 2).

In relation to the Intended Dose/Duration Population (IDP or 80/80 rule) 40 patients in the HDR group (76.9%) and 51 in the SDR group (87.9%) fulfilled the IDP criteria (continued prescription of at least 80% of peginterferon and ribavirin for at least 80% of the planned treatment duration). SVR occurred in 25 of the HDR (65.8%) and 25 of the SDR (50.0%) patients (p=0.14).

Table 2. **Logistic regression analysis in the modified intention to treat population**

	Univariate		Multivariate	
	OR	**P-value**	**OR**	**P-value**
Age	0.98 (0.94-1.0)	0.34	-	-
Male sex	0.95 (0.39-2.3)	0.90	-	-
BMI ≥30 kg/m^2	1.2 (0.43-3.4)	0.73	-	-
Advanced fibrosis (Metavir F3-F4)	0.56 (0.24-1.3)	0.18	-	-
HCV genotype 1	0.85 (0.24-3.0)	0.80	-	-
Baseline HCV RNA load (log IU/mL)	0.68 (0.33-1.4)	0.29	-	-
Caucasian	0.40 (0.15-1.0)	0.056	0.25 (0.084-0.76)	*0.014*
Il28B CC genotype (rs12979860)	5.2 (2.1-13.1)	*0.0004*	6.9 (2.5-18.9)	*<0.0002*
High-dose ribavirin	**1.4 (0.68-3.0)**	**0.35**	**2.4 (1.0-6.0)**	**0.051**

In patients with IL28B non-CC genotype, SVR occurred more often in the HDR (17/30; 56.7%) than in the SDR (10/32; 43.5%) patients (p=0.044). In multivariate logistic regression, Caucasian race was again inversely associated with SVR (OR 0.28; 95%CI 0.082-0.96) while Il28B CC-genotype was positively associated with SVR (OR 15.9; 95%CI 3.8-66.1). In patients who adhered to treatment, HDR was associated with SVR (OR 3.9; 95%CI 1.3-11.2, p=0.013) (table 3).

SAFETY

Hemoglobin patterns during treatment and follow up are given in figure 4. A polynomial fit with repeated statement to control for multiple measurement per study participant was applied. An overview of hematological adverse events is given in supplementary table 1. Per protocol epoetin requiring anemia (Hb<6.8 mmol/l) and grade 3 anemia (Hb<5.0 mmol/l) occurred more often in the HDR group (respectively p=0.004 and p=0.006). Only one patient in the HDR group had a grade 4 anemia. No anemia related serious adverse events occurred in this patient. Epoetin and transfusion was more often prescribed in the HDR group (respectively p<0.0004 and p=0.012). No difference between treatment groups was seen regarding dose reductions of ribavirin. However, per protocol, only 9 patients in the HDR and 1 patient in the SDR group required ribavirin dose reduction (due to a Hb<5.0 mmol/l), while more patients were

Table 3. Logistic regression analysis in the 80/80 rule population

	Univariate		Multivariate	
	OR	**P-value**	**OR**	**P-value**
Age	0.98 (0.94-1.0)	0.28	-	-
Male sex	0.68 (0.26-1.8)	0.43	-	-
BMI ≥30 kg/m^2	1.1 (0.35-3.5)	0.86	-	-
Advanced fibrosis (Metavir F3-F4)	0.54 (0.21-1.4)	0.20	-	-
HCV genotype 1	1.0 (0.26-4.1)	0.97	-	-
Baseline HCV RNA load (log IU/mL)	0.44 (0.18-1.1)	0.067	-	-
Caucasian	0.44 (0.16-1.2)	0.11	0.28 (0.082-0.96)	*0.042*
Il28B CC genotype (rs12979860)	9.9 (2.7-36.6)	*0.001*	15.9 (3.8-66.1)	*<0.0002*
High dose ribavirin	**1.7 (0.75-4.0)**	**0.20**	**3.9 (1.3-11.2)**	***0.013***

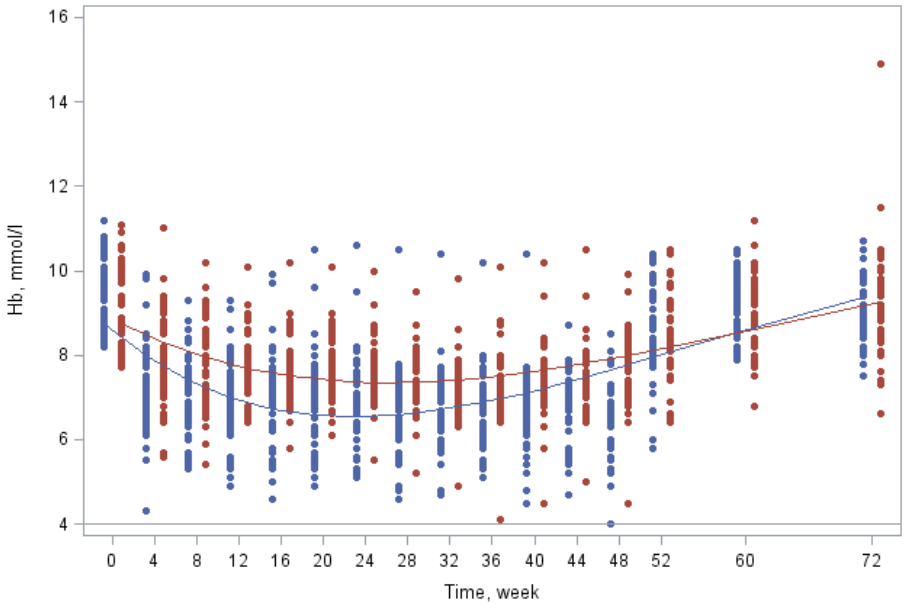

Figure 4. Fitted Hb patterns during therapy and follow up
Red: Standard-dose ribavirin; Blue: High-dose ribavirin

given per physicians discretion dose reductions for other indications.

Grade 4 leukopenia occurred in 3 patients in the SDR group and grade 4 neutropenia was more often seen in the SDR group (respectively p=not significant and p=0.013). No grade 4 thrombocytopenia was observed in this study.

The ten most registered adverse events are given in supplementary table 1. Registered cytopenia's are excluded from this overview. Details on serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) are given in supplementary table 2. A total of 31 SAEs were registered in 11 HDR (21.2%) and 16 (27.6%) SDR patients (p=0.43). Two patients in the HDR group had to discontinue treatment due to SAEs: one due to a colovesical fistula at week 16 and one due to fever of unknown origin at week 36. In the SDR group one patient died due to urosepsis.

DISCUSSION

Optimal ribavirin dosing plays a pivotal role in achieving SVR and preventing relapse in HCV infected patients treated with peginterferon-combination therapy. The current study was designed to investigate if high-dose ribavirin (HDR) improves SVR rates in difficult to treat patients with genotype 1 or 4 and high baseline viral load, compared to treatment with standard-dose ribavirin (SDR). We found that HDR in treatment adherent patients improves SVR rates. A trend towards higher SVR rates with HDR was seen in the total study population.

IL28B genotype was the strongest predictor for SVR in this cohort, consistent with previous studies [15, 16]. Especially patients with IL28B non-CC genotype seemed to benefit from HDR, with a trend towards higher SVR rates seen in the (modified) intention to treat population (p=0.067) and actual higher SVR rates in adherent patients (p=0.044). However, the study was not powered to investigate the difference between HDR and SDR in IL28B genotype subgroups and these results should be interpreted with caution.

HDR was relatively well tolerated in patients in this cohort, with only one patient experiencing grade 4 anemia. To avoid the development of (severe) anemia, Hb decline was actively managed with prescription of epoetin when Hb dropped below 6.8 mmol/l. Blood transfusion was more often needed in the HDR group, though only 17.3% of patients on HDR required transfusion.

Due to the arrival of boceprevir and telaprevir in 2012, the standard of care for genotype 1 patients changed and it was decided to end the current study. Target inclusion was 170 patients and therefore this study is underpowered. At the time of study design, the influence of IL28B on SVR was unknown and therefore, randomization did not take this influential factor into account. On the other hand, multiple

centers participated in this study and patients were randomized in blocks per region, increasing the generalizability of the effect of HDR on SVR.

The value of peginterferon and HDR for the treatment of HCV patients is limited now we have entered the era of direct-acting antiviral agents (DAAs). Though ribavirin remains part of several DAA-based treatment regimens, the current study results cannot be directly applied to these regimens since the mechanism of action of these compounds differ so greatly from peginterferon-ribavirin therapy. However, these new regimens are very costly and concerns have been raised that in certain parts of the world, DAAs will not be available for a considerable time [4]. For patients who cannot obtain these newer treatments, treatment with HDR may be a feasible option, especially when they are carrier of a IL28B non-CC genotype.

In conclusion, HDR is relatively well tolerated, and seems to improve SVR rates in patients who are adherent to treatment, though no significant effect on SVR was seen in the intention to treat group.

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Supplementary table 1. Adverse events

	High-dose ribavirin (N=52)	Standard-dose ribavirin (N=58)	Chi2; p-value
Anemia			
Grade 2 (Hb<6.8 mmol/l)	30 (57.7%)	27 (46.6%)	
Grade 3 (Hb<5.0 mmol/l)	8 (15.4%)	1 (1.7%)	
Grade 4 (Hb<4.0 mmol/l)	1 (1.9%)	0 (0.0%)	8.06; 0.006*†
Anemia intervention			
Epoetin use	39 (75.0%)	24 (41.4%)	12.7; <0.0004
Transfusion	8 (15.4%)	1 (1.7%)	6.81; 0.012*
Ribavirin dose reduction	15 (28.8%)	10 (17.2%)	2.10; 0.15
Leukocytopenia			
Grade 2 (L<3.0x10^9/l)	25 (48.1%)	21 (36.2%)	
Grade 3 (L<2.0x10^9/l)	20 (38.5%)	17 (29.3%)	
Grade 4 (L<1.0x10^9/l)	0 (0.0%)	3 (5.2%)	0.19; 0.67†
Neutropenia			
Grade 2 (N<1.5x10^9/l)	16 (30.8%)	13 (22.8%)	
Grade 3 (N<1.0x10^9/l)	29 (55.8%)	22 (38.6%)	
Grade 4 (N<0.5x10^9/l)	2 (3.8%)	11 (19.3%)	0.033; 0.86†
Thrombocytopenia			
Grade 2 (T<75x10^9/l)	4 (7.7%)	6 (10.3%)	
Grade 3 (T<50x10^9/l)	2 (3.8%)	2 (3.4%)	0.012; 1.00*†
Grade 4 (T<25x10^9/l)	0 (0.0%)	0 (0.0%)	
Adverse events			
Total	52 (100%)	57 (98.1%)	0.91; 1.00*
Fatigue	44 (84.6%)	44 (75.9%)	1.31; 0.25
Decreased appetite	27 (51.9%)	24 (41.1%)	1.22; 0.27
Headache	23 (44.2%)	24 (41.4%)	0.091; 0.76
Insomnia	22 (42.3%)	25 (43.1%)	0.0071; 0.93
Pruritis	25 (48.1%)	21 (36.2%)	1.59; 0.21
Influenza-like illness	18 (34.6%)	24 (41.4%)	0.53; 0.47
Myalgia	20 (38.5%)	19 (32.8%)	0.39; 0.53
Cough	18 (34.6%)	17 (29.3%)	0.36; 0.55
Nausea	20 (38.5%)	13 (22.4%)	3.36; 0.067
Dizziness	15 (28.8%)	15 (25.9%)	0.12; 0.73
SAE/SUSAR	11 (21.2%)	16 (27.6%)	0.613; 0.43

*Fisher's exact test

† Grade 3 or 4 hematologic event versus no grade 3 or 4 hematologic event

SAE=Serious Adverse Event; SUSAR=Suspected Unexpected Serious Adverse Reaction

Supplementary table 2A. **Serious adverse events (SAEs) in the High-Dose Group**

	Age	Advanced fibrosis (F3-F4)	SAE description	Effect on study medication	Outcome
1. Male	49	No	Colovesical fistula	Discontinuation treatment	Resolved
2. Male	42	No	Allergic angioedema	No changes	Resolved
3. Male	66	Yes	Anemia	Interruption RBV + PEG	Resolved
4. Male	51	No	Pneumonia	No changes	Resolved
5. Male	39	No	Appendicitis	No changes	Resolved
			Neutropenia	No changes	Resolved
6. Male	54	Yes	Poisoning*	No changes	Resolved
			Exacerbation psoriasis	No changes	Improved
			Exacerbation psoriasis	Dose reduction PEG	Unresolved
7. Male	50	Yes	Neutropenia	No changes	Resolved
8. Female	59	No	Urinary tract infection	No changes	Resolved
			Fever of unknown origin	Discontinuation treatment	Resolved
9. Male	55	Yes	Syncope	No changes	Improved
10. Male	56	No	Pneumonia	No changes	Resolved
11. Male	59	Yes	Embolism lung	No changes	Improved

*With benzodiazepine-based tranquilizers

SAE=Serious Adverse Event; SUSAR=Suspected Unexpected Serious Adverse Reaction; RBV= Ribavirin; PEG=Peginterferon alfa, EPO=Epoetin beta

Supplementary table 2B. Serious adverse events (SAEs) in the Standard-Dose Group

	Age	Advanced fibrosis (F3-F4)	SAE description	Effect on study medication	Outcome
1. Female	52	Yes	Neutropenia	Dose reduction PEG	Resolved
2. Male	51	No	Neutropenia	No changes	Resolved
3. Male	48	No	Neutropenia	Dose reduction PEG	Resolved
4. Male	51	Yes	Neutropenia	Dose reduction PEG	Resolved
5. Female	49	Yes	Pancytopenia	Dose reduction PEG	Resolved
6. Male	58	No	Neutropenia	No changes	Improved
7. Female	47	No	Neutropenia	No changes	Resolved
8. Female	21	No	Appendicitis	Interruption RBV + EPO	Resolved
9. Male	59	Yes	Neutropenia	No changes	Resolved
10. Female	41	No	Neutropenia	Interruption PEG	Resolved
11. Male	47	No	Interstitial pneumonia	No changes	Resolved
12. Male	42	No	Neutropenia	Dose reduction PEG	Resolved
13. Male	37	Yes	SUSAR: Urosepsis	Patient died	Death
14. Male	44	No	Pulmonary infiltration	No changes	Resolved
15. Male	42	Yes	Neutropenia	No changes	Resolved
16. Male	34	No	Neutropenia	Dose reduction PEG	Resolved

SAE=Serious Adverse Event; SUSAR=Suspected Unexpected Serious Adverse Reaction; RBV= Ribavirin; PEG=Peginterferon alfa, EPO=Epoetin beta

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CHAPTER 3

The ARRIBA concept: Adequate resorption of ribavirin

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ABSTRACT

BACKGROUND

Adequate ribavirin exposure is essential for optimal sustained virological response (SVR) rates in chronic HCV treatment. It has been proposed that the area under the concentration-time curve up to 4 h after intake of ribavirin ($AUC_{0–4\ h}$) of the first weight-based ribavirin dose should be ≥1.755 mg.h/l to guarantee the highest chance of SVR. Our ARRIBA concept comprises a test dose of ribavirin to select the optimal starting dose to achieve adequate exposure. This study aims to evaluate whether adequate exposure can be achieved after dose advice based on the $AUC_{0–4\ h}$ of a single weight-based ribavirin test dose.

METHODS

(Formerly) HCV-infected subjects received a single weight-based ribavirin test dose (<75 kg: 400 mg; ≥75 kg: 600 mg) and the $AUC_{0–4\ h}$ was calculated. If ribavirin $AUC_{0–4\ h}$ was ≥1.755 mg.h/l, subjects received the same dose 4 weeks later; if the $AUC_{0–4\ h}$ was <1.755 mg.h/l, an adjusted dose was administered. The ribavirin $AUC_{0–4\ h}$ was recorded again. The primary outcome was the proportion of subjects with an $AUC_{0–4\ h}$ ≥1.755 mg.h/l after the second dose.

RESULTS

A total of 26 subjects were included. The geometric mean (95% CI) ribavirin $AUC_{0–4\ h}$ was 1.67 (1.44–1.92) mg.h/l with 9 subjects (35%) reaching the target AUC on day 1. Thus, on day 29, 17 subjects (65%) received an adjusted dose. The geometric mean (95% CI) $AUC_{0–4\ h}$ increased to 1.90 (1.62–2.21) mg.h/l and then 16 subjects (62%) had an $AUC_{0–4\ h}$ ≥1.755 mg.h/l, which is significantly higher than day 1 (P<0.05).

CONCLUSIONS

Our ARRIBA concept of a ribavirin test dose, with dose adjustment if necessary, leads to an increased proportion of patients with an AUC≥1.755 mg.h/l compared to traditional weight-based ribavirin dosing.

INTRODUCTION

Ribavirin is a synthetic nucleoside (guanosine) analogue [1] and is used in combination with pegylated interferon for chronic HCV infections, but is also a component of various treatment options with newer direct-acting antivirals (DAAs) [2–8]. In particular the combination of sofosbuvir and ribavirin has become the first interferon-free gold standard therapy for patients with an HCV genotype-2 infection. An advantage of ribavirin is that physicians and nurses are familiar with the drug, including its side effects. Adverse events can therefore be recognized quickly and adequately managed. Ribavirin, when administered without pegylated interferon, results in fewer and less severe side effects [3]. Finally, ribavirin is cheap and generically available.

Adequate exposure to ribavirin in dual therapy consisting of ribavirin and pegylated interferon is essential for optimal sustained virological response (SVR) rates [9–15]. Studies have shown that ribavirin pharmacokinetics display small intra- but large inter-patient variability [11,16]. For this reason, therapeutic drug monitoring of ribavirin has been suggested in the literature [17–19]. Usually, therapeutic drug monitoring is performed at steady-state. However, due to the long elimination half-life of ribavirin, steady-state is not reached before week 8 of treatment [16] and therefore interventions based on measuring ribavirin concentrations at this point in treatment may come too late to influence treatment outcome. Loustaud-Ratti *et al*. [20] have proposed that the area under the concentration– time curve up to 4 h after intake of ribavirin ($AUC_{0–4\,h}$) of the very first weight-based dose of ribavirin should be ≥1.755 mg.h/l to guarantee the highest chance of SVR in patients treated with pegylated interferon and ribavirin. Here, we introduce the ARRIBA concept: Adequate Resorption of RIBAvirin. It comprises a test dose of ribavirin to select the optimal starting dose for each individual HCV-infected patient (Figure 1). The objective of this study is to evaluate whether adequate ribavirin exposure ($AUC_{0–4\,h}$ ≥1.755 mg.h/l) can be achieved after dose advice based on the $AUC_{0–4\,h}$ of a single weight-based dose of ribavirin.

METHODS

This open-label, prospective, multicentre, Phase IIa trial was conducted at Radboud University Medical Center, Nijmegen, and Erasmus University Medical Center, Rotterdam, both in the Netherlands, and at University Hospital Bonn, Bonn, Germany. The trial was approved by the Investigational Review Boards of the study sites and was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. The study was registered at The European Union Clinical Trials Register (EudraCT Number 2010-020371-22). All participants signed informed consent prior to screening evaluations.

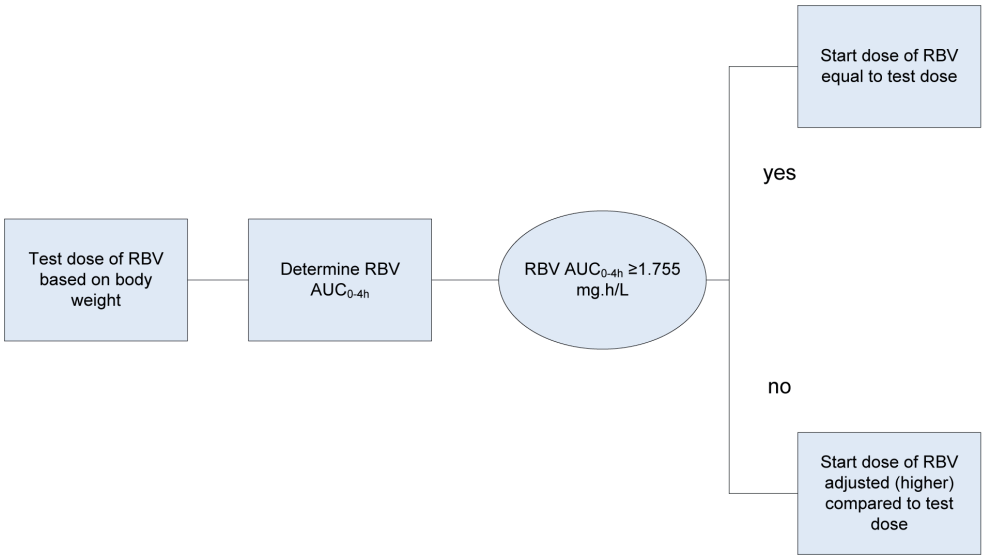

Figure 1. **The ARRIBA concept**

The study was designed to determine whether adequate exposure to ribavirin, that is ribavirin $AUC_{0-4\ h}$ ≥1.755 mg.h/l, can be achieved after dose advice based on the $AUC_{0-4\ h}$ of a single weight-based dose of ribavirin. The primary outcome was the proportion of subjects with an $AUC_{0-4\ h}$ ≥1.755 mg.h/l after the second dose of ribavirin. Secondary objectives were to evaluate the number of patients that need a dose adjustment, if there are subgroups of HCV-infected patients who more often need a dose adjustment to achieve sufficient exposure to ribavirin and to evaluate the safety and tolerability of an adjusted dose of ribavirin in (formerly) HCV-infected individuals.

HCV-treatment experienced patients were selected who were at least 18 years at screening, had tolerated ribavirin in the past and who were either cured or not yet eligible for subsequent HCV treatment. Main exclusion criteria were ribavirin use within 90 days prior to the first dose, pregnancy or breastfeeding, haemoglobinopathies, severe pre-existing cardiac disease, severe psychiatric condition, haemoglobin <7.5 mmol/l (female) or <8.5 mmol/l (male), $CD4^+$ T-cell count <200 cells/mm^3 within 3 months prior to screening for HIV positive patients, creatinine clearance <50 ml/min or signs of progressive liver disease.

On day 1 of the study, participants received a single dose of ribavirin based on their body weight (<75 kg: 400 mg ribavirin [two tablets of 200 mg Copegus®; Roche, Woerden, the Netherlands], ≥75 kg: 600 mg ribavirin [three tablets of 200 mg Copegus®; Roche]). Medication was taken at the study centre with a standardized breakfast (two pieces of brown bread, one slice of cheese and one slice of meat, one cup of custard and one cup of water [200 ml]). Blood samples were taken before and at

0.5, 1, 1.5, 2, 3 and 4 h after ribavirin intake to measure plasma ribavirin concentrations and to calculate $AUC_{0–4\ h}$. If ribavirin $AUC_{0–4\ h}$ was adequate, that is ≥1.755 mg.h/l, subjects received the same dose on day 29. If exposure to ribavirin was too low, that is an $AUC_{0–4\ h}$ <1.755 mg.h/l, an adjusted dose of ribavirin was administered on day 29, based on a predefined algorithm (Table 1). On day 29, after the second dose of ribavirin taken with the same standardized breakfast, blood samples were taken again at the same time points to measure plasma ribavirin concentrations and to calculate $AUC_{0–4\ h}$.

Blood samples for assessment of pharmacokinetic parameters of ribavirin were collected into heparinized tubes and centrifuged for 5 min at 2,500 *g* at 20°C within 5–6 h to obtain clear plasma. Plasma was transferred to polypropylene tubes and stored at -40°C until further bioanalysis. Plasma samples from all study sites were transported to the laboratory of the Pharmacy of the Radboud University Medical Center. Samples were prepared by a procedure previously described by Loregian *et al*. [21]. The concentrations of ribavirin in plasma were analysed by use of a validated reversedphase high-pressure liquid chromatography (HPLC) method with UV detection [22]. The linear calibration ranges in plasma were from 0.03 mg/l to 12.0 mg/l. Based on the individual plasma concentration–time data, the $AUC_{0–4\ h}$ was calculated by non-compartmental methods using the linear log trapezoidal rule in Winnonlin version 6.3. If a concentration of ribavirin was detected in the pre-dose sample on day 29, the $AUC_{0–4\ h}$ was corrected for this amount.

Table 1. Predefined dosing algorithm for the second ribavirin dose based on the $AUC_{0–4\ h}$ after the first weight-based dose of ribavirin

$AUC_{0–4\ h}$ after first dose	Adjusted second dose
After 400 mg (that is, body weight <75 kg)	
≥1.755 mg.h/l	400 mg (no adjustment)
1.20–1.76 mg.h/l	600 mg
0.90–1.20 mg.h/l	800 mg
<0.90 mg.h/l	1,000 mg
After 600 mg (that is, body weight ≥75 kg)	
≥1.755 mg.h/l	600 mg (no adjustment)
1.35–1.76 mg.h/l	800 mg
1.10–1.35 mg.h/l	1,000 mg
0.90–1.10 mg.h/l	1,200 mg
<0.90 mg.h/l	1,400 mg

$AUC_{0–4\ h}$=area under the concentration–time curve up to 4 h after intake of ribavirin.

Since the primary outcome was the proportion of subjects with an adequate $AUC_{0–4\ h}$ ≥1.755 mg.h/l after the second dose, the percentage of patients with an $AUC_{0–4\ h}$ ≥1.755 mg.h/l was calculated on day 1 and day 29 and the McNemar test was carried out to compare day 1 and day 29 data using SPSS for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). With a planned sample size of 50 subjects we would have sufficient power to demonstrate an increase in the proportion of subjects with an adequate AUC from 50% to 75%. We planned an interim analysis after 50% of the intended total number of patients had completed the study.

RESULTS

We present the results from the planned interim analysis. A total of 26 patients (17 males) completed both pharmacokinetic days. Median (range) age and body mass index (BMI) were 50 (20–70) years and 23 (18–35) kg/m^2, respectively. All subjects were Caucasian, 14 were HIV-coinfected and 16 had achieved an SVR prior to the study.

In total, 12 subjects received an initial weight-based dose of 400 mg and 14 subjects received 600 mg on day 1. The geometric mean (95% CI) ribavirin $AUC_{0–4\ h}$ on day 1 was 1.67 (1.44–1.92) mg.h/l with only 9 out of 26 (35%) subjects reaching the AUC target. Therefore, at day 29, 17 subjects (65%) received an adjusted dose of ribavirin. The distribution of adjusted ribavirin doses was: 600 mg for six patients, 800 mg for six patients, 1,000 mg for four patients and one patient received 1,200 mg on day 29. On day 29 the geometric mean (95% CI) ribavirin $AUC_{0–4\ h}$ increased to 1.90 (1.62–2.21) mg.h/l and now 16 subjects (62%) had an $AUC_{0–4\ h}$ ≥1.755 mg.h/l, which is significantly higher than on day 1 (P<0.05, McNemar; Figures 2 and 3).

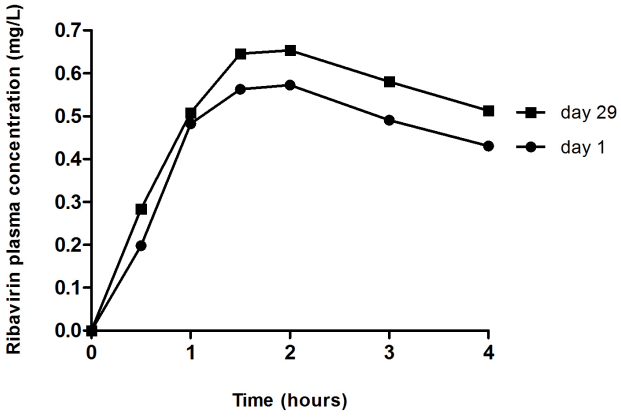

Figure 2. Mean ribavirin plasma concentrations versus time curve after a single dose based on body weight (day 1) and after a single dose based on individualized dose advice (day 29)

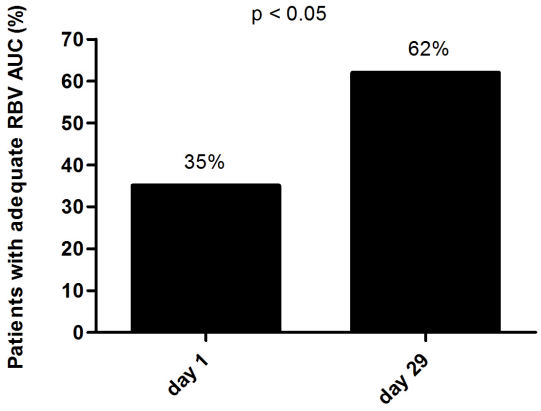

Figure 3. Percentage of patients with a ribavirin $AUC_{0–4\ h}$ ≥1.755 mg.h/l after a single dose based on body weight (day 1) and after a single dose based on individualized advice (day 29) AUC0–4 h, area under the concentration–time curve up to 4 h after intake of ribavirin (RBV).

$AUC_{0–4\ h}$=area under the concentration–time curve up to 4 h after intake of ribavirin.

In two patients ribavirin was detected in the pre-dose sample on day 29, their $AUC_{0–4\ h}$ was corrected for this. From the subjects with a dose intervention, eight (47%) had an adequate $AUC_{0–4\ h}$ on day 29. Eight patients with adequate exposure on day 1 were still on target after the second dose.

In a post hoc analysis there was no indication for a subgroup of patients not responding to the dose intervention, but numbers were small (Table 2). Overall, all subgroups demonstrated a 20–30% increase in adequate AUCs.

No serious adverse events were reported. In total, 46 adverse events were reported by 18 subjects. Sixteen adverse events were possibly related to the study drug. Two of these, headache in one patient and diarrhoea in another patient, were reported as grade 2 intensity. All other adverse events were reported grade 1.

DISCUSSION

With our ARRIBA concept, we were able to increase the percentage of subjects with adequate exposure to ribavirin, that is $AUC_{0–4\ h}$ ≥1.755 mg.h/l, from 35% to 62% after the dose advice based on the $AUC_{0–4\ h}$ of the single weight-based dose of ribavirin. Higher exposure to ribavirin is associated with a better response to treatment and therefore this ARRIBA approach can help optimize treatment with ribavirin for an individual patient. Individualizing therapy with ribavirin based on targeting higher ribavirin plasma concentrations does lead to high SVR rates as several studies have shown [12,23,24]. Usually, therapeutic drug monitoring, measuring ribavirin concentrations to reach and maintain these concentrations in a therapeutic range, is performed

Table 2. Percentage of subjects with adequate $AUC_{0-4\ h}$ on day 1 (after weight-based dose of ribavirin) and on day 29 (after dose intervention)

Subgroup	N	Adequate AUC on day 1 (%)	Adequate AUC on day 29 (%)	Increase (%)
HIV co-infection				
HIV positive	14	43	71	28
HIV negative	12	25	50	25
SVR				
SVR	16	31	63	32
No SVR	10	40	60	20
Genotype				
Genotype 1	20	40	60	20
Non genotype 1	6	17	67	50
Gender				
Male	17	41	65	24
Female	9	22	56	34
Ribavirin dose/kg bodyweight*				
< 6.61 mg/kg	13	23	46	23
> 6.61 mg/kg	13	46	77	31

*Median ribavirin dose/kg body weight was 6.61 mg/kg body weight.
$AUC_{0-4\ h}$=area under the concentration–time curve up to 4 h after intake of ribavirin; SVR=sustained virological response.

at steady state. In the case of ribavirin, interventions at steady state are probably too late to affect treatment outcome. With this ARRIBA concept (Figure 1) we have shown that it is possible to increase the exposure to ribavirin with a dose adjustment, thereby increasing the number of patients who can start HCV treatment with adequate exposure to ribavirin. As Loustaud-Ratti *et al*. [20] showed, this exposure is associated with realizing a higher chance for achieving SVR when patients are treated with pegylated interferon and ribavirin. This individualized concept is also more favourable than treating all patients with higher ribavirin doses. No significant effect of non-individualized higher doses of ribavirin on SVR rates has been seen in trials [25–27] and some patients are probably over-treated which can lead to more anaemia and other adverse events.

Because no resistance to ribavirin in HCV patients is described, it is very unlikely that a single ribavirin test dose will lead to resistant virus.

In the study from Loustaud-Ratti *et al*. [20], the percentage of patients with an $AUC_{0-4\ h}$ ≤1.755 mg.h/l was 58%, which is comparable to the 65% found in this study.

We expected to increase the percentage of patients with adequate ribavirin exposure by 25%, and we were able to increase this by 27%. On the other hand, there was still a proportion of patients who did not achieve adequate exposure to ribavirin and other dosing regimens should be explored. Our dosing algorithm was based on the linear relationship between the AUC from time zero to the last measurable concentration (AUC_{tf}) and single doses from 200 to 1,200 mg [28]. Even though the ribavirin AUCs increased in 13 of 17 (76%) subjects who received an increased dose, no more than 8 (47%) reached the target AUC. A possible explanation for this finding could be that although there is a linear relationship between AUC_{tf} and single doses, this linear relationship may not exist for $AUC_{0–4\ h}$. Ribavirin is a hydrophilic compound and requires nucleoside transporters for active transport into the cell [29,30]. The relationship between the ribavirin dose and the maximum plasma concentration is curvilinear, tending to asymptote above single doses of 800 mg, perhaps because of saturation of these nucleoside transporters [28]. It is possible that saturation of these transporters occurred with the doses used in our study. Therefore, other dosing algorithms or dosing frequencies should be explored and it was decided to terminate this study after the interim analysis. A limitation of our study is that the threshold for the ribavirin AUC was determined in HCV treatment naive patients treated with pegylated interferon and ribavirin [20]. The HCV landscape is changing rapidly and although ribavirin is still a component of various DAA-based treatment combinations, it remains to be determined whether the same threshold for ribavirin concentrations is valid. Preliminary studies from our group suggest, however, that a therapeutic range for ribavirin can also be defined when used as part of DAA-based HCV therapy [31].

In conclusion, our ARRIBA concept of a dose based on a test dose of ribavirin leads to an increased proportion of patients with an adequate AUC compared to the traditional weight-based dose of ribavirin. However, as there still remains a significant proportion of patients underdosed, alternative dosing algorithms should be explored.

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CHAPTER 4

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

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SUBMITTED

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 treatment with peginterferon alfa and ribavirin for chronic hepatitis C 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 $<15x10^6$/l) and asthenospermia (<32% moving spermatozoa) both occurred in 9 patients (39%). Median seminal volume decreased at week 12 of treatment (p=0.0012). No significant changes in progressive motility and sperm concentration were found. A significant increase of follicle stimulating hormone (p=0.012), but not of luteinizing hormone, free testosterone or inhibin was seen during treatment. Median DNA fragmentation index of hepatitis C patients before treatment was comparable with that of 22 healthy controls: 19% (range 2.6-43%) vs. 15.3% (range 6.4-26). Sperm DNA integrity was not altered during treatment and follow-up, even when corrected for the total dose of ribavirin.

CONCLUSIONS

Pre-existent 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

For years, treatment with peginterferon alfa and ribavirin (PEGIFN/RBV) has been the backbone of therapy for chronic hepatitis C virus (HCV) infection. Despite the arrival of direct acting antivirals (DAAs), especially RBV is still part of several HCV treatment regimens. The use of RBV is contraindicated during pregnancy and in men whose partners may become pregnant for up to seven months prior to conception. In animals, RBV has repeatedly been proven to be teratogenic. Malformation of limbs, spine, ribs, eyes and central nervous system have been shown in rodents [1-4] in addition to reductions in sperm count and alterations in morphology of spermatozoa [1, 2, 5, 6] after exposure to RBV. Contradicting results have been found in animal studies investigating the effect of interferon alfa on semen quality [7, 8]. In humans, many reports on direct maternal exposure to PEGIFN and/or RBV prior to or during pregnancy describe normal pregnancy outcomes [9-12], however there are some reports of miscarriage and birth defects where a possible association with RBV could not be ruled out [13, 14]. Little is known about the effects on offspring of males treated with PEGIFN and/or RBV. There are some reports of miscarriage, but most pregnancies resulted in normal live born infants [15-17]. Nonetheless, RBV is detectable in semen during antiviral treatment [18] and because 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.

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 [18, 19]. However, limited data are available on semen quality during follow up after completion of treatment and no data is available on sperm DNA integrity (sperm DNA damage) in men treated with PEGIFN/RBV.

Sperm concentration and sperm motility are important parameters for male fertility. Sperm DNA integrity is associated with a lower spontaneous conception rate and a possible higher miscarriage risk [20, 21]. Naturally, hormones play an important part in spermatogenesis. 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 [22] 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. Little is known about the effect of PEGIFN/RBV treatment on these endocrinological parameters in HCV infected men.

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.

MATERIALS AND METHODS

STUDY DESIGN

This study is a single center observational study. Treatment naive 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. This study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. The study protocol was reviewed and approved by the institutional review board. Written informed consent was obtained from all study subjects.

SEMEN ANALYSIS

Semen samples were obtained at baseline, week 12, 24 and 48 of treatment 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 volume, concentration and motility were analysed according to WHO standard criteria [23]. Sperm motility was assessed by categorizing the spermatozoa in 2 groups: progressively motile and non-progressively motile spermatozoa. The first group contains spermatozoa moving forward. The last group contains sperm cells that don´t move forward or don't move at all. Sperm morphology was not included in the study protocol, since this 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 [24], using a FACS (Fluorescense-Activated Cell Sortingcytometer (Becton Dickinson, San Jose, CA). In brief, frozen samples were quickly thawed, diluted to a concentration of 1-2 x 10^6 sperm cells/ml, exposed to acid detergent solution, and stained with acridine orange. Data collection of the fluorescence pattern in 5000 cells was performed at 3 minutes after acid treatment. Debris, bacteria and leukocytes were gated out during acquisition as recommended [24]. 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 and 48 of treatment 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. [25]. 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

Mann-Whitney U tests were used to compare baseline variables. These analysis were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).
To attain equal distributions continuous variables were log transformed before comparing variables at multiple time points using t-tests. These analysis were performed using the SAS 9.3 program (SAS Institute Inc., Cary, NC, USA).

RESULTS

PATIENTS

A total of 23 male patients were included in the analysis. Baseline samples were available in all patients. On-treatment and follow up samples were available in 19 patients. The remaining 4 patients dropped out of collecting samples (N=3) or hepatitis C treatment (N=1) after providing a baseline semen and blood sample. Baseline characteristics are summarized in Table 1.

Standard treatment consisted of peginterferon alfa-2a and RBV (10-15 mg/kg), except for 6 patients, who received high-dose RBV (25-29 mg/kg). Of the patients with follow up samples available, one was treated for 12 weeks, 9 patients for 24 weeks and 7 patients were treated for 48 weeks. One patient discontinued treatment at week 26, another had a viral breakthrough at week 37. 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 13 out of 23 patients.

SEMEN ANALYSIS

At baseline, semen abnormalities were common. Ten out of 23 patients (43%) had a low seminal volume (seminal volume <1.5ml), 9 patients (39%) had oligozoospermia (sperm concentration $<15x10^6$/ml) and 9 patients (39%) had asthenozoospermia

Table 1. Baseline characteristics

	N=23
Age (mean, range in years)	43 (22-62)
BMI (mean, range in kg/m2)	24 (18-33)
Cirrhosis (metavir score F4)	1 (4%)
HCV Genotype	
1	12 (52%)
2	4 (17%)
3	7 (30%)
Low seminal volume	10 (43%)
Oligozoospermia	9 (39%)
Asthenozoospermia	9 (39%)

(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, dropping from a median of 1.6 ml to 1.1 ml (p=0.0012), but no alteration from baseline was seen at week 24 (1.6 ml) and 48 (1.9 ml; Figure 1A). Median sperm concentration at baseline was $29x10^6$/ml (range 3-$344x10^6$/ml). Median sperm concentrations at week 12, 24, 48 and FU24 were 35, 29, 37 and 57 x 10^6/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 36%, 41%, 42% and 44% respectively. During treatment 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%). No change in percentage of normal spermatozoa during treatment or follow-up was observed (Figure 1D). Semen parameters during treatment did not differ significantly between patients receiving high-dose or standard-dose RBV. At follow up, semen parameters did not significantly differ between patients who had achieved SVR and those who had not achieved SVR.

SPERM DNA INTEGRITY ANALYSIS

Data on sperm DNA integrity were available in 21 patients. At baseline 4 out of 18 patients (22%) had a DFI >30%, which is considered abnormal. The DFI of 18 HCV patients at baseline did not significantly differ from the median DFI of 22 proven fertile healthy controls: 19%, range 2.6-43% vs. 15.3%, range 6.4-26% (p=0.51). The

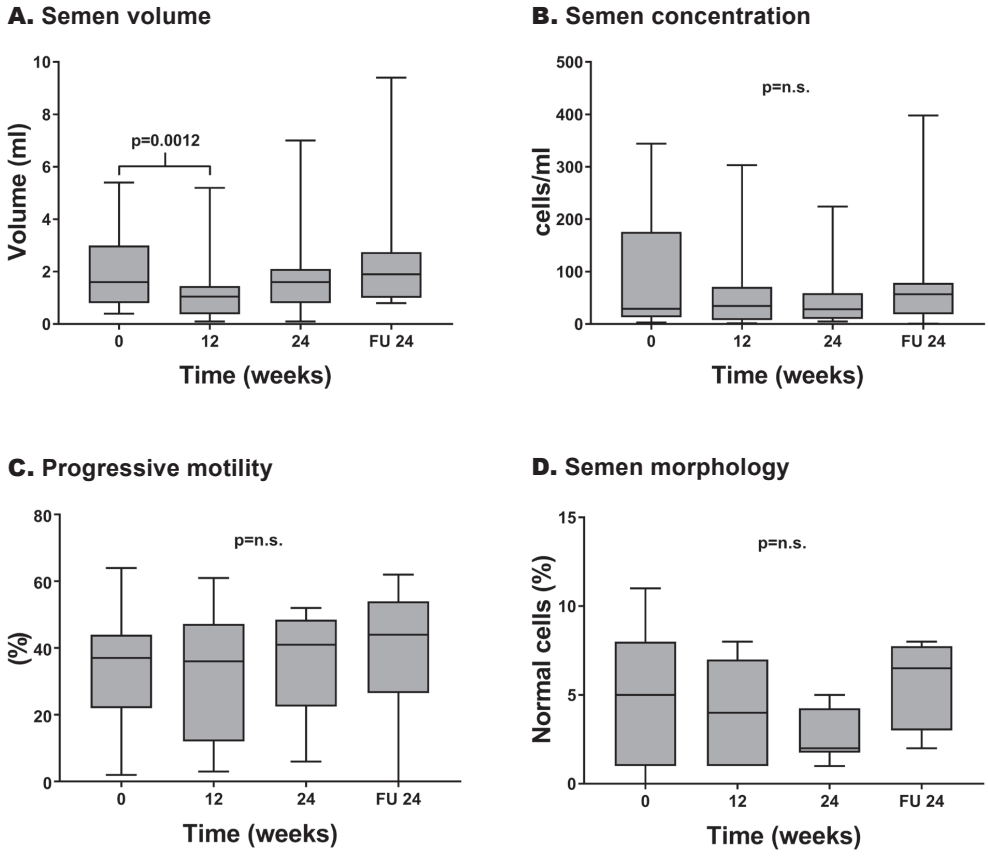

Figure 1. Semen analysis during hepatitis C treatment and follow up. Semen volume (A), Semen concentration (B), Progressive motility (C) and Semen morphology (D).

DFI did not significantly change during treatment and follow up (Figure 2); DFI was 15% (range 1.5-46%) at week 12, 19% (6.6-36%) at week 24, 13% (range 2.8-28%) at week 48 and 17% (range 2.6-74%) at week FU24. DFI during treatment did not differ significantly between patients receiving high-dose or standard-dose RBV. At follow up there was no difference in sperm DNA integrity between patients who had achieved SVR compared to those who had not achieved SVR.

ENDOCRINOLOGICAL PARAMETERS

Changes in gonadotrophic hormones, free testosterone and inhibin B are shown in Figure 3. An increase in FSH (Figure 3A) was seen during treatment which was significant at week 24 (p=0.012). During follow up FSH returned to baseline levels. No alterations in levels of LH, free testosterone or inhibin B were seen during treatment (Figure 3B-D).

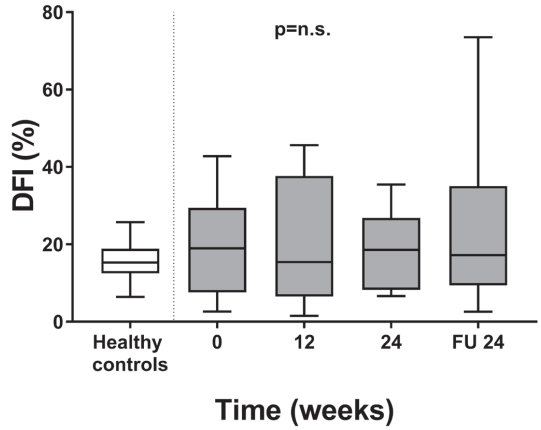

Figure 2. DNA fragmentation index (DFI) in healthy controls and in patients during hepatitis C treatment and follow up

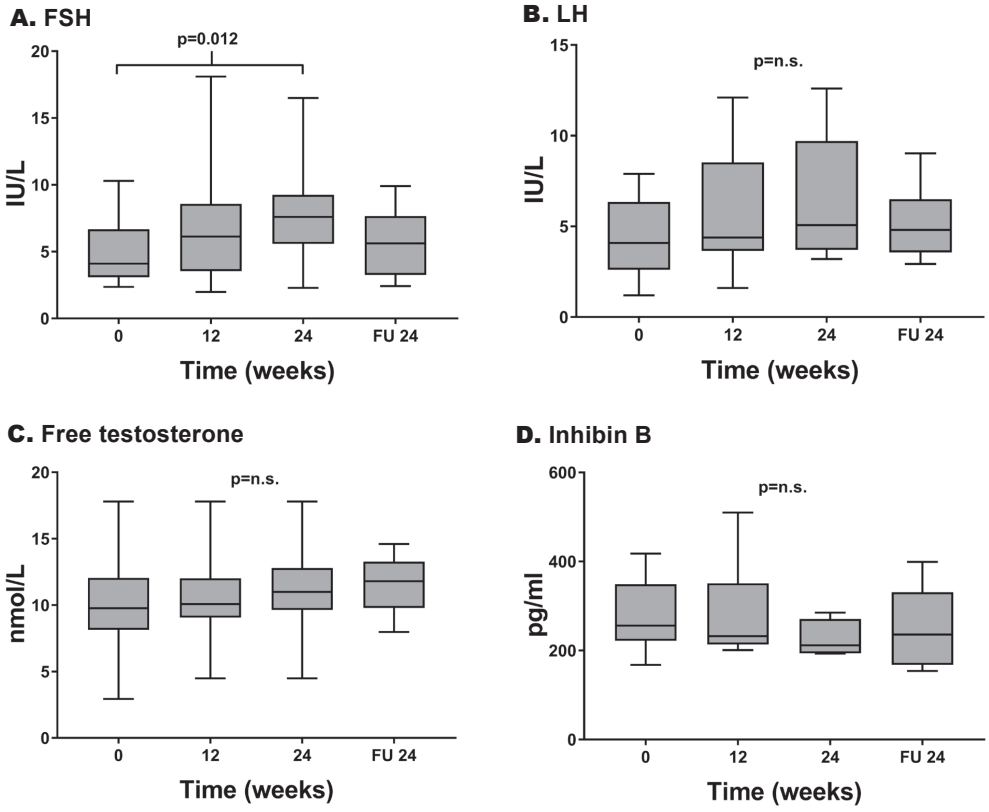

Figure 3. Endocronological parameters during hepatitis C treatment and follow up. FSH **(A)**, LH **(B)**, Free testosterone **(C)**, Inhibin B **(D)**

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. We found that 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 may be related to a decrease in sexual arousal during antiviral treatment [26]. Though we also report on semen morphology, this parameter is considered unreliable because of a great inter- and intra- observer variability and therefore 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 all patients.

Sperm DNA integrity is a predictor of spontaneous pregnancies and the occurrence of miscarriage. It is yet unclear if DNA integrity is also associated with an increased risk of birth defects, though theoretically it could be argued that DNA damage increases the risk of malformation during embryonic development [27]. The median DFI of HCV patients did not differ significantly from healthy controls and DFI levels were not altered during PEGIFN/RBV therapy. These findings are in disagreement with a case report on sperm DNA integrity during PEGIFN/RBV therapy for HCV [28]. 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. Of note, no difference in DFI was seen in the 5 patients receiving high-dose ribavirin, compared to patients on standard-dose ribavirin, nor did SVR influence DFI.

Finally, we investigated gonadotrophic and gonadal hormone levels during antiviral treatment. An increase in FSH was observed during treatment together with a non-significant decrease of 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 [19]. However, a decrease in the number of patients with semen abnormalities after achieving SVR would then be expected, which we did not observe. 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 substances or specific medication [29, 30].

Our findings are not entirely in agreement with previous findings by Hofer et al. [18]. They found a significant decrease in sperm concentration at week 4 and a

significant decrease in normal cell morphology at week 12 in a cohort of 15 patients. No significant changes in motility were found. They concluded that antiviral treatment leads to substantial alterations in both quantitative and qualitative parameters. However, changes in semen quality were only minimal, not consistent and only measured at week 4, 12 and 24 of treatment and not during follow up. Furthermore, sperm DNA integrity, a more reliable parameter of semen quality and teratogenicity, was not assessed.

RBV is excreted in semen; Hofer et al. found a twofold higher RBV concentration in semen compared to serum concentrations at week 4 and 12 of antiviral treatment. The clinical significance of detectable RBV in semen remains unclear; if RBV in semen is transmitted to female partners during sexual intercourse, blood concentrations will 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 is questionable whether double contraception during treatment and up to seven months after treatment cessation is necessary for male patients. Semen abnormalities were present at baseline in a considerable proportion of HCV patients and considering the fact that these abnormalities did not resolve after achieving SVR, it is likely that these abnormalities are due to other contributing factors in the HCV population.

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CHAPTER 5

Interferon induced low blood cell counts are associated with infection and bleeding in liver transplant recipients on hepatitis C treatment

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ABSTRACT

BACKGROUND

Despite the arrival of IFN-free HCV therapies, due to limited availability in certain parts of the world, LT recipients patients may still be treated with IFN. When HCV infected Liver Transplant (LT) recipients are treated with interferon (IFN) often suboptimal doses are given due to either low platelet count (PC) or low white blood cell count (WBC). Our aim was to investigate if low blood cell counts during HCV treatment with IFN are predictive of bleeding and infection in LT recipients.

METHODS

IFN-based HCV treatments in LT recipients from the Mayo Clinic in Rochester, Minnesota (US) were included. Bleeding and infections were correlated with lowest PC and lowest WBC during 4-weekly intervals.

RESULTS

We studied 178 treatments in 135 LT recipients. Thirty bleeding episodes in 20 patients (15%) and eighty-four infections in 47 patients (35%) were observed. In multivariate analysis the OR (bleeding) for log-PC was (0.031; 95%CI 0.0085-0.11) when corrected for treatment for diabetes mellitus and calcineurin inhibitor use and the OR (infection) for log-WBC was 0.14 (95% CI 0.044-0.45) when corrected for PEG-IFN versus standard IFN and use of MMF or AZA. In the prediction model, risk of bleeding and infection increased exponentially when PC dropped below $50x10^9$/l and WBC dropped below $2.0x10^9$/l, respectively.

CONCLUSION

IFN-induced thrombocytopenia and leukopenia are associated with bleeding and infection in this difficult-to-treat group of HCV infected patients. This study suggests that the common practice to reduce IFN doses when cytopenia occurs, prevents complications and should not be abandoned in LT recipients.

INTRODUCTION

Interferon-free treatment regimens for hepatitis C virus (HCV) infection have become widely available in developed countries. However, in certain areas around the world, due to high costs, reimbursement strategies and logistics, still not every HCV patient has access to interferon-free regimens [1, 2]. As an alternative, these patients will have to rely on more affordable options including treatment with peginterferon (PEG) and ribavirin or even standard interferon (sIFN). These drugs are known to have many side effects often requiring dose reductions leading to a decrease in sustained virological response (SVR; determined as undetectable HCV RNA at 24 weeks after therapy). One of the most well-known side effects of PEG/sIFN and ribavirin are cytopenias. Several studies have shown that the occurrence of leukopenias and thrombocytopenia during antiviral treatment of HCV is not correlated with the incidence of (severe) infections and (significant) bleeding, respectively [3-14]. Only one study described a relationship between neutropenia and infection [15] and in another study in patients with advanced fibrosis, a platelet count <50x10^9/l was associated with mild bleeding [16]. However, these studies were performed in non-transplant patients, while HCV infected organ transplant recipients, especially liver transplant recipients, often need antiviral treatment for HCV recurrence, while being on immunosuppressive medication. This immunosuppressive state may lead to a different risk profile regarding hematological side effects. Little is known about the risks of cytopenia in liver transplant patients with reports of cytopenia varying widely among cohorts [17-31]. In the current study we investigated the occurrence of hematological complications in a large cohort of liver transplant patients treated for recurrent hepatitis C.

METHODS

Data was collected from the Mayo Clinic in Rochester, Minnesota (US). All patients who received a liver transplant between 1995 and 2010 and underwent interferon-based hepatitis C treatment post-transplant were included in this retrospective study. Interferon-based treatments included either administration of pegylated interferon (PEG) or standard interferon (sIFN). The use of ribavirin was optional. Exclusion criteria were co-infection with HIV or hepatitis B, treatment including protease-inhibitors and treatment with (low-dose) interferon maintenance therapy.

Bleeding was defined as any episode during which a patient had non-physiological blood loss, excluding normal menstruation. Infection was defined as any episode during which a patient was treated with antibiotics for a suspected bacterial infection and/or any episode during which a patient had a proven bacterial infection (diagnosed through means of bacteriology or diagnostic imaging).

Data on bleeding, infection, lowest PC, lowest WBC, other variables thought to be associated with bleeding and infection (including immunosuppressive medication and comorbidities), interferon dose adjustment and/or discontinuation, hospital admission and death were collected per 4-week treatment interval.

STATISTICS

SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) was used for descriptive statistics. SAS 9.3 program (SAS Institute Inc., Cary, NC, USA) with the application of the repeated statement was used for multiple regression analysis to identify variables associated with infection or bleeding. All variables with a p-value <0.20 in univariate analysis were included in the multivariate regression analysis. Using backward elimination, only variables with a p-value <0.05 were included in the final model. Finally, a prediction model for infection and bleeding was developed using the following formula:

$$\frac{e^{(\beta 0+\beta 1*\log WBC\ or\ \log PC)}}{1+e^{(\beta 0+\beta 1*\log WBC\ or\ \log PC)}}$$

RESULTS

A total of 178 treatments in 135 patients were included. Baseline characteristics of these 178 treatments are given in table 1.

The average treatment duration was 42 weeks (SD +/- 26). A total of 57 patients (42%) experienced bleeding and/or infection on treatment.

PLATELET COUNT AND BLEEDING

PC dropped from $139x10^9$/l (SD +/- $57x10^9$/l) at baseline to $81x10^9$/l (SD +/- $45x10^9$/l) on treatment. In 7 treatments (4%) IFN dose was reduced and 6 treatments (3%) were discontinued due to low PC count (without signs of bleeding).

A total of 30 bleedings were registered in 21 treatments in 20 patients (15%). An overview of bleeding characteristics is given in table 2. Due to bleeding one patient received IFN dose reduction and two patients discontinued therapy. The two patients who stopped therapy due to bleeding both developed idiopathic thrombocytopenic purpura (ITP) on treatment. One suffered from bleeding from the nose and gums and had hematoma of the skin. The other patient had lower gastrointestinal bleeding. In 13 out of 28 bleedings (46%), patients were hospitalized (no data on hospitalization was available in 2 bleeding episodes). None of the patients died due to bleeding.

Table 1. Baseline characteristics per HCV treatment in 135 liver transplant recipients

	HCV Treatment (N=178)
Age (years; SD)	54 (+/- 8.3)
Male sex	130 (73%)
Cirrhosis	15 (9%)
Number of LT	
1st	157 (88%)
2nd	18 (10%)
3rd	3 (2%)
Number of HCV treatment	
1st	134 (75%)
2nd	30 (17%)
3rd	9 (5%)
4th	4 (2%)
5th	1 (1%)
Mean Time from LT to treatment (years; SD)	2.5 (+/-2.6)
Interferon use	
Pegylated interferon (PEG)	140 (79%)
Standard interferon (sIFN)	38 (21%)
Use of diabetes medication	62 (35%)
Use of MMF or AZA	20 (11%)
Calcineurin inhibitor used	
Tacrolimus	123 (69%)
Cyclosporine	45 (25%)
Other	7 (4%)
None	3 (2%)
Use of prednisone	47 (26%)

WHITE BLOOD CELL COUNT AND INFECTION

WBC dropped from $4.7x10^9$/l (SD +/- $1.9x10^9$/l) at baseline to $2.1x10^9$/l (SD +/- $1.1x10^9$/l) on treatment. In 25 treatments (14%) IFN dose was reduced and 10 treatments (6%) were discontinued due to low WBC (without signs of infection).

A total of 84 infections were registered in 55 treatments in 47 patients (35%). An overview of infection characteristics is given in table 2. None of the patients received an IFN dose reduction because of infection, but in 12 cases IFN therapy was discontinued. In 19 out of 78 infections (24%), patients were hospitalized (no data on hospitalization was available in 6 infection episodes).

Two patients died after treatment discontinuation for severe infections. The first patient had cirrhosis and developed liver failure and SBP on treatment. This patient died three months post-treatment. The second patient had cholestatic HCV and also developed SBP on treatment. This patient died one month post-treatment.

Table 2. Characteristics of bleedings and infections during HCV treatment

	Bleeding (N=30)
Bleeding category	
Urinary/renal tract	7 (23%)
Genital tract	6 (20%)
ENT	5 (17%)
Lower gastrointestinal	3 (10%)
Traumatic bleed	2 (7%)
Upper gastrointestinal	0 (0%)
Other	7 (23%)
	Infection (N=84)
Infection category	
Upper respiratory (including sinusitis)	21 (25%)
Wound infections	17 (20%)
Pulmonary	9 (11%)
Urogenital	8 (10%)
Dermatological	7 (8%)
Gastrointestinal	4 (5%)
Multiple infections	3 (4%)
SBP	3 (4%)
Other	12 (14%)

ENT=Ear, Nose, Throat; SBP=Spontaneous Bacterial Peritonitis

MULTIVARIATE ANALYSIS AND PREDICTION MODEL

To attain an equal distribution, PC and WBC were log-transformed. In univariate analysis, lower (log) PC (<0.0001), cyclosporine use (p=0.001) and not using medication for diabetes mellitus (p=0.039) were associated with bleeding (table 3). Lower (log) WBC (p=0.0006), the use of MMF/AZA (p=0.013) and age (p=0.043) were associated with infection (table 4). In multivariate analysis all variables with a p<0.02 were included. Lower (log) PC (<0.0001), cyclosporine use (p=0.0038) and not using medication for diabetes mellitus (p=0.046) were associated with bleeding (table 3). Lower (log) WBC (p=0.0009), the use of MMF/AZA (p=0.0009) and the use of PEG (versus sIFN) (p=0.032) were associated with infection (table 4).

Subsequently, a prediction model for PC and bleeding and WBC and infection was created (figure 1). The risk of bleeding increases exponentially at a PC <50x10^9/l and the risk of infection increases exponentially at a WBC <2x10^9/l.

Table 3. Logistic regression analysis for episodes of bleeding

	Univariate		Multivariate	
	OR (95%-CI)	**P-value**	**OR (95%-CI)**	**P-value**
Log PC (x10^9/l)	**0.027 (0.0086-0.083)**	***<0.0001***	**0.031 (0.0085-0.11)**	***<0.0001***
MMF or AZA	3.40 (0.89-13.2)	0.076	NS	NS
sIFN vs. PEG	0.55 (0.12-2.40)	0.42	-	-
Diabetes medication	0.28 (0.084-0.93)	*0.039*	0.30 (0.089-0.98)	*0.046*
Cyclosporine vs. tacrolimus	5.11 (1.93-13.5)	*0.001*	4.29 (1.60-11.5)	*0.0038*
Prednisone	0.57 (0.14-2.36)	0.44	-	-
Female sex	1.47 (0.48-4.53)	0.50	-	-
Age	1.02 (0.97-1.08)	0.35	-	-
Cirrhosis	2.14 (0.55-8.33)	0.27	-	-
Week of treatment	0.99 (0.97-1.00)	*0.053*	NS	NS

CI=Confidence Interval; NS=Not significant (p≥0.05); PEG=Peginterferon; sIFN=standard Interferon

DISCUSSION

Cytopenias are common in HCV infected liver transplant recipients on PEG/sIFN (and ribavirin).

In this cohort of 135 patients who received a total of 178 treatments with PEG or sIFN, dose reduction for thrombocytopenia occurred in 4% and for leukopenia in 14%. Respectively 3% and 6% of patients discontinued treatment due to thrombocytopenia and leukopenia without them having signs of bleeding or infection at the time of discontinuation. Because these patients have reduced chances of achieving an SVR, it is important to know whether on-treatment cytopenia is associated with classic cytopenia-related side effects.

Bleeding and infection were common in this cohort with up to 42% of patients experiencing either or both of these complications during HCV treatment, often leading to hospitalization (respectively in 46% and 24%), but these hematological complications

Table 4. Logistic regression analysis for episodes of infection

	Univariate		Multivariate	
	OR (95%-CI)	P-value	OR (95%-CI)	P-value
Log WBC (x10^9/l)	**0.14 (0.045-0.43)**	**0.0006**	**0.14 (0.044-0.45)**	**0.0009**
MMF or AZA	2.57 (1.22-5.43)	0.013	3.44 (1.66-7.15)	0.0009
sIFN vs. PEG	0.36 (0.12-1.08)	0.069	0.30 (0.12-1.01)	0.032
Diabetes medication	0.64 (0.34-1.20)	0.17	NS	NS
Cyclosporine vs. tacrolimus	1.37 (0.72-2.61)	0.33	-	-
Prednisone	0.81 (0.39-1.69)	0.58	-	-
Female sex	1.29 (0.72-2.32)	0.39	-	-
Age	1.03 (1.00-1.06)	0.043	NS	NS
Cirrhosis	1.25 (0.51-3.08)	0.62	-	-
Week of treatment	1.00 (0.99-1.01)	0.84	-	-

CI=Confidence Interval; NS=Not significant (p≥0.05); PEG=Peginterferon; sIFN=standard Interferon

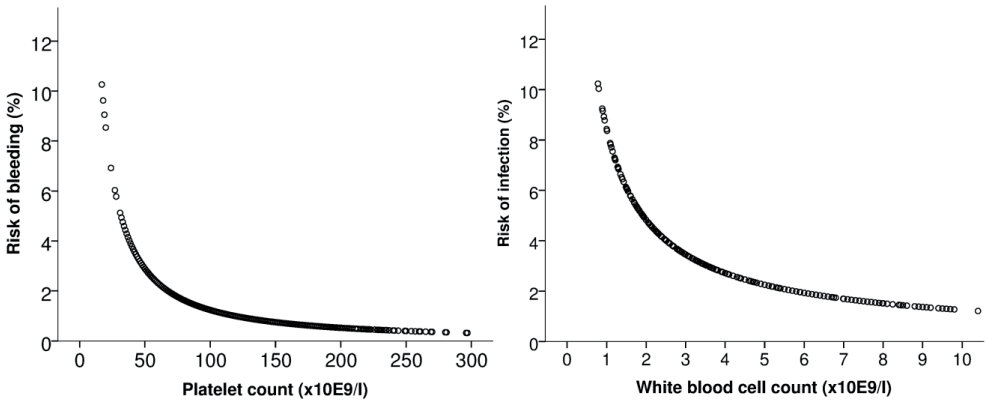

Figure 1. Risk of bleeding and infection with increasing (respectively) platelet and white blood cell count.
PC >300 and WBC >11 were censored in this figure to improve visuals.

were rarely associated with death. The two patients who discontinued treatment due to severe infection and died after one and three months post-treatment, most likely died from HCV recurrence rather than treatment-related infection.

Nonetheless, hospitalizations for bleeding and infection reflect a severe course with a risk of mortality and possible long-term morbidity. A well thought-out decision should be made between the risk of these side effects during PEG/sIFN treatment versus the benefits of SVR. It is therefore necessary to know whether cytopenias are associated with bleeding and infection before dose reductions for cytopenias are recommended.

In this study we indeed found that lower PC and lower WBC are associated with respectively bleeding ($p<0.0001$) and infection ($p=0.0009$) in multivariate analysis. The risk of bleeding increases exponentially at a PC $<50x10^9$/l and the risk of infection increases exponentially at a WBC $<2x10^9$/l in our prediction model. This confirms the findings of a previous published smaller cohort of 55 liver transplant treated with PEG and ribavirin, where a relationship was found between severe neutropenia and infection [29].

Immunosuppressive medication is associated with cytopenias and may therefore also be associated with bleeding and infection. In the multivariate analysis, cyclosporine was associated with bleeding. No studies have been published that reveal a more profound association between cyclosporine and bleeding compared to other calcineurin inhibitors. A possible explanations for the association between bleeding and cyclosporine use in this cohort may be that patients on cyclosporine are at a higher risk of developing an unfavourable cardiovascular profile [32], which may also increase the risk of bleeding. Diabetes mellitus is associated with an increased thrombogenic state [33] which may explain the inverse association of patients being on diabetes medication and bleeding in the multivariate analysis in this study.

As expected, MMF/AZA use, both immunosuppressives known to cause bone marrow suppression, was associated with infection. In the registration trials, PEG was associated with more profound drop in platelet count compared to sIFN, though (severe) bleeding did not occur more often in the PEG-groups [34, 35]. However, these non-transplant patients likely had less comorbidity and co-medication that may have a synergistic effect on the side effects of PEG-induced bone marrow suppression in this cohort of liver transplant recipients.

Surprisingly, though in 26% of treatments patients were on prednisone, its use was not associated with infection on treatment. We do not have an explanation of this lack of association other than that a stronger association existed between infection and the other factors we found in the multivariate analysis.

A limitation of this study is that we did not include an untreated control group. We consciously chose not to compare untreated patients to treated patients since un-

treated patients are often either too sick to receive treatment or have a mild post-transplant course not requiring treatment. Therefore treated and untreated groups are not comparable. It remains unclear whether cytopenias (especially thrombocytopenia) are part of a natural post-transplant course due to medication use and/or progression to cirrhosis or are PEG/sIFN induced. Of note, several case reports have been published describing PEG induced idiopathic thrombocytopenic purpura (ITP), a condition that occurred in two of the patients in this population. When a patient develops ITP on treatment, immediate discontinuation of PEG is warranted.

In conclusion, bleeding and infection are common in HCV infected liver transplant patients on PEG/sIFN based antiviral therapy and leukopenia and thrombocytopenia during treatment are associated with respectively infection and bleeding. Since we have entered the era of direct acting antiviral agents, interferon-free regimens are now the preferable course of HCV treatment. However, in some parts of the world access to these interferon-free regimens is limited and treatment with interfon-based treatments are still standard of care [1, 2]. This study suggests that the common practice to reduce IFN doses when cytopenia occurs, prevents complications and should not be abandoned in LT recipients.

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CHAPTER 6

Multicenter experience using telaprevir or boceprevir with peginterferon and ribavirin to treat hepatitis C genotype 1 after liver transplantation

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ABSTRACT

The safety, efficacy, and effect on immunosuppression levels of telaprevir (TVR) or boceprevir (BOC) in combination with peginterferon (PEG-IFN) and ribavirin (RBV) in recipients of liver transplantation (LT) with hepatitis C virus (HCV) genotype 1 have not been defined. We report our 3 centers' preliminary experiences with administering triple antiviral treatment protocols containing PEG-IFN, RBV, and TVR or BOC. Patients with biopsy-proven HCV recurrence (METAVIR grade ≥3 and/or stage ≥2) received TVR with PEG-IFN/RBV for 12 weeks and then PEG-IFN/RBV for 36 weeks or BOC with PEG-IFN/RBV for 44 weeks after 4 weeks of lead-in PEG-IFN/RBV. Maintenance immunosuppression was changed to cyclosporine whenever possible, and the levels were followed closely. PEG-IFN/RBV dose adjustments were based on patients' tolerance. Sixty patients started triple antiviral treatment, and they were followed for up to 66 weeks (mean 35 weeks); all were followed at least 12 weeks. Thirty of the 35 patients treated with TVR (86%) achieved undetectable HCV RNA levels after an average of 6 weeks, whereas 12 patients (48%) in the BOC-treated group achieved undetectable HCV RNA levels after a mean of 11 weeks. According to an intention-to-treat analysis, 14 of 21 TVR-treated patients (67%) and 10 of 22 patients who received BOC (45%) achieved undetectable HCV RNA levels at week 24 without viral breakthrough at the last follow-up. Cytopenias complicated both regimens; all patients required dose reductions of PEG-IFN and/or RBV or the administration of hematological growth factors. One death occurred in each group on triple antiviral treatment. In conclusion, TVR or BOC combined with PEG-IFN/RBV achieved on-treatment virological response rates of approximately 50% to 60% in patients with recurrent HCV after LT, but significant side effects were common.

INTRODUCTION

Hepatitis C virus (HCV) infection is the leading indication for liver transplantation (LT) in the United States [1]. HCV recurrence is universal and occurs immediately after LT [2]. Although the severity and clinical course of HCV infections after LT are variable, severe histological recurrence is the most common cause of death and graft loss in LT recipients with HCV infections and accounts for approximately half of deaths and two-thirds of graft losses by the tenth postoperative year [3-5]. Graft and patient survival can be improved with successful antiviral treatment.[3-5] Unfortunately, treatment with peginterferon (PEG-IFN) and ribavirin (RBV) is less effective and more poorly tolerated in the posttransplant setting in comparison with nontransplant patients, with sustained virological response (SVR) rates of 24% to 45%[5,6].

Recently, 2 protease inhibitors, telaprevir (TVR) and boceprevir (BOC), were approved by the Food and Drug Administration for the treatment of HCV genotype 1 infections. The administration of these protease inhibitors in combination with PEG-IFN/RBV resulted in substantial improvements in SVR rates in comparison with treatment with PEG-IFN/RBV alone in treatment-naive patients[7-9]. The SVR rates were also higher in patients who were nonresponders to previous treatment with PEG-IFN/RBV and in prior relapsers [10,11]. Although there is an urgent need for effective treatments for HCV recurrence after LT, significant concerns have been expressed about the safety and efficacy of HCV protease inhibitors in this setting because of the side effect profile and the potential for drug-drug interactions with immunosuppressive agents [12]. Both cyclosporine and tacrolimus are substrates of cytochrome P450 3A and P-glycoprotein. The coadministration of TVR, a potent cytochrome P450 3A4 substrate and inhibitor with the potential to saturate or inhibit intestinal P-glycoprotein, substantially increases the blood levels of the calcineurin inhibitors (CNIs) cyclosporine and tacrolimus [13]. Lesser drug-drug interactions between BOC and both CNIs have also been observed [14]. Additionally, levels of sirolimus and everolimus would also be expected to increase significantly if they were taken with protease inhibitors through the same mechanisms. Recently, Werner et al. described their initial 12-week experience in a pilot study using TVR with tacrolimus, cyclosporine, or sirolimus in 9 patients [15].

We report our preliminary experience with both pro-tease inhibitors in combination with PEG-IFN/RBV for the treatment of recurrent HCV genotype 1 infections after LT in 3 LT centers.

PATIENTS AND METHODS

Clinical treatment protocols were prospectively developed for LT recipients with HCV genotype 1 who had histological evidence of HCV recurrence at Mayo Clinic in Jack-

sonville, FL; at Mayo Clinic in Scottsdale, AZ; and at Mayo Clinic in Rochester, MN. Staff from each site agreed to both protocols, and a retrospective review of the safety and efficacy of these investigational protocols was approved by the Mayo Clinic institutional review board. All patients considered for triple antiviral treatment (an HCV protease inhibitor combined with PEG-IFN/RBV) had an allograft biopsy sample that showed significant fibrosis (stage ≥2) and/or moderate to severe lobular hepatitis (grade ≥3) according to the METAVIR system [16].

Exclusion criteria for antiviral treatment included evidence of biopsy-proven acute rejection (BPAR) in the past 2 months and/or any medical contraindication for PEG-IFN/RBV use. Mycophenolate mofetil and corticosteroids were discontinued before the initiation of antiviral treatment whenever possible.

Figure 1 is a timeline comparison of the immunosuppressive agent and triple antiviral treatment used in the treatment protocols.

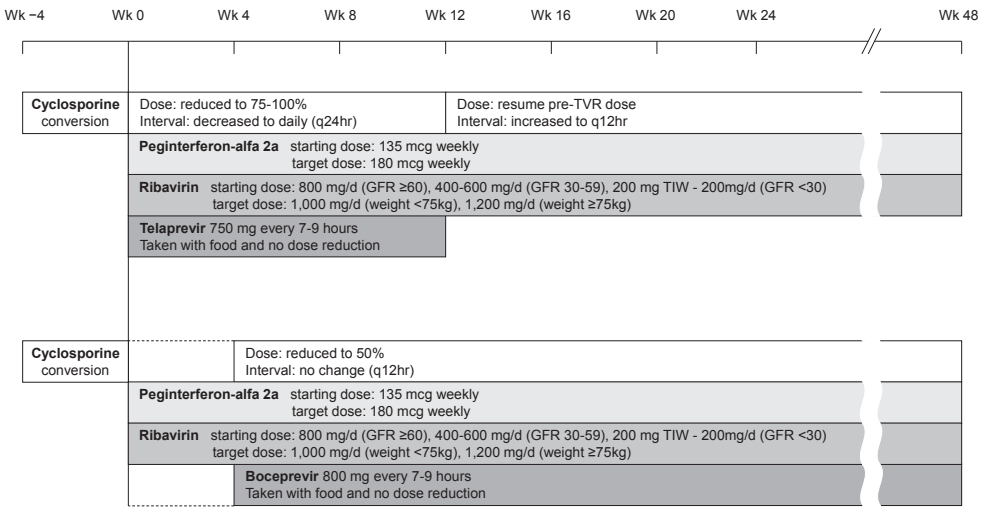

Figure 1. Timeline comparison of the immunosuppressive agent and triple antiviral treatment used in the 2 treatment protocols.

In both protocols, the maintenance immunosuppressive agent was converted to cyclosporine. A stable trough level of cyclosporine for 4 weeks was required before the initiation of antiviral treatment. Individual patients who did not tolerate the conversion to cyclosporine were considered for antiviral treatment while they were on either tacrolimus or sirolimus. Close monitoring of trough levels of the immunosuppressive agent was mandated during the first 14 days after the initiation of the protease inhibitor and again during the first 7 days of its discontinuation.

Once trough level of the immunosuppressive agent was stable, it was performed every 1 to 2 weeks throughout the triple antiviral treatment and every 2 to 4 weeks during the dual antiviral treatment with PEG-IFN/RBV.

The treatment regimen consisted of PEG-IFNa2a (Pegasys), RBV, and either TVR or BOC. TVR was used at Mayo Clinic in Jacksonville, FL, whereas BOC was selected at Mayo Clinic in Scottsdale, AZ and at Mayo Clinic in Rochester, MN with the plan of performing an interim analysis assessing the safety and efficacy of the 2 protocols after they were implemented.

In the TVR-based regimen, PEG-IFN was administered subcutaneously at 135 μg/week, and RBV was prescribed orally at 800 mg/day for the patients with a glomerular filtration rate (GFR) ≥60 mL/minute, at 400 to 600 mg/day for those with a GFR of 30 to 59 mL/ minute, and at 200 mg thrice weekly up to 200 mg/ day for those with a GFR <30 mL/minute. TVR was prescribed orally at 750 mg every 7 to 9 hours with food for the first 12 weeks without a lead-in phase (except for 3 patients who were on PEG-IFN/RBV for 4 to 8 weeks before TVR initiation to ensure tolerability). In the BOC-based regimen, the 4-week lead-in was initiated with PEG-IFN (135 μg/ week subcutaneously) and RBV (at a dose adjusted for renal function similarly to the TVR-based regimen). BOC was prescribed orally at 800 mg every 7 to 9 hours with food for 44 weeks after the lead-in phase. At both centers, PEG-IFN and RBV doses were adjusted according to patients' tolerance, side effects, and laboratory results, and they were planned to be used for a total of 48 weeks.

Hematological growth factors were used aggressively in our protocols. An erythropoiesis-stimulating agent [epoetin alfa (Procrit) at 40,000 U/week or darbepoetin alfa (Aranesp) at 100 μg/week] was administered subcutaneously to patients who had hemoglobin levels <10 g/dL and/or symptoms, and an agent was initiated whenever the hemoglobin level dropped >2 g/dL from the baseline in patients with a history of coronary artery disease. Filgrastim (Neupogen) was administered subcutaneously at 300 or 480 μg/week (depending on the body weight) to patients with absolute neutrophil counts <500/mm^3. Eltrombopag (Promacta) was prescribed orally at 25 to 75 mg/day to select patients without cirrhosis who developed severe thrombocytopenia to maintain platelet counts ≥20,000/mm^3.

Plasma HCV RNA levels were measured with the COBAS TaqMan HCV test, version 2.0 (Roche Molecular Systems, Inc.), with a lower limit of quantification (LLOQ) of 43 IU/mL and a lower limit of detection (LLOD) of 10 IU/mL. In the TVR protocol, HCV RNA was monitored weekly until the first instance of undetectable HCV RNA levels and then every 4 weeks for the duration of the treatment. TVR was discontinued in patients with HCV RNA levels ≥1000 IU/mL at week 4 (with a confirmed increase in HCV RNA at week 5), with HCV RNA levels ≥1000 IU/mL at week 12, or with detectable HCV RNA levels at week 24. In the BOC protocol, HCV RNA

was monitored every 4 weeks. BOC was discontinued in patients with HCV RNA levels ≥100 IU/mL at week 12 (week 8 after the initiation of BOC) or with detectable HCV RNA at week 24 (week 20 after the initiation of BOC). The option of continuing PEG-IFN/RBV was considered on the basis of patients' tolerance and biochemical responses on treatment along with the fibrosis stage.

RESULTS

At the time of this writing, 60 eligible patients had been enrolled and started on triple antiviral treatment. Thirty-five of these patients were started on the TVR-based regimen, whereas the remaining 25 patients were treated with the BOC-based regimen. Three patients were considered to have experienced cholestatic HCV recurrence: 2 in the TVR cohort and 1 in the BOC cohort. These patients were followed for a median of 35 weeks (mean 35 weeks, range 12-66 weeks). Table 1 summarizes the demographics and patient characteristics for both treatment groups. There were no differences in terms of age, sex, race, time from LT, HCV genotype 1 subtype, recipient interleukin-28B rs12979860 (IL-28B) polymorphism, history of previous treatment with PEG-IFN/RBV, fibrosis stage on last liver biopsy, or duration of treatment at the last follow-up. Figure 2 is a flow diagram of the patients enrolled in the 2 treatment protocols.

TVR-BASED TREATMENT REGIMEN

PRETREATMENT PREPARATION

Thirty-three of the 35 patients who were started on TVR combined with PEG-IFN/RBV were successfully changed to cyclosporine or were on cyclosporine as a maintenance immunosuppressive agent before antiviral treatment. The remaining 2 patients did not tolerate the conversion to cyclosporine: one developed evidence of posterior reversible encephalopathy syndrome necessitating a conversion to sirolimus, whereas the other patient had severe thrombocytopenia on cyclosporine, which completely resolved when tacrolimus was reintroduced in its place.

IMMUNOSUPPRESSION MODIFICATION WHILE ON TVR

On the first day of TVR, the cyclosporine dose was reduced to approximately 75% to 100% of the original twice daily (every-12-hour) dose, and the dosing interval was reduced to once daily. Subsequently, the dose was adjusted on the basis of trough levels. In the steady state, the cyclosporine dose to achieve target trough levels (100 ng/mL for patients with renal insufficiency and 150 ng/mL for others) was approximately 50% to 100% (mean 70%) of the original every-12-hour dose administered

Table 1. Demographics and patient characteristics according to the treatment groups

	TVR (n=35)	BOC (n=25)
Recipient age (years): mean ± SD	58 ± 8	60 ± 6
Sex: male [n (%)]	22 (63)	19 (76)
Race [n (%)]		
Caucasian	28 (80)	23 (92)
African American	2 (6)	0
Hispanic	1 (3)	2 (8)
Other	4 (11)	0
Time from LT (months)		
Mean ± SD	51 ± 36	71 ± 72
Median (range)	40 (1-135)	38 (3-283)
HCV genotype [n (%)]		
1a	23 (66)	17 (68)
1b	11 (31)	8 (32)
Unable to subtype	1 (3)	0
Recipient IL-28B polymorphism [n (%)]		
CC	3 (9)	1 (4)
CT	23 (66)	12 (48)
TT	9 (26)	6 (24)
Unknown	0	6 (24)
History of any prior PEG-IFN/RBV treatment [n (%)]		
Null responders	20 (57)*	15 (60)†
Partial responders	12 (34)‡	4 (16)§
Relapsers	0	2 (8)ᵏ
History of post-LT PEG-IFN/RBV treatment [n (%)]		
Null responders	11 (31)	11 (44)
Partial responders	9 (26)	3 (12)
Relapsers	0	1 (4)
Fibrosis stage on last liver biopsy [n (%)]		
F0-F2	15 (43)	12 (48)
F3-F4	20 (57)	13 (52)
HCV RNA at baseline		
Mean ± SD (log_{10} IU/mL)	6.47 ± 1.37	6.96 ± 0.64
≥800,000 IU/mL [n (%)]	29 (83)	22 (88)
Duration of treatment (weeks)		
Mean ± SD	32 ± 14	39 ± 13
Median (range)	30 (12-58)	41 (15-66)

*Nine patients only before LT, 2 patients only after LT, and 9 patients both before LT and after LT. †Four patients only before LT, 2 patients only after LT, and 9 patients both before and after LT. ‡Three patients only before LT, 2 patients only after LT, and 7 patients both before and after LT. §One patient only before LT, 2 patients only after LT, and 1 patient both before and after LT. ᵏOne patient only before LT and 1 patient only after LT.

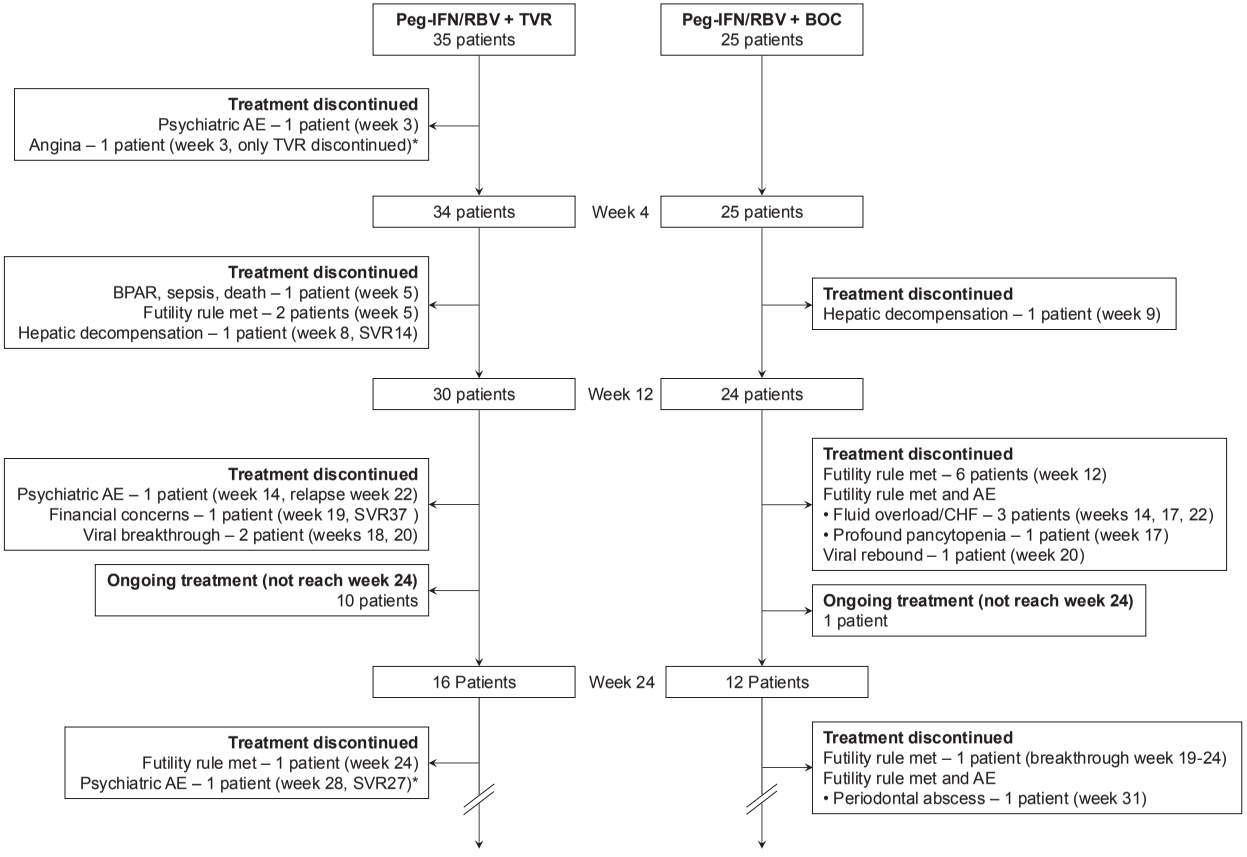

Figure 2. Flow diagram of the patients enrolled in the 2 treatment protocols.
*Same patient. SVR[n] represent sustained virological response at [n] weeks after discontinuation of treatment.

once daily. The sole patient on sirolimus required a significant dose reduction and took only 0.5 mg orally every 4 days, whereas the patient on tacrolimus required only 0.5 mg orally every 7 days. After the discontinuation of TVR, the pre-TVR every-12-hour dose and interval for cyclosporine were resumed. Subsequently, 6 patients changed from cyclosporine back to tacrolimus because of renal insufficiency (3 patients), neuropsychiatric side effects (1 patient), severe gingival hyperplasia (1 patient), or personal preference (1 patient).

ADVERSE EVENTS

Table 2 summarizes the adverse events that occurred during antiviral treatment. Cytopenias were very common, and all patients required dose reductions of PEG-IFN and/or RBV or the administration of hematological growth factors. Mild transient increases in serum creatinine from the baseline were noted in almost all patients during the first 12 weeks of TVR administration (range 0-1.4 mg/dL, mean 0.6 mg/dL, median 0.5 mg/dL). Figure 3 presents chronological changes in the average estimated GFRs during antiviral treatment.

BPAR developed in 2 patients with subtherapeutic cyclosporine levels, with the first episode occurring in a patient at week 3 of the protocol. This was treated success-

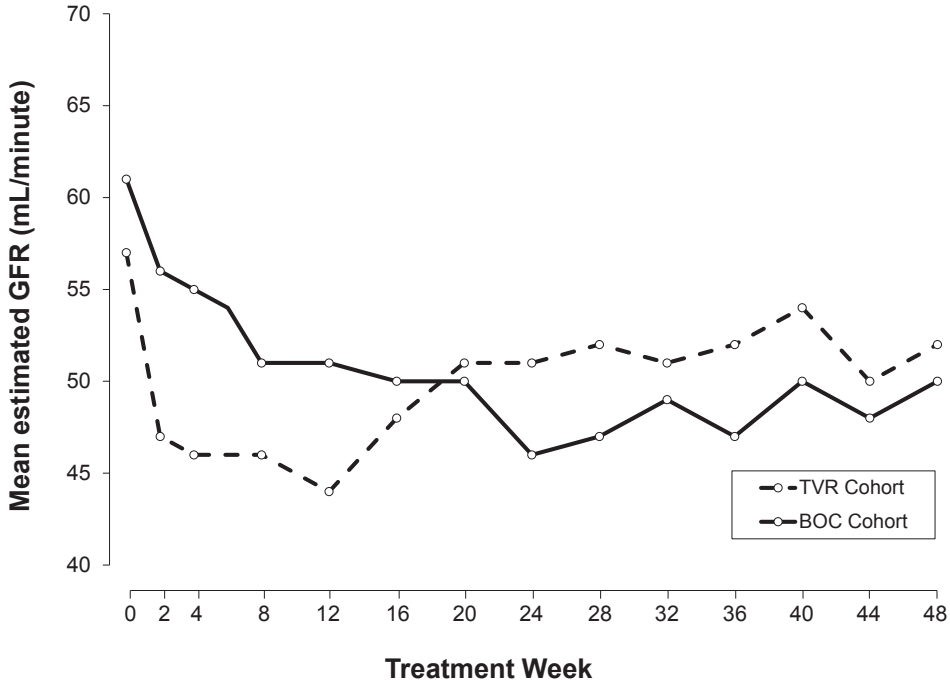

Figure 3. Chronological changes in the average estimated GFRs of the 2 cohorts during the triple antiviral treatment.

Table 2. Adverse events according to the treatment groups

	TVR (n=35)	BOC (n=25)
Leukopenia		
PEG-IFN dose reduction [n (%)]	27 (77)	19 (76)
Filgrastim use [n (%)]	6 (17)	14 (56)
Initiating point: average in weeks (range)	5 (0-15)	8 (0-21)
Anemia		
RBV dose reduction [n (%)]	31 (89)	25 (100)
Temporary RBV interruption	1 (3)	1 (4)
Permanent RBV discontinuation	0	3 (12)
Erythropoiesis-stimulating agent use [n (%)]	27 (77)	24 (96)
Initiating point: average in weeks (range)	4 (0-14)	7 (0-20)
Packed red blood cell transfusion [n (%)]	16 (46)	16 (64)
Units per patient: average (range)	5 (2-12)	11 (4-22)
Thrombocytopenia [n (%)]		
Temporary PEG-IFN interruption	2 (6)	2 (8)
Eltrombopag use (25-75 mg/day)	3 (9)	0
Renal insufficiency		
Increase in creatinine (mg/dL): average	0.6	0.5
Increase in creatinine (mg/dL): median (range)	0.5 (0-1.4)	0.4 (0.1-1)
Increase in creatinine > 0.5 mg/dL [n (%)]	14 (40)	9 (36)
Symptomatic skin rashes [n (%)]*	4 (11)	2 (8)
Anorectal complaints [n (%)]*	2 (6)	2 (8)
Dysgeusia [n (%)]	1 (3)	2 (8)
Infectious complications [n (%)]	6 (17)†	1 (4)‡
BPAR [n (%)]	2 (6)	1 (4)
Hepatic decompensation [n (%)]		
Ascites and/or fluid overload	2 (6)	3 (12)
Hepatic encephalopathy	1 (3)	1 (4)
Cardiovascular complications [n (%)]	1 (3)	1 (4)
Death [n (%)]	1 (3)	1 (4)

*Effectively treated with oral antihistamines and/or topical steroids. †Two patients with a urinary tract infection (weeks 4 and 23), 1 patient with sinusitis (week 11), 1 patient with lower extremity cellulitis (week 7), 1 patient with pneumonia (week 5), and 1 patient with trigeminal herpes zoster reactivation (week 5). ‡One patient with a periodontal abscess (week 31).

fully with intravenous corticosteroids and an adjustment of the cyclosporine dose. The patient continued on triple antiviral treatment and became HCV RNA–negative at week 12, but subsequently, viral breakthrough was noted at week 20; PEG-IFN/RBV was discontinued at week 24. The second patient received antiviral treatment for cholestatic HCV recurrence and developed BPAR at week 3, which was treated with intravenous corticosteroids. Allograft function declined precipitously despite a significant HCV RNA reduction; the patient developed multi-organ failure from sepsis and died 1 week later.

Because of significant anemia, 1 patient developed unstable angina at week 3 and required coronary angiography and single-vessel angioplasty. TVR was discontinued, but the patient requested to continue PEG-IFN/RBV and achieved undetectable HCV RNA levels at week 6. PEG-IFN and RBV were subsequently discontinued at week 28 because of psychological side effects, but HCV RNA remained undetectable at the last follow-up (week 55, SVR at 27 weeks after discontinuation of treatment).

Four additional patients discontinued antiviral treatment prematurely (Fig. 2). Six individual patients developed infectious complications, and all were treated successfully with appropriate treatments (Table 2). The triple antiviral treatment was not interrupted. Three patients with pretreatment cirrhosis developed hepatic decompensation (Table 2).

PRELIMINARY VIROLOGICAL RESPONSE

Figure 4A presents the HCV RNA levels of all 35 patients treated with the TVR-based regimen. At week 1, the mean HCV RNA reduction was 3.14 $\log_{10}$ IU/mL (range=1.07-4.95 $\log_{10}$ IU/mL), with no difference in the degree of HCV RNA reduction between patients with IL-28B polymorphisms CC (mean 3.01 $\log_{10}$ IU/mL) and CT/TT (mean 3.15 $\log_{10}$ IU/mL, P=0.7). At week 4 of triple antiviral treatment, 30 of the 35 patients (86%) had HCV RNA levels <1000 IU/mL, and HCV RNA was undetectable [rapid virological response (RVR)] in 6 patients (17%) and detectable at <43 IU/mL in 18 patients (51%). A patient who discontinued treatment at week 3 due to adverse events was considered a failure based on an intention-to-treat analysis. Two of the 4 remaining patients with HCV RNA levels ≥1000 IU/mL at week 4 discontinued TVR at week 5, whereas 2 patients continued triple antiviral treatment and achieved an undetectable HCV RNA level by weeks 7 and 14. At week 12 of triple antiviral treatment, 28 of the 35 patients (80%) achieved undetectable HCV RNA levels [a complete early virological response (cEVR)]. One of the 3 patients with a detectable HCV RNA level <43 IU/mL never achieved undetectable HCV RNA levels, and antiviral treatment was discontinued at week 24. The other 2 patients achieved undetectable HCV RNA levels at weeks 14 and 16: HCV RNA remained undetectable

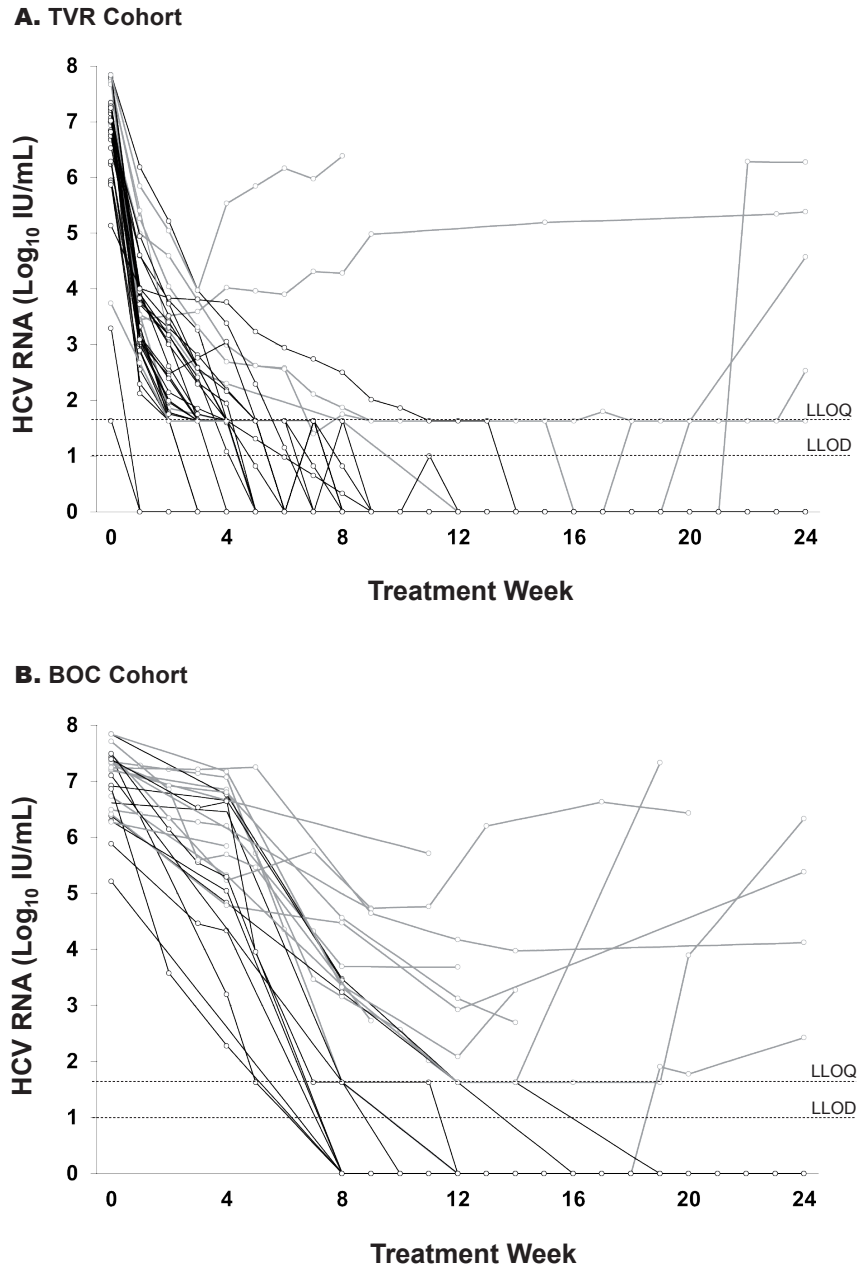

Figure 4. HCV RNA levels in (A) the TVR-based triple antiviral treatment group and (B) the BOC-based triple antiviral treatment group.

Each black line represents an individual patient with an on-treatment virological response. Each solid grey line represents an individual patient with treatment failure. The LLOQ was 43 IU/mL. The LLOD was 10 IU/mL.

at the last follow-up (week 34) in the former patient, whereas the latter patient developed viral breakthrough at week 18. Twenty-four weeks after the initiation of triple antiviral treatment, 14 of 21 patients (67%) achieved undetectable HCV RNA levels without viral break-through at the last follow-up. One of the 7 remaining patients (all genotype 1a) died after BPAR, sepsis, and multi-organ failure (discussed previously); 2 patients discontinued antiviral treatment at week 5; and after meeting treatment futility rules, another patient discontinued antiviral treatment at week 24. Three patients developed viral breakthrough at weeks 18, 20, and 22 after they achieved undetectable HCV RNA levels at weeks 16, 12, and 5, respectively.

Overall, 30 patients (86%) achieved undetectable HCV RNA levels on average at week 6 (median=week 6, range=weeks 1-16). At the last follow-up, 27 patients still had undetectable HCV RNA levels.

BOC-BASED TREATMENT REGIMEN

PRETREATMENT PREPARATION

All except 2 patients were successfully changed to or were on cyclosporine as a maintenance immunosuppressive agent before antiviral treatment. The remaining 2 patients did not tolerate the conversion to cyclosporine because of uncontrolled hypertension and migraine exacerbation, and they resumed taking tacrolimus.

IMMUNOSUPPRESSION MODIFICATION WHILE ON BOC

On the first day of BOC, the cyclosporine dose was reduced to approximately 50% of the original every-12-hour dose, but the dosing interval remained every 12 hours. Subsequently, the dose was adjusted on the basis of trough cyclosporine levels. In the steady state, the cyclosporine dose to achieve target trough levels (100 ng/mL for patients with renal insufficiency and 150 ng/mL for others) was approximately 33% to 100% (mean 56%) of the original every-12-hour dose administered twice daily. The 2 patients on tacrolimus required significant dose reductions from 1 mg orally twice daily to 0.5 mg every other day and from 0.5 mg orally every 12 hours to 0.5 mg orally twice weekly. After the discontinuation of BOC, the pre-BOC dose and interval of cyclosporine or tacrolimus were resumed.

ADVERSE EVENTS

Table 2 summarizes adverse events that developed during triple antiviral treatment. Cytopenias were very common, with all patients requiring dose reductions of PEG-IFN and/or RBV or the administration of hematological growth factors. Serum creatinine increased from the baseline throughout the 44 weeks of BOC administration (range 0.1-1.0 mg/dL, mean 0.5 mg/dL, and median 0.4 mg/dL). Figure 3 demon-

strates chronological changes in the estimated GFRs during antiviral treatment.

No BPAR developed during antiviral treatment. One patient had BPAR following BOC discontinuation after the futility rule was met because the cyclosporine dose was not appropriately increased; BPAR was treated successfully with intravenous corticosteroids. Six patients prematurely discontinued antiviral treatment because of adverse events (Fig. 2). Three patients with pre-existing advanced fibrosis or cirrhosis developed hepatic decompensation (Table 2). One patient who discontinued antiviral treatment at week 9 because of worsening hepatic encephalopathy subsequently died from allograft dysfunction and multi-organ failure.

PRELIMINARY VIROLOGICAL RESPONSE

Figure 4B presents the HCV RNA levels of all 25 patients treated with the BOC-based regimen. At week 4 after the lead-in phase of PEG-IFN/RBV, the mean HCV RNA reduction was 1.31 $\log_{10}$ IU/mL (range=0.03-4.58 $\log_{10}$ IU/mL). At week 8 of antiviral treatment (week 4 after the initiation of BOC), 10 of 25 patients (40%) had HCV RNA levels <100 IU/mL: HCV RNA was undetectable (RVR) in 6 of these patients (24%) and detectable at <43 IU/mL in 4 patients (16%). Eleven of the 15 remaining patients with HCV RNA levels ≥100 IU/mL at week 8 discontinued all treatment after week 12 because the HCV RNA level was ≥100 IU/mL with or without side effects, whereas 4 patients with detectable HCV RNA at <43 IU/mL at week 12 continued triple antiviral treatment. At week 12 of antiviral treatment (week 8 after the initiation of BOC), 10 of 25 patients (40%) achieved undetectable HCV RNA levels (cEVR), whereas the remaining 4 patients had detectable HCV RNA levels <43 IU/mL. One of the 10 patients with undetectable HCV RNA levels developed viral break-through at week 19 and discontinued antiviral treatment. Two of the 4 patients with detectable HCV RNA levels <43 IU/mL at week 12 never achieved undetectable HCV RNA levels, and antiviral treatment was discontinued at weeks 17 and 20, respectively. The other 2 patients achieved an undetectable HCV RNA level at weeks 16 and 19, and HCV RNA remained undetectable at the last follow-up. At week 24 of antiviral treatment, 10 of 22 patients (45%) were HCV RNA–negative without viral breakthrough at the last follow-up. Five of the 12 remaining patients prematurely discontinued antiviral treatment because of side effects (discussed previously), 5 patients (4 with genotype 1a) discontinued antiviral treatment after they met futility rules at week 12, 1 patient (genotype 1a) never achieved HCV RNA and discontinued antiviral treatment at week 20 after viral rebound, and 1 patient (genotype 1a) developed viral breakthrough at week 19 after achieving undetectable HCV RNA levels at week 11. Overall, 12 patients (48%) achieved undetectable HCV RNA levels at week 11 on average (median 5 week 9, range 5 weeks 8-19). At the end of follow-up, 11 patients still had undetectable HCV RNA levels, whereas 1 patient developed viral breakthrough.

INTENTION-TO-TREAT ANALYSIS OF VIROLOGICAL RESPONSES

Table 3 presents preliminary data for on-treatment virological responses based on an intention-to-treat analysis.

Table 3. Intention-to-treat analysis of on-treatment virological responses according to the treatment group

	TVR	BOC
HCV RNA at week 8 of treatment plan [n/N (%)]		
<1000 IU/mL (TVR)	30/35 (86)	
<100 IU/mL (BOC)		10/25 (40)
Undetectable	6/35 (17)	6/25 (24)
HCV RNA at week 12 of treatment plan [n/N (%)]		
<1000 IU/mL (TVR)	31/35 (89)	
<100 IU/mL (BOC)		14/25 (56)
Undetectable	28/35 (80)	10/25 (40)
HCV RNA at week 24 of treatment plan [n/N (%)]		
Undetectable	14/21 (67)	10/22 (45)
Genotype 1a	7/14 (50)	6/14 (43)
Genotype 1b	7/7 (100)	4/8 (50)
F0-F2	7/11 (64)	7/12 (58)
F3-F4	7/10 (70)	3/10 (30)
Baseline HCV RNA <800,000 IU/mL	2/3 (67)	1/2 (50)
Baseline HCV RNA ≥800,000 IU/mL	12/10 (67)	9/20 (45)

Because of the small number of patients with recipient IL-28B polymorphism CC, no meaningful analysis could be performed to assess the correlation between the recipient IL-28B polymorphism and the on-treatment virological response. In the TVR group, the patients with genotype 1b achieved undetectable HCV RNA levels at week 24 at a significantly greater rate than the patients with genotype 1a (100% versus 50%, P=0.047). In the BOC group, there was no significant difference in the rates of patients achieving undetectable HCV RNA levels at week 24 between genotypes 1a and 1b (43% versus 50%, P 5 1.0). There was no difference in the numbers of patients achieving undetectable HCV RNA levels at week 24 between the patients with early fibrosis (F0-F2) and those with advanced fibrosis (F3-F4) in both the TVR-based regimen (64% versus 70%, P=1.0) and the BOC-based regimen (58% versus 30% P=0.2).

In addition, we found no difference in the achievement of undetectable HCV RNA levels at week 24 between the patients with low baseline HCV RNA levels (<800,000 IU/mL) and the patients with high baseline HCV RNA levels (≥800,000 IU/mL) in both the TVR group (67% versus 67%, P= 1.0) and the BOC group (50% versus 45%, P= 1.0).

DISCUSSION

This report describes our initial experience in treating recurrent HCV in a large series of LT recipients using either TVR or BOC in combination with PEG-IFN/ RBV. This multicenter analysis has enabled us to present our experience using standardized treatment protocols with both protease inhibitors. Our study has produced some important insights into the management of immunosuppression while patients are on these medications and into the frequency and severity of adverse events as well as early virological response data. We have demonstrated that TVR- or BOC-containing antiviral protocols can be used after transplantation and result in moderately successful early virological responses, but these regimens are associated with important toxicities that mitigate their potential benefit. In addition, we have identified several factors that affect an early virological response.

Concerns have been expressed about the safety of protease inhibitors in the posttransplant setting because of the potential for drug-drug interactions documented in healthy volunteers [12-14]. All 3 centers in this report decided that using cyclosporine instead of tacrolimus would pose less risk for adverse consequences from subtherapeutic or supratherapeutic CNI levels because of the lack of any clinical data during protocol development. We determined that drug-drug interactions could be managed with careful monitoring. Drug-drug interaction data for TVR and BOC were derived from studies in patients with HCV infections and with single or few drug doses. One of the key contributions of our study is that it provides data on dose adjustments for CNIs in LT recipients. For patients placed on TVR, the dose of cyclosporine needed to be reduced to approximately 75% to 100% of the original every-12-hour dose, and the dosing interval was reduced to once daily. Patients treated with BOC required their cyclosporine dose to be reduced to approximately 50% of the original every-12-hour dose, although the dosing interval remained every 12 hours. These changes produced CNI trough levels that were comparable to those obtained before the initiation of protease inhibitors. The incidence of BPAR during antiviral treatment was low (5%) and similar to the rates previously reported (3%-7%) in studies using antiviral treatment with PEG-IFN/RBV [5]. Despite the consistent control of CNI trough levels, we observed frequent declines in GFR during the administration of protease inhibitors in both patient cohorts. The effect was greatest during the first 12 weeks in the TVR

cohort and throughout the latter 44 weeks in the BOC cohort. Severe renal dysfunction (grade III or higher according to the Risk, Injury, Failure, Loss, and End-Stage Kidney Disease criteria) was not observed. Because neither TVR nor BOC has been reported to be nephrotoxic, we suspect that the deterioration in renal function may have been related to changes in CNI pharmacokinetics during antiviral treatment. Our report supports the initial experience of other groups demonstrating that CNIs can be co-administered relatively safely with either TVR or BOC as long as careful attention is paid to CNI dosing at the initiation and discontinuation of the protease inhibitor and there is ongoing, close follow-up [17-21]. Further studies will be required to determine the optimal combination of protease inhibitors with CNIs that maximizes the virological response while minimizing treatment-related adverse events.

As seen in all studies using PEG-IFN/RBV in the treatment of recurrent HCV after LT, we observed a high frequency of cytopenias in comparison with nontransplant populations [5]. In our current study, the frequencies of cytopenias were similar in the TVR and BOC cohorts, with dose reductions of PEG-IFN/RBV almost universal. Erythropoiesis-stimulating agents and packed red cell transfusions were required in more than half of the patients (Table 2) in order to continue RBV therapy with acceptable hemoglobin levels, whereas smaller numbers of patients required filgrastim and eltrombopag to prevent PEG-IFN discontinuation. Although recent data suggest that RBV dosing can be modestly lowered in nontransplant patients treated with TVR without a loss of efficacy [22], the doses of RBV that were tolerated in our patients might be expected to attenuate efficacy. The decline in renal function seen in both the TVR cohort and the BOC cohort may have contributed to the poor tolerability of RBV, which is primarily renally excreted. The optimal doses of both PEG-IFN and RBV in the post-transplant setting when a protease inhibitor is used will require additional investigation.

The addition of a protease inhibitor to PEG-IFN/RBV in the posttransplant setting resulted in rapid suppression of HCV replication. In the TVR cohort, 86% of patients achieved HCV RNA levels <1000 IU/ mL at week 4, whereas only 17% had undetectable HCV RNA levels at week 4 (RVR). By week 12, 80% of this group had undetectable HCV RNA levels (cEVR), and this represents a significant improvement in response rates in comparison with previously published studies using only PEG-IFN/RBV in LT recipients. Futility rules were met in 3 patients who had IL-28B polymorphism CT or TT and were infected with genotype 1a. Viral breakthrough occurred in 3 patients with genotype 1a who completed 12 weeks of the triple treatment and were on the PEG-IFN/RBV dual treatment. In the BOC cohort, 40% and 56% achieved HCV RNA levels <100 IU/mL after 4 and 8 weeks, respectively, of triple antiviral treatment, whereas 40% achieved undetectable HCV RNA levels at week 12 (cEVR). By week 24, 45% of the patients were HCV RNA–negative. After the exclusion of those

patients who prematurely discontinued the BOC-based regimen because of adverse events, all but 1 patient who met futility rules or developed viral break-through were infected with genotype 1a and had IL-28B polymorphism CT or TT. Thus, we found that triple antiviral treatment using either protease inhibitor combined with PEG-IFN/RBV had limited efficacy in patients with HCV genotype 1a and IL-28B polymorphism CT or TT. In contrast to the nontransplant setting or dual PEG-IFN/RBV treatment, no correlation was demonstrated between the on-treatment virological response and the fibrosis stage or baseline HCV RNA levels for either regimen.

We observed that effective monitoring of patients on treatment was resource-intensive and required the input of experienced mid-level providers and physicians at regularly scheduled clinic visits to address patient concerns. Despite this structured approach at all 3 centers, 2 patients (3%) died while they were receiving triple antiviral treatment. The patient who died on the TVR-based regimen had recurrent cholestatic HCV and developed septic complications after the treatment of BPAR. The death in the BOC group occurred in a patient with established hepatic decompensation before the initiation of antiviral treatment. In addition, 6 patients with pre-existing advanced fibrosis or cirrhosis developed hepatic decompensation during triple antiviral treatment. The effort required to follow patients on triple antiviral treatment with the currently available protease inhibitors and the observed outcomes highlight the challenges of such demanding treatment regimens in these patient populations and counterbalance the likely benefit of successful therapy among those responding to treatment.

There are several limitations to our report. First, SVR data were not available because the majority of the patients were still undergoing antiviral treatment. A future analysis of SVR data at weeks 12 and 24 after the discontinuation of antiviral treatment will be required to assess overall treatment efficacy. Second, our treatment protocols were not developed to be directly compared to PEG-IFN/RBV dual therapy. There is no Food and Drug Administration–approved treatment for recurrent HCV after LT. More effective treatments of recurrent HCV are urgently needed, and we believe that our protocols serve as pragmatic and patient-centered responses to the issue of severe and progressive HCV recurrence. Waiting for the results from large-scale, randomized studies is simply not an option for patients affected in this way. Although our preliminary results are encouraging, we recognize the inherent risks associated with the use of these new agents. Third, our treatment protocols were not designed to compare the efficacy of TVR- and BOC-based regimens.

Our data appear to indicate that both regimens may be considered in treating recurrent HCV, with the choice of agent resting with the treating physician and the patient.

In conclusion, we have reported our preliminary experience with TVR and BOC combined with PEG-IFN/RBV to treat recurrent HCV after LT. These medications could be administered with a CNI as long as there were appropriate adjustments in CNI dosing and close monitoring during protease inhibitor exposure. On treatment, virological response rates of approximately 50% to 60% were achieved, but numerous side effects (particularly cytopenias) as well as 2 deaths highlight the potential hazards of these treatment regimens. Determining the overall efficacy in achieving SVR of either TVR or BOC combined with PEG-IFN/RBV to treat recurrent HCV in LT recipients requires longer follow-up.

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CHAPTER 7

Post-transplant Boceprevir Use is Associated with Profound Anemia and Renal Impairment Despite Stable Cyclosporine and Low Ribavirin Levels

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ABSTRACT

BACKGROUND

The use of direct-acting antiviral agents in liver transplant recipients with recurrent hepatitis C is challenging due to drug-drug interactions and risk of nephrotoxicity and hemotoxicities. We studied toxicities related to pharmacokinetic profiles of cyclosporine A and ribavirin during boceprevir based antiviral therapy in liver transplant recipients.

METHODS

N=17 liver transplant recipients with chronic hepatitis C were treated with boceprevir based antiviral therapy. All patients were placed on cyclosporine prior to initiation of boceprevir. Whole blood cyclosporine levels and ribavirin troughs were measured.

RESULTS

Initiation of boceprevir therapy required sequential cyclosporine dose reductions of 49% on average. Ribavirin levels were below desired therapeutic ranges (≥2500 ng/ml) in 75% of patients. Despite low ribavirin levels all patients developed anemia (hemoglobin <10 g/dl), with 71% developing severe anemia (Hb <8.5 g/dl), despite erythropoietin use (71% of patients), blood transfusions (94% of patients) and ribavirin dose reductions (76% of patients). Renal function declined with 22% on average after initiation of boceprevir. Four patients had to discontinue antiviral therapy due to side effects.

CONCLUSIONS

The apparent clearance of cyclosporine is dynamic during post-liver transplant boceprevir based antiviral therapy, requiring sequential dose reductions. Post-liver transplant boceprevir based antiviral therapy is associated with frequent and progressive anemia and renal impairment despite stable cyclosporine levels and suboptimal ribavirin levels. We propose a model explaining the triple therapy-induced cascade leading to renal impairment and anemia, suggesting whole blood cyclosporine levels might not be representative of true cyclosporine bioavailability during antiviral triple therapy.

INTRODUCTION

Hepatitis C virus (HCV) related end-stage liver disease remains the most common indication for adult liver transplantation (LT) in the United States and Europe [1, 2]. Recurrence of HCV post-LT is almost universal, leading to the development of cirrhosis within 5 years in 20-40% of LT recipients and is associated with increased risk of allograft failure and death [3, 4]. Successful therapy (defined by a sustained virological response (SVR)) of recurrent HCV post-LT significantly improves histology and 5-year survival [5]. Unfortunately, post-LT antiviral therapy with peginterferon and ribavirin (RBV) leads to SVR in about only 20-30% of treated LT recipients [6]. Recently, the protease-inhibitors boceprevir (BOC) and telaprevir (TVR) were approved for the treatment of HCV genotype-1 infected patients. A significant increase in SVR rates in HCV patients, none of whom were liver transplant recipients, was observed in the registration studies for both direct antiviral agents (DAAs) when combined with peginterferon and RBV. For BOC, SVR rates are 63%-66% in naïve patients and 59%-66% in previously treated patients. In the TVR registration studies SVR rates were 69%-75% in naïve patients and 64%-66% in treatment experienced patients [7-10]. Neither telaprevir nor boceprevir are specifically labelled for use in LT recipients and both agents have the potential for important drug-drug interactions with immunosuppressive agents. BOC and TVR are substrates as well as strong inhibitors of cytochrome P450 3A4 (CYP3A4), subsequently increasing the systemic exposure to calcineurin inhibitors cyclosporine (CsA) and tacrolimus (TAC), both of which are primarily metabolized by CYP3A4. In pharmacokinetic studies, a 17-fold increase in the TAC AUC_{inf} was seen after a single dose of TAC coadministered with BOC and a 70-fold increase in the TAC AUC_{inf} when given with TVR [11, 12]. The CsA AUC_{inf} increased 2.7-fold versus 4.6-fold when combined with respectively BOC and TVR [11, 12]. Due to the narrow therapeutic index of TAC and CsA, physicians face major challenges treating LT recipients with DAA-based antiviral therapy in order to avoid severe side effects of under- and overexposure such as respectively organ rejection and nephrotoxicity.

In the current study we present an in-depth analysis of safety and pharmacokinetics, including ribavirin level monitoring of BOC-based antiviral therapy in LT recipients. Per protocol, we chose to treat our HCV-infected LT recipients with the combination of BOC and CsA because of the relatively more favourable interaction pattern.

METHODS

A clinical treatment protocol for LT recipients with virological proven HCV recurrence was prospectively developed and used at the Mayo Clinic in Rochester, Minnesota and the Erasmus Medical Center in Rotterdam, The Netherlands.

The treatment protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of the Mayo Clinic in Rochester. Patients considered for antiviral treatment had either an allograft biopsy that showed significant fibrosis (stage ≥2) and/or moderate to severe lobular hepatitis (grade ≥3), using the METAVIR system (20) and/or abdominal ultrasound with transient elastography findings consistent with advanced fibrosis or cirrhosis. A total of 17 patients were selected for treatment. Efficacy data of thirteen of these patients have been previously discussed in a separate paper [13].

IMMUNOSUPPRESSIVE AND ANTIVIRAL THERAPY

Due to the more favourable interaction profile, BOC was chosen as the DAA given alongside RBV and peginterferon α-2a (Pegasys®) or peginterferon α-2b (Peg-Intron®). Because of expected drug-drug interactions all patients were converted to a CsA based immunosuppressive regimen and refrained from using mycophenolate mofetil and steroids before introduction of BOC. CsA dose was reduced by 50% at the time of (same day or day before) initiation of BOC and adjusted based on CsA whole blood levels. RBV and peginterferon were incrementally dosed and when needed reduced based on hematology and/or renal function. RBV whole blood levels were ordered at week 4 of treatment. Target RBV levels for efficacy were between 2500-3500 ng/ml. After a lead in period of at least four weeks, BOC was administered in a fixed dose of 800mg three times daily.

SAFETY ASSESSMENT

CsA levels, clinical chemistry and hematological parameters were assessed at least weekly up to the first four weeks of BOC treatment and four-weekly afterwards. Renal function was determined by the estimated creatinine clearance rate (ml/min) using the Cockroft-Gault formula. Growth factors were prescribed when Hb dropped below 12 g/dl (erythropoietin or EPO) or when WBCs dropped below $1.5x10^9$/l and/or absolute neutrophil count dropped below $0.75x10^9$/l (granulocyte colony-stimulating factor or G-CSF). Blood transfusion was given when hematocrit dropped below 21% and/or when patients developed symptomatic anemia.

ON-TREATMENT EFFICACY ASSESSMENT

Plasma HCV RNA levels were measured every 4 weeks using the COBAS Taqman HCV test, version 2.0 (Roche Molecular Systems, Inc.), with a lower limit of quantification of 43 IU/ml and a lower limit of detection of 10 IU/ml or the COBAS Ampliprep/ COBAS Taqman HCV test version 2.0 (Roche Molecular Systems, Inc.) with a lower limit of detection and quantification of 15 IU/ml. Patients were to discontinue therapy if HCV RNA was ≥100 IU/ml at week 8 or detectable at week 20 of BOC treatment.

RESULTS

The first 16 weeks of treatment in the 17 BOC treated patients are included in the current analysis. Patient characteristics are given in table 1. Three patients had undergone hepatic allograft retransplantation, of which one patient also received a combined renal transplant.

Table 1. Baseline characteristics of LT recipients on BOC-based antiviral therapy

	N=17
Median age in years (range)	59 (47-74)
Gender	
Male	11
Female	6
Ethnicity	
Caucasian	14
Hispanic	2
Arabic	1
Median time from LT to HCV treatment in years (range)	3.4 (0.3-24.3)
Fibrosis stage (Metavir)	
F0-F2	9
F3-F4	8
HCV genotype	
1a	10
1b	5
Other	2

BOC=boceprevir; HCV=hepatitis C virus; LT=liver transplant

IMMUNOSUPPRESSIVE THERAPY

Four patients switched from tacrolimus-based immunosuppression to CsA during the lead-in period of the antiviral treatment. Seven patients were switched to CsA in the year preceding the start of antiviral therapy, of which three within four weeks of start of antiviral therapy. The other six patients had been on CsA based therapy for more than one year. Average dosages of CsA and trough (C0) levels are given in table 2 and figure 1a. Individual CsA dose was reduced by on average 40% (range: -8%-77%) at initiation of BOC. By week 16, the average dose of CsA had been reduced by 49% (range: 15%-88%) compared to the lead-in period. Individual CsA C0 levels increased with 17% during the first four weeks after initiation of BOC, but by week 16 CsA levels had decreased by 4%, compared to the lead-in period. Of note, target C0 levels were per protocol between 100-150 ng/mL and the average CsA levels were therefore at the low end of the therapeutic range.

Table 2. **Medication, safety and efficacy parameters in LT recipients on BOC-based antiviral therapy**

	Baseline	Week 4	Week 8	Week 12	Week 16
Immunosuppressive and antiviral therapy					
Mean CsA C0 level in ng/ml (range) (N=15)	84 (29-168)	108 (67-256)	118 (50-198)	103 (40-180)	94 (40-155)
Mean CsA dose in mg/day* (range)	163 (100-270)	210 (100-800)	115 (28-211)	98 (50-200)	94 (50-183)
Mean Peginterferon α-2a dose in mcg/week (range) (N=16)	N/A	144 (45-180)	152 (45-180)	154 (45-180)	150 (45-180)
Mean Peginterferon α-2b dose in mcg/week (range) (N=1)	N/A	120 (N/A)	120 (N/A)	120 (N/A)	120 (N/A)
Mean RBV dose in mg/day (range)	N/A	546 (200-1200)	597 (200-1200)	531 (86-1200)	323 (0-1029)
Safety assessment					
Mean Hb in g/dL (range)	12.2 (8.5-15.1)	10.6 (7.4-12.3)	8.8 (7.1-11.0)	8.3 (6.2-10.3)	7.9 (4.9-11.1)
Mean WBCs $x10^9$/l (range)	4.2 (0.9-10.3)	2.5 (0.8-8.4)	2.2 (1.1-5.6)	2.0 (0.7-3.4)	2.0 (1.0-3.7)
Mean Platelets $x10^9$/l (range)	145.0 (88-199)	98.1 (34-151)	85.5 (28-128)	90.9 (17-170)	83.8 (6-144)
Mean GFR in ml/min (range)	69.4 (36.8-103.1)	69.1 (36.8-95.8)	57.7 (30.5-95.6)	55.2 (32.2-79.3)	52.4 (23.4-78.9)
Mean ALT in IU/l (range)	86 (19-317)	47 (15-141)	35 (9-108)	28 (12-69)	28 (14-62)
Mean Bilirubin in mg/dl (range)	2.2 (0.2-15.5)	1.8 (0.5-4.9)	1.2 (0.4-2.8)	0.9 (0.4-1.9)	0.7 (0.4-1.6)
Efficacy assessment (N=15)					
HCV RNA <100IU/mL (N)	N/A	1	7	9	8
HCV RNA <LLOD (N)	N/A	0	5	8	8
HCV RNA >2log drop from baseline (N)	N/A	5	12	12	11
HCV RNA >3log drop from baseline (N)	N/A	2	10	11	10

Medication results are average values calculated over the 4 preceding weeks. Safety assessment are values taken at or around baseline, week 4, week 8, week 12 and week 16. An exception was made for Hb, WBCs and platelets since the majority of patients received blood transfusions and/or growth factors, resulting in a rise in these parameters not reflecting real haematological decline. Therefore, the lowest Hb, WBCs and platelets in the 4 preceding weeks was taken.
BOC=boceprevir; CsA=Cyclosporine A; C0 level=trough level; Hb=haemoglobin; WBCs=white blood cells; GFR=glomerular filtration rate according to Cockroft Gault; LT=liver transplant; LLOD=lower limit of detection (10-15IU/ml); N/A=not applicable; N=number

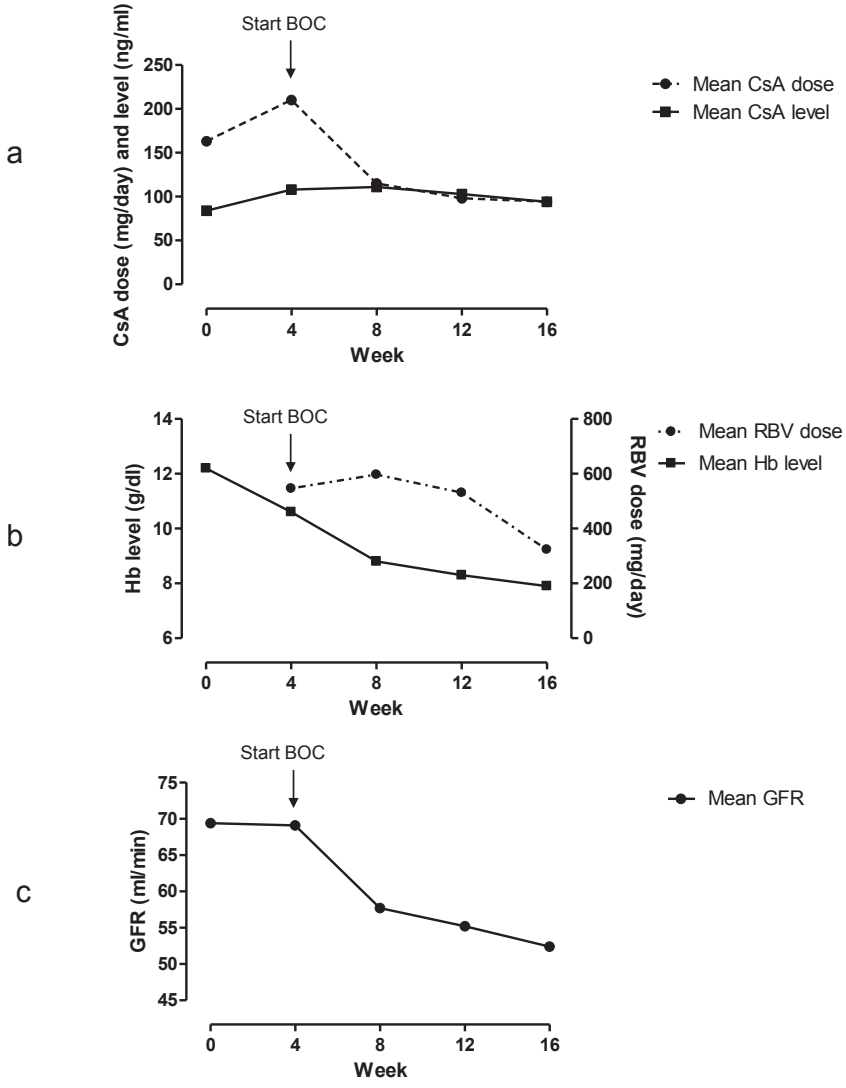

Figure 1. Medication, Hb decline and renal function in LT recipients on BOC-based triple therapy

A. Mean CsA dose was on average reduced by 49% at week 16 after addition of BOC. Mean CsA whole blood levels were maintained at the low end of the therapeutic range (100-150ng/ml)

B. RBV was reduced to 61% of the mean lead-in period dose by week 16. Mean Hb levels declined continuously during the first 16 weeks of therapy with mean levels after week 8 corresponding with severe anemia (<8.5g/dl)

C. GFR decline was seen in all patients after the addition of BOC. By week 16, mean GFR had dropped with 22% from baseline.

BOC=boceprevir; CsA=Cyclosporine A; GFR=glomerular filtration rate according to Cockroft-Gault; Hb=hemoglobin; HCV=hepatitis C virus; LT=liver transplant; RBV=ribavirin

Four out of 15 patients experienced a level above 200 ng/mL on at least one occasion (maximum levels respectively 213, 215, 219 and 325 ng/ml) after the introduction of BOC.

ANTIVIRAL THERAPY

On average, the lead-in period lasted 38 days (median: 28 days; range: 20-99 days). There were three patients with a prolonged lead-in period of more than 6 weeks; one due to anemia (75 days) and two due to delayed insurance approval (51 and 99 days). Only one patient received peginterferon α2b, the other 16 patients received peginterferon α2a. Peginterferon dosages were maintained in all but one patient who received a dose reduction after week 12 (see also table 2). Maximum doses per peginterferon label were given in 7 patients from day 1 on and in three patients who were on a dose escalating schedule after week 4. Due to incremental dosing in five patients, average RBV doses increased slightly after week 4 (table 2, figure 1b). RBV doses were reduced on average 39% (range: -36%-100%) by week 16 of treatment, compared to the lead-in period. One patient started with a low RBV dose of on average 294 mg/day in the lead-in period which was increased to on average 736 mg/day in the first four weeks of BOC treatment and subsequently reduced to 400 mg/day from week 12 to 16, accounting for the only patient with a dose increase (of 36%) by week 16 of treatment. Of the four patients that did not undergo RBV dose reduction, one received 800 mg/day, two received 200 mg/day and one was on a daily dose of 400 mg/day before discontinuing treatment after four weeks of BOC due to a worsening mental status.

At initiation of BOC at week 4, a time point at which a steady state would be predicted to have occurred, RBV levels were measured in 8 patients (median 2185 ng/ml; range: 1230-4460 ng/ml), of which only 2 had a therapeutic target level of >2500 ng/ml (respectively 3260 and 4460 ng/ml). The patient with the highest RBV level had been on a lead-in period of 75 days (with an average RBV dose of 600 mg/day) due to persistent anemia. The other patient received 1200 mg RBV per day.

Patients were instructed to maintain BOC dosing at 2400 mg/day. Three patients missed doses. Two of these patients discontinued treatment due to side effects and one patient missed one dose of BOC between week 12 and 16.

SAFETY

Two patients were anemic (Hb <10 g/dl) at baseline (Hb respectively 7.9 g/dl and 8.5 g/dl). Despite generally low RBV blood levels at week 4, individual Hb levels declined rapidly over the first 8 weeks of treatment, with an average 12% (range: -19%-34%) drop from baseline at week 4 and a subsequent 16% (-16%-38%) drop in the first 4 weeks after initiation of BOC. One patient had an unexplained increase in Hb in the

first four weeks from 8.5 g/dl to 10.1 g/dl. Three other patients had an increase in Hb in the first 4 weeks after the initiation of BOC, however all three had received blood transfusions in the preceding period. By week 16, individual Hb levels had decreased by an average of 34% from baseline (range: 8%-63%). Mean results in Hb decline are given in table 2 and figure 1b. All 17 patients experienced anemia on treatment, while 12 patients experienced severe anemia (Hb <8.5 g/dl). No less than 16 patients received blood transfusions; 3 during the lead in period and 15 after initiation of BOC. EPO was used in 12 patients, of which 3 already started during the lead in period. A decline in white blood cells (N=15) and platelets (N=16) was near ubiquitous, with the largest reduction occurring during the lead-in period (table 2). G-CSFs were used in 4 patients.

Despite stable CsA levels at the lower end of the therapeutic range, individual glomerular filtration rate (GFR) declined by 16% (range: -9%-65%) on average in the first four weeks of BOC treatment. By week 16, average GFR had dropped with 22% (range: -12%-44%) from baseline function (Table 2, figure 1c). GFR decline from baseline up to week 16 was almost universal, except for one patient, who suffered from cholestatic HCV resulting in renal insufficiency at baseline with a GFR of 37.4 ml/min. Renal function had improved by week 4 (GFR 78.6 ml/min) in this patient, but subsequently declined to 41.8 ml/min by week 15 of treatment, at which point the patient had to discontinue treatment due to anemia and thrombocytopenia. The average maximum GFR decline after BOC initiation compared to baseline was 27% (range -12%-52%). In the four patients with CsA C0 levels of >200 ng/ml after the introduction of BOC, maximum median GFR decline was 32% (range 17%-44%) compared to patients with levels below 200 ng/ml that had an average decline of 26% (-12%-52%).

On average, ALT and bilirubin levels gradually declined through the first 16 weeks of treatment (table 2). There was no suspicion of acute rejection in any of the 17 patients.

However, four patients had to discontinue therapy after the initiation of boceprevir due to severe side effects and/or progression of liver disease (table 3).

EFFICACY

A total of 6 out of 15 genotype 1 patients reached week 12 futility rules for BOC treatment (table 2). Of these, two had already discontinued treatment due to severe side effects. After week 12, one of the responders had to discontinue treatment due to anemia and thrombocytopenia. Based on intention to treat, in this cohort SVR rates will not exceed 53%.

Table 3. Characteristics of LT recipients discontinuing BOC-based antiviral therapy due to side effects

	Patient 1	Patient 2	Patient 3	Patient 4
Baseline characteristics				
Gender	Male	Female	Male	Female
Age (years)	74	69	54	54
Fibrosis (Metavir)	F4	F2	F3	F0
Ascites	Yes	No	Yes	Yes
HCV RNA load (IU/ml)	$16.3x10^6$	$22.1x10^6$	$>69.0x10^6$	$51.0x10^6$
Weeks on BOC at discontinuation	2	10	11	4
Last HCV RNA load (IU/ml)	$5.41x10^5$	$4.93x10^2$	Positive/<43	$5.27x10^2$
Reason for discontinuation	Worsening of ascites	Anemia with development of heart failure	Anemia and thrombo-cytopenia	Worsening mental status in patient witch cholestatic HCV

BOC=boceprevir; HCV=hepatitis C virus; LT=liver transplant; Positive, positive, but under lower limit of quantification

DISCUSSION

The aim of this study was to provide an in-depth analysis of safety and pharmacokinetics of BOC, RBV and peginterferon in combination with CsA for the treatment of recurrent HCV in a group of LT recipients in the clinical setting. This study has resulted in several important observations that have practical significance for the management of post-transplant HCV infection.

The apparent clearance of CsA is dynamic through at least the first 16 weeks of post-LT BOC-based antiviral therapy, requiring planned dose reductions of ~50% after the initiation of BOC. The magnitude of CsA reduction is consistent with the impact of BOC on CsA metabolism and pharmacokinetics that was observed in a healthy, nontransplant population without HCV infection. The translatability of the pharmacokinetic results from the nontransplant to the transplant setting is reassuring and was not a foregone conclusion. From a hematological perspective peginterferon was well tolerated, with over half of patients able to sustain a full dose and only one dose reduction required after the 12^{th} week of treatment. In contrast, RBV doses were reduced by 39% on average by week 16 of treatment. Of note, on average, RBV dose reduction mainly occurred after week 8 of treatment (11 out of 13 patients). RBV target C0 levels (2500-3500 ng/ml) were pragmatically based on efficacy and safety

data [14]. Presumably, RBV C0s were often beneath target levels at week 4 due to the relative low dosing of RBV (<600 mg/day on average). Nonetheless, Hb dropped steadily over time, with average levels at week 8 corresponding with anemia (Hb <10 g/dl) and average levels at week 12 and 16 corresponding with severe anemia (<8.5 g/dl), despite aggressive management with EPO (71% of patients) and blood transfusions (94% of patients). BOC is thought to induce bone marrow suppression, leading to an additional Hb drop of 0.7 g/dL from week 4 to 8 in non-transplanted patients [15, 16]. However, anemia occurs in only 50% of non-transplanted patients on BOC-based triple therapy, while just 7% develops severe anemia [16], compared to respectively 100% and 71% in this cohort of LT recipients. Moreover, WBCs and platelets appeared to reach a steady state by week 8, making it unlikely that BOC is solely accountable for the ongoing Hb decline after week 8. Instead, although anemia in this specifically difficult to treat group is presumably multifactorial, an important contribution to the ongoing Hb decline might be found in the diminishing renal function. Since RBV is excreted via urine, the almost ubiquitous GFR decline in this group of patients will likely have affected RBV blood levels, resulting in increased hemolysis.

These results suggest that, in LT recipients on antiviral triple therapy, RBV blood levels should also be monitored after week 4, especially in patients with increasing creatinine levels. Assessment of RBV blood levels in patients with substantial Hb decline may also be considered. While RBV level monitoring is typically carried out in patients with normal renal function, the high prevalence of renal insufficiency and the dynamic state of renal function in liver transplant recipients makes sequential RBV level monitoring of substantial potential utility.

Another factor that could potentially increase RBV blood levels after the addition of BOC is the routinely provided instruction to take BOC with sufficient food to facilitate intestinal uptake. The bioavailability of RBV increases upon intake with fatty foods [17]. Since treatment with BOC is new to most physicians and patients, they might emphasize the importance of food intake more when BOC is added to the treatment, subsequently also increasing the bioavailability of RBV. Increased RBV blood levels following initiation of BOC may exacerbate hemolysis. This might not apply to all patients, since antiviral triple therapy can also cause a loss of appetite. Moreover, BOC-evoked dysgeusia could also decrease appetite, although only 4 patients in this study reported dysgeusia-like side effects.

The most important and unanticipated finding of this study was the high frequency of GFR decline, which was almost ubiquitous and substantial with a maximum GFR decline after the introduction of BOC of 27% compared to baseline. This occurred despite whole blood CsA trough levels that were at or below low target trough ranges (100-150 ng/ml). Seven of the current patients switched from TAC to CsA within

four weeks after the start of antiviral therapy or during the lead-in period, which may have accounted for CsA induced nephrotoxicity. However, the median maximum GFR decline in this group was 26% (range: -12%-39%), compared to a median GFR decline of 34% (range: 3%-52%) in the 10 patients already on CsA. Moreover, in the patients that consistently had CsA C0 levels <200 ng/ml (N=11), median GFR decline was still 26% (range: -12%-52%), compared to a median decline of 32% (range: 17%-44%) in patients with at least one CsA C0 level >200 ng/ml (N=4). Since average CsA C0 levels on treatment were maintained at the low end of the therapeutic range, calcineurin inhibitor induced nephrotoxicity seems, superficially, to be unlikely. However, CsA levels are measured in whole blood in the clinical setting. This approach to CsA (and tacrolimus) blood level monitoring is safe in the context of stable hemoglobin levels since the majority of the CsA and TAC fraction in blood is sequestered in erythrocytes, while the biologically active fraction is unbound/free [18]. Hematocrit has been shown to be inversely related to plasma concentrations of CsA and subsequently the unbound fraction of CsA in plasma [18]. Due to RBV induced hemolysis a shift of the erythrocyte-bound CsA fraction to plasma will occur. Anemia will be exacerbated by peginterferon and BOC induced bone marrow suppression. Based on our observations and the whole blood method employed in CsA monitoring, we suspect that the cascade of treatment-induced hemolysis and bone marrow suppression in LT recipients leads to a vicious circle of CsA induced renal dysfunction and subsequent aggravated RBV-induced hemolysis, as shown in figure 2. Full blood CsA levels might not be representative of real CsA bioavailability in LT recipients on antiviral triple therapy. Again, to effectively interfere with this process, monitoring of RBV levels and/or subsequent dose reductions of RBV might be vital to restore renal function.

Nonetheless, other factors contributing to renal dysfunction should be considered, since GFR decline in LT recipients can have multiple causes. Of note, a two-fold increase of the AUC of the inactive SCH 629144 metabolite of BOC was observed after coadministration of BOC and CsA in healthy volunteers [11]. It is unclear whether this metabolite could potentially increase side effects of any kind. Moreover, it was recently shown that in non-LT recipients on HCV therapy with a baseline eGFR of >60 ml/min, eGFR at week 12 had decreased to <60 ml/min in 4.7% in patients on BOC and in 6.6% of patients on TVR compared to only 0.9% in patients on peginterferon/RBV duotherapy [19]. Renal function had improved by week 24 of treatment in the TVR treated group, suggesting a causal relationship between DAAs and renal dysfunction in a subgroup of patients.

Last, special attention should also be paid to patients with ascites. Unfortunately, four patients in our cohort had to discontinue antiviral treatment within 12 weeks after BOC initiation. This includes three (out of six) patients who had been diagnosed with

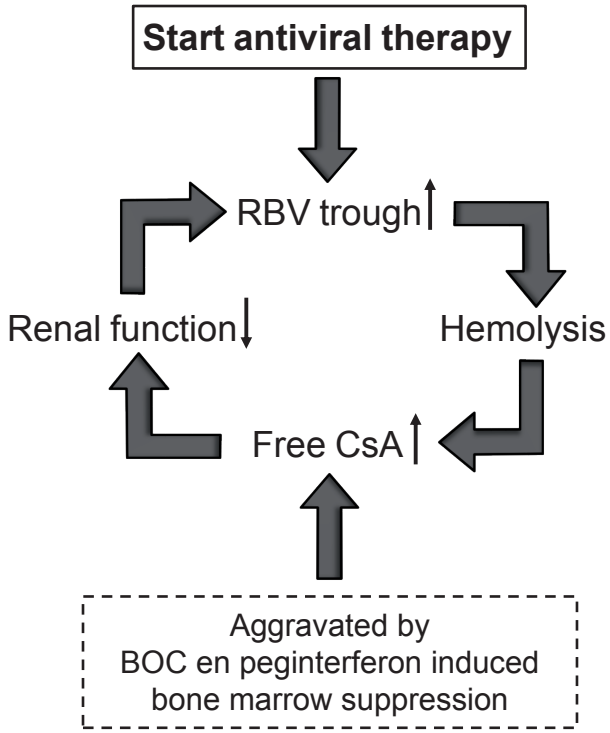

Figure 2. Suggested mechanism contributing to anemia and renal dysfunction in LT recipients on BOC-based triple therapy.
After starting antiviral therapy, RBV causes hemolysis leading to release of erythrocyte-bound CsA into plasma, thus increasing the biological active (or unbound) fraction of CsA. Increased bioavailability of (free) CsA leads to nephrotoxicity. Due to the decline in renal function RBV excretion is also impaired, causing higher blood levels of RBV and subsequently progressive hemolysis. This vicious circle is aggravated by the peginterferon and BOC induced bone marrow suppression and consecutive drop in hematocrit. Bioavailability of RBV might also increase upon start of BOC due to more emphasis on food intake.
BOC=boceprevir; CsA=Cyclosporine A; LT=liver transplant; RBV=ribavirin

ascites pre-treatment. This emphasizes that treatment of patients with ascites should be managed very carefully.

In conclusion, efficacy of BOC-based antiviral therapy in LT recipients is compromised by extensive side effects, which hamper viral clearance due to dose reduction(s) and/or early discontinuation of antiviral medication. In particular anemia and renal dysfunction were frequently observed and both worsened over time. Prevention of (severe) anemia and renal dysfunction is best carried out by repetitive measurement of ribavirin concentrations, followed by aggressive dose reductions when appropriate. In addition, CsA trough levels may need to be monitored by alternative means, e.g.

free drug levels or adjusted for degree of hemolysis.

Future research should focus on possible mechanisms causing renal function to decline in order to improve safety of DAA based antiviral therapy in LT recipients.

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PART 2

RIBAVIRIN IN HEV INFECTED PATIENTS

CHAPTER 8

Prevalence and clinical consequences of Hepatitis E in patients who underwent liver transplantation for chronic Hepatitis C in the United States

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ABSTRACT

BACKGROUND

Infection with hepatitis E virus (HEV) in immunocompromised patients can lead to severe liver disease. Treatment options for HEV include peginterferon or ribavirin, routinely also used for the treatment of hepatitis C virus (HCV) infection.

AIM

To determine the prevalence and clinical consequences of HEV in United States (US) based patients who underwent liver transplantation (LT) for chronic HCV.

METHODS

Seroprevalence of HEV in 145 US LT recipients with a history of chronic HCV was determined pre-LT, 1, 3 and 5 years post-LT. All last available samples and all samples in IgM positive patients and post-LT IgG seroconverters were tested for HEV RNA.

RESULTS

Overall anti-HEV seroprevalence was 42%. Five patients were HEV IgM positive pre-LT, one patient had IgM seroconversion post-LT and eight patients had IgG seroconversion post-LT. None of the tested samples were positive for HEV RNA. Eight out of nine of the post-LT seroconverters had been treated for HCV recurrence before or at the moment of seroconversion.

CONCLUSIONS

LT recipients in the US are at risk of acquiring HEV. Post-LT HCV treatment with interferons and/or ribavirin may have protected patients against chronic HEV. With the arrival of new direct antiviral agents for the treatment of HCV and the elimination of peginterferon and ribavirin from HCV treatment regimens, the prevalence of chronic HEV in this population may rise again.

INTRODUCTION

Hepatitis E virus (HEV), an enterically transmitted virus that is known for causing acute hepatitis, was first isolated in 1990 [1]. HEV is a non-enveloped virus with a single-stranded, positive sense RNA genome of approximately 7,500 base pairs and three partially overlapping open reading frames (ORF 1-3) [2]. Up to date, there are four genotypes prevalent known to infect humans. Genotype 1 and 2 are endemic in developing countries and mainly transmitted through contaminated water, while genotypes 3 and 4 are sporadically seen in industrialized countries and thought to be zoonotic of origin [3, 4].

While in most cases HEV infection is usually a self-limiting disease, in the past years multiple reports have emerged on immunocompromised patients, especially solid organ transplant (SOT) recipients, developing chronic HEV infection [5-7]. Reports on HEV infections in SOT recipients in the United States are scarce. Almost all well-defined cohort studies in industrialized countries on HEV infection in SOT recipients up to now have been done in Europe and these results cannot be automatically extrapolated to the situation in the US due to geographical and demographic differences. One study in HIV-infected liver and kidney transplant candidates in the US showed a pre-transplant seroprevalence of almost 20% [8]. Estimates of the seroprevalence of HEV in the general population (including blood donors) in the US are reaching up to 22% [9-13]. Swine-human contact is seen as an important possible source for HEV transmission in the US: HEV infection occurs in more than 80% of some pig herds in the US [14] and HEV RNA has been found in pig feces on multiple US farms and pig livers sold in local US grocery stores [15-17]. Veterinarians working with swine are 1.5 times more likely to be HEV IgG positive than blood donors from the same area [10]. In US blood donors, an HEV seroprevalence of up to 22% has been reported, making blood transfusions a likely mode of transmission as well [9-11].

Consequently, SOT recipients in the US are at risk of acquiring HEV infection. Early detection of HEV infection in these patients is crucial, since chronic HEV in SOT recipients can lead to rapid fibrosis and even cirrhosis [18-20].

Chronic HEV can be adequately managed by dose reduction of immunosuppressive medication or treatment with peginterferon alfa or ribavirin (RBV), compounds also used for the treatment of hepatitis C virus (HCV) infection [21-24]. Currently, no studies on HEV infection in HCV infected SOT recipients are available. On one hand, there is a risk of development of progressive or even fulminant liver disease when co-infection with HEV in these patients occurs. On the other hand, chronic HEV infection may be less prevalent due to treatment of HCV.

To gain more insight into the prevalence of hepatitis E infection in SOT recipients in the US and the influence of HCV treatment on the incidence and clinical course of HEV we conducted the current study in a cohort of patients with a history of HCV that underwent a liver transplantation in the Mayo Clinic in Rochester, Minnesota.

MATERIALS AND METHODS

PATIENTS AND SAMPLES

From 1997 through 2010 serum samples were prospectively collected according to a standard protocol in patients with a history of chronic hepatitis C infection that underwent liver transplantation for end stage liver disease or hepatocellular carcinoma in the Mayo Clinic in Rochester, Minnesota (US). No donor organs were obtained from executed prisoners or other institutionalized persons. Samples were collected in 145 patients at four time points: pre-transplantation (baseline), 1 year, 3 years and 5 years post-liver transplantation and subsequently stored at -70°C. Each enrolled subject had consented in future testing of archived bio-samples. Samples were shipped to the Erasmus University Medical Center in Rotterdam (The Netherlands) for hepatitis E testing. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of the Mayo Clinic in Rochester, Minnesota (US).

HEV SPECIFIC ANTIBODY DETECTION

First, all collected samples were tested for both HEV specific IgM and HEV specific IgG with the commercially available enzyme-linked immunosorbent assay (ELISA) (Wantai, Beijing, China), used according to the manufacturer's instructions.

HEV-RNA DETECTION

Second, all samples in patients with HEV specific IgM antibodies and patients with HEV specific IgG seroconversion post-LT were tested for the presence of HEV RNA by an internally controlled quantitative real-time RT-PCR, described previously [6]. The RT-PCR has a lower limit of detection (95% hit rate) of 143 IU/ml as determined by the 1st WHO standard for HEV RNA NAT-Based assays (6329/10, Paul Ehrlich Institute, Germany). Finally, all last available samples in the 145 patients were tested for HEV RNA to detect any ongoing active HEV infection at the last follow up point, since seroconversion in immunocompromised patients is often delayed and these patients might not have seroconverted yet.

RESULTS

A total of 370 samples in 145 LT-recipients were available for analysis. Distribution of samples over time and serology test results are given in figure 1. Baseline characteristics are given in table 1.

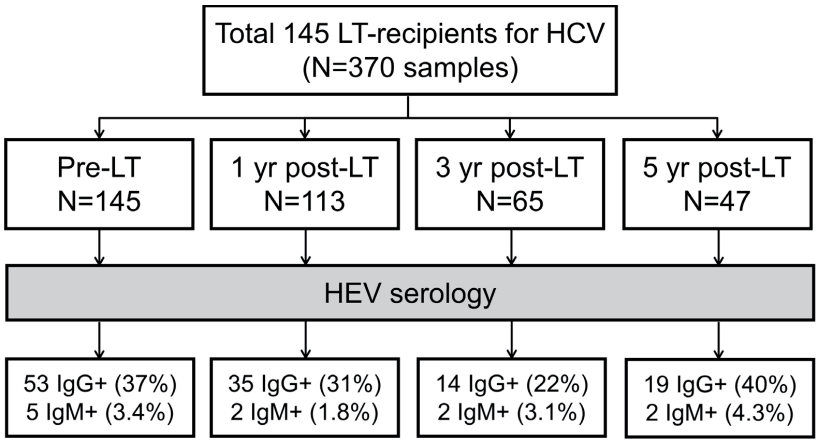

Figure 1. Sample distribution and hepatitis E serology in 145 liver transplant recipients
LT=Liver transplant; HEV=Hepatitis E virus

Table 1. Baseline characteristics in LT recipients with a history of HCV infection

	HEV IgG positive N=53	HEV IgG negative N=92	p-value
Mean age, years	53 ± 7	51 ± 8	0.153
Male sex	45 (85%)	64 (70%)	0.047
Caucasian ethnicity	47 (88%)	79 (84%)	0.615
Residing in Minnesota	29 (53%)	35 (37%)	0.077
History of alcohol abuse	26 (49%)	34 (37%)	0.166
HCV genotype 1	28 (67%)	63 (83%)	0.066

LT=liver transplant; HCV=Hepatitis C virus; HEV=Hepatitis E virus

IGG SEROCONVERSION

A total of 61 patients (42%) had HEV specific IgG antibodies at some point from baseline up to last follow up. Seroprevalence of HEV at baseline was higher in men compared to women, however, overall seroprevalence from baseline up to last follow up did not differ between sexes: N=60 (40%) for men and N=18 (33%) for women (p=0.418). Of the 53 patients that had HEV specific IgG antibodies at baseline, 46 had at least one follow-up sample post-LT. Fifteen of these patients (33%) with IgG

antibodies at baseline had IgG loss at some point post-LT. A total of 125 patients had a baseline sample and at least one follow up sample available. Eight patients (6,4%) were IgG negative at baseline and showed IgG seroconversion post-LT; four at year 1, one at year 3 and three at year 5 post-LT. Two of these 8 patients had IgG loss after post-LT seroconversion.

IGM SEROCONVERSION

Only 6 out of 145 patients (4.1%) had IgM antibodies against HEV. Five patients had IgG and IgM antibodies against HEV at baseline and one patient, who had been IgG positive from baseline up to 5 years post-LT, seroconverted IgM at year 5 post-LT (see also table 2). One of the 6 IgM-positive patients had IgG and IgM loss post-LT, another patient experienced IgG loss but remained IgM positive.

POST-LT HEV INFECTION

None of the last available samples in the 145 patients or any of the samples in the IgM positive and IgG seroconverters were positive for HEV RNA. We hypothesized that HCV infected LT-recipients were protected against chronic HEV infection due to concurrent treatment with either (peg)interferon or RBV for the treatment of HCV. To test this hypothesis we selected the patients that were most likely to have had active infection post-LT, namely: the patients that had IgM and/or IgG seroconversion post-LT. All 8 IgG seroconverters and the single IgM positive patient that seroconverted after LT met this definition and were further explored (see table 2).

All but one patient were treated with (peg)interferon and/or RBV before or at the time of seroconversion.

DISCUSSION

In this study we examined the prevalence and impact of serological evidence of HEV infection among a well-defined cohort of US liver transplant recipients with a history of HCV infection. The impact of HEV infection among solid organ transplant recipients is likely to be greatest among patients with pre-existing liver injury such as caused by chronic HCV, since acute on chronic infection will most likely lead to an aggravated clinical course. Post-transplant HCV infection is the most common cause of graft loss among liver transplant recipients, raising the possibility that the consequences of post-transplant HEV infection might be most readily apparent in this group of patients. Our analysis of HEV infection in a relatively large cohort of liver transplant recipients with HCV infection has produced several potentially important results that merit detailed consideration. The first important observation is that in this cohort, more than four out of ten liver transplant recipients with a history of HCV

Table 2. Characteristics of nine post-LT HEV seroconverting patients with a history of HCV infection

	Age at LT	Gender	Ethnicity	LT year	Type of serologic conversion	Conversion year (from LT)	Post-LT HCV treatment	Treatment year post-LT	Treatment medication
Pt 1	46	Male	Caucasian	1998	IgM	5	Yes	1-5	IFN+RBV
Pt 2	48	Male	Caucasian	1998	IgG	1	Yes	0-1	IFN+RBV
Pt 3	39	Male	Native Hawaiian	2000	IgG	5	Yes	2-3	IFN+RBV
Pt 4	36	Female	Caucasian	2001	IgG	1	Yes	0-1	IFN+RBV
Pt 5	47	Male	Other NS	2001	IgG	1	Yes	0	IFN
Pt 6	51	Male	Caucasian	2001	IgG	5	Yes	2-4	IFN+RBV
Pt 7	47	Male	Caucasian	2003	IgG	3	Yes	1-4	IFN+RBV
Pt 8	51	Male	Caucasian	2004	IgG	5	Yes	0-3	IFN+RBV
Pt 9	54	Male	Caucasian	2009	IgG	1	No	N/A	N/A

LT=Liver transplant; HEV= Hepatitis E virus; HCV=Hepatitis C virus; IFN=(peg)interferon; RBV=ribavirin; Other NS=Other than Caucasian, Hispanic, Black or Asian, not specified, N/A=not applicable

infection have been in contact with HEV. This finding highlights that these patients are at considerable risk of exposure to HEV, which may lead to chronic infection or accelerated liver disease. Indeed, we showed that transplant recipients in the US are at risk of acquiring HEV infection following liver transplantation, considering that 9 out of 125 patients (7.2%) with at least one follow up sample had HEV seroconversion post-LT.

There are several explanations why over four out of ten patients in this cohort of US liver transplant recipients with a history of HCV infection have HEV antibodies. Since HCV is a blood borne disease, HEV infection may share the same transmission route as HCV. An association between intravenous drug use in HCV infected patients and the prevalence of HEV IgG antibodies has been inconsistently reported [25, 26]. Another possible transmission route is through transfusion with blood products. There is a considerable seroprevalence of HEV in blood donors in the US with anti-HEV antibodies of up to 22%, though no active infection in US cohorts has been found [9-11]. In contrast, in European cohorts, HEV RNA was found in plasma and blood donors [27-33] leading to HEV infection in patients receiving those blood components [33]. As long as blood products are not routinely screened for HEV RNA, US physicians should be aware of this possible transmission route. LT recipients usually have received multiple blood products over the course of their disease. HCV treated LT recipients are even more likely of receiving blood transfusion due to treatment-induced anemia, further increasing the chance of receiving blood products from an HEV viremic donor. Patients are also at risk of acquiring HEV through infection of the donor organ as a recent report on a liver transplant recipient showed [34]. Finally, the average age in this cohort was 51.8 years and several studies have shown that HEV seroprevalence increases with age [10, 11, 35, 36].

One of the unexpected observations in our analysis was that, despite a substantial number of patients seroconverting to anti-HEV IgM and/or anti-HEV IgG following liver transplantation, we did not find any active HEV infection in this cohort. It is possible that HEV viremia was missed in our study due to the time between serum draws that were available for analysis. HEV seroconversion may have occurred with (spontaneous) clearance of active HEV infection and subsequent loss of HEV antibodies over time due to immunosuppressive therapy, as illustrated by HEV IgG loss post-LT in one third of patients with HEV IgG antibodies at baseline. We may also have missed patients that did not seroconvert at all despite infection due to their immunosuppressive state, since HEV seroconversion is often delayed or even absent in these patients [6, 24, 37, 38]. Patients that obtained HEV infection after the last follow up sample and subsequently died will have been missed as well. Our results may, therefore, have underestimated the severity of acute HEV infection.

We used the most sensitive HEV serologic test available at the moment [39-41] and

an internally controlled quantitative real-time RT-PCR, which has been an effective and reproducible tool for detecting HEV RNA in other cohorts [35, 42]. The effects of immunosuppression, which include suppression of antibody production and an increase of levels of viremia (e.g. for HCV, HBV, CMV), may have resulted in an underestimation of the seroprevalence of anti-HEV in this cohort but should not have affected the numbers regarding prevalence of active HEV infection. Our results should generally be viewed as reassuring in that no active HEV infection was found in any of the patients at last follow up. Moreover, HEV infection in SOT recipients does not always progress to chronicity [37]. A further consideration in the lack of active HEV infection in our analysis is that eight out of nine post-LT anti-HEV seroconverters were treated with either RBV or (peg)interferon. Both of these agents are known to be effective therapies for HEV infection and may have contributed to HEV clearance. As HCV treatment initiation is often based on clinical parameters indicating (recurrent) hepatitis, it is possible that superinfection with HEV may have contributed to the likelihood of initiation of anti-HCV therapy. Soon, new antiviral agents, including interferon- and ribavirin-free protocols, against HCV will become available for the treatment of HCV infection. The disappearance of interferon and RBV from HCV treatment regimens may increase the risk of acquiring chronic HEV and subsequent development of fibrosis and cirrhosis, as these new agents will most likely not have activity against HEV. HCV liver transplant recipients not treated for HEV are currently still at risk of developing chronic HEV.

Limitations of this study include a lack of a comparative non-HCV infected group of liver transplant recipients, the retrospective nature of the study and patients that were lost to follow up due to their transferring care to a local hospital.

In conclusion, LT recipients in the US are at risk of acquiring HEV. Therefore, screening of HEV in LT recipients should be carried out routinely, especially when there are clinical signs of (progressive) hepatitis. Evaluation of immunocompromised patients should include HEV RNA testing, since antibody detection is often delayed in these patients. Due to the lack of FDA-approved HEV RNA tests and high interlaboratory variability in PCR performance [43], we recommend to have samples tested in a laboratory with extensive experience and up to date assays. HCV infected LT recipients may be protected against the development of chronic HEV through treatment against HCV. However, HEV infection should be best managed through dose reduction of immunosuppressive medication and/or treatment with low-dose RBV [44], avoiding overtreatment of HCV infected patients. Due to the arrival of new interferon- and RBV-free HCV regimens not active against HEV, HCV infected LT recipients will again be at risk of acquiring (chronic) HEV in the nearby future.

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CHAPTER 9

Clinical implications of chronic Hepatitis E virus infection in heart transplant recipients

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ABSTRACT

BACKGROUND

Recent reports have shown that hepatitis E virus (HEV) infection can become chronic in solid-organ transplant recipients, but few studies have systematically investigated the clinical consequences of this chronic HEV infection in solid-organ transplant (SOT) recipients.

METHODS

We have undertaken an in-depth study of 6 chronic HEV-infected heart transplant recipients to gain further insight into the clinical, biochemical and virologic presentation of this disorder.

RESULTS

In 6 patients (2.3%) chronic HEV infection, genotype 3, was identified. Immunosuppression in these patients was tacrolimus-based, combined with either everolimus or prednisolone and/or mycophenolate mofetil. Median follow-up after case detection was 26 months (range 21 to 40 months). All chronic HEV cases had elevated liver enzyme values. IgM antibodies at presentation were positive in 2 of 6 (33%) patients. Liver histology in 4 of 6 (67%) patients showed advanced fibrosis within 2 years after infection. One patient spontaneously cleared the HEV infection: 1 after dose reduction of immunosuppressive therapy and 3 during ribavirin therapy. One patient has yet to clear the virus and remains on ribavirin therapy.

CONCLUSIONS

Chronic HEV infection in heart transplant (HTx) recipients may lead to rapid fibrosis of the liver. We advise additional HEV RNA screening in solid-organ transplant recipients with elevated liver enzymes, because antibody production is often delayed, as demonstrated in these patients. Dose reduction of immunosuppressive therapy should be the first intervention strategy to achieve viral clearance in chronic HEV-infected immunocompromised patients. Ribavirin treatment should be considered in cases of chronic HEV

INTRODUCTION

Hepatitis E virus (HEV) is a non-enveloped virus with a single-stranded, positive-sense RNA genome of approximately 7,500 basepairs and 3 partially overlapping open reading frames (ORF 1–3) [1]. There are 4 mammalian genotypes prevalent: genotypes 1 and 2, endemic in developing countries causing waterborne outbreaks; and genotypes 3 and 4, seen in sporadic cases in industrialized countries and thought to be of zoonotic origin [1,2].

In The Netherlands, the prevalence of genotype 3 hepatitis E virus in fecal tanks on pig farms is estimated to be about 55% [3], whereas HEV RNA was found in 6.5% of commercial porcine livers [4], suggesting a role for undercooked pig meat in pig-to-human infection. Contributing to this theory is the homology between strains detected in pigs that were also found in Dutch patients of up to 100% [5,6]. Close nucleotide identity to strains isolated in Dutch pigs was also found in HEV-infected patients in Germany and Scandinavia [8,9], which implies a shared distribution of genotype 3 hepatitis E virus in these countries and possibly the rest of Western Europe. Although hepatitis E virus has long been considered to cause solely acute infection, more recently there have been reports on persistent chronic infection with genotype 3 in immunocompromised patients, mostly with solid-organ transplantation (SOT) [7–13]. Moreover, chronic HEV infection in these patients can lead to rapid fibrosis and even cirrhosis [8,9,14]. Therefore, diagnosis of chronic HEV infection in solid-organ transplant recipients is vital in order to start early intervention and prevent irreversible liver damage. Although some case studies have addressed intervention with ribavirin or peginterferon alfa treatment, there is no current guideline or standardized treatment protocol available [13,15–21]. Few studies have systematically investigated the clinical consequences of this chronic HEV infection in SOT recipients. In one study, we found an HEV point prevalence of 1% in 1,200 SOT recipients [12]. Half of HEV-infected patients were heart transplant (HTx) recipients.

METHODS

CASE DEFINITION AND SAMPLE COLLECTION

In a previous study we identified 1 patient with a transient viremia and 6 chronically HEV-infected HTx recipients during the 2000 to 2011 time frame [12]. A case of HEV infection was defined as a patient with an HEV RNA-positive serum or EDTA (ethylenediamine tetraacetic acid) plasma sample and was confirmed either by showing HEV-specific serum IgM or IgG antibody or by showing the presence of HEV RNA in sequential serum or plasma samples. Chronic infection was defined as having HEV RNA in serum or EDTA plasma for 46 months and diagnosed by retrospective

testing of stored samples. These samples were collected during routine visits to our outpatient clinic for clinical assessment and were stored at 201C (serum) and 801C (EDTA plasma or feces). To verify excretion of HEV RNA via stool, available fecal samples were screened by reverse transcript– polymerase chain reaction (RT-PCR). In addition, spouses of identified cases were asked to donate a serum sample for serologic screening of HEV-specific antibodies. Each enrolled subject had consented to future testing of archived biosamples.

Our study protocol was in accordance with the ethics guidelines of the 1975 Declaration of Helsinki as reflected in the prior approval from the local medical ethics committee (MEC-2011-277).

HEV-SPECIFIC ANTIBODY DETECTION

For both HEV-specific IgM and HEV-specific IgG detection in serum or EDTA plasma samples, a commercially available enzyme-linked immunosorbent assay (ELISA; Wantai, Beijing, China) was used according to the manufacturer's instructions.

HEV RNA DETECTION

All samples were screened for the presence of HEV RNA by an internally controlled quantitative real-time RT-PCR, as described elsewhere [12]. The RT-PCR had a lower limit of detection (95% hit rate) of 143 IU/ml as determined by the first World Health Organization (WHO) standard for HEV RNA nucleic acid testingbased assays (6329/10, Paul Ehrlich Institute, Germany) [26].

SEQUENCE ANALYSIS

For phylogenetic analyses, ORF1 RdRp (nt 4254 to 4560) sequences of 306 bp were generated using the primer set MJ-C, as described elsewhere [22]. Detailed methods have also been described [12]. The sequences of all isolates were deposited into GenBank (Accession Nos. JQ15418, JQ15423-JQ15425 and JQ15427-JQ15428).

LIVER BIOPSY

Liver biopsies were performed using either a 14- or 18-gauge needle in combination with a plugged biopsy. All samples were fixed with formalin, embedded in paraffin and then stained. Staining included hematoxylin and eosin (HE), periodic acid– Schiff (PAS), PAS-diastase (PAS-D), Sirus red, copper, iron, reticulin and CK7. All biopsies were evaluated by the same pathologist (F.J.t.K.). Necroinflammatory activity was scored using the Histology Activity Index (HAI) [23], whereas grade of steatosis and iron deposition were classified according to Brunt et al. [24,25].

RESULTS

PATIENTS

A total of 1,200 SOT recipients were screened previously, of whom 263 were HTx recipients (Figure 1) [12]. Of the HTx recipients, 4 were multiple SOT recipients with a kidney– heart transplant. All HEV PCR-positive cases were retrospectively tested for HEV RNA, anti-HEV IgM and IgG to characterize the course of the infection in relation to the date of transplantation.

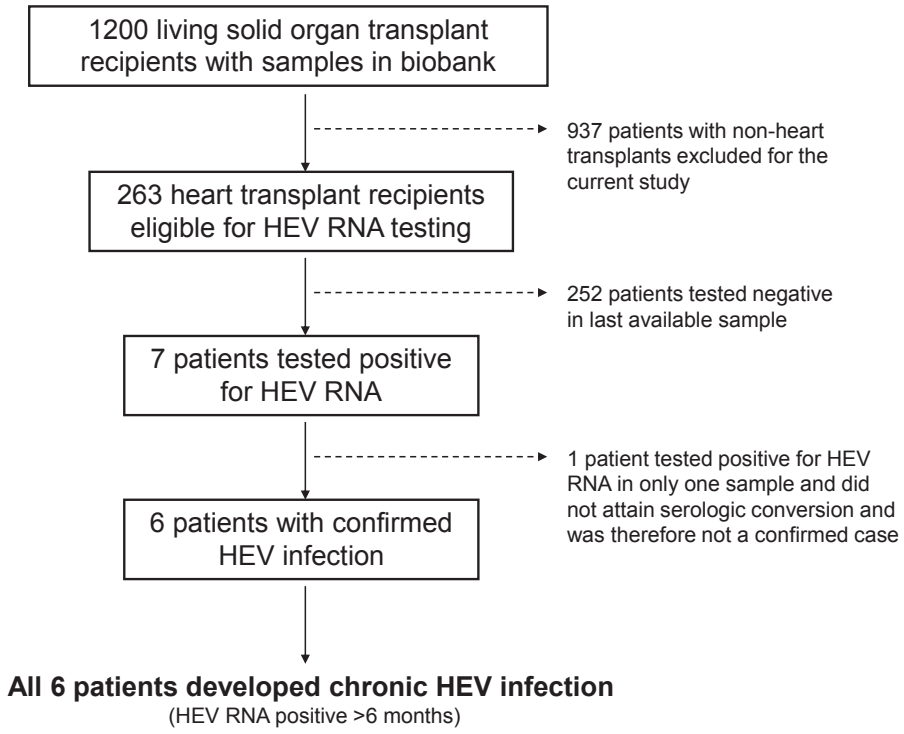


Figure 1. Flowchart of patient selection

In 7 (2.7%) patients, HEV RNA was detectable in at least 1 sample. HEV infection could be confirmed in 6 patients, including 5 heart transplant (HTx) recipients and 1 kidney–heart transplant (HTx-NTx) recipient. In 1 patient, HEV RNA was only detected in a single sample and could not be confirmed by either detectable HEV RNA or HEV-specific IgG or IgM antibodies in any previous or subsequent samples available; therefore, this patient did not fulfill the case definition. Moreover, no elevation of liver enzymes was documented in this patient.

All 6 confirmed cases developed chronic HEV infection (HEV RNA detectable for at least 6 months). The median age of all HEV-infected patients at the time of infection was 50.0 years (range 38.3 to 62.4 years) and 83% (n = 5) were male. Median time from transplantation to HEV infection was 7.5 years (range 1 to 20 years). In the samples studied, all chronic patients were infected after 2008. All 6 chronic HEV-infected patients received tacrolimus-based immunosuppression (Table 1).

Table 1. Baseline* characteristics of six HTX recipients with chronic HEV infection

	Age (years)	Gender	Year of Tx	Year of first HEV RNA + sample	Time from HTX to infection (years)	Immuno-suppression therapy	IgM	IgG
1	51.3	M	2008	2010	2	P/T/MMF	+	+
2	55.7	M	1997	2010	13	P/T	-	-
3	47.2	F	2009	2010	1	P/T	+	-
4	62.4	M	2008	2010	2	P/T	-	-
5	49.3	M	HTX: 1996 NTX: 2008	2009	13	E/T	-	-
6	38.3	M	1990	2010	20	P/T	-	-

*At time of first HEV RNA-positive sample
C=cyclosporin; E=everolimus; F=female; HTX=heart transplant, M=male; MMF=mycophenolate mofetil; NTX=kidney transplant, P=prednisolone, T=tacrolimus; Tx=transplant

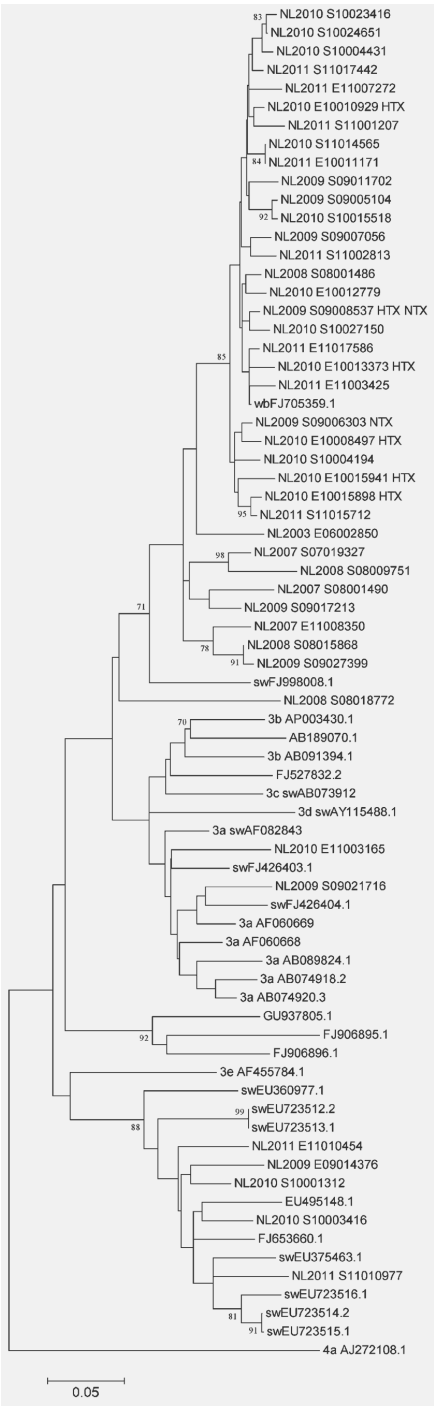
VIROLOGIC PARAMETERS

All chronically infected patients produced HEV-specific IgM antibodies at some point after HEV RNA became detectable in serum, although only 2 (33%) had positive IgM antibodies at time of first HEV RNA-positive sample. The median time from the first HEV RNA-positive sample to IgM seroconversion was 122 days (range 0 to 301 days). The median time to IgG seroconversion varied widely from 127 days before seroconversion of IgM to 475 days after seroconversion of IgM.

Fecal HEV shedding was found in all chronic HEV infected patients during viremia. Spouses of patients were tested for HEV infection. None of them had detectable HEV RNA or underwent anti-HEV IgM or IgG seroconversion in their serum.

PHYLOGENETIC ANALYSIS

The ORF1b sequences generated from the 6 chronic cases showed that all virus isolates grouped within genotype 3. Both HEV genotype 3 GenBank sequences (acces-



LEGEND

The phylogenetic relation of the 306-bp ORF1 region was calculated using maximum-likelihood K2P analysis with bootstrapping (n = 1,000). Branch lengths are proportional to the evolutionary relationship between the sequences, and internodal confidence of >70% is depicted in the tree. Heart transplant (HTx) recipients and heart–kidney (HTx-NTx) transplant recipients are indicated in the taxa. HEV sequences of Dutch origin[12] and year of infection are indicated as NLyyyy-isolate number, GenBank accession numbers are JQ15401, JQ15406-JQ15417, JQ15419-JQ15422, JQ15426 and JQ15429-JQ15448. No indication for a common origin nor for nosocomial HEV transmission was found.

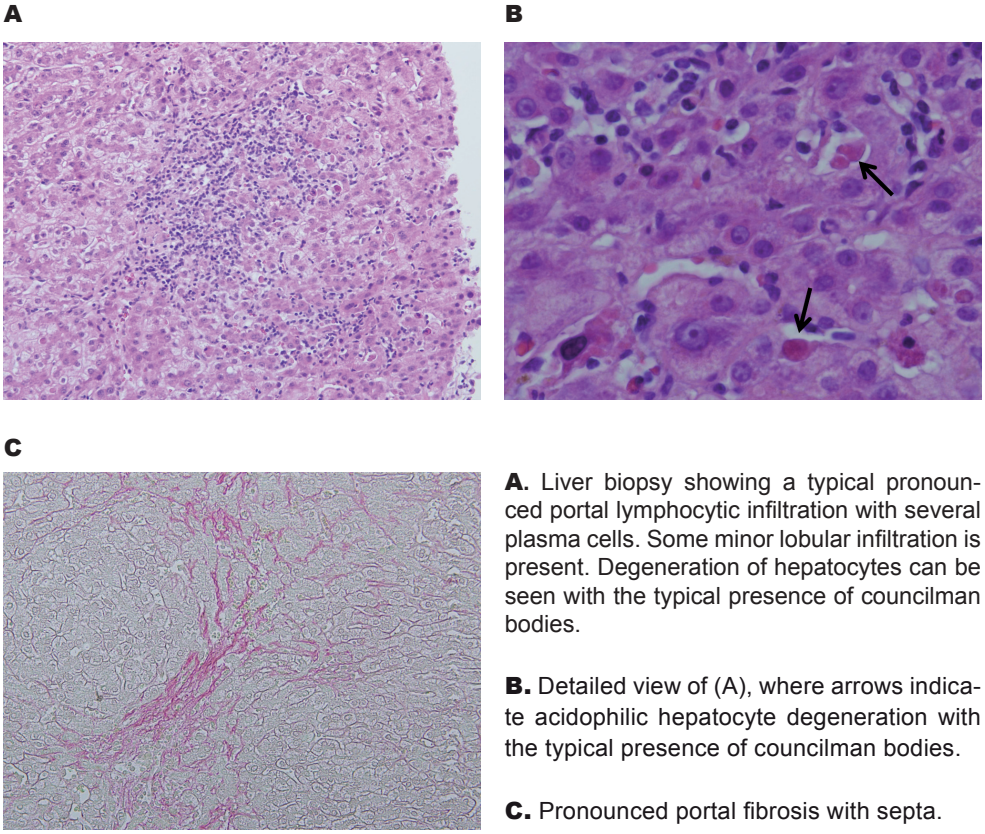
Figure 2. Phylogenetic tree of ORF1 HEV sequences in 6 chronic HEV-infected heart transplant recipients

sion nos. are taxon names; Figure 2) and HEV ORF1b sequences published previously [12] (Accession Nos. JQ15401, JQ15406JQ15417, JQ15419-JQ15422, JQ15426 and JQ15429-JQ15448) as reference. The latter samples were retrospectively tested to disclose the year of infection, as indicated in the taxon names (NLyyyy-specimen number; Figure 2).

Phylogenetic analyses revealed neither indications of a common origin nor evidence of nosocomial HEV transmission.

LIVER HISTOLOGY

All chronic HEV-infected patients underwent liver biopsy. Typical signs of acute viral hepatitis were seen with inflammatory activity, councilman bodies and acidophilic degeneration (Figure 3). Inflammatory activity was predominantly located in the periportal area (Figure 3A). HAI scores [23] were calculated for all biopsies (Table 2). Ad-



A. Liver biopsy showing a typical pronounced portal lymphocytic infiltration with several plasma cells. Some minor lobular infiltration is present. Degeneration of hepatocytes can be seen with the typical presence of councilman bodies.

B. Detailed view of (A), where arrows indicate acidophilic hepatocyte degeneration with the typical presence of councilman bodies.

C. Pronounced portal fibrosis with septa.

Figure 3. Histologic presentation of chronic hepatitis E infection in heart transplant recipients

Table 2. Liver biopsy results in six chronic HEV infected HTX recipients

	Infection time (months)*	HAI-Knodell score				Total HAI score	Steatosis†	Cholestasis	Iron‡
		Periportal necrosis	Intralobulair inflammation	Portal inflammation	Fibrosis				
1	9	1	3	2	3	9	0	none	1+
2	5	1	2	1	3	7	1	focal	none
3	7	3	3	3	3	12	0	none	none
4	5	0	1	0	0	1	1	none	none
5	22	3	3	3	3	12	1	none	none
6	8	1	1	1	1	4	1	none	none

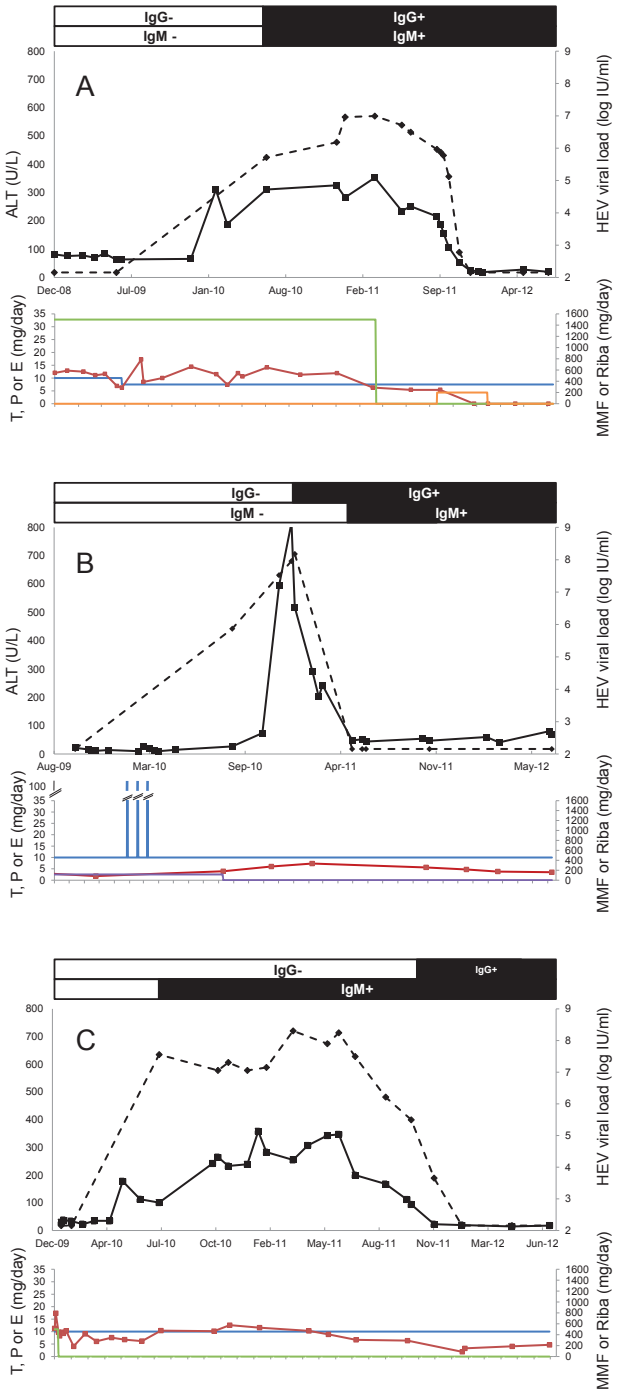
* Calculated from time-point at which first sample was positive for HEV RNA up to biopsy date
† According to Brunt et al. [24]
‡ According to Brunt et al. [25]

HAI=Histology Activity Index [23]

vanced fibrosis was seen in 4 patients (67%), of whom 3 had been infected with HEV for o1 year. The lowest HAI score (sum = 1) was from a patient whose liver biopsy was taken only 5 months after the first HEV RNA-positive sample. All biopsies displayed some steatosis, but in 4 of the 6 biopsies a marked steatosis, ranging from 5% to 25%, was seen.

CLINICAL COURSE AND MANAGEMENT

An overview of the clinical course and management of the chronic HEV-infected patients, including serum trough levels of tacrolimus, dose reduction of concomitant immunosuppressant therapy, and treatment with orally administered ribavirin, is presented in Figure 4. Liver enzyme values were elevated in all cases, with a median peak alanine aminotransferase (ALT) level of 356 (range 81 to 817) U/liter, aspartate transaminase (AST) level of 230 (range 66 to 672) U/liter, alkaline phosphatase level of 170 (range 80 to 278) U/liter and gamma-glutamyltranspeptidase (g-GT) level of 308 (range 196 to 1,740) U/liter. Bilirubin levels were elevated in 3 patients (17, 18 and 95 mmol/liter, respectively). All patients that had peak ALT levels of more than 4 times the upper limit of normal (ULN; males 40 U/liter, females 30 U/liter) had advanced liver fibrosis on presentation. One patient (Figure 4D) had only moderate elevation of liver enzymes throughout the whole course of HEV infection with peak ALT (81 U/liter) of 2 times the upper limit of normal. Interestingly, Patient 2 had a peak ALT level of >20 times the ULN (817 U/liter), but was the only patient to clear the chronic HEV infection spontaneously (Figure 4B). No dose reduction of immunosuppressive therapy was possible or necessary in this patient. Moreover, Patient 2 was the only patient with IgG seroconversion before IgM seroconversion. IgG seroconversion occurred 127 days before IgM seroconversion and was accompanied by a subsequent rapid increase of HEV viral load. At the time of IgM seroconversion HEV RNA had almost become undetectable. Immunosuppressive therapy was reduced in the other patients. Patient 3 managed to clear the HEV infection after dose reduction of tacrolimus (Figure 4C). A decrease in HEV viral load was seen after mycophenolate mofetil (MMF) was stopped in Patient 1 (Figure 4A) and, after a subsequent 3-month course of low-dose ribavirin therapy, this patient was able to rapidly clear the HEV infection. A similar course was observed in Patients 4 and 6 (Figure 4D and F, respectively). In these 2 patients, the tacrolimus dose was reduced slightly, but both cleared the virus only after a stepwise dose increase of ribavirin and were thus treated for 9 and 8 months, respectively. Interestingly, Patient 6 became IgM antibody negative immediately after clearing the HEV infection, but remained IgG antibody positive during follow-up. Patient 5 (Figure 4E) did have a drop in ALT after dose reduction of tacrolimus, but no effect was seen with respect to the HEV viral load. After introduction of ribavirin, viral load dropped initially, but increased for unknown reasons after 3 months. There were no indications that this



LEGEND

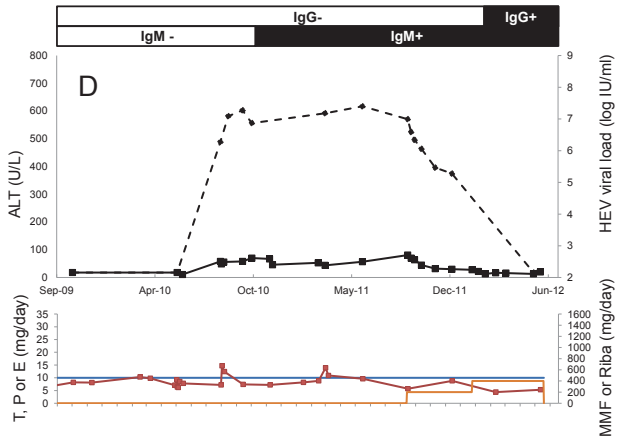
- ALT (U/L)
- HEV viral load (log IU/mL)
- Prednison dose (mg/day)
- Everolimus dose (mg/day)
- Ribavirin dose (mg/day)
- MMF dose (mg/day)
- serum Tacrolimus through levels (µg/L)

A. Patient 1.

B. Patient 2, who received 3 times rituximab-cyclophosphamide, hydroxydaunorubicin, oncovin, prednisolone (R-CHOP) treatment for lymphoma, which explains the prednisolone peaks.

C. Patient 3.

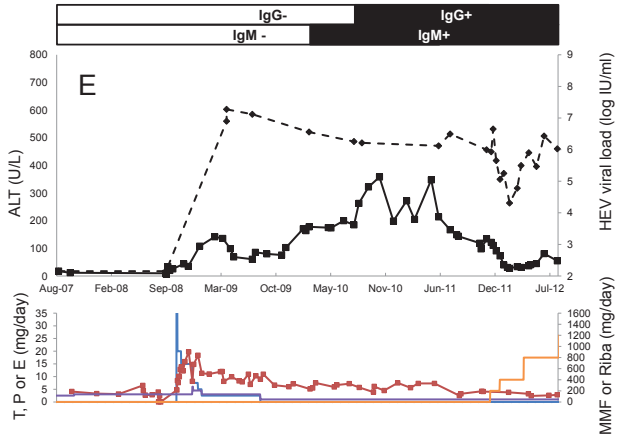
Figure 4. A-C. Clinical course of chronic HEV infection in 6 heart transplant recipients. The ALT upper limit of normal is 30 U/liter and 40 U/liter for females and males, respectively. The HEV RNA lower limit of detection is 143 (2.16 log) IU/ml.



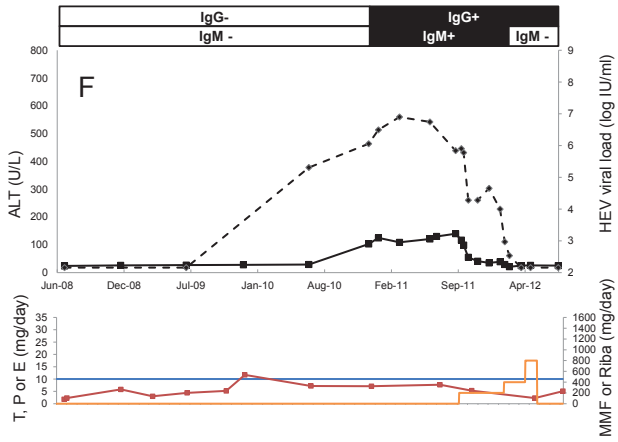
LEGEND

- ALT (U/L)
- HEV viral load (log IU/mL)
- Prednison dose (mg/day)
- Everolimus dose (mg/day)
- Ribavirin dose (mg/day)
- MMF dose (mg/day)
- serum Tacrolimus through levels (µg/L)

D. Patient 4.



E. Patient 5, who received a kidney transplant in 2008, which explains the increase in immunosuppressant therapy.



F. Patient 6.

Fig. 4. D-F. Clinical course of chronic HEV infection in 6 heart transplant recipients. The ALT upper limit of normal is 30 U/liter and 40 U/liter for females and males, respectively. The HEV RNA lower limit of detection is 143 (2.16 log) IU/ml.

patient was non-compliant with the ribavirin therapy. At the time of this writing, Patient 6 had been treated for 9 months with ribavirin, with several dose increases, although HEV RNA was still detectable in the most recent follow-up sample.

DISCUSSION

In this study we investigated 6 chronic HEV-infected heart transplant recipients to obtain greater insights into the clinical, biochemical and virologic presentation. The 6 patients represented 2.3% of 263 HTx recipients of whom HEV infection was identified and confirmed by real-time RT-PCR and serology. All patients developed chronic HEV infection and phylogenetic analysis classified all isolates within genotype 3. No direct relations between the isolated viruses were observed, thereby excluding a common source in our patients.

Production of HEV-specific IgM and IgG antibodies was delayed up to 301 and 539 days, respectively, after HEV RNA was detectable for the first time. This finding supports previous reports that testing for HEV infection in transplant recipients with elevated liver enzymes should be performed by HEV RNA (real-time RT-PCR) rather than screening for antibodies [8,10,12].

Sixty-seven percent of the chronically infected patients had already progressed to advanced fibrosis within 2 years of initial infection. Rapid progression of fibrosis in HEV infected transplant recipients was described in other studies [8,9,14]. We cannot exclude the possible presence of some pre-existing liver disease, such as that due to right-sided heart failure before transplantation or as a consequence of drug-induced liver disease after transplantation. However, 4 of 6 patients had normal liver enzyme values before the first positive HEV PCR. Our study further emphasizes that early detection and, when possible, intervention is needed to prevent severe liver damage.

All (chronic) HEV-infected patients had elevated liver enzyme values shortly after HEV RNA was first detectable in serum. Therefore, in patients on immunosuppressive drugs, hepatitis E virus infection should always be part of the differential diagnosis of all raised liver enzymes. Interestingly, a high peak ALT level (>20 times the ULN) was seen in the only patient who spontaneously cleared the chronic HEV infection. Previously, in a group of 85 HEV-infected transplant recipients, clearance of HEV infection within 6 months was associated with higher peak ALT levels compared with transplant recipients who did not clear the HEV infection within 6 months [27]. Our study has also shown that, even after an infection duration of 46 months, HEV can be cleared spontaneously in immunocompromised patients.

Previous studies have indicated that the non–travel associated HEV infection route is thought to be zoonotic of origin [1,2]. We found that the non-immunocompromised spouses of the HEV-infected patients in this study did not have an active in-

fection or had seroconversion, in accordance with the theory that person-to-person infection is unlikely.

In our cohort, all patients received tacrolimus-based immunosuppressive therapy. Previously, tacrolimus has been described as a risk factor for developing chronic HEV infection in SOT recipients [27].The first step in the treatment of chronic HEV-infected patients is, whenever possible, a reduction of immunosuppressive therapy. In this study, dose reduction led to clearance of the HEV infection in 1 patient and in a decline in the viral load of HEV in other patients. However, in the majority of patients, complete clearance of HEV infection only followed treatment with ribavirin. Low-dose ribavirin was sufficient in only one out of four patients. Therefore, an approach with stepwise dosing of ribavirin seems reasonable to minimize side effects and to optimize the dose effectively. Low-dose ribavirin was able to normalize liver enzymes within weeks, but in all patients viral clearance took longer. The time correlation between ribavirin therapy and reduction in viral load and liver parameters further emphasizes the effectiveness of ribavirin in HEV infection in this patient group.

A clear limitation of our study is that infections cleared earlier may have been missed.

In conclusion, chronic HEV infection in heart transplant recipients may lead to rapid fibrosis of the liver. Our study has highlighted the need for early detection of HEV infection in immunocompromised patients and the importance of early medical intervention, if possible, by reducing immunosuppressive therapy and, if insufficient, by introduction of ribavirin. We advise additional HEV RNA screening in immunocompromised patients with elevated liver enzymes because antibody production is often delayed.

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CHAPTER 10

Summary and future perspectives

BACKGROUND

Since the broad spectrum antiviral activity of ribavirin was discovered in 1972, the use of this nucleoside analogue has played a major role in the changing landscape of treatment for viral hepatitis. Currently, ribavirin is used in treatment regimens for patients infected with hepatitis C virus (HCV) as well as hepatitis E virus (HEV). The global health burden of HCV and HEV infection is considerable. Chronic HCV infection leads to liver cirrhosis in at least 20% of patients[1], which often leads to liver failure or the development of hepatocellular carcinoma. In fact, chronic HCV infection is one of the leading indications for liver transplantation in the western world. Chronic HEV infection is seen in immunocompromised patients, in particular transplant recipients. HEV infection in immunocompromised patients may lead to rapid fibrosis and results in cirrhosis in around 14% of solid organ transplant recipients [2-4]. In this thesis the evolving role of ribavirin treatment in patients with HCV and/or HEV infection is evaluated, with the purpose to optimize treatment regimens and reduce side effects.

Part one of this thesis focuses on multiple aspects of ribavirin therapy in patients infected with HCV

In **chapter 2** the results of the Dutch multicentre randomized controlled trial 'VIRID' are reported. For years, ribavirin in combination with peginterferon has been the standard of care for HCV treatment. Especially patients infected with HCV genotype 1 or 4 with a high viral load before start of this duo therapy, have notoriously low sustained virological response (SVR; HCV RNA negativity 24 weeks after discontinuation of treatment) rates. Around half of these patients with 'unfavourable' baseline characteristics does not achieve SVR [5-7]. Since multiple studies have shown that optimal and/or higher dosing of ribavirin increases SVR rates [8-14], the VIRID study was designed to evaluate whether genotype 1 and 4 patients with a high baseline viral load who received high-dose weight-based ribavirin (HDR; 25-29mg/kg) in combination with peginterferon (alfa-2a) achieved better SVR rates after 48 weeks of treatment compared to patients on standard-dose weight-based ribavirin (SDR; 12-15mg/kg) in combination with peginterferon. Eighteen different treatment sites in the Netherlands, divided over 14 treatment regions, included 110 patients in the VIRID study. A total of 28/52 (56.0%) patients in the HDR group and 26/58 (45.6%) patients in the SDR group achieved SVR (p=0.28). In the multivariate logistic regression, we found that HDR in treatment adherent patients (continued prescription of at least 80% of peginterferon and ribavirin for at least 80% of the treatment duration), improves SVR rates (OR 3.9; 95%CI 1.3-11.2, p=0.013). As expected, significantly more patients in the HDR group required epoetin to treat ribavirin-induced anemia (75% compared to 41% in the SDR group; p<0.0004). With the use of epoetin, the development of

severe anemia was rare; only one patient in the HDR group had a Hb-drop below 4.0 mmol/l. We therefore conclude that HDR may be a feasible option in patients who do not have access to the newer treatment regimens with direct acting antivirals (DAAs). Another way of ensuring patients are treated with optimal ribavirin doses during peginterferon and ribavirin duo therapy, is monitoring of ribavirin serum concentrations. Adequate ribavirin serum levels are essential for optimal SVR rates [15-20]. However, ribavirin pharmacokinetics show a large inter-patient variability [17, 21]. Therapeutic drug monitoring followed by dose adjustments could be the solution for optimizing individual duo therapy. However, since it takes weeks before ribavirin steady-state is reached [21], adjustment and optimization of ribavirin doses may come too late to influence treatment outcome. However, it has been proposed that the area under the concentration time curve up to 4 hours after intake of ribavirin ($AUC_{0\text{-}4h}$) of the very first weight-based dose of ribavirin should be ≥1.755mg.h/L to guarantee the highest chance of SVR [22]. If this optimal dose is determined before start of treatment, true individualized duo therapy can be achieved. Therefore, in **chapter 3** we introduce the ARRIBA concept: Adequate Resorption of RIBAvirin. Twenty-six patients were given a single weight-based dose after which the $AUC_{0\text{-}4h}$ was measured in serial blood samples taken up to 4 hours after ingestion. Sixty-five percent of patients did not achieve an adequate $AUC_{0\text{-}4h}$ of ≥1.755mg.h/L. On day 29, these patients received an adjusted dose. This led to 62% of the total population achieving an adequate $AUC_{0\text{-}4h.}$ Individualizing optimal ribavirin dosing through calculating $AUC_{0\text{-}4h}$ before treatment instead of advocating higher dosing of ribavirin for all patients, may decrease side effects such as anemia, since not all patients will be in need of high ribavirin doses due to the great inter-patient variability in ribavirin pharmacokinetics.

Patients who are initiated on ribavirin containing therapy are, according to treatment guidelines, advised to use double contraception. This is because ribavirin has been proven to be teratogenic in animal models [23-26]. In addition, changes in animal sperm count and morphology have been observed after exposure to ribavirin [23, 24, 27, 28]. Therefore, the use of ribavirin is contraindicated during pregnancy and in men whose partners may become pregnant for up to seven months prior to conception. In humans, the effect of ribavirin on pregnancy outcomes are less well studied due to ethical reasons. In **chapter 4** the effect of therapy with ribavirin and peginterferon on semen is studied. A total of 23 HCV infected male patients provided semen samples of which 19 had on-treatment and follow up samples available. 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 peginterferon and ribavirin treatment and during follow up. We did find a significant decrease of semen volume at week 12 of treatment, which may be related to a decrease in sexual arousal during antiviral treatment [29]. We also assessed the DNA

fragmentation index, a predictor of spontaneous pregnancies and the occurrence of miscarriage. It is yet unclear if DNA integrity is also associated with an increased risk of birth defects, though theoretically it could be argued that DNA damage increases the risk of malformation during embryonic development [30]. The DNA fragmentation of HCV treated patients did not significantly differ from 22 proven fertile healthy controls, nor did they alter during peginterferon and ribavirin therapy. These findings suggest that the need for male contraception during treatment and up to seven months after treatment cessation is questionable. On top of that, most pregnancies after paternal exposure to ribavirin result in normal live born infants [31-33]. Male patients whose female partners become pregnant during the advised double contraception period, can be cautiously reassured.

Ribavirin treatment in transplant recipients is challenging due to comorbidity and especially co-medication that can lead to considerable interactions or synergistic side effects. For example, bone marrow suppression is a common side effect of the immunosuppressive medication patient receive to prevent rejection. Since peginterferon and ribavirin treatment is notorious for the induction of cytopenias, treating HCV infected transplant patients with this duo therapy may lead to serious complications. Cytopenias require per protocol dose reductions of the antiviral medication, which increases the risk of therapy failure. However, the occurrence of cytopenias during HCV treatment with peginterferon and ribavirin does not necessarily have to lead to an increased risk of bleeding and infection. In fact, in non-transplant patients, multiple studies did not find an association between leukopenia and thrombocytopenia versus infection and bleeding respectively [34-45]. In **chapter 5** we investigated whether cytopenias in transplant patients on HCV treatment with (peg)interferon and/or ribavirin were associated with bleeding and infection. A total of 135 liver transplant recipients received 178 treatments with peginterferon or standard interferon and/or ribavirin. Bleeding and infection were common in this cohort with up to 42% of patients experiencing either or both of these complications during HCV treatment, often leading to hospitalization (respectively in 46% and 24%). Two patients died, most likely due to HCV recurrence rather than treatment-related infection. Thrombocytopenia (lower platelet count) and leukopenia (lower white blood cell count) were associated with respectively bleeding ($p<0.0001$) and infection ($p=0.0009$) in the multivariate analysis. We created a prediction model for the risk of bleeding and infection. Bleeding increased exponentially when the platelet count dropped below $50x10^9$/l and the risk of infection increased exponentially when the white blood cell count dropped below $2x10^9$/l in our prediction model. This study shows that the per protocol dose reduction of antiviral medication to prevent bleeding and infection, should not be abandoned in transplant recipients.

On top of the side effects of (peg)interferon and ribavirin therapy in liver trans-

plant recipients, the efficacy is moderate at best. Approximately a quarter of liver transplant recipients achieves an SVR on this HCV duo therapy [46]. When the first generation protease inhibitors boceprevir and telaprevir became available as an add-on to peginterferon and ribavirin therapy for patients with genotype 1 HCV infection, great concerns were raised regarding the interaction with calcineurin inhibitors. Boceprevir and telaprevir are substrates as well as strong inhibitors of cytochrome P450 3A4 (CYP3A4). Inhibition of CYP3A4 increases the systemic exposure to the calcineurin inhibitors *cyclosporine* and *tacrolimus*, both of which are primarily metabolized by CYP3A4. Due to the narrow therapeutic index of tacrolimus and cyclosporine, under- and overexposure due to interaction with boceprevir or telaprevir, may result in respectively organ rejection and nephrotoxicity. In **chapter 6** the real life experience of the treatment of 60 HCV genotype 1 infected liver transplant patients with boceprevir (N=25) or telaprevir (N=35) based antiviral therapy is reported. Since pharmacokinetic studies showed that boceprevir or telaprevir administration in combination with tacrolimus leads to far greater increase in tacrolimus serum concentrations, compared to cyclosporine, patients were, where possible, initiated on cyclosporine therapy before start of HCV treatment [47, 48]. To achieve target trough levels cyclosporine was reduced to approximately 35% in telaprevir based treatment and 50% in boceprevir based treatment.

At week 24 67% (N=14/21) of telaprevir treated patients were HCV RNA negative, compared to 45% (N=10/22) of boceprevir treated patients. These patients were eligible for treatment continuation and still had a chance of achieving an SVR. Cytopenias were very common in this treatment cohort with all patients requiring dose reductions of peginterferon and/or ribavirin or the administration of haematological growth factors. Decline in renal function was common during protease inhibitor therapy.

In **chapter 7** we provide an in-depth analysis of the possible pathways that lead to renal insufficiency during triple therapy in liver transplant recipients. Seventeen liver transplant recipients on cyclosporine, of which 13 were included in the analysis in chapter 6, were treated with boceprevir based HCV treatment. The first 16 weeks of treatment (first 4 weeks of peginterferon and ribavirin duo therapy, after which boceprevir was added) were analysed. Anemia was universal and progressive during treatment with an average 12% haemoglobin drop during peginterferon and ribivarin duo therapy followed by an additional drop of 16% in the first four weeks of boceprevir addition. Ribavirin doses were reduced by 39% on average by week 16 of treatment, even though start doses were relatively low (<600 mg/day on average). Ribavirin levels at week 4 of treatment, the time point at which a steady state was expected, were below the therapeutic target level in 6 out of 8 patients. Despite stable cyclosporine levels at the lower end of the therapeutic range, the decrease in glom-

erular filtration rate was progressive during boceprevir treatment. Based on all these factors, we propose a theoretical model where boceprevir and ribavirin induced anemia leads to an increase in the unbound fraction of cyclosporine in plasma. Despite apparent normal cyclosporine troughs measured in whole blood, this free plasma fraction causes nephrotoxicity. A decrease in glomerular filtration rate, will lead to a diminished clearance of ribavirin, thus leading to higher ribavirin serum levels, inducing hemolysis and anemia. We suggest that sequential ribavirin level monitoring followed by aggressive ribavirin dose reductions may prevent this cycle of progressive anemia and nephrotoxicity.

Part 2 of this thesis focuses on multiple aspects of ribavirin treatment in patients infect with HEV

Since in 2008 the first reports emerged on chronic HEV infection in immunocompromised patients, ribavirin, due to its broad antiviral activity, was soon tried as a treatment option [49-51]. Since ribavirin is also used for the treatment of chronic HCV, a chronic HEV infection in HCV infected liver transplant recipients may have falsely been diagnosed as a (severe) recurrence of HCV and subsequently be cured after initiation of HCV therapy with peginterferon and/or ribavirin. In **chapter 8** we set out to investigate the prevalence of (chronic) HEV infection in 145 HCV infected liver transplant recipients. Baseline seroprevalence was 37% and seroconversion was lost in 33% of these patients after liver transplantation. Post-transplant, eight patients (6.4%) had IgG seroconversion and 1 patient (0.6%) had IgM seroconversion. These patients were thought to have been infected with HEV post-transplant. However, additional HEV PCR testing did not reveal an active (or chronic) HEV infection. All but one of the post-transplant seroconverters were treated with (peg)interferon and/or ribavirin before or at the moment of seroconversion. This study showed that, since the HEV seroprevalence in HCV infected liver transplant recipients is high, post-transplant infection with HEV should always be part of the differential diagnosis of elevated liver enzymes. Treatment with peginterferon and ribavirin in liver transplant recipients leads to serious side effects (see also **chapter 5**) and can be easily avoided if liver enzyme elevation is not due to HCV recurrence, but to HEV infection. Since calcineurin inhibitors are found to stimulate HEV replication in vitro [52], the first step in HEV management should not be aggressive antiviral therapy, but dose reduction of immunosuppressive medication,ss demonstrated in **chapter 9**, where we describe the clinical course of six heart transplant recipients with chronic HEV. Four of these patients had progressed to advanced fibrosis within 2 years of HEV infection. One patient was able to clear the chronic HEV infection spontaneously and an-

other after dose reduction of immunosuppressive medication. One patient was able to clear HEV on low-dose ribavirin therapy, while two other patients required stepwise increase of ribavirin dose to attain clearance. One patient had several dose increases of ribavirin, but had not been able to clear HEV after 9 months of treatment. Since this study showed that chronic HEV may lead to rapid fibrosis, early detection and intervention is important. We advise a stepwise approach, with reduction of immunosuppressive medication and if insufficient, low and stepwise dosing of ribavirin.

The future of ribavirin for the treatment of chronic HCV

Interferon-free regimens for the treatment of chronic HCV have become the standard of care in most countries. The value of the findings in this thesis regarding peginterferon and ribavirin duo therapy cannot be extrapolated to treatments containing direct acting antiviral agents. However, in resource constrained countries, patients will still have to rely on classic duo therapy for the considerable future [53-56]. Optimizing treatment for patients with poor health care infrastructure should preferably be obtained through the provision of interferon-free regimens that have much better efficacy and considerable less side effects. In the meantime, optimizing ribavirin doses could be a means through which better SVR rates can be achieved in these patients. A pragmatic treatment approach with high dose ribavirin in patients who are able to tolerate such doses, should be considered.

Nonetheless, ribavirin is expected to remain part of several interferon-free treatment regimens for the foreseeable future, especially in the setting of direct acting antivirals with a low barrier to resistance or in patients that are notoriously hard to treat, such as patients who previously failed on peginterferon and ribavirin duo therapy or therapy with direct-acting antivirals and patients with liver cirrhosis [57]. Fortunately, ribavirin side effects are quite tolerable in interferon-free regimens.

The future of ribavirin for the treatment of chronic HEV

If dose reduction of immunosuppressive medication fails to induce HEV clearance in transplant recipients, the first step remains treatment with ribavirin. However, ribavirin treatment failure associated with HEV mutations has been described [58-60], though the occurrence of ribavirin resistant HEV mutations does not necessarily have to lead to treatment failure [61]. Alternative treatment options for patients who develop ribavirin resistant HEV mutations or cannot be treated with ribavirin, for example due to pregnancy or allergies, are necessary. It seems that sofosbuvir, a direct acting antiviral agent currently also used for the treatment of chronic HCV, may be a serious candidate since it inhibits HEV in vitro [62] and led to HEV viral load decline in a patient who

developed ribavirin resistant mutations [63]. Nonetheless, the combination of ribavirin and sofosbuvir was more potent than sofosbuvir monotherapy [62]. Currently, ribavirin remains the most effective compound for the treatment of chronic HEV.

In the long run, ribavirin may become obsolete for the treatment of chronic HEV and HCV. However, don't be surprised when due to the versatility of this broad antiviral active nucleoside analogue, it appears again in the future as part of treatment regimens of other viral infections. Better understanding of the mode of action of ribavirin will be instrumental for finding new and optimizing old indications. More research in this field should therefore be embarked upon.

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Nederlandse samenvatting

INLEIDING

Sinds in 1972 werd ontdekt dat ribavirine een breed spectrum van antivirale activiteit heeft, is deze nucleoside-analoog een belangrijk onderdeel geweest in het veranderende behandellandschap van virale hepatitis. Momenteel wordt ribavirine gebruikt in behandelregimes voor patiënten geïnfecteerd met zowel het hepatitis C-virus (HCV) als het hepatitis E-virus (HEV). HCV- en HEV-infecties hebben een grote impact op de wereldwijde gezondheid. Chronische HCV-infectie leidt tot levercirrose bij minstens 20% van de geïnfecteerde patiënten[1], vaak resulterend in leverfalen of het ontstaan van hepatocellulair carcinoom (leverkanker). Een chronische HCV-infectie is zelfs een van de meest voorkomende indicaties voor levertransplantatie in de westerse wereld.

Chronische HEV-infectie komt voor bij immuungecompromitteerde patiënten, in het bijzonder patiënten met een orgaantransplantatie in de voorgeschiedenis. HEV-infectie bij immuungecompromitteerde patiënten kan leiden tot snelle ontwikkeling van leverfibrose, uiteindelijk resulterend in cirrose bij ongeveer 14% van de patiënten die een orgaantransplantaat hebben ontvangen [2-4]. In dit proefschrift wordt de rol van ribavirine in het veranderende behandellandschap van patiënten met een HCV- en/of HEV-infectie geëvalueerd, met als doel behandelregimes te optimaliseren en bijwerkingen te verminderen. In **hoofdstuk 1** wordt een algemene inleiding en uiteenzetting gegeven van de epidemiologie en behandeling van HCV- en HEV-infecties.

Deel één van dit proefschrift focust op diverse aspecten van ribavirinetherapie bij patiënten geïnfecteerd met HCV.

In **hoofdstuk 2** worden de resultaten besproken van de Nederlandse multicenter gerandomiseerde, gecontroleerde 'VIRID'-studie. Jarenlang is ribavirine in combinatie met peginterferon de standaardbehandeling voor HCV-infectie geweest. Helaas hebben patiënten met HCV-genotype 1 of 4 en een hoog aantal virusdeeltjes in het bloed voor aanvang van de behandeling (baseline virale load) notoir slechte behandelresultaten. Ongeveer de helft van de patiënten met deze ongunstige aanvangskenmerken behaalt geen *sustained virological response* (SVR; het afwezig zijn van virusdeeltjes in het bloed 24 weken na het staken van de behandeling) [5-7]. Aangezien meerdere studies hebben aangetoond dat het optimaliseren of verhogen van de dosis van ribavirine SVR-percentages verbetert [8-14], is de VIRID-studie ontworpen om te evalueren of genotype 1 en 4 patiënten met een hoge baseline virale load behandeld met een hoge dosis ribavirine (HDR) aangepast aan gewicht (25-29 mg/kg) in combinatie met peginterferon (alfa-2a) betere SVR-percentages behalen na 48 weken behandeling in vergelijking met een standaard dosis ribavirine (SDR) aangepast

aan gewicht (12-15 mg/kg) in combinatie met peginterferon. In totaal 110 patiënten uit 18 behandelcentra in Nederland, verspreid over 14 behandelregio's, deden mee aan de VIRID-studie. In de HDR-groep behaalden 28 van de 52 patiënten (56.0%) en in de SDR-groep 26 van de 58 patiënten (45.6%) een SVR, maar dit was niet significant. Uit de multivariate logistische regressie bleek dat patiënten die zich aan de behandeling hielden (dat wil zeggen tenminste 80% van de behandelduur minimaal 80% van de peginterferondosis en minimaal 80% van de ribavirinedosis kregen) een hoger SVR-percentage hadden (OR 3.9; 95%CI 1.3-11.2, p=0.013). Zoals verwacht hadden significant meer patiënten in de HDR-groep erytropoëtine nodig om ribavirine-geïnduceerde anemie te behandelen (75% versus 41% in de SDR groep; p<0.0004). Met het gebruik van erytropoëtine kwam ernstige bloedarmoede zelden voor; slechts bij één patiënt in de HDR-groep daalde het hemoglobinegehalte onder de 4.0 mmol/l. Gezien deze bevindingen zou HDR een geschikte optie kunnen zijn voor HCV-patiënten met ongunstige aanvangskenmerken die geen toegang hebben tot de nieuwere behandelregimes met Direct Acting Antivirals (DAA's).

Een andere manier om een optimale ribavirinedosering te garanderen gedurende duotherapie met peginterferon en ribavirine, is het monitoren van de serumconcentratie van ribavirine. Adequate serumconcentraties zijn essentieel om optimale SVR-percentages te behalen [15-20]. Helaas is de farmacokinetiek van ribavirine tussen patiënten onderling erg verschillend [17, 21], waardoor patiënten onderling niet goed met elkaar vergeleken kunnen worden. Het vervolgen van de serumconcentraties tijdens therapie (therapeutische geneesmiddelmonitoring) gevolgd door dosisaanpassingen bij de individuele patiënt zou een goede oplossing kunnen zijn voor het optimaliseren van duotherapie. Het is echter lastig om gedurende duotherapie de dosis van ribavirine te optimaliseren door middel van therapeutische geneesmiddelmonitoring, omdat een stabiele concentratie van ribavirine in het bloed pas na weken wordt bereikt [21]. Het tijdstip van aanpassen en optimaliseren van de ribavirinedosis kan dan te ver in de behandelperiode liggen om de uitkomst nog te kunnen beïnvloeden. In een eerdere studie is geopperd dat de ideale 'Area Under the Curve' tot 4 uur na inname van een enkele dosis ribavirine (AUC_{0-4h}) meer dan 1.755mg.h/L moet zijn om de beste kans op SVR te garanderen [22]. Als deze optimale dosis vóór de start van duotherapie wordt bepaald, kan daadwerkelijk geïndividualiseerde HCV-behandeling worden gecreëerd. Daarom introduceren we in **hoofdstuk 3** het ARRIBA-concept: Adequate Resorptie van RIBAvirine. Zesentwintig patiënten kregen een eenmalige dosis ribavirine, gebaseerd op hun lichaamsgewicht, waarna de AUC_{0-4h} werd gemeten in opeenvolgende bloedafnames tot 4 uur na inname. Zesenvijftig procent van de patiënten behaalde niet de streef-AUC_{0-4h} van ≥1.755mg.h/L op deze aanbevolen dosis. Op dag 29 kregen deze patiënten een aangepaste dosis. Hierop steeg het aantal patiënten met een adequate AUC_{0-4h} naar 62%. Het optimaliseren

van de ribavirinedosering bij individuele patiënten (door het berekenen van de AUC_{0-4h} voor aanvang van duotherapie), in plaats van het aanraden van hogere doseringen ribavirine voor alle patiënten, kan bijwerkingen zoals bloedarmoede reduceren, omdat niet alle patiënten een hoge dosis ribavirine nodig hebben gezien de grote verschillen in ribavirinefarmacokinetiek tussen patiënten onderling.

In behandelrichtlijnen voor HCV wordt patiënten geadviseerd om dubbele anticonceptie te gebruiken als zij worden behandeld met ribavirine. Dit advies is gebaseerd op bewezen teratogeniciteit (het vermogen om schade aan de ongeboren vrucht toe te brengen) van ribavirine in proefdiermodellen [23-26]. Uit diverse studies is gebleken dat het aantal spermacellen en de morfologie veranderen bij dieren die bloot zijn gesteld aan ribavirine. [23, 24, 27, 28]. Op grond hiervan is het gebruik van ribavirine gecontra-indiceerd gedurende zwangerschap en door mannen die een kind willen verwekken vanaf start van de behandeling tot 7 maanden na blootstelling aan ribavirine.

Bij mensen is het effect van ribavirine op zwangerschapsuitkomsten minder goed onderzocht, mede vanwege ethische implicaties. In **hoofdstuk 4** is het effect van behandeling met ribavirine en peginterferon op semenkwaliteit onderzocht. In totaal 23 HCV-geïnfecteerde mannen verstrekten semenmonsters voor aanvang van duotherapie en 19 mannen verstrekten ook monsters gedurende de behandeling en tijdens follow-up. Ongeveer 40% van de mannen had afwijkingen in het sperma, al vóór aanvang van de behandeling. Spermaconcentratie en motiliteit, beide voorspellers van vruchtbaarheid, veranderden niet gedurende duotherapie met peginterferon en ribavirine of tijdens follow-up. Wel werd een significante daling in semenvolume geobserveerd na 12 weken behandeling, die mogelijk wordt veroorzaakt door een afname in seksuele opwinding gedurende duotherapie [29]. Daarnaast werd de DNA-fragmentatie-index bepaald, een voorspeller van spontane zwangerschappen en het optreden van miskramen. Het is overigens nog onduidelijk of verminderde DNA-integriteit is geassocieerd met het risico op geboortedefecten, hoewel theoretisch kan worden beredeneerd dat DNA-schade het risico op malformaties gedurende de embryonale ontwikkeling verhoogt [30]. De DNA-fragmentatie van patiënten geïnfecteerd met HCV verschilde niet van 22 bewezen vruchtbare en gezonde controledeelnemers, noch veranderde deze gedurende therapie met peginterferon en ribavirine. Deze resultaten suggereren dat het advies aan mannen om dubbele anticonceptie te gebruiken gedurende ribavirinetherapie en tot 7 maanden daarna in twijfel kan worden getrokken. Bovendien resulteren de meeste zwangerschappen waarbij de vader is blootgesteld aan ribavirine in gezonde baby's [31-33]. Mannelijke patiënten wiens vrouwelijke partners zwanger worden gedurende de periode dat oorspronkelijk dubbele anticonceptie wordt geadviseerd, kunnen derhalve voorzichtig worden gerustgesteld.

De behandeling van transplantatiepatiënten met ribavirine wordt gecompliceerd door comorbiditeit en in het bijzonder co-medicatie die kan leiden tot aanzienlijke interacties of synergistische effecten. Beenmergsuppressie bijvoorbeeld, leidend tot een laag aantal bloedcellen (cytopenieën), is een veelvoorkomende bijwerking van de immuunonderdrukkende medicatie die patiënten nemen om afstoting van het transplantaat te voorkomen. Aangezien peginterferon en ribavirine beide ook cytopenieën veroorzaken, kan de behandeling van HCV-geïnfecteerde transplantatiepatiënten met duotherapie leiden tot ernstige complicaties. Cytopenieën vereisen protocolgereguleerde dosisreducties van de antivirale medicatie, waardoor het risico op therapiefalen weer toeneemt. Het ontstaan van cytopenieën gedurende HCV-behandeling met peginterferon en ribavirine hoeft echter niet noodzakelijk te leiden tot een verhoogd risico op bloedingen en infecties; uit meerdere studies onder niet-getransplanteerde HCV-patiënten blijkt dat een associatie ontbreekt tussen leukopenie (een laag aantal witte bloedcellen) en trombocytopenie (een laag aantal bloedplaatjes) en respectievelijk infectie en bloedingen [34-45]. In **hoofdstuk 5** onderzoeken we of cytopenieën bij transplantatiepatiënten, die met (peg)interferon en/of ribavirine worden behandeld tegen een HCV-infectie, zijn geassocieerd met bloedingen en infecties. In totaal 135 ontvangers van een levertransplantatie werden in totaal 178 keer behandeld met peginterferon of standaard interferon en/of ribavirine. Bloedingen en infecties kwamen veelvuldig voor in dit cohort; tot 42% van de patiënten had één of beide van deze complicaties gedurende HCV-behandeling, vaak leidend tot opname in het ziekenhuis (respectievelijk 46% en 24%). Twee patiënten overleden, waarschijnlijker door complicaties van herinfectie van het levertransplantaat met HCV dan complicaties van de behandeling. In de multivariate analyse werd een associatie gevonden tussen trombocytopenie en leukopenie en respectievelijk bloedingen (<0.0001) en infecties (p=0.0009). We creëerden een predictiemodel voor het risico op bloedingen en infecties. In ons predictiemodel neemt het risico op bloedingen exponentieel toe als het aantal bloedplaatjes daalt onder de $50x10^9$/l en het risico op infecties exponentieel toe als het aantal witte bloedcellen daalt onder de $2x10^9$/l. Deze studie toont aan dat protocolgereguleerde dosisreducties van antivirale medicatie teneinde bloedingen en infecties te voorkomen niet moeten worden gewijzigd voor transplantatiepatiënten.

Naast dat levertransplantatiepatiënten veel bijwerkingen ervaren van (peg)interferon- en ribavirinetherapie, is de effectiviteit in deze populatie op zijn best matig te noemen. Ongeveer een kwart van de levertransplantatiepatiënten bereikt een SVR met HCV-duotherapie [46].

Toen de eerste generatie proteaseremmers *boceprevir* en *telaprevir* op de markt kwamen als onderdeel van tripletherapie met peginterferon en ribavirine van patiënten met genotype 1 HCV-infectie, werden er zorgen geuit over de interactie

met specifieke immuunonderdrukkende medicijnen, de zogenaamde calcineurineremmers. Boceprevir en telaprevir zijn zowel substraat als sterke remmers van het eiwit 'cytochroom P450 3A4' (CYP3A4). Remming van CYP3A4 verhoogt de concentratie van de calcineurineremmers *cyclosporine* en *tacrolimus* in het bloed, beide immuunonderdrukkende medicijnen die door CYP3A4 worden gemetaboliseerd. Gezien de nauwe therapeutische breedte van tacrolimus en cyclosporine, is er een groot risico op te lage of juist te hoge spiegels in het bloed door de interactie met boceprevir en telaprevir, resulterend in respectievelijk afstoting van het transplantaat en nierschade. In **hoofdstuk 6** wordt de klinische ervaring beschreven van 60 met HCV-genotype 1 geïnfecteerde levertransplantatiepatiënten die antivirale therapie kregen met boceprevir (N=25) of telaprevir (N=35). Omdat farmacokinetische studies hebben aangetoond dat boceprevir of telaprevir in combinatie met tacrolimus leidt tot een veel sterkere stijging in tacrolimus-serumconcentraties in vergelijking met cyclosporine [47, 48], werden patiënten zo mogelijk voor aanvang van de antivirale therapie overgezet op cyclosporine. Teneinde streefdalspiegels te behalen werd de cyclosporinedosering gereduceerd tot gemiddeld 35% bij patiënten die telaprevir kregen en 50% bij patiënten die boceprevir kregen. Na 24 weken therapie was 67% (N=14/21) van de telaprevir-behandelde patiënten HCV-RNA-negatief, in vergelijking met 45% (N=10/22) van de boceprevir-behandelde patiënten. Dit waren de patiënten die in aanmerking kwamen voor doorbehandeling, omdat zij nog kans hadden op het behalen van een SVR. Cytopenieën kwamen veelvuldig voor; alle patiënten hadden dosisreducties van peginterferon en/of ribavirine nodig, dan wel behandeling met hematologische groeifactoren. Afname van de nierfunctie werd ook regelmatig geobserveerd gedurende tripletherapie met proteaseremmers.

In **hoofdstuk 7** worden de mogelijke mechanismen die schuilgaan achter afname van de nierfunctie gedurende tripletherapie met proteaseremmers bij levertransplantatiepatiënten uitgediept. Zeventien HCV-geïnfecteerde patiënten die een levertransplantatie hebben gehad en cyclosporine kregen voorgeschreven, werden behandeld met tripletherapie met boceprevir. Dertien van deze patiënten werden ook geïncludeerd in de analyse in hoofdstuk 6. De eerste 16 weken van behandeling (waarvan de eerste 4 weken duotherapie met peginterferon en ribavirine betrof, waarna boceprevir werd toegevoegd) werden geanalyseerd. Bloedarmoede kwam in de gehele groep voor en was progressief gedurende de behandeling met een gemiddelde daling in hemoglobine van 12% gedurende de eerste 4 weken duotherapie met peginterferon en ribavirine, gevolgd door een extra daling van 16% in de eerste 4 weken na toevoeging van boceprevir. De ribavirinedosering werd gereduceerd tot gemiddeld 39% ten tijde van week 16, terwijl de aanvangsdosering al relatief laag lag (gemiddeld <600mg/dag). De ribavirinespiegel na 4 weken behandeling, het moment waarop een stabiele spiegel bereikt zou moeten zijn, was onder de streef-

waarde bij 6 van de 8 patiënten. Ondanks stabiele cyclosporinespiegels tegen de ondergrens van de therapeutische streefwaarde was er sprake van een progressieve daling van de glomerulaire filtratiesnelheid (nierfunctie) gedurende tripletherapie. Al deze factoren in acht nemend, presenteren we een theoretisch model waarin boceprevir- en ribavirine-geïnduceerde bloedarmoede leidt tot een toename in de ongebonden fractie van cyclosporine in plasma.

Ondanks ogenschijnlijk normale dalspiegels van cyclosporine, die worden gemeten in volbloed, veroorzaakt de vermoedelijk veel hogere vrije plasmafractie nierschade. Een daling in de glomerulaire filtratiesnelheid zal bovendien leiden tot een verminderde klaring van ribavirine, leidend tot hogere serumspiegels van ribavirine, waardoor hemolyse en bloedarmoede verergeren. We stellen dat het herhaaldelijk meten van de ribavirinespiegels gevolgd door een streng beleid met betrekking tot dosisreducties van ribavirine de cyclus van progressieve bloedarmoede en nierschade kan voorkomen.

Deel 2 van dit proefschrift focust op diverse aspecten van ribavirinetherapie bij patiënten geïnfecteerd met HEV.

Sinds in 2008 de eerste publicaties verschenen over chronische HEV-infectie bij immuungecompromitteerde patiënten, werd ribavirine, vanwege de breedspectrum antivirale activiteit, al gauw geïntroduceerd als behandeloptie [49-51]. Omdat ribavirine ook wordt gebruikt voor de behandeling van chronische HCV, zou een chronische HEV-infectie bij levertransplantatiepatiënten abusievelijk gediagnosticeerd kunnen zijn als een (ernstige) herinfectie met HCV en vervolgens genezen kunnen worden doordat HCV-therapie met peginterferon en/of ribavirine wordt gestart. In **hoofdstuk 8** beschrijven we de prevalentie van (chronische) HEV-infectie bij 145 HCV-geïnfecteerde levertransplantatiepatiënten. Vóór transplantatie was de seroprevalentie 37%, maar bij 33% van deze patiënten verdwenen de antilichamen weer na levertransplantatie. Na transplantatie vond er bij acht patiënten (6.4%) IgG-seroconversie plaats en bij één patiënt (0.6%) vond er IgM-seroconversie plaats. Dit zijn patiënten die zeer waarschijnlijk na transplantatie geïnfecteerd zijn geraakt met HEV. Aanvullende bepaling van de HEV-RNA-load in het bloed doormiddel van PCR toonde echter geen actieve (of chronische) infectie aan. Op één na werden deze patiënten behandeld met (peg)interferon en/of ribavirine vóór of op het moment van seroconversie na transplantatie. Gezien de hoge HEV-seroprevalentie bij HCV-geïnfecteerde levertransplantatiepatiënten, toont deze studie aan dat HEV-infectie altijd in de differentiaaldiagnose moet staan bij patiënten met verhoogde leverenzymen na transplantatie.

De behandeling van levertransplantatiepatiënten met peginterferon en ribavirine

gaat gepaard met ernstige bijwerkingen (zie ook **hoofdstuk 5**) en kan voorkomen worden als verhoogde leverenzymen correct gediagnosticeerd worden als HEV-infectie, in plaats van HCV-herinfectie. Aangezien calcineurineremmers HEV-replicatie in vitro lijken te stimuleren [52], is de eerste stap in de behandeling van een HEV-infectie niet het starten van antivirale therapie, maar het toepassen van dosisreductie van immuunonderdrukkende medicijnen, zoals in **hoofdstuk 9**, waar we het klinisch beloop beschrijven van zes harttransplantatiepatiënten met een chronische HEV-infectie. Vier van deze patiënten hadden gevorderde leverfibrose ontwikkeld binnen twee jaar na HEV-infectie. Eén patiënt klaarde de chronische HEV-infectie spontaan en een tweede patiënt na dosisreductie van immuunonderdrukkende medicatie. Eén patiënt klaarde de HEV-infectie met een lage dosis ribavirine, terwijl twee andere patiënten stapsgewijze verhoging van de ribavirinedosis nodig hadden om het virus te klaren. Eén patiënt had al meerdere malen een dosisverhoging van ribavirine gehad, maar had na 9 maanden behandeling de HEV-infectie nog niet geklaard. Deze studie toont aan dat chronische HEV-infectie in relatief korte tijd kan leiden tot fibrose en daarom is vroege detectie en interventie belangrijk. We adviseren een stapsgewijze aanpak van een chronische HEV-infectie in deze populatie: beginnend met het verlagen van de dosis van immuunonderdrukkende medicatie en, indien dit niet voldoende werkt, behandeling met ribavirine, te starten met een lage dosis die stapsgewijs kan worden opgehoogd.

De toekomst van ribavirine voor de behandeling van chronische HCV

Chronische HCV-infectie wordt in de meeste landen inmiddels behandeld met interferonvrije behandelcombinaties. De resultaten in de studies in dit proefschrift op het gebied van duotherapie met peginterferon en ribavirine kunnen niet zomaar worden toegepast op behandelregimes met DAA's. Helaas zijn er nog steeds landen waar patiënten zijn aangewezen op duotherapie met peginterferon en ribavirine, omdat daar geen mogelijkheid is tot het verkrijgen van de nieuwere middelen [53-56]. Het optimaliseren van behandeling van patiënten in landen met een minder goed georganiseerde gezondheidszorg moet bij voorkeur plaatsvinden door het verstrekken van interferonvrije therapieën die veel effectiever zijn en aanzienlijk minder bijwerkingen hebben. Tot die tijd kan het optimaliseren van de ribavirinedosering bijdragen aan het verbeteren van SVR-percentages in deze populatie. Een pragmatische aanpak waarbij een hoge dosis ribavirine wordt gegeven aan patiënten die dit kunnen verdragen zou derhalve moeten worden overwogen.

Ribavirine zal in de nabije toekomst onderdeel blijven uitmaken van diverse interferonvrije behandelcombinaties, voornamelijk DAA's met een lage resistentie-

barrière, maar ook bij patiënten die notoir moeilijk zijn te behandelen, zoals zij die eerder niet genezen zijn na behandeling met duotherapie met peginterferon en ribavirine en/of DAA's en patiënten met levercirrose [57]. Gelukkig zijn de bijwerkingen van ribavirine goed te verdragen in combinatie met interferonvrije regimes.

De toekomst van ribavirine voor de behandeling van chronische HEV

Als dosisreductie van immuunonderdrukkende medicatie niet leidt tot het klaren van een chronische HEV-infectie bij transplantatiepatiënten is de eerste stap nog altijd behandeling met ribavirine. Helaas komt het voor dat behandeling met ribavirine faalt, waarschijnlijk door mutatie van het Hepatitis E-virus [58-60], hoewel het ontstaan van ribavirineresistente HEV-mutaties niet hoeft te leiden tot het falen van therapie [61]. Het is belangrijk dat er andere behandelmogelijkheden komen voor patiënten die ribavirineresistente HEV-mutaties ontwikkelen of om andere redenen niet met ribavirine kunnen worden behandeld, bijvoorbeeld vanwege zwangerschap of allergieën. Het lijkt erop dat sofosbuvir, een DAA die momenteel ook wordt gebruikt voor de behandeling van chronische HCV, mogelijk een serieuze kandidaat is aangezien het HEV in vitro onderdrukt [62] en leidt tot een daling in het aantal HEV-deeltjes in het bloed van patiënten die ribavirineresistente mutaties hadden ontwikkeld [63]. De combinatie van ribavirine met sofosbuvir was echter potenter dan monotherapie met sofosbuvir [62]. Momenteel blijft ribavirine het meest effectieve medicijn voor de behandeling van chronische HEV.

Op langere termijn zou ribavirine overbodig kunnen worden voor de behandeling van chronische HEV en HCV. Wees echter niet verbaasd als vanwege de veelzijdigheid van dit breedspectrum antivirale middel, ribavirine in de toekomst opeens opduikt als onderdeel van de behandeling van andere virale infecties. Meer inzicht in het werkingsmechanisme van ribavirine is noodzakelijk om nieuwe indicaties te ontdekken en oude te optimaliseren. Het is daarom belangrijk dat toekomstig onderzoek op het gebied van ribavirine zich hierop focust.

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Dankwoord

DANKWOORD

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Bettina, dank voor je ondersteuning op het gebied van de statistiek, wat zouden wij promovendi toch zonder jou moeten… Mijn eerste keer New York met jou, **Jeoffrey** en de Liver Ladies was een fantastische ervaring.

Het is een waar genoegen als je pad mag kruisen met coauteurs die net als jij een promotietraject aan het volgen zijn. **Klaartje**, dank voor de fijne ARRIBA-samenwerking. Het was heel leerzaam om alle aspecten van een studie, van indiening tot afronding, te mogen organiseren als tweede deelnemend centrum. **Suzan**, dankzij jou hebben IgG- en IgM-cut-offs geen geheimen meer voor mij. Ik ken ook niemand die zo bedreven is in Powerpoint. Dank voor de fijne samenwerking.

Dear colleagues at the MDL-lab: it was a pleasure to start at your welcoming department. The worst part about moving to clinical research was leaving you behind, I loved the inclusivity and diversity. In het bijzonder wil ik **Hanneke**, **Buddy**, **Jan** en **Thonie** bedanken voor de vele (VIRID)-samples die ze verwerkt hebben; er zijn prachtige resultaten uit voortgekomen.

Alle research nurses zonder wie er geen klinisch onderzoek mogelijk was geweest, in het bijzonder **Melek**, **Heleen** en **Lucille**; uit ervaring weet ik nu dat geen bloedvat veilig is voor jullie! Samen hebben we menig investigator-meeting bezocht door heel Europa, in een vliegveldhotel ver weg van alle toeristische hotspots; het leven van een onderzoeksteam gaat niet over rozen…

De harde werkers van het Clinical Research Bureau: **Edith**, **Irene** en **Wanda**, dank voor jullie ondersteuning bij mijn projecten. **Judith**, dankzij jou bleef het VIRID-treintje op de rails, en samen met **Elke** was er altijd ruimte voor strikt werkinhoudelijke besprekingen of snoep met dubieuze houdbaarheidsdatum. Fijn dat de deur nog steeds voor me openstaat nu ik terug ben in het Erasmus MC.

Alle poli-assistenten en in het bijzonder **Wilma**; als eerste aanspreekpunt voor de patiënt zijn jullie van onschatbare waarde. Dank voor jullie ondersteuning.

Marion en **Margriet**, door de jaren heen hebben jullie me met menig regelding geholpen. Marion, dank voor al je tips en tricks voor het logistieke deel rond mijn promotie.

Promoveren op het dak, oftewel de duiventil, is een continu gevecht. Met de elementen welteverstaan. Het is een wonder dat die keet ondanks tropische zomers en barre winters nog altijd rechtop staat. En het is werkelijk waar een genoegen, een eer en het beste dat me is overkomen sinds mijn terugkeer als AIOS MDL, dat ik er nu weer een bureaustoel mag bezetten.

Allereerst wil ik mijn roomies bedanken: **Leonie**, **Vincent de J**, **Veerle**, **Cokkie**, **Pauline** ('Ik heb een heel zwaar leven, echt waar'), **Edith** (roomies again, wij mazzelaars!), **Esmée** en **Priscilla**; dank voor alle gezelligheid en noodzakelijke werkontwijkende onderbrekingen.

Hepatitis C-buddies, **Robert**, **Daphne**, **Ad**, **Raoel**, en **Michelle** vanaf het lab uiteraard! Robert; de VIRID is af, mission accomplished. Daphne, ik herinner me dat ik zo blij was dat jij mijn collega werd toen ik naar het dak kwam; je hebt me wegwijs gemaakt in het onderzoek en me enthousiast gemaakt voor al die fantastische hepatitis C-patiënten; wat een voorrecht dat wij hun arts mochten zijn! Je schreef ooit dat we heel anders zijn, maar het feit wil dat als je maanden in Londen woont het simpelweg onethisch is om maar één keer een musical te bezoeken… Ik hoor nog je twijfel: een musical over een religieus boek, is dat een goed idee? Maar nu weet je wat je moet doen met je gevoelens als het in het leven tegenzit: *Turn it off, like a light switch*!

Natuurlijk mogen de andere hepa-buddies met wie ik menig hepacongres bezocht heb ook niet ongenoemd blijven: **Heng**, **Lisanne**, **Margo**, **Milan**, **Roeland**, **Vincent R**, **Willem-Pieter** en **Wim**, dank voor alle gezelligheid.

Alle andere dakduiven en collega-onderzoekers met wie ik lief en leed, congressen, borrels en skireizen gedeeld heb; dank voor de fantastische tijd. In het bijzonder wil ik de volgende personen noemen: Veerle, eindelijk had ik een plek op het dak

gevonden waar ik wat langer mocht blijven. En dan met jou als fijne kamergenoot. Het was fantastisch om met jou onze geheel verschillende promotietrajecten te kunnen delen en te filosoferen over de mooie en minder mooie dingen van het leven. **Jihan**, nooit gedacht dat ik op het dak iemand tegen zou komen met net zo'n brede smaak in cultureel vermaak. Hoogtepunt op dit gebied was natuurlijk de smaakvolle Sing-a-Long van Purple Rain-de-film. Daarnaast zijn wij het levende bewijs dat aspartaam onschadelijk is, getuige de hoeveelheid cola light die er doorheen is gegaan. Ik moet vaak vreselijk met je lachen en kan lief en leed met je delen, ik kijk uit naar je terugkomst naar het Erasmus MC!

Al mijn musicalgenoten uit de regio en vér, vér daar buiten: jullie gaven me een fantastische uitlaatklep naast alle promotieperikelen. In het bijzonder wil ik **Elzo** bedanken; jij wierp je op als mijn mentor en zonder jouw wijze inzichten, raad en onze etentjes was ik nooit zover gekomen als promoverende projectleider. Daarnaast is het gewoon ontzettend leuk om met jou te kletsen. **Ite**, dank voor alle gezelligheid, je scherpe analyses en je luisterend oor. Ik heb veel bewondering voor hoe jij bewust je leven vormgeeft. **Merel**, wat we verschillen in lengte maken we goed in praatvaardigheden. Wat is het fijn om met jou het leven onder de loep te nemen. Door onze saunadates is dit promotietraject een stuk lichter geworden.

Mijn paranimfen:

Lieve **Wendeline**, een hart van goud, ongelofelijke planningsvaardigheden en de enige die gek genoeg is om een week lang alleen maar musicals te kijken in New York: je bent de ideale vriendin. Ook als we elkaar even niet hebben kunnen zien door omstandigheden pakken we de draad meteen weer op; het is altijd thuiskomen bij jou. Het blijft ongelofelijk dat je tijdens je eerste dag aan de universiteit toevallig in dezelfde collegebank gaat zitten en daar een vriendschap voor het leven aan overhoudt. Onze levens hebben sinds die tijd niet stilgestaan; het was een eer om naast je te mogen staan bij jouw grote mijlpalen. Vandaag zijn de rollen omgedraaid en ben ik vereerd dat jij naast mij staat.

Lieve **Hanna**, mijn grote kleine zusje. Wat herken ik veel van jou in mezelf. Het is fijn om niet altijd mijn sarcastische grappen toe te hoeven lichten, maar iemand te kennen die er nog een schepje bovenop doet. Sinds we gestopt zijn met elkaar prikkeldraad te geven en elkaars dekbedkleur af te kraken, ben je mijn steun en toeverlaat en hoop ik dat ook voor jou te zijn, zeker nu de promotieperikelen vandaag tot een einde komen. Hoe ouder we worden, hoe meer ik besef wat een eer het is om je op te hebben mogen zien groeien tot de getalenteerde, slimme, empathische vrouw die je nu bent. Ik kijk uit naar het tweede avontuur dat je begin 2018 verwacht. Natuurlijk sta jij naast mij vandaag.

De rest van mijn fantastische familie: **Willem**, wie had gedacht dat toen je een afbeelding van een mij onbekende structuur op de familie-app gooide, deze de inspi-

ratie zou worden voor de cover van dit proefschrift? En hoe interessant is het dat je na 7 jaar onderzoek de structuurformule van ribavirine eigenlijk nog nooit gezien hebt en mijn eerste gedachte dan ook was: wat een gek hondje met een hoedje… Je bent mijn duetpartner in crime; dat de Four Kings nooit meer compleet zijn geweest sinds het eerste optreden wordt gewoon onze running gag. **Wouter**, het is fijn om een broer te hebben die net zo'n onredelijke liefde heeft voor actiefilms van het niveau Marvel en co. Ik kijk altijd uit naar onze (sushi-)etentjes vooraf waarin we de zaken des levens de revue laten passeren. Dank voor je hulp bij de vele wik- en weegmomenten gedurende mijn promotietraject. **Franca**, mijn Badass Bonuszus, ik heb veel bewondering voor hoe jij alle ballen hooghoudt. Dank voor al je hulp en wijze woorden. **Johannes**, vaak heb ik mijn hart bij jullie thuis mogen luchten en kwam jij met scherpe inzichten. Nu er gezinsuitbreiding aan zit te komen, wil ik wel natuurlijk wel oppassen op de...
Oculus Rift…

Zonder deze geweldige broers en zussen was ik nooit tante geweest van de leukste neefjes en nichtjes die voor een hoop vrolijke afleiding hebben gezorgd gedurende mijn promotietraject: **Sanne**, **Dewi**, **Jasper**, **Niels**, **Tirza** en haar toekomstige zusje.

Lieve **papa** en **mama**, wat jullie voor mij betekenen valt niet in een paar zinnen samen te vatten. Van jullie heb ik de liefde voor de muziek en het begrip voor de medemens meegekregen. Jullie hebben me onvoorwaardelijk gesteund tijdens dit promotietraject en me gestimuleerd mezelf te blijven. Het is een ongekend voorrecht dat jullie er altijd voor me zijn. Ik had me geen betere ouders kunnen wensen.

Over de auteur

Curriculum vitae
PhD portfolio
Bibliografie

CURRICULUM VITAE

Ludi Koning werd op 20 november 1984 geboren te Harderwijk. Na het cum laude behalen van haar eindexamen aan het *Studiehuis Molenplein* te Den Helder, koos zij voor de studie Geneeskunde aan de *Vrije Universiteit* van Amsterdam vanwege de mogelijkheid tot deelname aan het wetenschappelijke Honours Programme. Op basis van haar propedeusecijfers werd zij uitgenodigd voor het Honours Programme en koos ze voor onderzoek bij de Kinder- en Jeugdpsychiatrie. Onder begeleiding van dr. Lisette 't Hart-Kerkhoffs, prof. dr. Robert Vermeiren en prof. dr. Theo Doreleijers deed zij gedurende haar doctoraal onderzoek naar jeugdige zedendelinquenten. Haar eerste publicatie-ervaring deed zij op nadat haar essay over Encephalitis Lethargica gekozen werd tot Top-essay, waarop zij werd uitgenodigd om onder begeleiding van prof. dr. Toine Pieters het essay om te schrijven tot een wetenschappelijk artikel. Haar schrijfvaardigenheden kon zij verder ontwikkelen toen zij na deelname aan een columnistenwedstrijd een vaste column kreeg in *Ad Valvas*, het weekblad van de *Vrije Universiteit*, waarin zij met veel plezier haar belevenissen als student, coassistent en later als arts optekende.

Tijdens haar coschappen werd haar interesse voor de Maag-, Darm- en Leverziekten gewekt in het ziekenhuis met het mooiste uitzicht van Nederland, het voormalige *Gemini Ziekenhuis* te Den Helder. Op aanraden van prof. dr. Chris Mulder deed zij een extra coschap Hepatologie in het *Queen Elizabeth Hospital* te Birmingham, waar haar keuze definitief werd. In 2010 begon zij haar promotieonderzoek aan het *Erasmus MC* te Rotterdam onder begeleiding van dr. Rob de Knegt en prof. dr. Harry Janssen en later prof. dr. Herold Metselaar. Een periode van haar promotietijd bracht zij door in de *Mayo Clinic* in Rochester, Minnesota (VS) waar zij onder begeleiding van Michael Charlton onderzoek deed onder levertransplantatiepatiënten. In 2014 startte zij met de vooropleiding Interne Geneeskunde in het *Franciscus Gasthuis* te Rotterdam (opleider: Arie Rietveld). Zij won hier tweemaal een Posterprijs tijdens de jaarlijkse Wetenschapsdag en de Best Clinical Case Award op het 14e *European Congress of Internal Medicine* in Moskou. In 2016 keerde zij terug naar het *Erasmus MC* voor de vervolgopleiding tot Maag-, Darm-, Leverarts (opleider: prof. dr. Rob de Man en later prof. dr. Janneke van der Woude).

In haar vrije tijd is zij verbonden aan diverse muzikale projecten, speelt zij gitaar en acteert in musicals. In 2016 heeft ze haar organisatorische kwaliteiten ingezet als productieleider van de jubileummusical 'Follies' van *Musicalgroep Rits* te Delft.

PHD PORTFOLIO

Name PhD student:	Ludi Koning
Erasmus MC Department:	Gastroenterology and Hepatology
PhD period:	2010-2017
Promotor:	Prof. dr. H.J. Metselaar
Co-promotor:	Dr. R.J. de Knegt

1. PhD training	Year	Workload
General courses		
Classical methods of data analysis. Netherlands Institute for Health Sciences, Rotterdam	2011	5.7 ECTS
Integrity in Research. department of Medical Ethics and Philosophy. Erasmus MC, Rotterdam	2011	2.0 ECTS
Good Clinical Practice (BROK). Erasmus MC, Rotterdam	2013	30 hours
Recertification Good Clinical Practice (BROK). Erasmus MC, Rotterdam	2016	4 hours
Seminars and workshops		
Course in Virology. MolMed, Rotterdam	2010	1.0 ECTS
Abdominal ultrasound course. Dutch Liver Week, Amsterdam	2010	10.5 hours
25th Erasmus Liver Day. Rotterdam	2010	6 hours
8th Post AASLD Symposium. Rotterdam	2010	2 hours
3e Lagerhuisdebat Hepatitis B en C. Utrecht	2010	4 hours
Hepatitis Masterclass. Virology Education, Utrecht	2010-2011	18 hours
Hepatology journal club. Erasmus University MC, Rotterdam	2011-2014	30 hours
26th Erasmus Liver Day. Rotterdam	2011	6 hours
9th Post AASLD Symposium. Rotterdam	2011	2 hours
Postgraduate Course: Personalized Medicine and the Practice of Hepatology. AASLD, Boston, MA	2012	7 hours
10th Post AASLD Symposium. Rotterdam	2012	2 hours
4e Lagerhuisdebat Hepatitis B en C. Utrecht	2012	4 hours
De 24 uur van Vanenburg. Putten	2013	8 hours
Postgraduate Course: A New Era of Diagnostics Therapeutics and Intervention in Hepatology. AASLD, Washington, DC	2013	7 hours
28th Erasmus Liver Day. Rotterdam	2013	6 hours
11th Post AASLD Symposium. Rotterdam	2013	2 hours
Hot Topics Masterclass. EASL, Bordeaux	2013	16 hours
29th Erasmus Liver Day. Rotterdam	2014	6 hours
12th Post AASLD Symposium. Rotterdam	2014	2 hours
Postgraduate Course: Managing Liver Disease From the Clinic to the Community. AASLD, San Francisco	2014	7 hours

	Year	Workload
Oral presentations		
New insights into toxicity and pharmacokinetics of ribavirin and cyclosporine in liver transplant recipients on boceprevir-based antiviral therapy - evidence of need for new target troughs for CsA and lower dosing of ribavirin. Annual meeting of the Netherlands Association of Hepatology (NVH), Zeist	2012	12 hours
New insights into toxicity and pharmacokinetics of ribavirin and cyclosporine in liver transplant recipients on boceprevir-based antiviral therapy - evidence of need for new target troughs for CsA and lower dosing of ribavirin. 63th Annual meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA	2012	36 hours
Reduced risk of chronic hepatitis E infection post liver transplantation in HCV infected patients. Evidence for HCV treatment protection against development of chronic HEV infection. 19th Annual International Congress of the Internatonal Liver Transplantation society (ILTS), Sydney	2013	36 hours
Hepatitis E. Pros & cons: relevant or over diagnosed? Hot topics in Hepatology. Masterclass EASL,	2013	36 hours
Update on HCV. 12th Post AASLD Symposium, Rotterdam	2014	12 hours
Poster presentations		
High pre-transplant HEV seroprevalence in HCV infected liver transplant recipients; Evidence for HCV treatment protection against development of chronic HEV infection. 48th Annual Meeting of the European Association for the Study of the Liver (EASL), Amsterdam	2013	36 hours
High-dose versus standard-dose weight-based ribavirin in combination with peginterferon alfa-2a for patients infected with hepatitis C virus genotype 1 or 4. 64th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), Washington, DC	2013	36 hours
High dose ribavirin influences early viral kinetics and improves SVR rates in chronic HCV patients who adhere to therapy (VIRID study). 49th Annual Meeting of the European Association for the Study of the Liver (EASL), London	2014	36 hours
Leukopenia during interferon based hepatitis C treatment in liver transplant recipients is associated with increased infection risk. 20th Annual International Congress of the International Liver Transplantation society (ILTS), London	2014	36 hours
Interferon induced low blood cell counts are associated with bleeding and infection in liver transplant recipients on hepatitis C treatment. 65th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, MA	2014	36 hours
Ribavirin levels and transporters in the early phase of hepatitis C therapy. 66th Annual meeting of the American Association for the Study of Liver Diseases (AASLD), San Francisco, CA	2015	36 hours

	Year	Workload
International conferences		
46th Annual Meeting of the European Association for the Study of the Liver (EASL). Berlin	2011	28 hours
62th Annual meeting of the American Association for the Study of Liver Diseases (AASLD). San Francisco, CA	2011	28 hours
63th Annual meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA	2012	28 hours
48th Annual Meeting of the European Association for the Study of the Liver (EASL). Amsterdam	2013	28 hours
19th Annual International Congress of the International Liver Transplantation society (ILTS). Sydney	2013	28 hours
64th Annual meeting of the American Association for the Study of Liver Diseases (AASLD). Washington, DC	2013	28 hours
49th Annual Meeting of the European Association for the Study of the Liver (EASL). London	2014	28 hours
20th Annual International Congress of the International Liver Transplantation society (ILTS). London	2014	28 hours
65th Annual meeting of the American Association for the Study of Liver Diseases (AASLD). Boston, MA	2014	28 hours
66th Annual meeting of the American Association for the Study of Liver Diseases (AASLD). San Francisco, CA	2015	28 hours
Peer review activities		
American Journal of Transplantation, Hepatology, Journal of Clinical Virology		

2. Teaching	Year	Workload
Diagnosis and treatment of chronic Hepatitis C. Third year Erasmus MC medical students. Rotterdam	2014	12 hours
What to do with Hepatitis E. Scientific update for the Departments of Internal Medicine, Reumatology, Gastroenterology, Pulmonology and Cardiology. Franciscus Gasthuis, Rotterdam	2016	16 hours

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Hou J, Groothuismink ZM, **Koning L**, Roomer R, van IJcken WF, Kreefft K, Liu BS, Janssen HL, de Knegt RJ, Boonstra A. Analysis of the transcriptome and immune function of monocytes during IFNα-based therapy in chronic HCV revealed induction of TLR7 responsiveness. Antiviral Res. 2014;109:116-24

Koning L, de Knegt RJ, Metselaar HJ. Living donor liver transplantation in HCV-infected patients: improvement of the donor risk-recipient benefit ratio is around the corner. Transpl Int. 2014;27:765-6

Pungpapong S, Aqel BA, **Koning L**, Murphy JL, Henry TM, Ryland KL, Yataco ML, Satyanarayana T, Rosser BG, Vergas HE, Charlton MR, Keaveny AP. Multicenter experience using telaprevir or boceprevir with peginterferon and ribavirin to treat hepatitis C genotype 1 after liver transplantation. Liver Transpl. 2013 Jul;19:690-700

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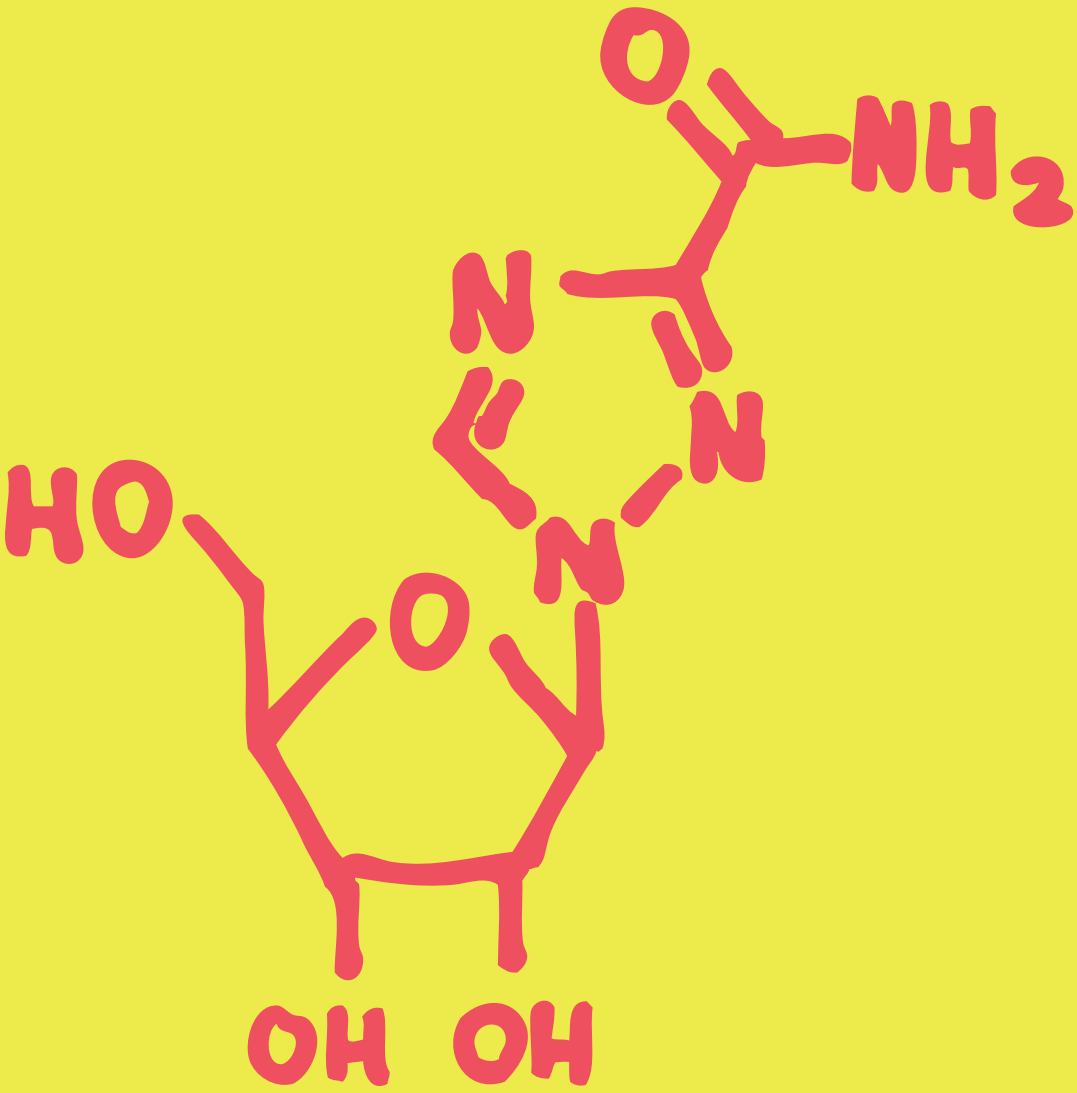
Koning L. [Encephalitis lethargica in the Netherlands; renewed interest in a mysterious disease] Encephalitis lethargica in Nederland: hernieuwde belangstelling voor een mysterieuze ziekte. Ned Tijdschr Geneeskd. 2009;153:B326.

Life really does begin at forty.
Up until then, you are just doing research

Carl Gustav Jung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