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**NATURAL COURSE AND TREATMENT OUTCOME IN
HEPATITIS C RECURRENCE AFTER
LIVER TRANSPLANTATION**

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Natural course and treatment outcome in Hepatitis C recurrence after liver transplantation

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ABSTRACT

Hepatitis C virus (HCV) infection post-liver transplantation (post-LT) is associated with an increased rate of fibrosis progression compared to non-transplanted patients. Thus, 25% of the recipients will progress to cirrhosis within 5 years after LT. Antiviral treatment after LT with peg-IFN and ribavirin (RBV) yields lower sustained viral response (SVR) than in non-transplanted patients. In many LT-recipients non-response to treatment will eventually lead to progression to cirrhosis.

The aim of this thesis was to study the natural course and treatment outcome in liver transplant patients with hepatitis C recurrence, and the influence of baseline factors on the course of the HCV recurrence and antiviral treatment, with particular emphasis on HCV genotype and IL28B gene polymorphism.

In **paper I** we conducted a pilot-study on 21 hepatitis C LT recipients with the aim to increase adherence and tolerance to antiviral treatment. All recipients were pre-treated with Darbepoetin (EPO) starting 2 weeks before the initiation of Peg-IFN and RBV. RBV was dosed taking weight and kidney function into account, with a target serum concentration set to 10 $\mu\text{M/L}$ by using a formula to calculate the dose. A majority of patients achieved the target concentration, and 90% could stay adherent to a full treatment course. SVR was reached in 18% recipients with genotype 1 and 60% with genotype non-1. Recipients with mild fibrosis achieved SVR in 67%. In **paper II** we studied the influence of IL28B gene polymorphism on fibrosis progression and treatment outcome in 54 LT recipients, who had received antiviral treatment, and in 45 of their donors. The most favorable IL28B genotype CC was associated with slower fibrosis progression and better treatment outcome. Patients with HCV genotype non-1 and the IL28B CC gene achieved SVR in 71%, whereas patients with genotype 1 and IL28B non-CC did so in only 23%, $p < 0,016$. Patients with mild fibrosis (F1-2) had better treatment outcome than patients with advanced fibrosis. In **paper III** we treated 46 Swedish and 8 Norwegian patients with the treatment regimen evaluated in paper I. 94% stayed adherent to the treatment course. SVR was achieved in 82% of recipients with HCV genotype 2/3 versus in only 22% with genotype 1, $p < 0.002$. Patients with IL28B CC achieved SVR in 73% and patients with non-CC in 33%, $p < 0.001$. Patients with mild fibrosis achieved SVR in 56% and patients with advanced fibrosis in 26% $p < 0.01$. Thus, with favorable HCV genotype and IL28B genotype, LT recipients have a good chance to achieve SVR, when treated before advanced fibrosis has developed. In **paper IV** we evaluated the utility of an early liver biopsy post-LT to detect and predict fibrosis progression of recurrent HCV infection post-LT. 35 HCV RNA positive, and 11 HCV RNA negative LT recipients, who underwent protocolled liver biopsies 6 and 12 months post-LT, were studied. Histological recurrence with fibrosis stage \geq F1 was noted in 56% of the HCV positive LT recipients at 6 months, and in 82% 12 months post-LT. Acute cellular rejection (ACR) and IL28B genotype CC were associated with a more pronounced fibrosis progression 12 months post-LT. Fibrosis was absent in all eleven recipients who were HCV RNA negative directly after LT. Thus, a 6 months biopsy post-LT is a valuable tool for detection of an early HCV recurrence, which makes an early treatment intervention for HCV possible.

LIST OF SCIENTIFIC PAPERS

- I. **Ackefors M**, Gjertsen H, Wernerson A, Weiland O. Concentration-guided ribavirin dosing with darbepoetin support and peg-IFN alfa-2a for treatment of hepatitis C recurrence after liver transplantation. *J Viral Hepat.* 2012 Sep;19(9):635-9.
- II. **Ackefors M**, Nystrom J, Wernerson A, Gjertsen H, Sonnerborg A, Weiland O. Evolution of fibrosis during HCV recurrence after liver transplantation - influence of IL-28B SNP and response to peg-IFN and ribavirin treatment. *J Viral Hepat.* 2013 Nov;20(11):770-8
- III. **Ackefors M**, Castedal M, Dahlgard O, Verbaan H, Gjertsen H, Wernerson A, Weiland O. Cost-effective treatment for genotype 2 and 3 Hepatitis C Recurrence after Liver Transplantation. *Submitted manuscript*
- IV. **Ackefors M**, Wernerson A, Gjertsen H, Weiland O. The utility of an early liver biopsy to predict fibrosis progression of recurrent hepatitis C after liver transplantation. *Submitted manuscript*

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LIST OF ABBREVIATIONS

AASLD	American Association for the Study of Liver Disease
AFP	alpha fetoprotein
ALT	alanine aminotransferase
AST	aspartate aminotransferase
cEVR	complete Early Virological Response
CHC	chronic hepatitis C
CNI	Calcineurin inhibitor
CT	computed tomography
CyA	Cyklosporin A
DAA	direct acting antiviral agents
DRI	Donor Risk Index
EASL	European Association for the Study of the Liver
ELISA	enzyme-linked immunosorbent assay
ETR	Early Treatment Response
EVR	Early Virological Response
FCH	Fibrosing cholestatic hepatitis
HAV	hepatitis A virus
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IVDU	intravenous drug use
IFN	Interferon
LDLD	living donor liver transplantation
LSM	liver stiffness measurement

LT	liver transplantation
MELD	Model for End-Stage Liver Disease
MRI	magnetic resonance imaging
mTOR	mammalian target of Rapamycin
NANB hepatitis	Non-A, Non-B hepatitis
NK cell	natural killer cell
NS protein	Non-structural protein
Peg-IFN	Pegylated interferon
Post-LT	post-liver transplantation
PWID	people who inject drugs
Re-LT	re-liver transplantation
RBV	Ribavirin
RCT	randomized controlled trial
RNA	Ribonucleic acid
SOC	standard of care
SVR	sustained virological response
TAC	Tacrolimus
TE	transient elastography
UNOS	United Network for Organ Sharing
US	ultrasound

INTRODUCTION HEPATITIS C

1.1 HISTORY

In the 1950s and 1960s the field of viral hepatitis evolved from the observations of so called serum-hepatitis (1), later proven to be caused by infections with hepatitis A virus (HAV) and hepatitis B virus (HBV) (2). In the mid-1970s, when serological tests were introduced, analysis of earlier stored sera from transfusion studies made it clear that neither of these known viruses caused the majority of blood-transmitted hepatitis cases (3). This unknown hepatitis, with slowly progressing fibrosis, was named non-A, non-B (NANB) hepatitis. When the genome of NANB virus was characterized in 1989 (4), it showed similarities with Flavivirus (flaviviridae), and was named hepatitis C virus (HCV). An assay was developed to detect antibodies, anti-HCV (5), and general blood donor screening became possible in the early 90s.

1.2 VIROLOGY AND GENOTYPES

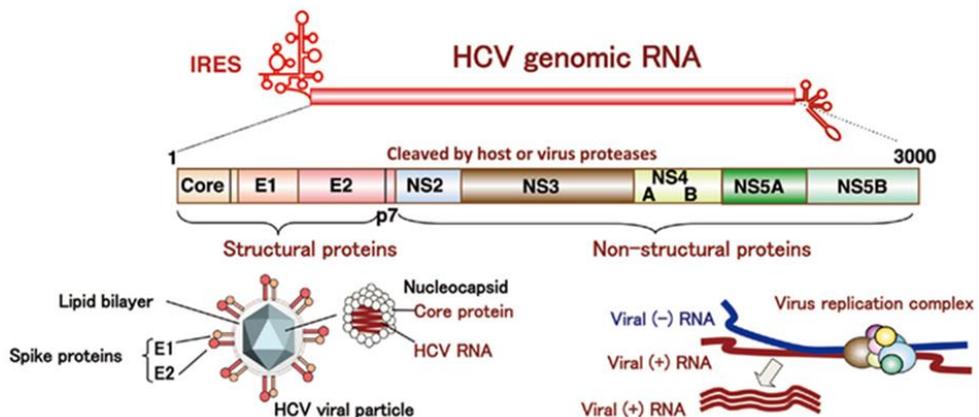


Figure 1: Structure of Hepatitis C virus

HCV is a single-stranded RNA virus and the genome consists of approximately 9 600 nucleotides which encodes a single glycoprotein cleaved into three structural proteins and seven non-structural proteins (6). The virus replicates rapidly in the cytoplasm of the hepatocytes and because the HCV RNA polymerase lacks proofreading, multiple quasispecies are generated and circulate simultaneously (7).

HCV is divided in seven major genotypes (8), and each genotype in several subtypes with varied global distribution. HCV genotypes 1-4 are most common. In Sweden, genotype 1a and 3a are most common, approximately 30% each, followed by genotype 2 with about 20% (9).

1.3 EPIDEMIOLOGY

HCV is endemic in most parts of the world, with an estimated 180 million people being infected worldwide, comprising about 3% of the global population (10, 11). Some 350 000 persons die each year from HCV related liver disease. The highest prevalence, 5-10%, has been reported from Africa and the Middle East (12), in Egypt as high as 12.5% due to iatrogenic spread during Schistosomiasis treatment campaigns (13). In the majority of developed countries the prevalence is below 2%.

In Sweden the prevalence of anti-HCV positive individuals is approximately 0.6%, whereof 77% have a chronic hepatitis C (14, 15). Hence, there are approximately 55 000 individuals infected with HCV in Sweden, and the annual rate of newly diagnosed individuals some 1500. The majority of the HCV infected individuals in Sweden are born in the 1950s and 1960s, and probably became infected in the 1970s and 1980s, when IVDU started to become more widely spread among the young population (16).

The transmission route is parenteral exposure, including transfusions before blood screening became mandatory in the early 90s, injections, household exposures and perinatal transmission (17). IVDU is the main route of transmission in the Western Countries. The risk of transmission by an infected needle has been calculated to 1.8 %, and in Stockholm more than 80% of PWID (people who inject drugs) are anti-HCV positive (18). In the recently initiated needle exchange program in Stockholm, 83% of the individuals in the program were anti HCV positive, and 74% of these also HCV RNA positive. (M Kåberg, personal communication). The sexual transmission rate in heterosexual couples is low (1.5%). Among MSM (men who have sex with men) the rate is dependent on sexual practices which lead to transmission of blood, the viral load, use of intravenous drugs, and concomitant STIs (sexually transmitted infections) (19). In Sweden the incidence of co-infection with HIV is low. Only 7% of the HIV infected cohort is co-infected with HCV according to the national InfCare HIV register (15)(Stenkvist et al 2014 in press)

1.4 THE NATURAL COURSE OF HEPATITIS C INFECTION

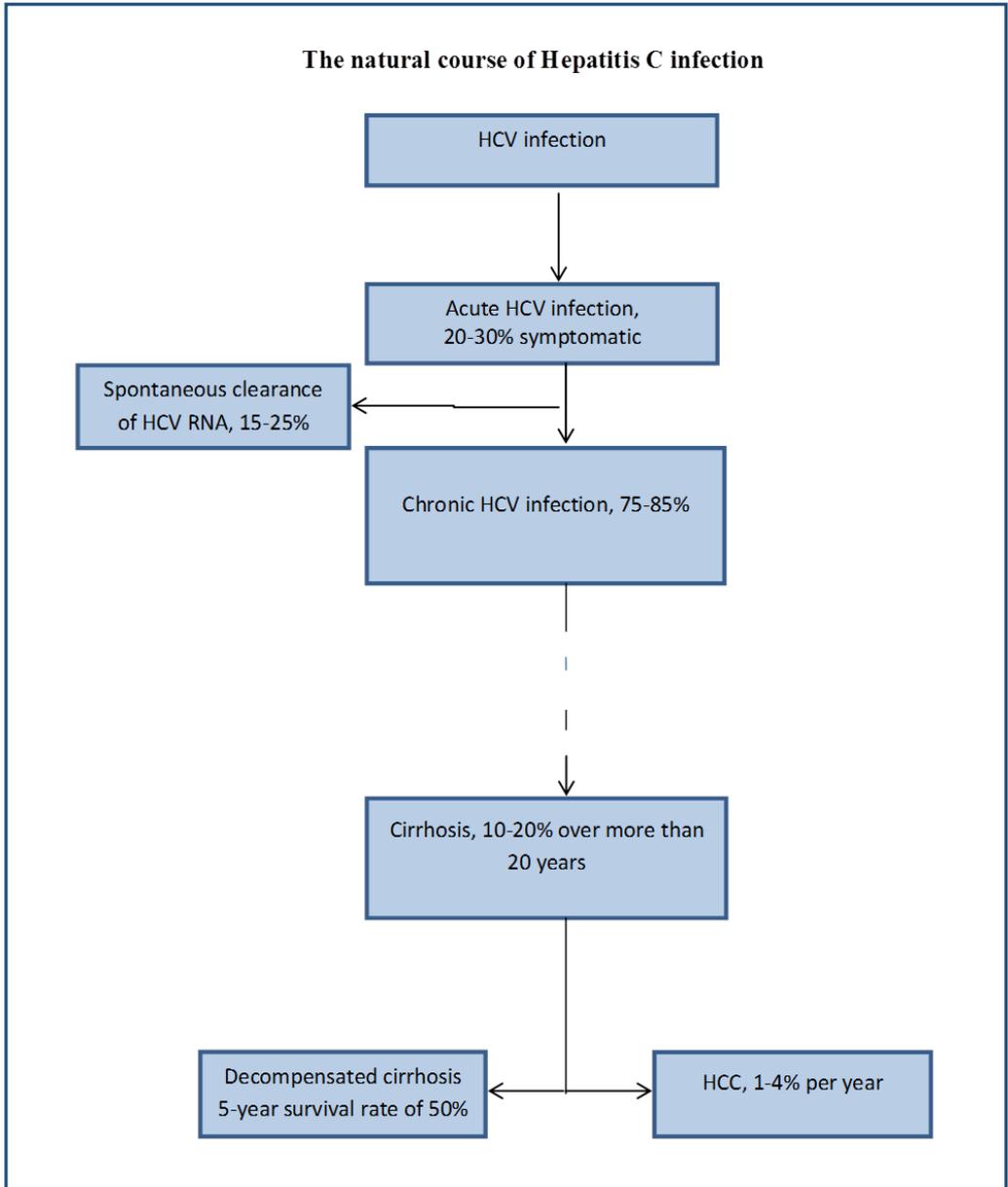


Figure 2: *The Natural course of Hepatitis C infection*

Only some 20% of HCV infected individuals will develop symptoms of acute hepatitis with jaundice, malaise and anorexia 1-3 months after being infected. The majority, will have an asymptomatic disease. The chance to achieve a spontaneous clearance is approximately 25%,

hence 75% will go on to develop chronic HCV and risk HCV-related end stage complications, such as liver cirrhosis and hepatocellular carcinoma (HCC). Approximately 10 -15 % will eventually develop cirrhosis over 20 years, which in turn carries a 2-4% yearly risk for HCC. Factors associated with a risk to develop chronic HCV infection are age > 25 years at the time of infection, male gender, asymptomatic disease during the acute infection, IL28B genotype non-CC, HIV co-infection and immunosuppression (20, 21).

1.4.1 Factors influencing the natural course

Generally, hepatitis C progresses slowly over 20-25 years, then turning into a more rapid progression. The course, however, is highly variable, and efforts have been made to identify risk factors associated with a more rapid fibrosis progression. A major risk factor for progression to cirrhosis is alcohol. Further risk factors include older age at time of infection, male gender, IL28B non-CC, higher degree of inflammation and fibrosis present, co-infection with human immunodeficiency virus (HIV) or hepatitis B virus (HBV), all factors that is associated with a more rapid progression of fibrosis. HCV genotype, on the other hand, does not seem to be associated with fibrosis progression. (20, 22, 23).

1.5 DETECTION OF HCV RELATED FIBROSIS AND CIRRHOSIS

Scarring of the liver, evaluated as fibrosis stage, is a slowly evolving process during a chronic HCV infection progressing towards cirrhosis. Evaluation of the extent of fibrosis and its progression is thus very important.

1.5.1 Liver biopsy

Liver biopsy has been the gold standard for grading and staging the liver disease. In mild cases, fibrosis is limited to the portal tracts, whereas more advanced fibrosis extends from one portal area to another, also known as "bridging fibrosis". In cirrhosis, nodules of liver parenchyma are formed surrounded by fibrotic tissue. The inflammatory activity is graded based on the extent of inflammation. It is evaluated by the amount of inflammatory cells present in the border of the portal tract causing cell death, also named "interface activity". One of the few validated scoring systems used for classification of fibrosis in HCV patients is the METAVIR scoring system (24). The fibrosis stage is divided in four levels, F0-F4, ranging from F0, absence of fibrosis, to F4 corresponding to cirrhosis. The necro-inflammatory activity is classified in four levels, A0 – A3, where A0 means no inflammatory activity and A3 corresponds to severe activity. At Karolinska University Hospital the liver biopsies are evaluated according to Batts and Ludwig, a scoring system similar to METAVIR, where fibrosis stage is divided into F0-F4 and inflammatory activity is classified into five levels, A0-A5 (25).

1.5.2 Liver stiffness measurement by transient elastography (TE)

In recent years, liver biopsy has gradually been replaced by non-invasive methods, such as transient elastography (TE), a non-invasive method measuring the liver stiffness in kilo Pascal (kPa) (FibroScan®, Echosens, Paris, France) (26). Measuring liver elasticity with TE has demonstrated a high accuracy to detect advanced fibrosis where a cut-off of more than 9,5 kPa is used to define advanced fibrosis (\geq F3)

1.6 ANTIVIRAL TREATMENT IN HCV INFECTED NON-TRANSPLANT PATIENTS

The main goal with treatment is viral eradication, leading to stabilization and improvement of liver function, and a diminished risk to develop end-stage liver complications and hepatocellular carcinoma (HCC).

1.6.1 Treatment regimens

In the mid 1980 reports on treatment for non-A, non-B hepatitis with IFN were published (27). In the late 1990 the addition of RBV was found to improve the outcome when combined with IFN, mainly by reducing the rate of relapses after treatment (28-31). The addition of a polyethyleneglycol molecule to interferon (Peg-IFN) renders IFN a longer half-life, allowing once-weekly dosing, with more stable IFN serum levels over time, resulting in a higher response rate of 50-80% depending on genotype (32, 33). Today with the recent development of direct acting antivirals (DAA) for treatment of chronic HCV infection, IFN based treatment will be replaced with IFN-free treatment options with shorter treatment courses and higher efficacy (34-40).

1.6.2 Predictors of treatment response

Patient related predictors of response to IFN-based therapy are age, sex, pre-treatment HCV RNA levels and fibrosis stage, and the most important are HCV genotype and IL28B gene polymorphism (41, 42). Furthermore, on-treatment predictors of response, in particular early virological response (EVR), is highly predictive of SVR, as is adherence to the antiviral treatment, both dosing and treatment length (43, 44).

2 LIVER TRANSPLANTATION IN HCV INFECTED PATIENTS

2.1 HISTORY

The first liver transplantation was performed in the 1960s. Hereafter, the surgical technique, immune-suppressive regimens, patient selection criteria, organ allocation, and the organ preservation techniques have undergone great improvement and refinement, resulting in better graft and patient survival (45). The indication for LT is mainly end-stage liver disease and HCC, when other therapies have failed or are judged to be inferior. Presently, the leading indication for LT in the Western world is cirrhosis caused by chronic hepatitis C (46).

2.2 IMMUNOSUPPRESSION

Immunosuppression (IS) after LT is necessary to prevent allograft rejection and is generally life-long. In a few recipients, however, complete withdrawal of IS has been possible (47). In general, during the IS induction phase, peri-operatively and early post-LT, when the risk of ACR is highest, high dose of IS is given. The IS dose is hereafter gradually tapered and finally given as lower doses in the maintenance phase, generally reached after some months post liver transplantation.

2.2.1 General principles in immunosuppression

Most regimens use Calcineurin inhibitors (CNI), either cyclosporine A (CyA) or Tacrolimus, as the major agents for maintenance IS. An antimetabolite, mainly Mycophenolate (MMF), is added to reduce the doses of CNI, in order to minimize the side-effects induced by CNI, mainly nephrotoxicity, diabetes mellitus and de-novo tumours, and some centers also use low dose steroids (48,49).

2.2.2 Immunosuppression in HCV infected recipients

In liver transplant recipients with HCV, there is no consensus regarding the optimal IS regimen. The strategy is to avoid over-immunosuppression and unnecessary use of steroid boluses, and slowly taper the IS doses, since high, or rapidly changing, doses of corticosteroids is associated with increased replication of HCV RNA and fibrosis progression (50, 51). Steroid-sparing regimens have been investigated with no clear benefit regarding patient and graft survival or ACR, but some centers prefer a steroid-sparing regimen to minimize co-morbidities associated with steroids, mainly diabetes mellitus, obesity and osteoporosis (52-54).

3 HCV RECURRENCE

3.1 KINETICS

Hepatitis C recurrence occur very early post-transplant, in fact, already during the reperfusion phase. By day 4 after transplantation, serum HCV RNA levels reach pre-transplant levels (55). The viral load increases over the following weeks to levels higher than in non-transplant patients, reaching a plateau 1-2 logs higher than pre-LT levels at approximately 1 month post-transplant (56).

3.2 MECHANISM

The proposed mechanism causing allograft injury in the liver during recurrent HCV infection is thought, at least in part, to be caused by the increased hepatitis C viral load which appears to overcome the inhibitory effect on the immune system caused by the immunosuppression. This results in an enhanced inflammatory response and an induction of antiviral interferon inducible genes leading to an HCV-driven enhanced proliferation, apoptosis and fibrosis response in the allograft (57).

3.3 THE NATURAL COURSE OF FIBROSIS PROGRESSION

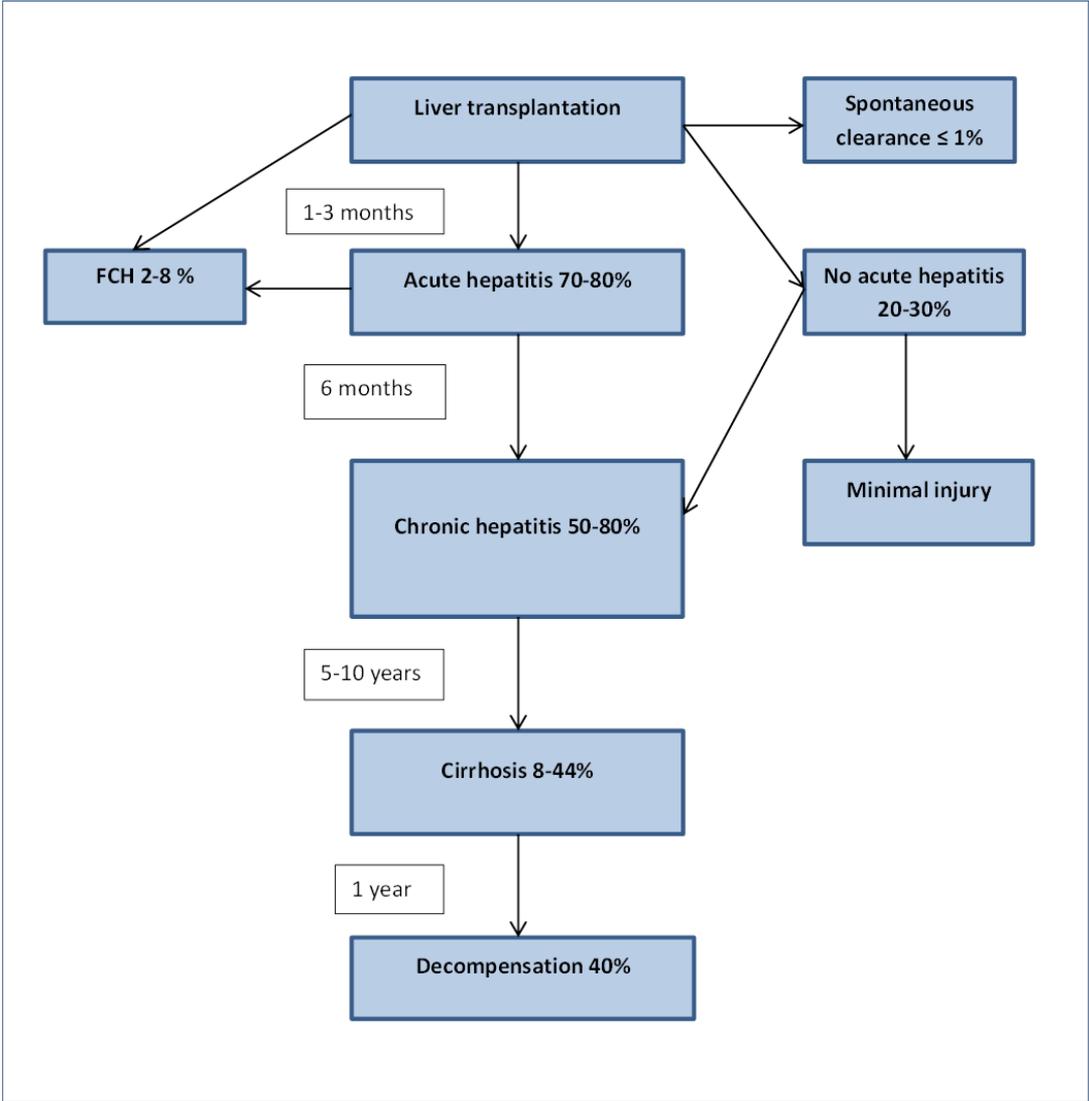


Figure 3: Natural course of HCV recurrence after liver transplantation

Biochemically and histologically, acute hepatitis after transplantation is usually detected between 1 and 3 months post-transplant (58). However, the course can be highly variable as chronic hepatitis develops either as a typical chronic hepatitis with late-onset fibrosis

progression, or as a more aggressive, sometimes severe cholestatic form (57, 59, 60). The most aggressive form causes fibrotic cholestatic hepatitis (FCH), which is a severe, rapidly progressive liver injury seen in < 10% of liver transplant patients (61). FCH is associated with high HCV RNA levels, jaundice and biochemical cholestasis. It is more often seen in patients who have received high doses of immunosuppression early post-transplant, including pulses of methylprednisolone or anti-lymphocyte therapy used for ACR episodes. Therefore, treatment of mild rejection with steroid boluses should be used cautiously, and mild rejections should rather be treated with increased dosing of the baseline IS drugs (62).

3.3.1 Fibrosis progression rate

Overall, the rate of fibrosis progression is accelerated in liver transplant recipients compared to what is observed in immune competent hosts. Thus, liver transplant recipients with hepatitis C relapse will develop cirrhosis in 20-40% within 5 years after transplantation (58, 63). Repeated biopsies have demonstrated an annual rate of fibrosis progression between 0.3-0.6 stages/year staged by the Metavir system, compared to only 0.1-0.2 stages/year in immune competent patients with hepatitis C (22, 64, 65). Hence, the median interval from transplantation to development of cirrhosis is 9.5 years versus 30 years in liver transplant recipients and non-transplanted immune competent hosts, respectively.

Once cirrhosis has developed the 1-year risk to develop hepatic decompensation is approximately 40% in immune competent patient, which is increased to more than 70% in liver transplant patients with established cirrhosis (66, 67, 68). Thus, identification of patients at risk to develop severe HCV recurrence after liver transplantation at an early stage, to be able to provide treatment, is very important.

3.3.2 Long-term survival

In several studies, graft and patient survival post-LT in HCV positive recipients are significantly lower than in LT recipients without HCV (63, 64, 69). Patient and graft survival rates have improved steadily in HCV negative recipients, however, the same trend is not seen in HCV positive patients (70, 71). Hence, the 5-year survival rate in HCV positive LT recipients is 60-65% as compared to 75% in HCV negative recipients with even lower survival rates seen in HCV infected recipients with concomitant HCC (63, 64, 69). In the Scandinavian registry, the 5-year survival rate in HCV positive recipients was 71% in recipients without HCC versus 46 % in recipients with HCC (69). This illustrates that HCV infection with HCC is associated with the least favorable survival rates. Older donors and donor liver histology, with findings of steatosis in the graft, has also been associated with lower patient and graft survival (69, 72, 73).

3.3.3 Graft survival

The graft survival in HCV positive LT recipients is impaired compared to that in LT recipients without HCV. The 5-year survival rate in HCV positive LT recipients is 55 % compared to 67-

71 % in LT recipients without HCV (63, 69). In re-transplanted LT recipients with HCV, the donor age is associated with an increased rate of graft failure (64).

3.4 DETECTION OF HCV RECURRENCE

3.4.1 Liver biopsy early post-transplant

A liver biopsy performed early (0-3 months) post-LT, often due to elevated liver enzymes and/or bilirubin, can be very difficult to evaluate. It is important to determinate whether recurrent HCV or rejection is the cause of the allograft dysfunction, or other causes. HCV is an uncommon cause of dysfunction during the initial months after liver transplantation, although occasional cases of severe acute HCV occurs early post-LT. In contrast, most acute rejection (ACR) episodes occur within the first 30 days (74, 75). It is of great importance to discriminate between ACR and HCV recurrence, since treatment for ACR with steroid bolus doses, or anti-lymphocyte therapy with OKT-3, is associated with a more rapid fibrosis progression (71, 76, 77).

3.4.2 Protocolled liver biopsy

Several studies have shown an association between early hepatitis C recurrence and disease progression. Hence, the activity and fibrosis extent observed in the biopsy taken 12 months after transplantation was found to be associated with the progression of fibrosis and survival (59, 60, 65). Thus, only 3-10% with mild fibrosis 1-year after transplantation will progress to cirrhosis, as opposed to 30-60% of recipients who have developed severe fibrosis. It was suggested that a 1-year biopsy would have the best ability to identify recipients with an increased risk to have a rapid fibrosis progression.

At Karolinska University Hospital, protocolled liver biopsies are performed already at 6 months post-LT, and hereafter continuously on a yearly basis, with the aim to detect HCV recurrence as early as possible.

Repeated liver biopsies, however, carries a risk, although small, for severe complications. Furthermore, they are suffering from sample variability and are not preferred by the patients (78). Hence, non-invasive methods for evaluation of fibrosis stage are preferred by most patients.

3.4.3 Liver stiffness measurement (LSM)

LSM is a non-invasive alternative to liver biopsy post-LT, described in detail in Methods. It is found to have a diagnostic accuracy for detection of significant fibrosis and cirrhosis, and to predict the clinical outcome post-LT (79-81). At 12 months post-LT, evaluation with LSM is useful for discrimination of recipients who will have rapid versus slow fibrosis progression, in particular if combined with clinical variables (82). In a recent review, a liver elasticity cut-off

value at 8.7 kPa was identified, which delineates significant fibrosis and cirrhosis (F3-4) (83). This cut-off level is now recommended and has a sensitivity and negative predictive value for detection of significant fibrosis of > 0.90 . Early post-LT, however, several confounding factors are operating which have a great influence on the kPa value, such as ischemia, acute cellular reaction, toxic reactions to immune suppressive drugs.

4 FACTORS ASSOCIATED WITH THE COURSE OF HCV RECURRENCE

In the transplant setting, many factors will have an impact on the disease progression. Efforts have been made to pinpoint the patients who will have the highest risk to develop a rapid and aggressive fibrosis progression post-LT.

4.1 VIRAL FACTORS

4.1.1 HCV viral load and genotype

A high pre-transplant viral load ($>10 \times 10^6$ IU/mL) is associated with an increased risk for fibrosis progression, graft loss, and mortality (71, 84). The significance of the HCV genotype is more controversial, but genotype 1, and in particular 1b, have been thought to be involved with a more rapid fibrosis progression, and higher risk to develop cirrhosis (59, 85).

4.1.2 Cytomegalovirus

Recurrence of cytomegalovirus (CMV) post-transplant has been associated with a more rapid fibrosis progression (86), probably due to high levels of immunosuppression. However, today, with highly effective prophylaxis regimens and surveillance with monitoring of CMV DNA serum levels, the impact of recurrent CMV on fibrosis progression in HCV recurrence has been reduced to a minimum.

4.1.3 HIV co-infection

Co-infection with HIV in transplant recipients is associated with a more aggressive and rapid fibrosis progression and a higher rate of severe HCV recurrence such as FCH, resulting in lower survival rates compared to recipients without HIV infection (87). The mechanisms are not fully understood. Treatment with ART (anti-retroviral therapy) for HIV seems to slow down the fibrosis progression. Hence, it is recommended that ART should be initiated early in co-infected patients (EACS guidelines 2013, www.eacsociety.org).

4.2 DONOR AND RECIPIENT-RELATED FACTORS

Several donor and recipient factors have been evaluated regarding the fibrosis progression in recipients with HCV recurrence after liver transplantation. Evaluating donor factors may help us to understand the mechanisms leading to more severe recurrent hepatitis C, and possibly to predict the clinical and histological outcome of hepatitis C in the graft recipients.

4.2.1 Donor and recipient age

In some studies it has been shown that older donor age affects the rate of fibrosis progression and patient survival, although the results are not uniform. A 10-year difference in donor age (40

vs 50 years) was associated with an increase in the fibrosis progression rate from 0.6 to 2.1 units/year, and donor age >33 years was independently associated with cirrhosis in recurrent HCV in another study (59, 88). A Swedish study showed a significant reduction in patient survival with older donor age (>60 years), whereas the graft survival was associated with the presence of inflammation in the donor graft (73). An increased rate in fibrosis progression post-transplant have been noted, and that progression was independently associated with the year of transplantation, the same group concluded that the increasing donor age, seen over time, due to shortage of organs, is believed to be one contributing factor to the decrease in survival among liver transplanted patients with HCV (71, 72). These findings contributed to the discussion whether allocating younger donor grafts should be done or not to HCV recipients.

The association between older recipient age and fibrosis progression is not firmly established. Thus, some authors have claimed that older recipients receiving grafts from older donors have both lower long-time graft and patient survival than older recipients receiving grafts from younger donors (89). This suggests that using older donors for older recipients should be avoided.

4.2.2 Liver graft steatosis

The influence of steatosis in the liver graft on long-term survival has yielded conflicting results from different groups. Some suggests that donor steatosis will lead to a more rapid fibrosis progression, whereas others fail to see this association (90-93).

4.2.3 IL28B gene polymorphism in donor and recipient

The impact of IL28B genotype on fibrosis progression in recipients with HCV recurrence after liver transplantation, both in the donor and recipient, is under current investigation. The IL28B genotype, however, has a great influence on the spontaneous clearance of an acute hepatitis C and on the treatment response in non-transplant patients (21, 42). The IL28B genotype also seems to play an important role for liver transplant recipients with recurrent HCV for prediction of disease progression. The IL28B genotype both in donor and recipient seem to be equally important to study. The results so far have been inconsistent, especially concerning the donor genotype, and further studies are needed. Regarding IL28B in the recipient, the IL28B genotype TT seems to be correlated with a more rapid recurrence, whereas genotype CC seems to be associated with a more slow progression of fibrosis post-transplant (23, 94-96). The results regarding the donor IL28B genotype, however, are not conclusive. Two different groups have stated that IL28B CC in the donor seems to be associated with a more rapid course of HCV recurrence indicated by higher ALT and HCV RNA levels post-transplant, and an earlier recurrence of hepatitis in the liver (96, 97). All studies so far have failed to show a clear survival benefit induced by the favorable IL28B genotype both in the donor and recipient. However, the IL28B CC genotype is associated with higher SVR rates in liver transplant recipients treated

with peg-IFN and ribavirin, where the response to treatment leads to higher long-term survival rates (94, 98)

4.3 IMMUNOSUPPRESSION

The extent of the immunosuppression used is correlated to the progression of hepatitis C after liver transplantation. This is at least in part due to the increase in viral load which it causes. It seems that high levels of immune suppression, and in particular sudden changes in the IS drug dosing, is detrimental and causes accelerated progression of the liver injury. Hence, a delicate balance exists between the risk for ACR with appropriate dosing of the IS drugs and the risk for accelerated HCV progression with over-immunosuppression.

4.3.1 Steroids

Steroid boluses are given early post-transplant to prevent ACR, but boluses used to treat ACR are associated with a more severe course of the HCV recurrence (71, 84), and induce higher HCV RNA levels and shorten the time to HCV recurrence. However, the strategy of rapid and early steroid withdrawal may also lead to an increase in the rate of fibrosis progression, possibly causing an immunological rebound (57), and improvement in outcome is shown if rapid steroid tapering is avoided (50). This led to the recommendations not to change the level of immunosuppression rapidly, and not to use over-immunosuppression, and only give steroid pulses to liver transplant recipients with HCV when a severe ACR is properly diagnosed.

4.3.2 Calcineurin inhibitors

The possible different impact on HCV recurrence between cyclosporine (CyA) and tacrolimus (TAC) is controversial. In *in vitro* studies, CyA is found to suppress hepatitis C replication (99, 100) This, however, has not resulted a better outcome in CyA treated recipients with HCV recurrence. In a prospective, randomized study on 122 liver transplant recipients there was no significant difference in the rate of severe HCV recurrence one year post-LT or in the survival rate (101).

4.4 RISK FACTORS ASSOCIATED WITH DEVELOPMENT OF CIRRHOSIS

If advanced fibrosis is present already 12 months post-LT, the risk of rapid fibrosis progression to HCV-related graft cirrhosis is considerable (59, 65, 76). Treatment for ACR with boluses of methylprednisolone or anti-lymphocyte therapy, which increases the fibrosis progression post-LT, is also found to be associated with a higher risk of cirrhosis development post-LT.

4.5 RE-TRANSPLANTATION IN PATIENTS WITH HCV RECURRENCE

Re-transplantation (re-LT) is the only therapeutic option when decompensated liver disease due to HCV recurrence has developed after primary LT. However, re-LT due to HCV recurrence is controversial, because of its increased rate of graft loss and high patient mortality rates (102). The main reason for re-LT within the first 14 days after the primary transplantation is non-function in the graft, followed by vascular thrombosis, whereas HCV recurrence only accounts for 0.5%. The indication for re-LT caused by HCV recurrence increase to 5.3% between day 15 and 222, to 24.5% between day 223 and 1307, and to 20.2% after day 1308 post-LT. Thus, recurrent HCV is a leading cause for re-transplantation.

The one-year patient survival after re-transplantation due to HCV recurrence in the graft amounts to some 70%, whereas the outcome in HCV negative recipients undergoing re-LT is steadily improving and much better (70). Graft and patient survival is found to be significantly lower in HCV infected recipients as compared to HCV-negative patients who underwent re-LT after within 90 days after the primary LT (103).

The best approach to prevent re-LT in recipients with HCV recurrence is antiviral treatment, which can eradicate HCV and stabilize fibrosis progression. Up till today, IFN-based therapy was the only option. With the introduction of direct acting antivirals (DAAs), it is now possible to treat patients with HCV recurrence easier and safer both prior to and after transplantation with highly improved SVR rates (104, 105).

5 ANTIVIRAL TREATMENT WITH PEG-IFN AND RBV IN LIVER TRANSPLANT PATIENTS WITH HCV RECURRENCE

5.1 INTRODUCTION

Due to the often severe impact HCV recurrence has on the graft and patient survival, causing cirrhosis in 30% of the recipients within 5 years, treatment for hepatitis C recurrence is important to improve the outcome post-LT (63, 66). Several treatment strategies have been evaluated both pre- and post-LT. In general, antiviral therapy with peg-IFN and RBV is less effective in transplant recipients than in non-transplant patients, and the tolerability less good.

Initial studies based on IFN mono-therapy yielded poor results, with very low SVR rates (106). The addition of RBV improved SVR rates somewhat to reach 20% (107). With the introduction of peg-IFN in combination with RBV, viral clearance rates improved to 26%-45%. In a review from 2008, the average SVR rate in 19 evaluated studies was 30% (108-110). The low response rate is partly explained by the large proportion of HCV genotype 1 patients, premature discontinuation of therapy due to side-effects, and the high proportion of patients with advanced fibrosis and cirrhosis. In recent years, the impact of a non-favorable IL28B genotype is also found to be a contributing factor, since an accumulation of the unfavorable IL28B genotype non-CC is found in HCV infected liver transplanted recipients (94, 97). Although treatment results have clearly improved during recent years with peg-IFN and ribavirin, the side-effects are still a major limitation.

In order to optimize treatment response and minimize side-effects, the most important aspects to study have been timing of the initiation of antiviral therapy, optimal dosing of Peg-IFN and RBV, whether to use growth factors or not, and how to diminish the risk for allograft rejection.

5.2 TIMING OF TREATMENT

5.2.1 Treatment prior to transplantation

Antiviral therapy administered before transplantation, in order to clear the infection pre-transplant, with the aim of preventing recurrence, is attractive. However, treatment with Peg-IFN and RBV during 24-28 weeks has not been successful, due to the frequent adverse events causing many withdrawals and serious complications including deaths. The alternative is a shorter course leading to an “on-treatment” negative serum HCV RNA at time of transplantation. Studies have shown that HCV recurrence was prevented in all patients who achieved SVR before transplantation. The SVR rate, however, was low, approximately 20% (111, 112), and the treatment caused many side-effects and life-threatening complications, including infection and decompensation (113). The virological response with the shorter treatment pre-transplant was 30 %, however, due to HCV persistence in peripheral mononuclear

cells, the SVR rate dropped to 20% (114). In practice, treatment prior to transplantation with Peg-IFN and RBV is only feasible in patients with Child-Pugh A cirrhosis, making this strategy applicable in only some 50% of the patients.

5.2.2 Treatment after transplantation

The main goal in treating liver transplant recipients with HCV recurrence is clearance of HCV. The stabilization, or even improvement, of fibrosis seen after a successful treatment is also a reason to offer antiviral therapy. The treating physician has to balance the efficacy against the tolerability before a treatment decision is taken, in order to maximize the usefulness of treatment. A major challenge when treating HCV-infected transplant recipients is to improve SVR rates and minimize side effects. For this, timing of treatment initiation and dosing is important to study.

5.2.3 Pre-emptive treatment

Pre-emptive treatment has been used very early after transplantation, before recurrent hepatitis has developed, but after re-infection has occurred immediately after reperfusion, with high HCV RNA levels (55). Pre-emptive therapy may be more effective than treatment started only after HCV recurrence histological and biochemically has occurred, optimally within one month post-LT. However, early post-transplant recipients have higher doses of immunosuppressive drugs and the tolerability to peg-IFN and RBV treatment is limited. The risk of acute cellular rejection is higher during this period, and will increase with IFN (115-117), and the SVR rates are found to be low, only 8-20%. Hence, pre-emptive treatment with peg-IFN and RBV is applicable in few transplant recipients.

5.2.4 Treatment of established recurrent HCV

Treatment with Peg-IFN and RBV for recurrent HCV infection was until recently the only treatment regimen available. SVR and prevention of disease progression was the main goals. SVR rates varied between 20-45% depending on genotype, thus in HCV genotype 1 only 15-30% achieved SVR as reviewed 2008 by Berenguer et al in (110).

From 2008 and onwards, the main strategy has been to start therapy earlier, when the initial signs of histological recurrence are evident in a liver biopsy, due to the low SVR rate in transplant patients with advanced fibrosis/cirrhosis (109, 118, 119). In a study from 2012, SVR according to fibrosis stage ranged from 52% in patients with mild fibrosis (F1-F2) to 35.5% in patients with advanced fibrosis/cirrhosis (F3-F4) (118).

5.3 TREATMENT IN PATIENTS WITH FIBROSING CHOLESTATIC HEPATITIS (FCH)

Antiviral treatment with Peg-IFN and RBV in recipients with FCH, is associated with low SVR rates, risk for ACR, and serious side-effects. In a study from 2006, 10 patients with FCH treated with peg-IFN and RBV achieved an SVR rate of 20 % (120). Five out of 10 patients achieved biochemical response, but remained HCV RNA positive after 48 weeks of treatment, 3 died with liver failure, and one due to acute rejection, high-lighting the difficulties faced when treating FCH.

5.4 ADHERENCE TO TREATMENT

Adherence to antiviral therapy with Peg-IFN and RBV is of great importance since it enhances SVR in both transplant and non-transplant patients (43, 44, 108, 121). A good adherence to antiviral therapy is defined as patients who receive 80% or more of the total Peg-IFN dose, and 80% or more of the total RBV dose, and have completed 80% or more of the expected duration of therapy. In transplant patients, the tolerance to antiviral treatment is low with frequent side-effects, primarily RBV induced anemia, which leads to frequent dose reductions (70%), and treatment discontinuations (30%) (122).

5.5 SIDE EFFECTS IN ANTIVIRAL TREATMENT WITH PEG-IFN AND RBV

5.5.1 Hematological complications

Hematological side-effects are largely attributable to IFN-related bone-marrow suppression affecting all three cell lines, and RBV-related, dose-dependent, hemolytic anemia (123, 124). In non-transplant patients, the hematological side-effects induced by the antiviral treatment also cause dose-reductions (32, 125). The hematological side-effects, however, are more pronounced in transplant patients.

5.5.2 Ribavirin

Ribavirin (RBV) is a purin nucleoside analogue with antiviral activity against DNA and RNA viruses (126). RBV mono-therapy has effect on ALT levels but does only reduce HCV RNA levels slightly (28) (124).

RBV induces a dose-dependent hemolytic anemia (31, 124). RBV is eliminated via renal excretion, which often is impaired in transplant patients, making them vulnerable to the hematological side-effects of RBV. The concentration of RBV correlates with the SVR rate, and concentrations needed for a sufficient viral response will thus cause anemia (127). The therapeutic target concentration is estimated to 10-15 $\mu\text{mol/L}$ to reach an SVR rate of 80% in non-transplant HCV genotype-1 patients (128).

5.5.3 Interferon

Interferon (IFN) is a potent immune modulator, affecting both the innate and the adaptive immune system (129). During antiviral treatment it is associated with a rapid suppression of hematopoiesis and causes leucopenia and thrombocytopenia (123).

5.6 MANAGEMENT OF HEMATOLOGICAL SIDE-EFFECTS

5.6.1 Anemia

The most notable adverse effect of RBV therapy is hemolytic anemia, and most patients receiving antiviral treatment with Peg-IFN and RBV experience a decrease in their hemoglobin levels, associated with fatigue, reduced exercise tolerance and decreased quality of life. To counteract the hematological side-effects of antiviral treatment, and increase adherence to the treatment course, erythropoietin (EPO) has been utilized. EPO in non-transplanted patients has increased the number of patients able to maintain their RBV dose throughout the treatment course (130, 131).

5.6.2 Leucopenia

A decrease in WBC counts, due to bone marrow suppression induced by Peg-IFN, is seen during treatment of HCV patients (123, 125). This has been discussed as a potential risk for bacterial infections (132), and recently, Peg-IFN and RBV in combination with 1st generation PIs, showed an increased frequency of serious bacterial infections (133). Filgrastim, a recombinant human granulocyte colony-stimulating factor (G-CSF), is used in several studies to counteract neutropenia, reviewed in 2008 (110). In most cases, however, neutropenia can be managed effectively with recommended dose modifications of Peg-IFN (134).

5.6.3 Thrombocytopenia

Thrombocytopenia is associated with advanced cirrhosis, with an insufficient hepatic production of thrombopoietin (TPO) and increased sequestration of platelets in the spleen. Patients with advanced cirrhosis have a higher risk of developing thrombocytopenia during treatment with IFN, and IFN dose modifications are frequent in such patients (123, 134).

5.6.4 Other side-effects

The most common other IFN induced side-effects are fever, flu-like symptoms, headache and depression (120). Other side-effects noted are thyroid disorders, ACR, liver failure and psychosis (110).

5.6.5 Rejection

The overall incidence of ACR in HCV transplanted patients varies between 30-50% (135). ACR is a rare but serious IFN induced complication during IFN based treatment for recurrent hepatitis C after liver transplantation. The reported incidence is ranging from 0 to 35% (129).

5.7 PREDICTORS OF RESPONSE

Predictors of response to peg-IFN and RBV treatment in immune-competent patients have been evaluated (41, 42). In transplanted patients, the recipient, donor and on-treatment factors have been evaluated in several treatment studies and reviewed (110). EVR at 12 weeks was the strongest on-treatment predictor of response in transplant patients. Monitoring of HCV RNA levels is therefore important for identification of patients who should discontinue treatment. Other important host-related predictors of treatment response are fibrosis pre-treatment, where treatment initiated at earlier stages of fibrosis predicts higher SVR rates (118). Concerning HCV genotype, higher SVR rates are associated with genotype 2 and 3. (136, 137, 138, 139). The IL28B genotype in the donor and recipient has a substantial impact on the outcome of peg-IFN and RBV treatment post-transplant (94, 98, 140). Higher SVR rates are noted in patients with the favorable IL28B CC genotype, the highest when the most favorable genotype CC is present in both the donor and recipient.

5.8 LONG-TERM BENEFITS OF TREATMENT

5.8.1 Treatment and fibrosis progression

The response to INF-based therapies is associated with improved outcome including improved histology with diminishing fibrosis. However, due to small sample size, and different scoring systems used, the association has not been as obvious as that seen in immuno competent patients (141). Treatment has often been initiated 3 months or more after the liver biopsy was performed, allowing further progression of fibrosis to occur before treatment is commenced, contributing to difficulties to show histological benefit from antiviral treatment. In studies with follow-up biopsies 3-5 years after treatment, the benefit of antiviral treatment on fibrosis progression seems to be more evident. In one study, the rate of fibrosis progression diminished after antiviral treatment, and progression of fibrosis seemed to be prevented when SVR was achieved (142). Another study showed that fibrosis stabilization and improvement became evident first in the biopsy performed at least 12 months post-treatment, with histological improvement in 92% in patients with SVR and only in 41% in non-responders to treatment (118).

5.8.2 Treatment and survival

Since SVR to antiviral treatment is associated with regression of fibrosis, treatment also plays a key role in the prevention of hepatitis C-related graft failure and for survival. Significantly

lower mortality was noted in recipients who achieved SVR (137). In another study, the survival was significantly higher in treated recipients than in matched untreated controls 7 years after LT, and patients with mild fibrosis had a more pronounced survival benefit from treatment (118). There is, thus, a significant improvement in survival among patients who achieve SVR, and patients with mild disease benefit most from antiviral therapy. Hence, antiviral treatment should be offered early in recipients with recurrent hepatitis C after LT.

5.9 RE-TREATMENT

Liver transplant patients with HCV recurrence who are non-responders to antiviral treatment will ultimately need a new liver transplant when end stage liver disease is reached. Data regarding re-treatment are scarce but in one study, SVR was achieved in 35% of the 79 retreated patients who were prior non-responders with cirrhosis present in 37% (143). Full-dose RBV was used with EPO-support. EVR was the strongest predictor of SVR followed by age, disease severity, and adherence. Thus, treatment response with re-treatment can be predicted by the same factors as response in naïve LT recipients, and SVR can be achieved in 1 of 3 patients, provided growth factors are given and the patients stay adherent to treatment.

6 NEW TREATMENT STRATEGIES

6.1 ANTIVIRAL TREATMENT WITH PEG-IFN AND RBV IN COMBINATION WITH 1ST GENERATION PROTEASE INHIBITORS (PIs)

There have been significant changes in the management of non-transplant patients with HCV genotype 1 infections with the approval of two HCV NS3/4A serine protease inhibitors (PIs), telaprevir (TVP) and boceprevir (BOC). The addition of these 1st generation PIs to the previous standard of care with Peg-IFN and RBV, increased SVR rates to approximately 75% in HCV genotype 1 (144-146).

In transplant patients however, hematological side-effects, infections, and drug-drug interactions with CNIs, made this treatment dangerous, and it did not gain broad acceptance (133, 147). Triple therapy with 1st generation PIs has now been abandoned.

6.2 TREATMENT WITH NEW DIRECT ACTING ANTIVIRALS (DAAS)

With the introduction of new DAAs, patients on the transplant waiting list with HCV cirrhosis can be treated more safely and with higher SVR rates. Pre-transplant Sofosbuvir and RBV have been given to patients with chronic HCV infection with compensated cirrhosis (104). At time of transplantation, 93% were HCV RNA negative and 64% were still HCV RNA negative 12 weeks post-transplant. Among the 25 patients who had been HCV RNA negative 4 weeks or more pre-transplant, there was only one relapse (4%).

Also, after transplantation, in recipients with HCV recurrence, the treatment possibilities are rapidly changing and IFN-free regimens are introduced which carries improved SVR rates.

Sofosbuvir in combination with RBV has been given to patients with HCV relapse during 24 weeks to patients with severe fibrosis (F3-4). The majority became HCV RNA negative already at treatment week 4 and continued to be so at end of treatment. Finally, the preliminary report stated that 77% achieved SVR12, indicating cure (105). No interaction with CNIs was noted and the treatment was well tolerated. Combination treatment with drugs from 2 DAA classes has been used successfully for treatment of FCH in a case report (148).

7 AIMS

The overall aim was to study the natural course of hepatitis C recurrence after liver transplantation, the influence of antiviral therapy with Peg-IFN and RBV and IL28B polymorphism in the recipient and donor on the long-term outcome.

7.1 SPECIFIC AIMS

- 1 To develop an optimal RBV dosing schedule in liver transplant recipients.
- 2 To evaluate a concentration guided Ribavirin dosing scheme in liver transplant recipients.
- 3 To study the influence of treatment adherence and compliance on the SVR rate.
- 4 To evaluate the utility for prediction of fibrosis outcome of protocolled 6 and 12 months liver biopsies after liver transplantation.
- 5 To study the impact of fibrosis stage prior to treatment, IL8B SNP (rs12979860) in donor and recipient, and host factors on treatment outcome.
- 6 To evaluate the progression of fibrosis in the liver graft after HCV recurrence according to treatment outcome and IL28 gene polymorphism.

8 MATERIALS AND METHODS

8.1 STUDY PARTICIPANTS

In paper I, II and IV all patients were liver transplanted at Karolinska University Hospital, Huddinge, due to end-stage liver disease caused by HCV with or without HCC. Data on liver transplant recipients and their donors are available in InfCare Hepatitis, Equator and local data bases at Karolinska University Hospital, described in detail below. In paper III, a Nordic Multicenter Study on treatment of recurrent hepatitis C, patients were recruited from each participating center.

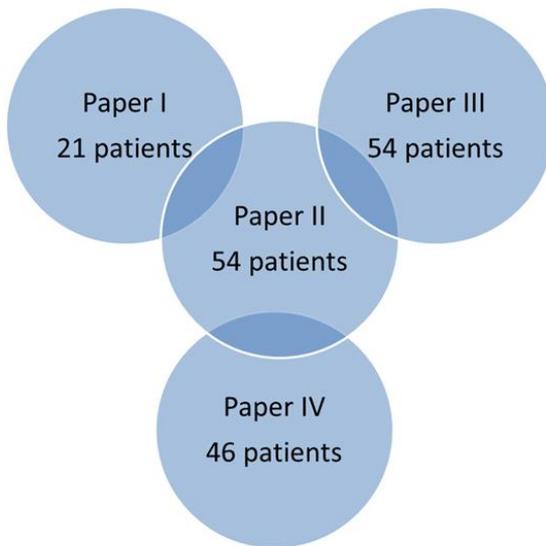


Figure 4: *Overwiev of study participants in paper I - IV*

8.1.1 Paper I

In a pilot study on concentration guided RBV-dosing with EPO support, 21 patients liver transplanted at Karolinska University Hospital, Huddinge due end-stage liver disease caused by HCV with or without HCC from 1999 to 2008 where included. The majority of patients where Caucasian. Eleven patients had HCV genotype 1 or 4 and 10 patients had HCV genotype 2 or 3. Patient characteristics are depicted in Table1 in paper I.

Twenty of 21 were also included in Paper II

8.1.2 Paper II

The influence of IL28B polymorphism on the natural course and treatment outcome in 54 liver transplant recipients with recurrent HCV, was studied. All the patients were transplanted at Karolinska University Hospital Huddinge between 1997-2010, due to end-stage liver disease caused by HCV, with or without HCC, and all were treated with Peg-IFN and RBV post-transplant, during 2001 to 2011. Pre-treatment with EPO and concentration-guided RBV dose was used, in 38/54 according to the protocol described in paper I. All 54 recipients were analyzed for IL28B genotype and 45 of their donors. The majority of the recipients were Caucasian, and 35 had HCV genotype 1 or 4 and 19 had genotype 2 or 3. Patient characteristics are depicted in Table I in study II.

8.1.3 Paper III

In a Nordic Multicenter Study on concentration-guided RBV-dosing with EPO support, 54 patients from the four participating centers were included; 29 patients from Karolinska University Hospital, 8 from Sahlgrenska University Hospital, 9 from Malmö University Hospital, and 8 from Akers Rikshospital, Oslo. All patients were transplanted due to end-stage liver disease caused by HCV, with or without HCC. 36 patients had HCV genotype 1 and 4 and 18 had HCV genotype 2 or 3. All patients were pre-treated with EPO, starting 2 weeks prior to the RBV and Peg-IFN treatment was initiated, and continued throughout the entire treatment. The formula used for calculating the RBV-dose is described in detail in paper I and III. Patient characteristics are depicted in Table 1 in paper III.

18 of these recipients were also included in paper II.

8.1.4 Paper IV

In this study, we evaluated the utility of protocolled liver biopsies performed 6 and 12 months post-transplant. We included 46 patients who had a liver transplant at Karolinska University Hospital, Huddinge due to end-stage liver disease, caused by HCV with or without HCC during 2008 to 2011. The majority of patients were Caucasians, and 36 had HCV genotype 1 or 4 and 18 genotype 2 or 3. Patient characteristics are depicted in table 1 in study IV.

7 of these recipients were also included in paper II.

8.2 METHODS

8.2.1 InfCare Hepatitis

All patients with hepatitis C monitored and treated at the Department of Infectious Diseases at Karolinska University Hospital are registered in the database InfCare Hepatitis. Demographics, virology, biochemical testing, histological findings, data on treatment regimen, and response to treatment were extracted in all our study patients from this data base.

8.2.2 Eqvator

All liver transplanted patients at the Department of Transplant Surgery at Karolinska University Hospital are registered in the database Eqvator. Data from donor, recipient, surgery, rejection episodes, immunosuppressive drugs, graft and patient survival was extracted.

8.2.3 Virological methods

HCV genotyping was performed by a line probe assay (Inno-LiPA HCV 2, Innogenetics NV, Gent, Belgium) or by an in-house method.

Hepatitis C virus RNA levels (HCV RNA) were measured at time of transplantation and during routine follow-up at the Department of Transplant Surgery, Karolinska University Hospital and at the Department of Infectious Diseases, Karolinska University Hospital, using the Roche TaqMan test with a sensitivity of 15 IU/mL. In the treatment studies (paper I and III), HCV RNA was measured at baseline, at 4 weeks, 12 weeks, at EOT, and during follow-up 6 months after stopping treatment.

8.2.4 Antiviral treatment

All treated patients in paper I-IV received Peg-IFN alfa 2a, 180 µg in patients with HCV genotype 1 and 4 and 135 µg in HCV genotype 2 or 3.

RBV formula and dose

All patients in paper I and III were pre-treated with EPO, starting 2 weeks prior RBV and Peg-IFN treatment was initiated, and continued throughout the entire treatment. The RBV dose was calculated with a formula taking body weight and renal function into account, initially developed and studied in patients with hepatitis C and renal insufficiency (149):

RBV dose = 0.244 x Ctarget x T x (0.122 x Clcreat + 0.0414 x body weight)

Ctarget set to 10 µmol/L and T set to dosage intervall 12 hours

0.244 is a scale factor to convert RBV dose from µmol to mg.

8.2.5 RBV concentration

The target RBV plasma concentration was set to 10 µmol/L, and was analyzed at week 4 and week 12 during the treatment.

8.2.6 Treatment outcome definitions

Rapid viral response was defined as a negative HCV RNA test at week 4, EVR and complete early viral response (cEVR) as a 2 log₁₀ drop in HCV RNA levels and a negative HCV RNA test, respectively, 12 weeks after starting treatment. Patients who did not achieve EVR stopped

treatment at week 12. End-of-treatment viral response (ETR) was defined as negative HCV RNA when treatment was stopped, and SVR as negative HCV RNA at treatment stop and after 24 weeks of follow-up.

8.2.7 Adherence to treatment

Adherence was defined as complete if treatment in patients with genotype 2 and 3 was carried on throughout the 24 weeks, and for genotype 1 and 4 throughout the 48 weeks. Adherence to treatment was also defined as complete if the treatment was withdrawn at week 12 owing to non-response, and at week 24 if HCV RNA had not become negative.

8.2.8 Hematological parameters

All hematological parameters were analyzed at accredited laboratories at Karolinska University Hospital.

8.2.9 DNA extraction and IL28B genotyping

Genomic DNA was extracted from peripheral blood or from spleen. The DNA from spleen was extracted in NucliSens, EasyMAG, Biomérieux. The DNA samples were genotyped for IL28B *rs12979860* polymorphism with TaqMan SNP genotyping assay (Applied Biosystems Inc, Foster City, CA, USA), using the ABI 7500 Fast equipment. All TaqMan probes and primers were designed and synthesised by Applied Biosystems Inc. Automated allele calling was performed using SDS software from Applied Biosystems. The primers and probes used were: *rs12979860* Forward primer: 5'GCCTGTCGTGACTGAACCA3', Reverse primer: 5'GCGCGGAGTGCAATTCAAC3', Vic probe: 5'TGGTTTCGCGCCTTC3', Fam probe: 5'CTGGTTTCACGCCTTC3'. Human genomic DNA was purified from 5 x10⁶ spleen cells according to the manufacturer's instructions using the QIAamp DNA Mini kit (Qiagen, Tokyo, Japan), except for one change in the elution step, elution was done using 100 microliter elution-buffer instead of 200 microliter. The result is presented as CC, C/T or TT genotype.

8.2.10 Allograft histology

All liver biopsies were fixed in 4% formaline in a phosphate buffer, dehydrated and embedded in paraffin. Tissue sections, 2-3 µm thick, were cut on a microtome and stained with hematoxylin/eosin (HE) and Sirius staining, 8 and 4 section levels respectively. The biopsy material was defined as representative if the number of portal zones were > 8. Inflammation was graded 1-4 (A 1-4) in HE staining and the fibrosis staged 1-4 (F 1-4) according to Ludwig and Batts (25). HCV recurrence was confirmed when a liver biopsy showed findings consistent with histological relapse and F 1 or more, provided HCV RNA was detectable in serum. The fibrosis was defined as mild when the fibrosis stage was $F \leq 2$ and severe when it was $F \geq 3$.

8.2.11 Liver stiffness measurement (LSM) by FibroScan

After 2009, liver elasticity by FibroScan, was performed routinely in patients with a biopsy showing HCV recurrence, and also during follow-up after antiviral treatment. The liver elasticity was measured in kPa, and the fibrosis staged as F1 – F4 according to Castera et al (26). 8.7 kPa was used as cut-off where $kPa \leq 8.7$ correlates to $F \leq 1$ and $kPa > 8.7$ to $F \geq 2$. (83).

8.2.12 Statistics

The Chi-Square test was used for categorical variables and the Wilcoxon Rank Sum test for continuous variables in paper I-IV. A P value < 0.05 was considered statistically significant. All data were analysed using JMP software version 9.0.0.

In paper II we performed an univariate analysis on factors associated with SVR. We included recipient and donor age, gender, HCV genotype, IL28B gene polymorphism, in both donor and recipient, baseline viral load, time from LT to treatment and fibrosis pre-treatment. The factors found to be significantly associated with SVR, HCV genotype and fibrosis pre-treatment, were included in a stepwise Cox regression model. In paper III we performed an univariate analysis on factors associated with SVR. We included age, gender, HCV genotype, IL28B gene polymorphism, fibrosis pre-treatment, and mean RBV concentration at week 4 and 12. The factors found to be significantly associated with SVR, HCV genotype, IL28B genotype and fibrosis pre-treatment, were included in a stepwise Cox regression model.

9 RESULTS AND DISCUSSION

9.1 Paper I: PILOT STUDY ON CONCENTRATION GUIDED RBV DOSING WITH EPO SUPPORT

21 HCV liver transplant recipients received Darbepoetin with start 2 weeks prior to treatment start and continued during the entire antiviral therapy.

9.1.1 Adherence and tolerance to treatment

In HCV genotype 1, 82% completed treatment and 90% in HCV genotype non-1. Thus, a majority of our patients stayed adherent to a full treatment course, an improvement compared to other studies with fixed RBV dose and without EPO support (109, 150). Only one patient ended treatment early due to anaemia. The majority of patients had reasonable fall in hemoglobin levels (mean hemoglobin fall, 20–25 g/L). To achieve this, however, dose escalation of Darbepoetin to 150 µg weekly was required in 40% of the patients. No serious adverse events occurred and no rejection episode was noted during treatment and follow-up. Thus, treatment with Peg-IFN and concentration guided RBV with EPO support can be considered safe, and for HCV genotype non-1 patients a cost-saving alternative compared to treatment with new DAA combinations.

9.1.2 RBV concentration

The RBV concentration of 10 µmol/L is generally correlated with favorable SVR rates in non-transplanted and haemodialysis patients (127, 151). This concentration was possible to achieve in the absolute majority of our patients, but only when we used pre-treatment with Darbepoetin. Dose adjustment of RBV was made in 48% of our patients, underlining the necessity of monitoring the RBV concentration during treatment.

	<u>Genotype 1/4</u>	<u>Genotype 2/3</u>		
	<u>Week 4</u>	<u>Week 12</u>	<u>Week 4</u>	<u>Week 12</u>
No tested/total no	11/11	11/11	7/10	9/10
RBV concentration	10.2	11.7	7.36	9.42
range (mikromol/L)	6.3-18.1	7.6-18	4.9-9.4	4.1-13.5

Table 1: Ribavirin concentrations week 4 and 12 according to HCV genotype 1/4 or 2/3

9.1.3 Treatment response

ETR was achieved in 36% and 80% in HCV recipients with genotype 1 and non-1 respectively ($p < 0.05$), and the corresponding figures for SVR was 18% versus 60% ($p < 0.05$). Two patients in each group relapsed after treatment. The overall SVR rate was thus not impressive. If treatment is initiated at earlier stages of fibrosis, the SVR rate would probably be higher (118). In accordance with these findings, patients with low-grade fibrosis (F 1-2) achieved SVR in 50 %, where no patient with advanced fibrosis (F 3-4) did ($P < 0.05$). If we excluded patients with advanced fibrosis, patients with HCV genotype 1 and non-1 achieved SVR in 29% versus 67%. Thus, almost two thirds of patients with the favorable combination of low grade fibrosis and HCV genotype non-1 achieved SVR, the same figure as seen in non-transplanted patients (32, 33, 44).

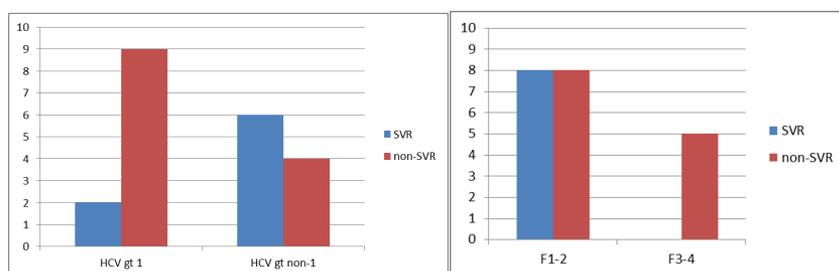


Figure 5: Treatment response according to HCV genotype and fibrosis stage

9.2 PAPER II: THE INFLUENCE OF IL28B GENE POLYMORFISM IN RECIPIENT AND DONOR ON FIBROSIS PROGRESSION AND TREATMENT RESPONSE

We analyzed the IL28B genotype *rs12979860* in 54 patients, all transplanted due to HCV, and in 45 of their donors.

9.2.1 Distribution of IL28B genotype

Among the 54 recipients, 12 had IL28B CC and 42 IL28B non-CC. In the 45 donors the IL28B CC genotype was more frequent, 30 versus 15 with IL28B CC and non-CC respectively. The difference in distribution was significant both in recipient and donor. This is seen also in previous studies (94, 97), and an explanation for this could be that the favorable genotype CC is associated with a higher spontaneous clearance rate, and that more HCV patients with genotype CT and TT will develop chronic HCV and cirrhosis, eventually leading to end-stage liver disease and liver transplantation (21). Similarly, as in non-transplanted patients, the IL28B CC

genotype in transplant recipients is associated with higher SVR rates after SOC treatment than the non-CC genotype (94, 97, 98). Hence, non-CC recipients are more likely to be prior non-responders to treatment with Peg-IFN and RBV, increasing the proportion even further of non-CC patients among liver transplant recipients.

9.2.2 Treatment response according to HCV genotype

The SVR rate in HCV genotype 1 versus non-1 was 9/35 (26%) and 13/19 (68%) ($p=0.002$), in agreement with earlier findings in IFN-based treatment regimens (110, 119).

9.2.3 Treatment response according to IL28B genotype

The SVR rate according to IL28B genotype was 58% for CC and for recipients with IL28B CC, 36% ($p=0.16$). The corresponding figures according to IL28B genotype in the donor was 43% versus 40%, respectively ($p=0.83$). Although not significant, a trend towards a more favorable treatment outcome was noted in both recipient and donor with IL28B CC genotype.

The effect on SVR rate in recurrent hepatitis C after liver transplantation, associated with the recipient IL28B genotype, has been shown earlier and was reviewed in 2012 (152). The influence of IL28B donor genotype alone, is not as clear. The combination of IL28B CC both in the donor and in the recipient has been found to be the most favorable (95, 152).

When we combined HCV genotype non-1 and IL28B CC in the recipient, SVR was achieved in 71% achieved SVR versus only 23% in the recipients with HCV genotype 1 and IL28B non-CC ($p= 0.02$).

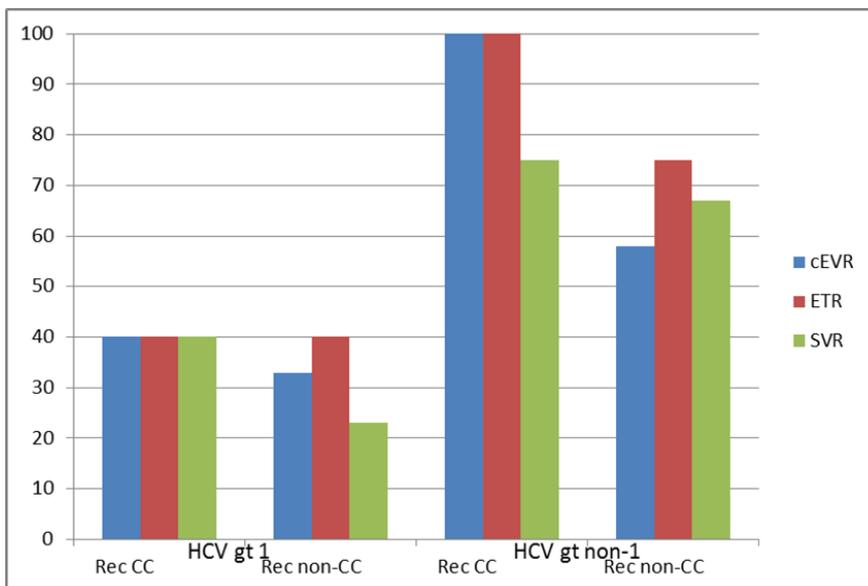


Figure 6: Treatment response according to HCV genotype 1 and non-1, and according to HCV genotype and IL28B genotype combined

9.2.4 Treatment response according to fibrosis stage pre-treatment

SVR in recipients with in mild fibrosis (F 1-2) and advanced fibrosis (F 3-4) at baseline, was 61% and 27% respectively, indicating the importance of treating patients at an early fibrosis stage of recurrent HCV, to increase the possibility to achieve SVR. (118, 119).

9.2.5 Fibrosis progression post-transplant according to IL28B genotype

Recipients with IL28 CC tended to have less advanced fibrosis prior to treatment initiation. Thus, mild fibrosis was seen in 64% of recipients with IL28B CC, versus 38% of recipients with IL28B non-CC, a non-significant difference, $p = 0.13$. Although earlier finding are not uniform, the IL28B CC genotype seems to be associated with a slower fibrosis progression post-LT (94). At follow-up after treatment, significantly more of our recipients with CC had mild fibrosis than recipients with non-CC, 75% versus 32%, respectively. All recipients with SVR who had IL28B CC, had mild fibrosis.

9.3 Paper III: NORDIC MULTICENTER STUDY ON TREATMENT FOR HCV RELAPSE AFTER LIVER TRANSPLANTATION

54 liver transplant patients from four Nordic centers were recruited. All received Darbepoetin with start 2 weeks prior to treatment initiation and throughout the antiviral therapy. RBV was given concentration guided with Peg-IFN.

9.3.1 Adherence and tolerance to treatment

Two patients with HCV genotype 1 withdrew from treatment prematurely, one at week 1 due to rash and pruritus, and one at week 4 due to myocardial infarction. Among HCV genotype non-1 infected recipients, one stopped treatment early due to severe cholangitis at week 7. The mean drop in hemoglobin level in the recipient was 37 g/L (range 0-87). The nadir hemoglobin level occurred at week 22 (range 4-56). The Darbepoetin dose had to be adjusted in 52% of the patients. It was raised due to anemia in 88%, and tapered due to high hemoglobin levels in 12%. 2 patients needed blood transfusions. Thus, both adherence and tolerance to the treatment course was excellent, as seen in paper I (119). To achieve these figures however, frequent monitoring and dose adjustments were necessary.

9.3.2 RBV dose and concentration

The RBV dose needed to achieve an intended serum concentration is highly individual and dependent on kidney function. By using the formula from paper I, developed for antiviral treatment with Peg-IFN and RBV in patients with kidney failure (127, 151), it was possible to individualize the dose also in liver transplant patients, who often suffers from impaired renal function, due to co-morbidities and immunosuppression with CNI (49). The mean RBV dose at treatment start was 800 mg (range 400-1400 mg). Dose adjustments were done dependent on the RBV concentrations noted at week 4 and week 12, or due to anemia, in 78% of the patients.

The intended serum RBV concentration of 10 $\mu\text{mol/L}$, was achieved in a majority of the patients. The mean serum RBV concentration at week 4 was 7.7 $\mu\text{mol/L}$ (range 3.1-15.9), and at week 12 11.2 $\mu\text{mol/L}$ (5.3-21.6), with no significant difference between HCV genotype 1 and non-1. Hence, the RBV dosing formula worked well in this study. Since new direct antiviral drugs (DAAs), in particular Sofosbuvir, have recently become available for treatment of HCV relapse in liver transplant patients, treatment options are changing rapidly. Presently, however, RBV is still used. Thus, an optimized RBV dose will continue to be of importance, also when Sofosbuvir is used in combination with RBV, a combination that has offered very promising results, when used both prior to and after LT (104, 105).

9.3.3 Virological response according to fibrosis stage pre-treatment, HCV genotype and IL28B genotype

Before 2008 antiviral therapy was not initiated at early stages of HCV recurrence. This led to poor SVR rates since treatment response is generally lower in patients with advanced fibrosis/cirrhosis (118, 119). In our study, 50% of the patients had mild (F 1-2) fibrosis at treatment start. Patients with mild (F 1-2) and advanced fibrosis (F 3-4), achieved SVR in 56%, and 26% respectively, $p = 0.025$.

As discussed earlier in paper II, the HCV genotype and the IL28B genotype have a profound influence on the treatment response in liver transplant patients (153). SVR was thus achieved in recipients with HCV genotype 1 in 22%, and with HCV genotype 2 and 3 in 78%. In patients with IL28B CC versus non-CC, SVR was achieved in 73% and 33% respectively.

9.3.4 Univariate and multivariate analysis of factors associated with SVR

Factors associated with SVR in univariate analysis were HCV genotype, IL28B gene polymorphism and fibrosis pre-treatment. Only HCV genotype ($p=0.0003$) and IL28B gene polymorphism ($p=0.007$) were significantly associated with SVR in the multivariate analysis, whereas fibrosis pre-treatment ($p=0.06$) did not reach full statistical significance. Our univariate analysis and earlier findings, however, have shown that less advanced baseline fibrosis stage predicts a better treatment response (118).

<u>Variable</u>	<u>Univariate</u>
Age over (n=28)/under (n=23) mean 56	p = 0.08
Sex M (n=40)/F (n=11)	p = 0.61
HCV gt 1 (n=34)/gt non-1 (17)	p = <0.0001*
IL28B CC (n=11)/non-CC (40)	p = 0.025*
F1-2 (n=26)/F3-4 (n=25)	p = 0.031*
F1-3 (n=40)/Cirrhosis (n=11)	p = 0.22
RBV under (n=27)/over (n=24) mean 7.7 μmol/L	p = 0.06
RBV under (n=27)/over (n=22) mean 11.2 μmol/L	p = 0.28

<u>Variable</u>	<u>Multivariate</u>
HCV gt 1 (n=34)/gt non-1 (17)	p=0.0003*
IL28B CC (n=11)/non-CC (40)	p=0.007*
F1-2 (n=26)/F3-4 (n=25)	p=0.06

Figure 7: *Univariate and multivariate analysis of baseline factors associated with favorable treatment outcome*

HCV genotype and IL28B gene polymorphism in the recipient were found to be the most important baseline predictors of treatment response to Peg-IFN and RBV in patients with HCV relapse after transplantation. When HCV genotype and IL28B gene polymorphism were combined for prediction of response, the highest SVR rate was found in recipients with HCV genotype 2 and 3 and IL28B CC, all three patients achieved SVR, as earlier noted by us and others (94, 153). However, the overall 43% SVR rate with peg-IFN and RBV was not impressive. In HCV genotype 2 and 3 patients, 82% achieved SVR whereas only 24% of HCV genotype 1 patients did, $p < 0.0001$. For recipients with HCV genotype 2 and 3, this is comparable to SVR rates in non-transplant patients with genotype 2 or 3 infections, and SVR

rates in liver transplant patients treated with Sofosbuvir and RBV (33, 105, 154). Since treatment with the new DAAs carries a high cost, this regimen can therefore still be used with reasonable treatment results in patients with HCV genotype 2 and 3. For HCV genotype 1 infected recipients, however, this treatment regimen is suboptimal and should be replaced with DAA combinations, including Sofosbuvir, in combination with Daclatasvir or Simeprevir (148).

9.4 Paper IV: EVALUATION OF EARLY BIOPSY 6 AND 12 MONTHS POST-TRANSPLANT

46 patients transplanted due to HCV, who underwent protocolled liver biopsies 6 and 12 months post-LT, were studied. Eleven recipients who were negative for HCV RNA, but anti-HCV positive post-LT, served as a control group.

9.4.1 Histological recurrence at 6 and 12 months post-transplant

Histological recurrence with fibrosis stage \geq F1 was noted in 56% at 6 months post-LT, the majority (18/19) with mild fibrosis (F1-2). It is thus possible to detect HCV recurrence early post-transplant, supporting the strategy to perform protocolled biopsies already 6 months post-transplant. When divided into slow (F0-F1) and rapid (F2-F4) fibrosers at 6 months post-LT, 76% recipients were slow and 24% rapid fibrosers. IL28B CC was more frequently noted in patients with rapid fibrosis ($p=0.05$)

The number of patients with histological evidence of HCV recurrence increased to 82% at 12 months post-LT, where 89% had mild fibrosis (F 1-2). At this time point 44% of the recipients were slow fibrosers ($F \leq 1$) and 56% were rapid fibrosers with $F \geq 2$.

9.4.2 Factors associated with rapid HCV recurrence

It has previously been shown that treatment of ACR with steroid boluses or anti-lymphocytic agents, will lead to an enhanced viral replication, higher HCV RNA levels, and an increased fibrosis progression (77, 155). This is in accordance with our findings where the 3 recipients with severe fibrosis progression at 12 months post-LT all had suffered from ACR.

The IL28B CC genotype was associated with rapid fibrosis progression also at 12 months post-LT, $p=0.01$. Earlier findings on fibrosis progression and IL28B CC have not yielded uniform results. Hence, some found a more pronounced inflammatory activity, in IL28B CC recipients, leading to a rapid fibrosis progression (95).

9.4.3 Liver transplant recipients with slow fibrosis progression

Some liver transplant recipients with HCV recurrence will have a slow fibrosis progression. In the 26 recipients with slow fibrosis progression at 6 months post-LT, 50% had F0-F1 also in the 12 months biopsy. This is an interesting finding, since this subgroup of patients may not need

antiviral treatment, at least not early post-LT. Due to the expected high costs with the new DAAs, identifying this group is also important concerning saving of costs.

9.4.4 Evaluation of fibrosis in the HCV RNA negative control group

In the HCV RNA negative control group, 18% had fibrosis in the graft at time of transplantation. However, none of the eleven recipients showed histological signs of HCV recurrence during follow-up. Up till now, IFN-based regimens given pre-transplant have been associated with poor SVR rates and a risk of complications (112, 114). With the new DAAs however, antiviral treatment pre-transplant, also in cirrhotic patients, has offered promising results (105).

10 CONCLUSIONS AND FUTURE ASPECTS

The formula we used for calculating the RBV dose, originally developed for patients with renal failure, worked well also in liver transplant patients, where the target concentration of 10 $\mu\text{mol/L}$ was achieved in the majority of our patients. By using Darbepoetin two weeks prior to start of treatment with Peg-IFN and RBV, and continuing throughout the whole treatment course, tolerance to treatment was improved, and the regimen enabled the patients to stay adherent to a full treatment course. Presently, new DAAs are being evaluated among liver transplant patients, where RBV in combination with Sofosbuvir have shown promising results. Thus, an optimized RBV dose will continue to be of importance in the treatment of HCV recurrence post-transplant.

IL28B gene polymorphism in liver transplant recipient and donors influences the natural course and treatment outcome. Among our recipients, the IL28B CC genotype was associated with a better treatment response to Peg-IFN and RBV. Recipients with IL28B CC genotype had less advanced fibrosis both pre and post-treatment, and all recipients with IL28B CC who achieved SVR had mild fibrosis at follow-up. The combination of IL28B CC and HCV genotype non-1 yielded the highest SVR rates. Even after the introduction of new DAAs in liver transplant patients, HCV genotype and IL28B gene polymorphism will still be baseline factors important to determine, although the extent needs to be evaluated.

In paper I+II+III, all patients were treated with Peg-IFN and RBV and treatment response was evaluated. Patients with HCV genotype non-1 and IL28B CC genotype had the highest SVR rates, and patients with this favorable combination, treated at early stages of fibrosis (F1-2) had SVR rates comparable to treatment results in non-transplant patients. Thus, treatment at earlier stages of fibrosis should be offered.

We evaluated protocolled liver biopsies post-transplant, and already at 6 months after transplantation about 50% of the recipients showed histological signs of recurrence with $F \geq 1$. The corresponding figures at 12 months were more than 80%. We also identified a group of liver transplant recipients with HCV recurrence with slow ($F \leq 1$) fibrosis progression post-transplant. An early liver biopsy is therefore both useful in identifying patients with rapid fibrosis progression in order to offer treatment early, and to monitor patients with slow fibrosis progression that might not need antiviral treatment, a cost-saving strategy.

None of the liver transplant recipients, who were HCV RNA negative at time of transplantation, showed histological signs of HCV recurrence during follow-up. Therefore, if possible, antiviral treatment should be offered pre-transplant, a strategy which will be both safer and more efficient with newer treatment regimens.

11 SAMMANFATTNING PÅ SVENSKA

Hepatit C virus (HCV) infektion efter levertransplantation (LT) leder till en snabbare ärrbildning än hos icke transplanterade. 25 % av patienterna utvecklar skrumplever (cirrhos) inom 5 år efter LT. Antiviral behandling efter LT med Peg-Interferon (Peg-IFN) och ribavirin (RBV) har lägre utläkningsgrad (SVR) än hos icke-transplanterade patienter, delvis beroende på ökad frekvens av biverkningar, framförallt anemi. Patienter som inte svarar på behandling (NR) löper stor risk för att utveckla skrumplever.

Syftet med denna avhandling var att studera naturalförlopp och behandlingsutfall hos levertransplanterade patienter med återfall av hepatit C. Vi studerade även hur basala värdfaktorer påverkar förloppet av hepatit C återfallet, och svaret på antiviral behandling, med fokus på HCV genotyp och IL28B polymorfism.

I det första arbetet utförde vi en pilot-studie på 21 patienter, levertransplanterade på grund av hepatit C. Alla förbehandlades med Darbeoetin (EPO) med start 2 veckor före den antivirala behandlingen med Peg-IFN och RBV. Syftet var att öka följsamhet och tolerans för den antivirala behandlingen. RBV doserades enligt en formel baserad på vikt och njurfunktion, en önskad serumkoncentration på 10 µM eftersträvades. Majoriteten av patienterna uppnådde målkoncentrationen och 90 % fullföljde den planerade behandlingen. 60 % av HCV genotyp non-1 uppnådde SVR, men bara 18 % av de med genotyp 1. 67% av patienterna med mild ärrbildning (F1-2) uppnådde SVR. I andra arbetet studerade vi IL28B genpolymorfismens betydelse för ärrbildning och behandlingssvar bland 54 LT patienter med hepatit C återfall, som alla erhållit antiviral behandling, samt bland 45 av deras donatorer. Patienterna med kombinationen IL28B CC och HCV genotyp non-1 uppnådde SVR i 71 % mot endast 23% med kombinationen IL28B non-CC och HCV genotyp 1. Patienter med mild ärrbildning (F1-2) uppnådde oftare SVR. I tredje arbetet behandlade vi 46 svenska och 8 norska patienter med koncentrationsstyrd RBV dos, enligt formeln i arbete 1, i kombination med Peg-IFN. Alla förbehandlades med EPO. 94 % fullföljde behandlingen. SVR uppnåddes av patienter med HCV genotyp 2/3 i 82 % men endast av 22 % med HCV genotyp 1. Patienter med IL28B CC uppnådde SVR i 73 % och patienter med IL28B non-CC i 33 %. Patienter med mild ärrbildning (F1-2) uppnådde SVR i 56 % och patienter med avancerad ärrbildning (F3-4) i 26 %. Patienter med fördelaktig HCV genotyp och IL28B genotyp visade sig ha en bra möjlighet att läka ut sin HCV med denna behandlingsregim, framför allt vid mild ärrbildning. I fjärde arbetet utvärderade vi möjligheten att använda leverbiopsi tidigt efter LT för att upptäcka och förutsäga ärrbildning vid HCV recidiv. 46 HCV RNA positiva och 11 HCV RNA negativa LT patienter, som genomgått leverbiopsi 6 och 12 månader efter LT, studerades. Histologiskt HCV återfall sågs hos 56 % av HCV positiva patienter 6 månader efter LT, och hos 82 % efter 12 månader. Akut avstötning (ACR) och IL28B genotyp CC var faktorer som korrelerade till en mer uttalad ärrbildning. Ingen ärrbildning sågs hos de 11 HCV RNA negativa patienterna. Leverbiopsi efter LT kan användas för att upptäcka tidigt HCV återfall så att tidig behandling kan ges

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13 REFERENCES

1. Krugman S, Ward R, Giles JP. The natural history of infectious hepatitis. *Am J Med.* 1962 May;32:717-28.
2. Feinstone SM, Kapikian AZ, Purcell RH. Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. *Science.* 1973 Dec 7;182(4116):1026-8.
3. Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV. Transfusion-associated hepatitis not due to viral hepatitis type A or B. *N Engl J Med.* 1975 Apr 10;292(15):767-70.
4. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science.* 1989 Apr 21;244(4902):359-62.
5. Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science.* 1989 Apr 21;244(4902):362-4.
6. Moriishi K, Matsuura Y. Exploitation of lipid components by viral and host proteins for hepatitis C virus infection. *Front Microbiol.* 2012;3:54.
7. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science.* 1998 Oct 2;282(5386):103-7.
8. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment Web resource. *Hepatology.* 2014 Jan;59(1):318-27.
9. Westin J, Lindh M, Lagging LM, Norkrans G, Wejstal R. Chronic hepatitis C in Sweden: genotype distribution over time in different epidemiological settings. *Scand J Infect Dis.* 1999;31(4):355-8.
10. Global burden of disease (GBD) for hepatitis C. *J Clin Pharmacol.* 2004 Jan;44(1):20-9.
11. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology.* 2013 Apr;57(4):1333-42.
12. Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect.* 2011 Feb;17(2):107-15.
13. Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, et al. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet.* 2000 Mar 11;355(9207):887-91.
14. Sjoberg K, Widell A, Verbaan H. Prevalence of hepatitis C in Swedish diabetics is low and comparable to that in health care workers. *Eur J Gastroenterol Hepatol.* 2008 Feb;20(2):135-8.

15. Bruggmann P, Berg T, Ovrehus AL, Moreno C, Brandao Mello CE, Roudot-Thoraval F, et al. Historical epidemiology of hepatitis C virus (HCV) in selected countries. *J Viral Hepat.* 2014 May;21 Suppl 1:5-33.
16. Weiland O, Berg JV, Bjorvatn B, Flehmig B, Lundbergh P. Acute viral hepatitis A, B and non-A, non-B in Stockholm in the 1950s and 1970s: a comparison. *Infection.* 1981;9(6):268-74.
17. Luban NL, Colvin CA, Mohan P, Alter HJ. The epidemiology of transfusion-associated hepatitis C in a children's hospital. *Transfusion.* 2007 Apr;47(4):615-20.
18. Lidman C, Norden L, Kaberg M, Kall K, Franck J, Aleman S, et al. Hepatitis C infection among injection drug users in Stockholm Sweden: prevalence and gender. *Scand J Infect Dis.* 2009;41(9):679-84.
19. Yaphe S, Bozinoff N, Kyle R, Shivkumar S, Pai NP, Klein M. Incidence of acute hepatitis C virus infection among men who have sex with men with and without HIV infection: a systematic review. *Sex Transm Infect.* 2012 Nov;88(7):558-64.
20. Chen SL, Morgan TR. The natural history of hepatitis C virus (HCV) infection. *Int J Med Sci.* 2006;3(2):47-52.
21. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature.* 2009 Oct 8;461(7265):798-801.
22. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet.* 1997 Mar 22;349(9055):825-32.
23. Eurich D, Boas-Knoop S, Bahra M, Neuhaus R, Somasundaram R, Neuhaus P, et al. Role of IL28B polymorphism in the development of hepatitis C virus-induced hepatocellular carcinoma, graft fibrosis, and posttransplant antiviral therapy. *Transplantation.* 2012 Mar 27;93(6):644-9.
24. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology.* 1996 Aug;24(2):289-93.
25. Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol.* 1995 Dec;19(12):1409-17.
26. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol.* 2008 May;48(5):835-47.
27. Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Di Bisceglie A, Peters M, et al. Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon. A preliminary report. *N Engl J Med.* 1986 Dec 18;315(25):1575-8.
28. Reichard O, Andersson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. *Lancet.* 1991 May 4;337(8749):1058-61.
29. Dusheiko G, Main J, Thomas H, Reichard O, Lee C, Dhillon A, et al. Ribavirin treatment for patients with chronic hepatitis C: results of a placebo-controlled study. *J Hepatol.* 1996 Nov;25(5):591-8.

30. Reichard O, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Randomised, double-blind, placebo-controlled trial of interferon alpha-2b with and without ribavirin for chronic hepatitis C. The Swedish Study Group. *Lancet*. 1998 Jan 10;351(9096):83-7.
31. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alpha-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med*. 1998 Nov 19;339(21):1485-92.
32. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet*. 2001 Sep 22;358(9286):958-65.
33. Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med*. 2004 Mar 2;140(5):346-55.
34. Afdhal N, Reddy KR, Nelson DR, Lawitz E, Gordon SC, Schiff E, et al. Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med*. 2014 Apr 17;370(16):1483-93.
35. Afdhal N, Zeuzem S, Kwo P, Chojkier M, Gitlin N, Puoti M, et al. Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med*. 2014 May 15;370(20):1889-98.
36. Feld JJ, Kowdley KV, Coakley E, Sigal S, Nelson DR, Crawford D, et al. Treatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med*. 2014 Apr 24;370(17):1594-603.
37. Zeuzem S, Jacobson IM, Baykal T, Marinho RT, Poordad F, Bourliere M, et al. Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med*. 2014 Apr 24;370(17):1604-14.
38. Zeuzem S, Dusheiko GM, Salupere R, Mangia A, Flisiak R, Hyland RH, et al. Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med*. 2014 May 22;370(21):1993-2001.
39. Poordad F, Hezode C, Trinh R, Kowdley KV, Zeuzem S, Agarwal K, et al. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med*. 2014 May 22;370(21):1973-82.
40. Ferenci P, Bernstein D, Lalezari J, Cohen D, Luo Y, Cooper C, et al. ABT-450/r-ombitasvir and dasabuvir with or without ribavirin for HCV. *N Engl J Med*. 2014 May 22;370(21):1983-92.
41. Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med*. 2000 Dec 7;343(23):1666-72.
42. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*. 2009 Sep 17;461(7262):399-401.

43. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology*. 2003 Sep;38(3):645-52.
44. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology*. 2002 Oct;123(4):1061-9.
45. Dienstag JL, Cosimi AB. Liver transplantation--a vision realized. *N Engl J Med*. 2012 Oct 18;367(16):1483-5.
46. Adam R, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, et al. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J Hepatol*. 2012 Sep;57(3):675-88.
47. Benitez C, Londono MC, Miquel R, Manzia TM, Abraldes JG, Lozano JJ, et al. Prospective multicenter clinical trial of immunosuppressive drug withdrawal in stable adult liver transplant recipients. *Hepatology*. 2013 Nov;58(5):1824-35.
48. Creput C, Blandin F, Deroure B, Roche B, Saliba F, Charpentier B, et al. Long-term effects of calcineurin inhibitor conversion to mycophenolate mofetil on renal function after liver transplantation. *Liver Transpl*. 2007 Jul;13(7):1004-10.
49. Ojo AO, Held PJ, Port FK, Wolfe RA, Leichtman AB, Young EW, et al. Chronic renal failure after transplantation of a nonrenal organ. *N Engl J Med*. 2003 Sep 4;349(10):931-40.
50. Berenguer M, Aguilera V, Prieto M, San Juan F, Rayon JM, Benlloch S, et al. Significant improvement in the outcome of HCV-infected transplant recipients by avoiding rapid steroid tapering and potent induction immunosuppression. *J Hepatol*. 2006 Apr;44(4):717-22.
51. Lake JR. The role of immunosuppression in recurrence of hepatitis C. *Liver Transpl*. 2003 Nov;9(11):S63-6.
52. Klintmalm GB, Davis GL, Teperman L, Netto GJ, Washburn K, Rudich SM, et al. A randomized, multicenter study comparing steroid-free immunosuppression and standard immunosuppression for liver transplant recipients with chronic hepatitis C. *Liver Transpl*. 2011 Dec;17(12):1394-403.
53. Zaydfudim V, Feurer ID, Landman MP, Moore DE, Wright JK, Pinson CW. Reduction in corticosteroids is associated with better health-related quality of life after liver transplantation. *J Am Coll Surg*. 2012 Feb;214(2):164-73.
54. Neumann U, Samuel D, Truneka P, Gugenheim J, Gerunda GE, Friman S. A Randomized Multicenter Study Comparing a Tacrolimus-Based Protocol with and without Steroids in HCV-Positive Liver Allograft Recipients. *J Transplant*. 2012;2012:894215.
55. Garcia-Retortillo M, Fornis X, Feliu A, Moitinho E, Costa J, Navasa M, et al. Hepatitis C virus kinetics during and immediately after liver transplantation. *Hepatology*. 2002 Mar;35(3):680-7.
56. Powers KA, Ribeiro RM, Patel K, Pianko S, Nyberg L, Pockros P, et al. Kinetics of hepatitis C virus reinfection after liver transplantation. *Liver Transpl*. 2006 Feb;12(2):207-16.

57. McCaughan GW, Zekry A. Mechanisms of HCV reinfection and allograft damage after liver transplantation. *J Hepatol.* 2004 Mar;40(3):368-74.
58. Gane EJ, Portmann BC, Naoumov NV, Smith HM, Underhill JA, Donaldson PT, et al. Long-term outcome of hepatitis C infection after liver transplantation. *N Engl J Med.* 1996 Mar 28;334(13):815-20.
59. Neumann UP, Berg T, Bahra M, Seehofer D, Langrehr JM, Neuhaus R, et al. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. *J Hepatol.* 2004 Nov;41(5):830-6.
60. Berenguer M, Aguilera V, Prieto M, Carrasco D, Rayon M, San Juan F, et al. Delayed onset of severe hepatitis C-related liver damage following liver transplantation: a matter of concern? *Liver Transpl.* 2003 Nov;9(11):1152-8.
61. McCaughan GW, Zekry A. Pathogenesis of hepatitis C virus recurrence in the liver allograft. *Liver Transpl.* 2002 Oct;8(10 Suppl 1):S7-S13.
62. Wiesner RH, Sorrell M, Villamil F. Report of the first International Liver Transplantation Society expert panel consensus conference on liver transplantation and hepatitis C. *Liver Transpl.* 2003 Nov;9(11):S1-9.
63. Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology.* 2002 Apr;122(4):889-96.
64. Neumann UP, Berg T, Bahra M, Puhl G, Guckelberger O, Langrehr JM, et al. Long-term outcome of liver transplants for chronic hepatitis C: a 10-year follow-up. *Transplantation.* 2004 Jan 27;77(2):226-31.
65. Firpi RJ, Abdelmalek MF, Soldevila-Pico C, Cabrera R, Shuster JJ, Theriaque D, et al. One-year protocol liver biopsy can stratify fibrosis progression in liver transplant recipients with recurrent hepatitis C infection. *Liver Transpl.* 2004 Oct;10(10):1240-7.
66. Berenguer M, Prieto M, Rayon JM, Mora J, Pastor M, Ortiz V, et al. Natural history of clinically compensated hepatitis C virus-related graft cirrhosis after liver transplantation. *Hepatology.* 2000 Oct;32(4 Pt 1):852-8.
67. Firpi RJ, Clark V, Soldevila-Pico C, Morelli G, Cabrera R, Levy C, et al. The natural history of hepatitis C cirrhosis after liver transplantation. *Liver Transpl.* 2009 Sep;15(9):1063-71.
68. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology.* 1997 Feb;112(2):463-72.
69. Melum E, Friman S, Bjoro K, Rasmussen A, Isoniemi H, Gjertsen H, et al. Hepatitis C impairs survival following liver transplantation irrespective of concomitant hepatocellular carcinoma. *J Hepatol.* 2007 Dec;47(6):777-83.
70. Thuluvath PJ, Guidinger MK, Fung JJ, Johnson LB, Rayhill SC, Pelletier SJ. Liver transplantation in the United States, 1999-2008. *Am J Transplant.* 2010 Apr;10(4 Pt 2):1003-19.

71. Berenguer M, Ferrell L, Watson J, Prieto M, Kim M, Rayon M, et al. HCV-related fibrosis progression following liver transplantation: increase in recent years. *J Hepatol.* 2000 Apr;32(4):673-84.
72. Berenguer M, Prieto M, San Juan F, Rayon JM, Martinez F, Carrasco D, et al. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. *Hepatology.* 2002 Jul;36(1):202-10.
73. Ydreborg M, Westin J, Lagging M, Castedal M, Friman S. Impact of donor histology on survival following liver transplantation for chronic hepatitis C virus infection: a Scandinavian single-center experience. *Scand J Gastroenterol.* 2012 Mar 27.
74. Wiesner RH, Demetris AJ, Belle SH, Seaberg EC, Lake JR, Zetterman RK, et al. Acute hepatic allograft rejection: incidence, risk factors, and impact on outcome. *Hepatology.* 1998 Sep;28(3):638-45.
75. Demetris AJ, Adeyi O, Bellamy CO, Clouston A, Charlotte F, Czaja A, et al. Liver biopsy interpretation for causes of late liver allograft dysfunction. *Hepatology.* 2006 Aug;44(2):489-501.
76. Prieto M, Berenguer M, Rayon JM, Cordoba J, Arguello L, Carrasco D, et al. High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. *Hepatology.* 1999 Jan;29(1):250-6.
77. Bahra M, Neumann UP, Jacob D, Langrehr JM, Neuhaus P. Repeated steroid pulse therapies in HCV-positive liver recipients: significant risk factor for HCV-related graft loss. *Transplant Proc.* 2005 May;37(4):1700-2.
78. Rousselet MC, Michalak S, Dupre F, Croue A, Bedossa P, Saint-Andre JP, et al. Sources of variability in histological scoring of chronic viral hepatitis. *Hepatology.* 2005 Feb;41(2):257-64.
79. Carrion JA, Navasa M, Bosch J, Bruguera M, Gilibert R, Forns X. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. *Liver Transpl.* 2006 Dec;12(12):1791-8.
80. Rigamonti C, Donato MF, Fraquelli M, Agnelli F, Ronchi G, Casazza G, et al. Transient elastography predicts fibrosis progression in patients with recurrent hepatitis C after liver transplantation. *Gut.* 2008 Jun;57(6):821-7.
81. Crespo G, Lens S, Gambato M, Carrion JA, Marino Z, Londono MC, et al. Liver stiffness 1 year after transplantation predicts clinical outcomes in patients with recurrent hepatitis C. *Am J Transplant.* 2014 Feb;14(2):375-83.
82. Carrion JA, Torres F, Crespo G, Miquel R, Garcia-Valdecasas JC, Navasa M, et al. Liver stiffness identifies two different patterns of fibrosis progression in patients with hepatitis C virus recurrence after liver transplantation. *Hepatology.* 2010 Jan;51(1):23-34.
83. Adebajo CO, Talwalkar JA, Poterucha JJ, Kim WR, Charlton MR. Ultrasound-based transient elastography for the detection of hepatic fibrosis in patients with recurrent hepatitis C virus after liver transplantation: a systematic review and meta-analysis. *Liver Transpl.* 2012 Mar;18(3):323-31.

84. Charlton M, Seaberg E, Wiesner R, Everhart J, Zetterman R, Lake J, et al. Predictors of patient and graft survival following liver transplantation for hepatitis C. *Hepatology*. 1998 Sep;28(3):823-30.
85. Feray C, Caccamo L, Alexander GJ, Ducot B, Gugenheim J, Casanovas T, et al. European collaborative study on factors influencing outcome after liver transplantation for hepatitis C. European Concerted Action on Viral Hepatitis (EUROHEP) Group. *Gastroenterology*. 1999 Sep;117(3):619-25.
86. Rosen HR, Chou S, Corless CL, Gretch DR, Flora KD, Boudousquie A, et al. Cytomegalovirus viremia: risk factor for allograft cirrhosis after liver transplantation for hepatitis C. *Transplantation*. 1997 Sep 15;64(5):721-6.
87. Duclos-Vallee JC, Feray C, Sebah M, Teicher E, Roque-Afonso AM, Roche B, et al. Survival and recurrence of hepatitis C after liver transplantation in patients coinfecting with human immunodeficiency virus and hepatitis C virus. *Hepatology*. 2008 Feb;47(2):407-17.
88. Wali M, Harrison RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. *Gut*. 2002 Aug;51(2):248-52.
89. Selzner M, Kashfi A, Selzner N, McCluskey S, Greig PD, Cattral MS, et al. Recipient age affects long-term outcome and hepatitis C recurrence in old donor livers following transplantation. *Liver Transpl*. 2009 Oct;15(10):1288-95.
90. Briceno J, Ciria R, Pleguezuelo M, Naranjo A, Sanchez-Hidalgo J, Ruiz-Rabelo J, et al. Contribution of marginal donors to liver transplantation for hepatitis C virus infection. *Transplant Proc*. 2007 Sep;39(7):2297-9.
91. Briceno J, Ciria R, Pleguezuelo M, de la Mata M, Muntane J, Naranjo A, et al. Impact of donor graft steatosis on overall outcome and viral recurrence after liver transplantation for hepatitis C virus cirrhosis. *Liver Transpl*. 2009 Jan;15(1):37-48.
92. Bahra M, Jacob D, Neumann UP, Spies F, Langrehr JM, Berg T, et al. Influence of donor histology on outcome in patients undergoing transplantation for hepatitis C. *Transplantation*. 2007 Jul 27;84(2):144-8.
93. Burra P, Loreno M, Russo FP, Germani G, Galligioni A, Senzolo M, et al. Donor livers with steatosis are safe to use in hepatitis C virus-positive recipients. *Liver Transpl*. 2009 Jun;15(6):619-28.
94. Charlton MR, Thompson A, Veldt BJ, Watt K, Tillmann H, Poterucha JJ, et al. Interleukin-28B polymorphisms are associated with histological recurrence and treatment response following liver transplantation in patients with hepatitis C virus infection. *Hepatology*. 2011 Jan;53(1):317-24.
95. Duarte-Rojo A, Veldt BJ, Goldstein DD, Tillman HL, Watt KD, Heimbach JK, et al. The course of posttransplant hepatitis C infection: comparative impact of donor and recipient source of the favorable IL28B genotype and other variables. *Transplantation*. 2012 Jul 27;94(2):197-203.
96. Biggins SW, Trotter J, Gralla J, Burton JR, Jr., Bambha KM, Dodge J, et al. Differential effects of donor and recipient IL28B and DDX58 SNPs on severity of HCV after liver transplantation. *J Hepatol*. 2013 May;58(5):969-76.

97. Lange CM, Moradpour D, Doehring A, Lehr HA, Mullhaupt B, Bibert S, et al. Impact of donor and recipient IL28B rs12979860 genotypes on hepatitis C virus liver graft reinfection. *J Hepatol.* 2011 Aug;55(2):322-7.
98. Fukuhara T, Taketomi A, Motomura T, Okano S, Ninomiya A, Abe T, et al. Variants in IL28B in liver recipients and donors correlate with response to peg-interferon and ribavirin therapy for recurrent hepatitis C. *Gastroenterology.* 2010 Nov;139(5):1577-85, 85 e1-3.
99. Firpi RJ, Zhu H, Morelli G, Abdelmalek MF, Soldevila-Pico C, Machicao VI, et al. Cyclosporine suppresses hepatitis C virus in vitro and increases the chance of a sustained virological response after liver transplantation. *Liver Transpl.* 2006 Jan;12(1):51-7.
100. Watashi K, Hijikata M, Hosaka M, Yamaji M, Shimotohno K. Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. *Hepatology.* 2003 Nov;38(5):1282-8.
101. Berenguer M, Aguilera V, Prieto M, San Juan F, Rayon JM, Benlloch S, et al. Effect of calcineurin inhibitors on survival and histologic disease severity in HCV-infected liver transplant recipients. *Liver Transpl.* 2006 May;12(5):762-7.
102. Pelletier SJ, Schaubel DE, Punch JD, Wolfe RA, Port FK, Merion RM. Hepatitis C is a risk factor for death after liver retransplantation. *Liver Transpl.* 2005 Apr;11(4):434-40.
103. Ghabril M, Dickson R, Wiesner R. Improving outcomes of liver retransplantation: an analysis of trends and the impact of Hepatitis C infection. *Am J Transplant.* 2008 Feb;8(2):404-11.
104. Curry M, Forns X, Chung R, Terrault N, Bown R. Pretransplant Sofosbuvir and Ribavirin to Prevent Recurrence of HCV Infection after Liver Transplantation. *Hepatology.* [abstract 213]. 2013;58(Sept).
105. Charlton M, Gane E, Manns M, Brown RS, Jr., Curry M. Sofosbuvir and Ribavirin for the Treatment of Established Recurrent Hepatitis C Infection After Liver Transplantation: Preliminary Results of a prospective, Multicenter Study. Late Breaker Abstract Session AASLD 2013. 2013;LB-2.
106. Berenguer M, Lopez-Labrador FX, Wright TL. Hepatitis C and liver transplantation. *J Hepatol.* 2001 Nov;35(5):666-78.
107. Samuel D, Forns X, Berenguer M, Trautwein C, Burroughs A, Rizzetto M, et al. Report of the monothematic EASL conference on liver transplantation for viral hepatitis (Paris, France, January 12-14, 2006). *J Hepatol.* 2006 Jul;45(1):127-43.
108. Berenguer M, Palau A, Fernandez A, Benlloch S, Aguilera V, Prieto M, et al. Efficacy, predictors of response, and potential risks associated with antiviral therapy in liver transplant recipients with recurrent hepatitis C. *Liver Transpl.* 2006 Jul;12(7):1067-76.
109. Carrion JA, Navasa M, Garcia-Retortillo M, Garcia-Pagan JC, Crespo G, Bruguera M, et al. Efficacy of antiviral therapy on hepatitis C recurrence after liver transplantation: a randomized controlled study. *Gastroenterology.* 2007 May;132(5):1746-56.

110. Berenguer M. Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. *J Hepatol.* 2008 Aug;49(2):274-87.
111. Thomas RM, Brems JJ, Guzman-Hartman G, Yong S, Cavaliere P, Van Thiel DH. Infection with chronic hepatitis C virus and liver transplantation: a role for interferon therapy before transplantation. *Liver Transpl.* 2003 Sep;9(9):905-15.
112. Crippin JS, McCashland T, Terrault N, Sheiner P, Charlton MR. A pilot study of the tolerability and efficacy of antiviral therapy in hepatitis C virus-infected patients awaiting liver transplantation. *Liver Transpl.* 2002 Apr;8(4):350-5.
113. Carrion JA, Martinez-Bauer E, Crespo G, Ramirez S, Perez-del-Pulgar S, Garcia-Valdecasas JC, et al. Antiviral therapy increases the risk of bacterial infections in HCV-infected cirrhotic patients awaiting liver transplantation: A retrospective study. *J Hepatol.* 2009 Apr;50(4):719-28.
114. Fornis X, Garcia-Retortillo M, Serrano T, Feliu A, Suarez F, de la Mata M, et al. Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation. *J Hepatol.* 2003 Sep;39(3):389-96.
115. Chalasani N, Manzarbeitia C, Ferenci P, Vogel W, Fontana RJ, Voigt M, et al. Peginterferon alfa-2a for hepatitis C after liver transplantation: two randomized, controlled trials. *Hepatology.* 2005 Feb;41(2):289-98.
116. Bzowej N, Nelson DR, Terrault NA, Everson GT, Teng LL, Prabhakar A, et al. PHOENIX: A randomized controlled trial of peginterferon alfa-2a plus ribavirin as a prophylactic treatment after liver transplantation for hepatitis C virus. *Liver Transpl.* 2011 May;17(5):528-38.
117. Shergill AK, Khalili M, Straley S, Bollinger K, Roberts JP, Ascher NA, et al. Applicability, tolerability and efficacy of preemptive antiviral therapy in hepatitis C-infected patients undergoing liver transplantation. *Am J Transplant.* 2005 Jan;5(1):118-24.
118. Berenguer M, Aguilera V, Rubin A, Ortiz C, Jimenez M, Prieto M. Comparison of two non-contemporaneous HCV-liver transplant cohorts: strategies to improve the efficacy of antiviral therapy. *J Hepatol.* 2012 Jun;56(6):1310-6.
119. Ackefors M, Gjertsen H, Wernerson A, Weiland O. Concentration-guided ribavirin dosing with darbepoetin support and peg-IFN alfa-2a for treatment of hepatitis C recurrence after liver transplantation. *J Viral Hepat.* 2012 Sep;19(9):635-9.
120. Fernandez I, Meneu JC, Colina F, Garcia I, Munoz R, Castellano G, et al. Clinical and histological efficacy of pegylated interferon and ribavirin therapy of recurrent hepatitis C after liver transplantation. *Liver Transpl.* 2006 Dec;12(12):1805-12.
121. Dumortier J, Scoazec JY, Chevallier P, Boillot O. Treatment of recurrent hepatitis C after liver transplantation: a pilot study of peginterferon alfa-2b and ribavirin combination. *J Hepatol.* 2004 Apr;40(4):669-74.
122. Roche B, Samuel D. Hepatitis C virus treatment pre- and post-liver transplantation. *Liver Int.* 2012 Feb;32 Suppl 1:120-8.

123. Peck-Radosavljevic M, Wichlas M, Homoncik-Kraml M, Kreil A, Hofer H, Jessner W, et al. Rapid suppression of hematopoiesis by standard or pegylated interferon-alpha. *Gastroenterology*. 2002 Jul;123(1):141-51.
124. Bodenheimer HC, Jr., Lindsay KL, Davis GL, Lewis JH, Thung SN, Seeff LB. Tolerance and efficacy of oral ribavirin treatment of chronic hepatitis C: a multicenter trial. *Hepatology*. 1997 Aug;26(2):473-7.
125. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med*. 2002 Sep 26;347(13):975-82.
126. Sidwell RW, Huffman JH, Khare GP, Allen LB, Witkowski JT, Robins RK. Broad-spectrum antiviral activity of Virazole: 1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science*. 1972 Aug 25;177(4050):705-6.
127. Lindahl K, Schvarcz R, Bruchfeld A, Stahle L. Evidence that plasma concentration rather than dose per kilogram body weight predicts ribavirin-induced anaemia. *J Viral Hepat*. 2004 Jan;11(1):84-7.
128. Jen JF, Glue P, Gupta S, Zambas D, Hajian G. Population pharmacokinetic and pharmacodynamic analysis of ribavirin in patients with chronic hepatitis C. *Ther Drug Monit*. 2000 Oct;22(5):555-65.
129. Selzner N, Guindi M, Renner EL, Berenguer M. Immune-mediated complications of the graft in interferon-treated hepatitis C positive liver transplant recipients. *J Hepatol*. 2011 Jul;55(1):207-17.
130. Afdhal NH, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, et al. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. *Gastroenterology*. 2004 May;126(5):1302-11.
131. Dieterich DT, Wasserman R, Brau N, Hassanein TI, Bini EJ, Bowers PJ, et al. Once-weekly epoetin alfa improves anemia and facilitates maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alfa. *Am J Gastroenterol*. 2003 Nov;98(11):2491-9.
132. Soza A, Everhart JE, Ghany MG, Doo E, Heller T, Promrat K, et al. Neutropenia during combination therapy of interferon alfa and ribavirin for chronic hepatitis C. *Hepatology*. 2002 Nov;36(5):1273-9.
133. Coilly A, Roche B, Dumortier J, Leroy V, Botta-Fridlund D, Radenne S, et al. Safety and efficacy of protease inhibitors to treat hepatitis C after liver transplantation: A multicenter experience. *J Hepatol*. 2014 Jan;60(1):78-86.
134. Sulkowski MS. Management of the hematologic complications of hepatitis C therapy. *Clin Liver Dis*. 2005 Nov;9(4):601-16, vi.
135. Burton JR, Jr., Rosen HR. Acute rejection in HCV-infected liver transplant recipients: The great conundrum. *Liver Transpl*. 2006 Nov;12(11 Suppl 2):S38-47.
136. Rodriguez-Luna H, Khatib A, Sharma P, De Petris G, Williams JW, Ortiz J, et al. Treatment of recurrent hepatitis C infection after liver transplantation with combination of pegylated interferon alpha2b and ribavirin: an open-label series. *Transplantation*. 2004 Jan 27;77(2):190-4.

137. Picciotto FP, Tritto G, Lanza AG, Addario L, De Luca M, Di Costanzo GG, et al. Sustained virological response to antiviral therapy reduces mortality in HCV reinfection after liver transplantation. *J Hepatol.* 2007 Mar;46(3):459-65.
138. Oton E, Barcena R, Moreno-Planas JM, Cuervas-Mons V, Moreno-Zamora A, Barrios C, et al. Hepatitis C recurrence after liver transplantation: Viral and histologic response to full-dose PEG-interferon and ribavirin. *Am J Transplant.* 2006 Oct;6(10):2348-55.
139. Neumann U, Puhl G, Bahra M, Berg T, Langrehr JM, Neuhaus R, et al. Treatment of patients with recurrent hepatitis C after liver transplantation with peginterferon alfa-2B plus ribavirin. *Transplantation.* 2006 Jul 15;82(1):43-7.
140. Coto-Llerena M, Perez-Del-Pulgar S, Crespo G, Carrion JA, Martinez SM, Sanchez-Tapias JM, et al. Donor and recipient IL28B polymorphisms in HCV-infected patients undergoing antiviral therapy before and after liver transplantation. *Am J Transplant.* 2011 May;11(5):1051-7.
141. Berenguer M, Schuppan D. Progression of liver fibrosis in post-transplant hepatitis C: Mechanisms, assessment and treatment. *J Hepatol.* 2013 May;58(5):1028-41.
142. Bahra M, Neumann UP, Jacob D, Langrehr JM, Berg T, Neuhaus R, et al. Fibrosis progression in hepatitis C positive liver recipients after sustained virologic response to antiviral combination therapy (interferon-ribavirin therapy). *Transplantation.* 2007 Feb 15;83(3):351-3.
143. Berenguer M, Roche B, Aguilera V, Duclos-Vallee JC, Navarro L, Rubin A, et al. Efficacy of the retreatment of hepatitis C virus infections after liver transplantation: role of an aggressive approach. *Liver Transpl.* 2013 Jan;19(1):69-77.
144. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med.* 2011 Jun 23;364(25):2405-16.
145. Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, et al. Telaprevir for retreatment of HCV infection. *N Engl J Med.* 2011 Jun 23;364(25):2417-28.
146. Poordad F, McCone J, Jr., Bacon BR, Bruno S, Manns MP, Sulkowski MS, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med.* 2011 Mar 31;364(13):1195-206.
147. Charlton M. Telaprevir, boceprevir, cytochrome P450 and immunosuppressive agents--a potentially lethal cocktail. *Hepatology.* 2011 Jul;54(1):3-5.
148. Fontana RJ, Hughes EA, Bifano M, Appelman H, Dimitrova D, Hindes R, et al. Sofosbuvir and daclatasvir combination therapy in a liver transplant recipient with severe recurrent cholestatic hepatitis C. *Am J Transplant.* 2013 Jun;13(6):1601-5.
149. Bruchfeld A, Lindahl K, Schvarcz R, Stahle L. Dosage of ribavirin in patients with hepatitis C should be based on renal function: a population pharmacokinetic analysis. *Ther Drug Monit.* 2002 Dec;24(6):701-8.
150. Berenguer M. Treatment of chronic hepatitis C in hemodialysis patients. *Hepatology.* 2008 Nov;48(5):1690-9.

151. Bruchfeld A, Lindahl K, Stahle L, Soderberg M, Schvarcz R. Interferon and ribavirin treatment in patients with hepatitis C-associated renal disease and renal insufficiency. *Nephrol Dial Transplant*. 2003 Aug;18(8):1573-80.
152. Duarte-Rojo A, Deneke MG, Charlton MR. Interleukin-28B polymorphism in hepatitis C and liver transplantation. *Liver Transpl*. 2013 Jan;19(1):49-58.
153. Ackefors M, Nystrom J, Wernerson A, Gjertsen H, Sonnerborg A, Weiland O. Evolution of fibrosis during HCV recurrence after liver transplantation - influence of IL-28B SNP and response to peg-IFN and ribavirin treatment. *J Viral Hepat*. 2013 Nov;20(11):770-8.
154. Weiland O, Hollander A, Mattsson L, Glaumann H, Lindahl K, Schvarcz R, et al. Lower-than-standard dose peg-IFN alfa-2a for chronic hepatitis C caused by genotype 2 and 3 is sufficient when given in combination with weight-based ribavirin. *J Viral Hepat*. 2008 Sep;15(9):641-5.
155. Roche B, Samuel D. Risk factors for hepatitis C recurrence after liver transplantation. *J Viral Hepat*. 2007 Nov;14 Suppl 1:89-96.

