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HEPATITIS C IN CHRONIC KIDNEY DISEASE AND KIDNEY TRANSPLANTATION: WITH SPECIAL REFERENCE TO EPIDEMIOLOGY AND TREATMENT

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"Even when all the possible scientific questions have been answered, the problems of life remain completely untouched"

Ludwig Wittgenstein

Abstract:

Hepatitis C (HCV) is a clinical problem in infected kidney transplant patients but is also a cause of renal disease and vasculitis. Standard therapy for HCV is a combination of interferon and ribavirin. Renal patients have mostly been treated with interferon monotherapy since ribavirin has been contraindicated in renal insufficiency with a GFR (glomerular filtration rate) < 50 ml/min.

A cohort of 520 HCV-negative and 51 HCV-positive patients transplanted between 1989 and 1997 at Huddinge University Hospital was studied with last follow-up 2002. The methods used were univariate Kaplan-Meier survival analysis and multivariate Cox proportional hazards model. We confirmed that HCV is an independent risk factor for both patient ($p<0.0012$) and graft-survival ($p<0.0003$) after kidney transplantation in line with previous studies. After adjusting for age, gender, diabetes, previous transplants, type of transplant and time in renal replacement therapy (RRT) HCV remained significantly more important than time in RRT for long-term outcome after kidney transplantation. This has not been well characterized before. The results lend support to the suggestion that successful anti-viral HCV therapy prior to transplantation may be preferable to an early transplant for long-term patient and graft survival after kidney transplantation. However, this remains to be confirmed in prospective studies.

In order to optimise ribavirin therapy in patients with renal disease a high-performance liquid chromatography (HPLC) method for ribavirin plasma concentration measurement was developed. A trough level interval of 7-17µmol/L was found in 10 patients with normal renal function treated with interferon-alpha and ribavirin. The ribavirin monitoring method was used in 6 HCV infected dialysis patients in study and in 7 patients with renal insufficiency with HCV-related renal disease in study. The method enabled clinical use of ribavirin in combination with interferon-alpha in these patients with fair tolerability. However this required reduced ribavirin doses, close monitoring of ribavirin plasma concentrations and surveillance of side effects. Ribavirin-associated anaemia, the main side effect, was handled by means of medium-high doses of erythropoietin as well as low-dose iron therapy, thereby avoiding blood transfusions. Viral response during treatment in all 13 HCV replicating patients was 85%. Although the studies were small and uncontrolled ribavirin in addition to interferon-alpha is likely to have had an additive effect in renal insufficient patients.

Overall 383 ribavirin samples from 63 patients were collected, 44 with normal serum creatinine and 19 with renal impairment with an estimated GFR of 5-57 ml/min. A population pharmacokinetic analysis was performed using non-linear mixed effect modelling (NONMEM) evaluating population factors as age, gender, body weight, serum creatinine and estimated GFR. Estimated GFR was found to be a significantly better predictor of ribavirin clearance than body weight alone. A small non-renal clearance dependant on body weight and age and a significant inter-individual 40 % variability in ribavirin total clearance remained, not explained by GFR and body weight. The results indicate that ribavirin should mainly be dosed on renal function and not on body weight alone. A dosing schedule is proposed based on these findings.

Ribavirin monitoring is strongly recommended in renal patients. Further studies including patients with normal GFR should be of interest considering the inter-individual variability in ribavirin total clearance and in order to identify a possible therapeutic ribavirin target concentration for HCV infection.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I   Bruchfeld A, Wilczek H, Elinder C-G .
Hepatitis C infection, time in renal replacement therapy and outcome after kidney transplantation. Submitted for publication

II   Svensson J-O, Bruchfeld A, Schvarcz R, Ståhle L.
Determination of ribavirin in serum using highly selective solid-phase extraction and high-performance liquid chromatography. Therapeutic Drug Monitoring 2000; 22 : 215-218.

III   Bruchfeld A, Ståhle L, Andersson J, Schvarcz R.
Ribavirin treatment in dialysis patients with chronic hepatitis C virus infection - a pilot study. Journal of Viral Hepatitis, 2000; 8 : 287-292,2001.

IV   Bruchfeld A, Lindahl K, Ståhle L, Söderberg M, Schvarcz R.
Interferon and ribavirin treatment in patients with hepatitis C-associated renal disease and renal insufficiency. Nephrol Dial Transplant 2003; 18(8):1573-80.

V   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. Therapeutic Drug Monitoring 2002; 24 (6): 701-708.

CONTENTS

LIST OF ABBREVIATIONS

ALT	alanine aminotransferase
ANCA	anti neutrophil cytoplasmatic antibodies
AST	aspartate aminotransferase
CI	confidence interval
EIA	enzyme immunoassay
ESRD	end-stage renal disease
FSGS	focal segmental glomerulosclerosis
GFR	glomerular filtration rate
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HCV-RNA	hepatitis C virus ribonucleic acid
HD	haemodialysis
HGF	hepatocyte growth factor
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
IMPDH	inosine monophosphate dehydrogenase
IFN	interferon
MC	mixed cryoglobulinemia
MGN	membranous glomerulonephritis
MPGN	membranoproliferative glomerulonephritis
PBMC	peripheral blood mononuclear cells
RF	rheumatoid factor
RR	relative risk
RRT	renal replacement therapy
SVR	sustained viral response

1 GENERAL INTRODUCTION

In 1996 a joint meeting between transplant surgeons and nephrologists was held at Huddinge University Hospital. The topic was a severely ill kidney transplant patient with a primary diagnosis of Wegener´s granulomatosis but who was also infected with Hepatitis C virus (HCV). This patient had a vasculitic disease and the physicians decided to increase the patient´s immunosuppressive therapy. Being a junior doctor at the Division of Renal Medicine I asked whether they believed that the HCV infection could have influenced the condition of the patient, but I was told that HCV in transplanted patients had not been shown to have any negative effects. This intrigued me and I decided to find out more about this subject. No dialysis patients and very few renal patients had been treated for HCV infection at the hospital. However colleagues at the Infectious Diseases Department were pioneers in the field of interferon and ribavirin treatment for HCV and experts in pharmacological monitoring with a special interest in anti-viral therapy were working at the Clinical Pharmacology Department. I then formed a network together with these centres and transplant surgeons in order to study the potential influence of HCV in kidney transplantation and to evaluate and if possible improve therapy options for HCV infection in dialysis and renal insufficiency.

1.1 HEPATITIS C VIRUS (HCV)

HCV, an RNA virus which is the only member of the genus hepacivirus within the flaviviridae family, was isolated in the late 1980´s thereby identifying the virus as being the major cause of what was previously known as non-A, non-B hepatitis (1). The virus, shown in **Figure 1**, has a single stranded RNA genome of approximately 9600 nucleotides, consisting of a polyprotein which is cleaved into a nucleocapsid core (C), 2 envelope proteins E1 and E2 and non-structural proteins named NS2 - NS5. The RNA-dependant RNA polymerase activity needed for RNA virus replication is located in the NS5 region (2).
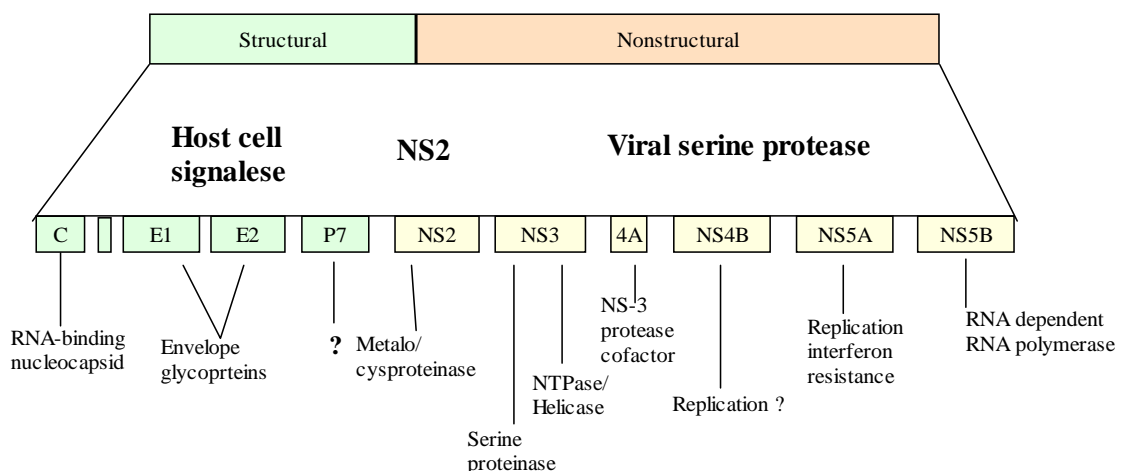

Figure 1. Schematic figure illustrating the organization of the HCV RNA genome. Locarnini S A, Journal of Gastroenterology and Hepatology, 2002. Reprinted with permission.

Soon after the discovery of the virus methods were developed to detect anti-viral antibodies thus making routine laboratory diagnosis feasible (3, 4).

HCV is a heterogenous virus with a genetic diversity. Six major HCV strains or genotypes have been identified as well as a large number of subtypes, with a geographical pattern of distribution. In Europe, the United States and Japan genotype 1a and 1b is prevalent, followed by genotype 2 and 3, whereas genotype 4 is common in Africa and the Middle East (5, 6)

The clinical importance of HCV genotype on disease manifestation and progression is unclear, although lately, genotype 3 has been associated with increased steatosis of the liver, which is a risk factor for progressive fibrosis (7, 8). It is however well established that HCV genotype is an important determinant of response to antiviral treatment with a significantly higher response rate for genotype 2 and 3 as compared to genotype 1 (9-12).

The genetic diversity of the HCV virus could influence the spontaneous cure rate after acute infection by evasion of the host immune response. Quasispecies, which are reservoirs of genetic variants within a genotype, can impair both humoral and cellular immune responses (13). HCV can infect dendritic cells thereby impairing their antigen-presenting function, which could have a negative influence on the activiation of anti-HCV-specific T-cells (14). Recently it was shown that HCV NS3/4A serine protease can block the action of interferon regulatory factor-3, which is a key cellular antiviral signalling molecule (15). Protective immunity has been shown to be lacking in both animal models and humans. When re-challenged with a different HCV strain superinfection has been reported indicating that immunity is genotype specific (16,17). The pattern of HCV quasispecies has also been shown to affect outcome of antiviral therapy (18). Due to the biological properties of the virus the development of a protective HCV vaccine has so far not been successful.

1.2   HCV TRANSMISSION AND TROPISM

Hepatitis C has predominantly been spread by blood and blood products. Before screening methods identifying hepatitis C carriers were developed in the early 1990´s transmission was fairly common in patients receiving blood transfusions. Other risk factors for HCV transmission have been: solid organ transplantation, dialysis, occupational exposure, intravenous drug abuse, sexual transmission and perinatal transmission, although the latter two are considered low-frequency events (19, 20). In developed countries the incidence of HCV infection is diminishing due to screening programs in the healthcare system but also due to a decline among intravenous drug users (21).

HCV preferentially infects hepatocytes. However during the last decade extrahepatic replication has been shown in peripheral blood mononuclear cells (PBMC) including lymphocytes, monocytes and dendritic cells, but also in granulocytes. Clinically this can be exemplified by reinfection of liver grafts after transplantation by an extrahepatic

HCV reservoir as well as by the presence of autoimmune manifestations associated with HCV infection (22, 23, 24).

1.3   HCV DIAGNOSTIC METHODS

In clinical practice a number of diagnostic virological tests are used to detect HCV infection.

a) *Indirect tests* identify anti-HCV antibodies by use of second- or third generation enzyme immunoassays (EIA) or enzyme-linked immunosorbent assays (ELISA). The current anti-HCV EIAs tests are highly specific but confirmatory recombinant immunoblot assays (RIBA) are often used to exclude false positive results.

b) *Direct tests* detect the presence of HCV RNA in peripheral blood. These tests can trace the virus within 1 to 2 weeks after infection by molecular amplification of viral genome copies. The most common tests are qualitative RNA tests (e.g Amplicor HCV v 2.0; Roche Molecular Systems, Cobas Amplicor HCV v 2.0, Roche) with cut-off levels of 50 IU HCV RNA per ml. Viral quantification, which has recently been standardised and is done by target or signal amplification techniques, represents the amount of HCV RNA (IU/ml) in a sample rather than the number of viral particles previously measured in the non-standardised commercial methods. HCV genotype determination is performed by direct sequencing of the NS5B or E1 region. (25).

Serological detection of HCV by anti-HCV EIAs are generally positive (>99%) in immunocompetent patients with detectable HCV RNA, but in special populations such as dialysis patients and others with immunodeficiences such as HIV EIAs can be negative (26, 27). A multicenter German study found the prevalence of HCV to be 7.0%, as diagnosed by HCV antibody and/or HCV-RNA positivity in a cohort of nearly 3000 haemodialysis patients. Of these 21.6 % were negative for HCV antibodies (28).

1.4   HCV PREVALENCE

According to the World Health Organization an estimated 170 million people are chronically infected with HCV worldwide. The burden to global health measured as disability adjusted life years (DALYs), which is a weighted calculation of factors measured as a lost year of "healthy" life, was 844.000 and 46.000 deaths have been attributed to HCV in 2001. An estimated global prevalence of 3 % has been reported, which in the European context varies between low-prevalence areas in Northern Europe (0.3 %) and high-prevalence in Southern Europe (1.5 %). A similar variation in prevalence is found in the United States, but is more strongly correlated to ethnicity and socio-economic status than geographic distribution. These estimates are based on testing of serum samples collected from a representative sample of the population of the United States during the Third National Health and Nutrition Examination – NHANES (29). In Egypt more than 15-20 % of the population are reported to be HCV antibody positive (30).The global geographical distribution and approximate HCV prevalence according to the World Health Organization are shown in **Figure 2**.
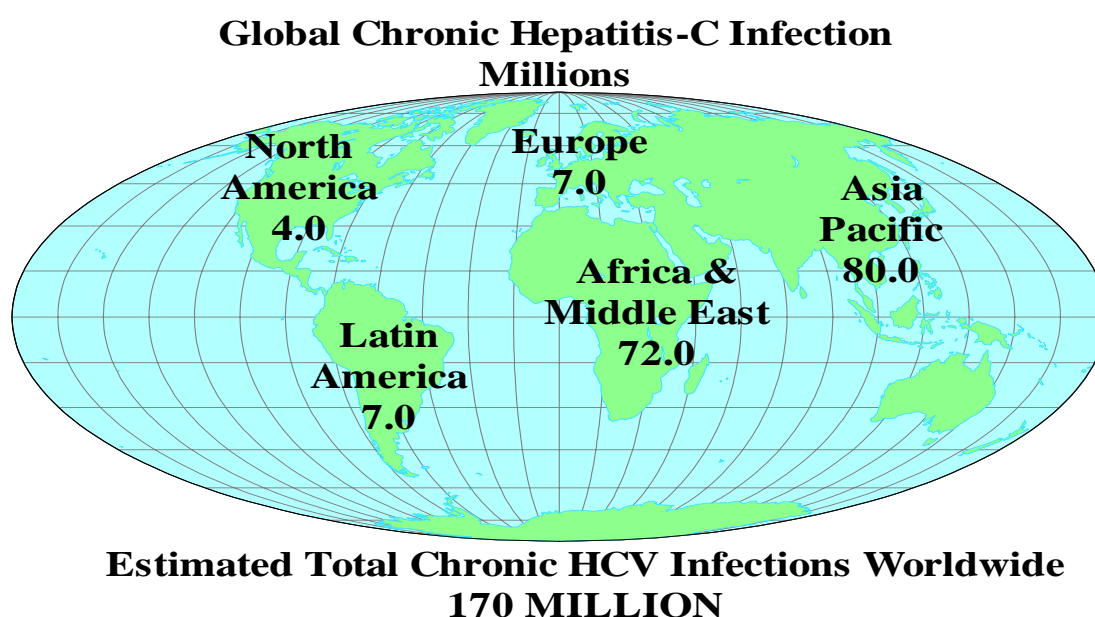
Global Chronic Hepatitis-C Infection Millions

North America 4.0

Europe 7.0

Asia Pacific 80.0

Africa & Middle East 72.0

Latin America 7.0

Estimated Total Chronic HCV Infections Worldwide 170 MILLION

Figure 2 Estimated HCV prevalence according to the World Health Organization, 2001

1.5 HCV PREVALENCE IN RENAL DISEASE

Hepatitis C infection (HCV) is an important cause of liver disease among patients with end-stage renal disease (ESRD) and in patients after kidney transplantation. Most patients have been infected through contaminated blood products, solid organ transplantation or nosocomial HCV transmission within dialysis units. Considerable variation between countries and centres has been reported with a prevalence ranging from 3 to 80 % for dialysis patients and from 10 to 41 % in kidney transplant recipients (31, 32). In a Swedish follow-up study, which included 184 dialysis and transplanted patients between 1989 –1997, the prevalence of anti-HCV antibodies was initially 10 %, but later dropped to 8 % during the latter part of the study (33). No other published prevalence figures are available from Sweden. However the transplant cohort presented in manuscript I consisted of 520 HCV-negative and 51 HCV-positive patients at the time of kidney transplantation resulting in a HCV prevalence of 9.8 %.

1.6 NATURAL HISTORY

Acute infection with hepatitis C is followed by the occurrence of HCV RNA within 1 to 2 weeks. The viremia is followed by the development of anti-HCV antibodies in immunocompetent subjects 4-6 weeks later. The antibody response can be weakened or even absent in immunocompromised patients (28, 31). Symptoms are often absent, but

if they occur jaundice is usually present as well as serum alanine transferase (ALT) and serum aspartate transferase (ALT) elevation (34, 35).

In a majority of cases the infection is not cleared. A large number of studies have assessed the persistence of chronic hepatitis C infection after the acute stage in different populations ranging from 54 to 86 %. In the lower range young children undergoing cardiac surgery and women who had received HCV-contaminated Rh immunoglobulin are found and the higher range is associated with transmission by transfusion in older patients (21). Immunocompromised individuals are generally at higher risk of developing chronic HCV infection most likely due to a deficient immune reactivity. Dialysis patients, patients with HIV and patients with agammaglobulinemia have been shown to have a rate of chronicity of 80-90 %, but recovery from infection is still possible in these groups (35). HCV is a leading cause of cirrhosis of the liver. In different studies 10 -30 % of the infected patients will over a 20-30 year period of chronic infection develop cirrhosis and these patients are at a higher risk (annual rate 1-4 %) of developing hepatocellular carcinoma (HCC)(36). The incidence of and mortality in HCC has doubled in the United States over the last 25 years. Given the current prevalence of HCV, the incidence of HCC is believed to double again over the next 10-20 years (37, 38, 39). In the United States and most of Europe HCV cirrhosis is today a leading indication for liver transplantation. The progression of liver disease is influenced by factors such as older age at the time of infection, male sex, abuse of alcohol, co-infection with HIV or Hepatitis B and environmental factors, as well as immunosuppression associated with organ transplantation (21, 40). HCV viral load has been proposed to promote HCV-related liver fibrosis. However in a review it was recently pointed out that there is currently no convincing proof that increased viral load influences disease progression (41). Whether there is a difference between immunocompetent and immunocompromised patients in this respect is not known.

2   HCV AND EXTRAHEPATIC MANIFESTATIONS

2.1   HCV AND CRYOGLOBULINEMIA

During the last decade mounting evidence has linked HCV to a number of extraheptic manifestations. HCV seems capable of triggering immune responses detected by autoantibodies, cryoglobulins, circulating immunocomplexes and rheumatoid factor (RF) (42, 43). Cryoglobulins are immunoglobulins that precipitate at temperatures below 37 ° C. The analysis of the precipitate is usually done by immunoelectrophoresis or immunofixation.

Cryoglobulins are usually classified as:

Type I, mainly found in patients with lymphoid tumours i.e Waldenström´s macroglobulinemia and multiple myeloma.

Type II and III, which are characterized by polyclonal IgG and mono- or polyclonal IgM RF respectively.

These "mixed" cryoglobulinemia (MC) type II and III can be associated with infections, autoimmune or neoplastic diseases (44-46). HCV has however been shown to be the causative agent in a majority (80-90%) of MC cases (45, 47). Epidemiological studies have found low levels of circulating cryoglobulins in up to 50 % of HCV patients, but the overt MC syndrome is present in a minority of patients (48).

The clinical manifestations of MC involve the skin with palpable purpura, also known as leukocytoclastic vasculitis, in which HCV has been detected in immunocomplexes. Arthralgia, peripheral neuropathy, sicca syndrome and Raynaud´s phenomenon are common (30-80 %). Liver involvement, with mildly elevated aminotransferase levels, as well as renal manifestations are fairly frequent (34-70 %). Auto-immune thyroid disease has been reported and an association to lichen planus and thyreoiditis has also been described. Most of these data are from France and Italy, where HCV prevalence is fairly high and where cryoglobulinemic vasculitis is a common form of vasculitis (46). Abdominal pain secondary to mesenteric vasculitis occurs and it has been reported that at least one-third of patients with cryoglobulinemia in this part of Europe have acute renal vasculitis (49).

Circulating autoantibodies in HCV-infected patients are quite common. In a prospective study of 321 patients, in whom cryoglobulins were present in 56 % , 70 % were found to have at least one autoantibody i.e anti-nuclear antibodies (ANA) 41 %, RF 38 %, cardiolipin-antibodies 27 %. Anti-smooth muscle cell and anti-liver kidney muscle antibodies were also detected (50). Anti-neutrophil cytoplasmatic antibodies (ANCA) have been described and proteinase 3 (PR 3) has recently been suggested to be a major auto-antigen for HCV. In a study from Taiwan 278 out of 516 patients (> 50 %) were positive for ANCA with anti-proteinase 3 specificity. These patients had a higher prevalence of skin involvement, anaemia, abnormal liver function and elevated alfa-feto protein (51). Complement activation is a feature of immunocomplex mediated

injury and decreased complement levels, primarily detected by low total haemolytic complement and C4, and to a lesser degree C3, are commonly found. Complement levels usually normalize after anti-viral treatment (52).

No clear correlation between HCV genotype and the occurrence of cryoglobulinemia has been shown, rather a correlation with genotype prevalence in individual countries (53-55). Some investigators have proposed a genetic predisposition linking the HLA B8-DR 3 and HLA DR 2 subtypes to the syndrome (56, 57). Cryoglobulinemia also appears to be a less common event in Northern Europe, probably mirroring the HCV prevalence in Northern Europe (58, 59). The occurrence of cryoprecipitates is likely to be a later event in the natural history of HCV. In a meta-analysis including more than 2000 patients, cryoprecipitates were found in 44 % and a highly significant association between biopsy-verified cirrhosis and cryoglobulinemia was found (60). Other authors have suggested that liver disease mirrored by ALT elevation is more benign in mixed cryoglobulinemia, but these studies are small and often lack complete histological data (61, 62).

2.2 HCV AND HAEMATOLOGICAL DISEASE

HCV has been associated with monoclonal gammopathy, also in the absence of cryoglobulinemia, indicating the ability of the virus to induce B-cell expansion (63, 64). Cryoglobulinemia can in some cases evolve into a B-cell non-Hodgkin lymphoma (B-NHL) and this association is found primarily in high prevalence areas like Italy, USA and Japan (65). Regression of the lymphoma has been demonstrated after anti-viral therapy (66).

2.3 HCV AND DIABETES

HCV infection has also been linked to type 2 diabetes. In a recent prospective case-cohort analysis from the USA, high-risk HCV infected patients defined as having an elevated Body Mass Index (BMI) > 25 and being older than 55 years of age had an increased relative risk of 11,6 (95% CI 1,39-96,6) compared to HCV infected patients of less than 55 years, with a normal BMI (67). Another interesting finding is an increased HCV association with post transplant diabetes in kidney transplant patients, found exclusively in patients immunosupressed with tacrolimus but not with cyclosporine (68).

2.4 HCV AND RENAL MANIFESTATIONS

Hepatitis C virus infection has been associated with glomerular disease, the most common form being membranoproliferative glomerulonephritis (MPGN) with or without mixed cryoglobulinemia (69, 70), but cases of membranous glomerulonephritis (MGN) and focal segmental glomerulosclerosis (FSGS) have also been reported (71-73). Again there is a geographical difference, with HCV infection reported to be present in about 60 % of patients with MPGN in Japan compared to 10 – 20 % of cases in the USA (74).

MPGN can occur with and without cryoglobulinemia and may be the presenting symptom of HCV infection. The glomerular disease presents with microscopic hematuria and proteinuria, in about 50 % of patients with mild to moderate renal insufficiency. Approximately 25 % of patients present with an acute nephritic syndrome with macroscopic hematuria, proteinuria and hypertension and in 25 % a nephrotic syndrome is present (70, 75, 76). Glomerular deposition of immunocomplexes containing HCV antigen and HCV-antibody, in cryoglobulinemia induces the production of IgM RF in the subendothelium and mesangium. A subsequent complement activation is likely to induce glomerular injury. The deposits within the mesangium and capillary walls can be demonstrated in renal biopsies by immunofluorescence. Other features are mesangial proliferation and later sclerosis, increased cellularity and accentuation of glomerular lobular structure, infiltration of mononuclear cells and a typical double contour of the basement membrane (70). A necrotizing glomerulonephritis may be present in severe cases (**Figure 3**). Non-enveloped HCV core antigen forming immunocomplexes with HCV-antibodies have been demonstrated in cryoprecipitates (77). However the detection of HCV virions in glomeruli in MPGN is still a matter of debate. By some authors it is suggested that this is merely an antigen trapping phenomenon, whereas others have proposed that the indirect methods used could react with RF in the deposits (70, 78). There is at present no routine method available for direct detection of HCV in renal biopsies.
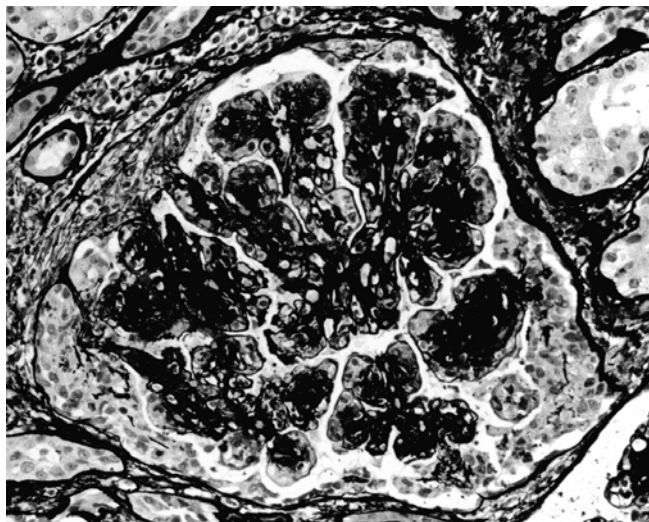

Figure 3 From Bruchfeld et al, NDT 2003;18:1573-1580. Light microscopial picture: MPGN with mesangial proliferation and segmental crescent formation.PAS-methenamine–silver stain.

The course of disease is fairly variable with only a minority reaching end-stage renal disease (ESRD). ESRD is, however, more likely to develop in more active disease and in older patients. However many patients with cryoglobulinemia and MPGN die of cardio-vascular disease, systemic vasculitis, infections and liver failure before reaching ESRD (79).

In most patients (> 80 %), HCV-associated MGN presents with overt nephrotic syndrome. In MGN normal complement and normal liver function is normally seen and cryoglobulinemia as well as RF are absent (76).

3 HCV THERAPY

3.1 INTERFERON

Interferon-alpha (IFN-α) and interferon-beta (IFN- β) are a family of proteins with important functions in the innate immune system and are potent activators of antiviral and anti-bacterial pathways. They are produced by most nucleated cells but mainly by macrophages, dendritic cells and fibroblasts. IFN also has anti-proliferative, anti-inflammatory, anti-angiogenetic and anti-oncogenic effect and exerts immune-regulating activity by modulating expression of cytokines and cytokine receptors. The immunomodulatory actions of IFN includes enhancement of cytotoxic activity of T cells and natural killer cells (NK cells). T helper 1 lymphocytes have been found to be induced by IFN in humans (80, 81). As previously mentioned HCV NS3/4A serine protease has been shown to block the action of interferon regulatory factor-3, which is a key cellular antiviral signalling molecule (15).

Both IFN-alpha and IFN-beta have been used in the treatment for HCV, first reported in 1989 (82). Interferon monotherapy has resulted in a sustained viral response (SVR), defined by undetectable serum HCV RNA more than six months after conclusion of treatment, in only 10-25 % of subjects. The least favourable outcome has been reported for genotype 1. More advanced liver disease such as cirrhosis, has also been associated with a reduced chance of SVR (9, 10).

3.2 RIBAVIRIN

Ribavirin (1-Beta-D-ribofuranosyl-1, 2, 4-triazole-3-carboxamide) is a purine nucleoside analogue with broad-spectrum in vitro antiviral activity (84, 85). The molecular weight is 244, and ribavirin does not bind to plasma proteins (86). The aerosol form was used to treat children with severe respiratory syncytial virus in the mid-80s. Ribavirin was later used in HIV trials, but a real therapeutic role for the compound was found when HCV was discovered in 1989 (87).

Intracellular phosphorylation is believed to be required for ribavirin to excert its pharmacological activity and 4 major possible mechanisms of action have been proposed (87-89).

Indirect
1. Enhancing T-cell mediated immunity against viral infection through switching the T-cell phenotype from type 2 to type 1.

2. Inhibition of the host enzyme inosine monphosphate dehydrogenase (IMPDH), which is an important target for immunosuppressive drugs.

Direct
3. Direct inhibition of HCV, including NS5B-encoded RNA-dependant polymerase.

4. Ribavirin as an incorporated RNA mutagen that drives the rapidly mutating RNA virus to an error catastrophe.

Ribavirin has been shown to modulate Th1/Th2 balance in vivo (87, 90) also at doses resulting in concentrations seen in treated patients (91).

IMPDH is the rate-limiting step in the *de novo* synthesis of guanosine triphosphate (GTP). The inhibition of the enzyme might theoretically suppress viral RNA replication by depletion of the intracellular GTP pool. Ribavirin monotherapy does however not affect serum viral load but a transient ALT reduction is commonly found (87). Other IMPDH inhibitors such as mycophenolate mofetil (MMF) used as an immunosuppressant in organ transplantation have been tested for anti-viral activity in patients after showing some antiviral activity in vitro. Preliminary data does not support that MMF in combination with interferon is as effective as ribavirin for the treatment of HCV, but no comparative studies have yet been published (92). If MMF has an effect it is likely to be immunomodulatory, possibly on the Th1/Th2 level (93).

Both direct inhibition of HCV replication and ribavirin as an RNA virus mutagen has been shown in vitro at inhibitory concentrations ranging from 50-150 µmol/L (87, 94, 95). There are three published in vivo pharmacokinetic studies in treated patients, examining ribavirin concentrations. The ribavirin concentrations found in these patient studies are however considerably lower than the levels used in the in vitro studies (96-98).

The recommended ribavirin dose is currently 800 mg, 1000 mg and 1200 mg according to a bodyweight of <65 kg, 65-85 kg and >85 kg respectively (ViraferonPeg, Summary of Product Characteristics, Schering-Plough). Ribavirin has been considered contraindicated in renal insufficiency (creatinine clearance < 50 ml/min) and in dialysis patients due to the reduced elimination rate in patients with reduced renal capacity, low dialysis clearance and the risk of severe anemia (99-101).

Therapeutic drug monitoring is not an established standard in ribavirin treatment. Nevertheless, there is an emerging interest in the relationship between ribavirin dose, ribavirin concentration and treatment effect. An optimal target concentration for ribavirin is however not yet determined, but in recent studies higher ribavirin concentrations in HCV-infected patients with normal renal function resulted in a higher rate of SVR (96, 97).

The major side effect of ribavirin is haemolysis and is believed to be related to the accumulation of phosphorylated forms of ribavirin in red blood cells at concentrations higher than those found in plasma. It is known that ribavirin is converted to its phosphorylated metabolites in all cell types, but the conversion to ribavirin through dephosphorylation occurs mainly in nucleated cells (87, 88).

3.3   COMBINATION THERAPY AND CURRENT OPTIMAL THERAPY

As early as 1991, ribavirin was suggested as a potential anti-HCV drug in combination with interferon-alpha (102). The initial trials confirmed that a synergistic effect existed (103-105) and large randomised international studies corroborated that the combination of interferon-alpha and ribavirin was superior to interferon monotherapy (9, 10). The SVR was approximately 65 % for non-1 genotypes and 30 % for genotype 1. It was established that optimal therapy for genotype 2 and 3 was 24 weeks, whereas genotype 1 warranted 48 weeks of treatment. In addition to genotype, low viral load, age 40 years or less, minimal fibrosis and female sex was associated with better response (9, 10). Recent studies combining pegylated interferon and ribavirin has further improved response rates, reaching almost 80 % in genotype 2 and 3 and 42-44 % in genotype 1 (11, 12). This combination is currently considered to be the optimal therapy for HCV infection.

Pegylated interferon, currently available as pegylated interferon-alpha-2a (Pegasys® 40 kD, Roche) and pegylated interferon-alpha-2b (PegIntron® 12 kD, Schering-Plough) are polyethylene glycol modified forms of interferon-alpha with an increased plasma half-life given as weekly injections.

3.4   TREATMENT OF HCV-ASSOCIATED RENAL DISEASE

Mixed cryoglobulinemia, which in most cases has a slowly progressive clinical course, has been treated with immunosuppressive therapy such as prednisone and cytotoxic drugs especially prior to the discovery of the association with HCV. A sustained clinical effect in the long-term is uncommon. The treatment outcome with immunosuppression for acute severe disease, such as progressive renal involvement, critical vasculitic ischemia and neuropathy has been more successful in the short-term especially in combination with plasmapheresis, which removes circulating cryoglobulins. In addition, these measures could prevent new antibody formation. However, there is a concern that rising HCV viral titers, commonly seen during immunosuppressive therapy, will result in a more active HCV infection with possible worse outcome with regard to liver disease in the long-term (46, 49, 75, 106).

In a randomised study interferon-alpha was added to immunosuppressive therapy with beneficial effects with clinical improvement as well as loss of HCV RNA in 60 % of patients in the interferon-alpha group, but both viremia and cryoglobulinemia reappeared after cessation of therapy (107). Further studies have confirmed these findings, but all in all very few patients have achieved SVR with interferon monotherapy alone (108, 109).

Combination therapy with interferon and ribavirin has recently been reported in several case-reports with cryoglobulinemia, many with an improved outcome when a SVR has been achieved (110-113). However in these reports treatment has been restricted to patients with a preserved renal function, since ribavirin has been contraindicated for patients with a creatinine clearance below 50 ml/min.

Another complication with anti-viral therapy is that it may induce auto-immune diseases, primarily thyroid disease. Other auto-immune manifestations such as arthritis and an SLE-like syndrome have also been reported (114, 115). In a recent review, it was pointed out that patients with pre-existing thyroid peroxidase antibodies and females have an increased risk of developing autoimmune thyroid disease during HCV therapy (116).

4 HCV IN DIALYSIS AND TRANSPLANTATION

4.1 HCV AND DIALYSIS

HCV is a major cause of liver disease in dialysis and transplantation (31). In patients on peritoneal dialysis, the prevalence of HCV is low and seroconversion is a rare event (117, 118). Apart from the obvious risks with blood transfusions before the early 1990´s, a number of factors have been investigated in the haemodialysis setting. HCV transmission between patients has been traced through phylogenetic analysis (119, 120). Contamination through HD machines is a controversial issue, but most clinicians argue that universal precautions by health staff are more important than isolation of HCV-infected patients, which is only practiced in a minority of centres (31, 121). The problem is likely to be partly related to the prevalence of the infection which could influence the risk for nosocomial transmission. It has also been shown that the seroprevalence of HCV increases with time on dialysis (122, 123).

The natural history of HCV on dialysis is for many reasons a difficult subject. Survival on dialysis is reduced compared to the general population, primarily due to an increased risk of cardiovascular disease (124, 125) and therefore the long-term effects over decades are difficult to assess. Furthermore, a substantial number of eligible HCV-infected patients have been transplanted and have not remained on dialysis. A prospective cohort study was conducted in Japan that included 1470 dialysis patients, followed over a 6- year period. Out of these, 276 patients were positive for anti-HCV antibodies, which was one of the risk factors for death as shown by the Cox´ proportional hazards model (RR 1.57; 95% CI 1.23-2.0, p<0,01). Cirrhosis of the liver and hepatocellular carcinoma (HCC) was significantly more frequent in the anti-HCV-antibody-positive group as a cause of death (126). A smaller American cohort study including patients on dialysis for at least 6 months was followed prospectively for 6 years. Out of 200 patients 34 were HCV RNA positive. Again HCV was significantly associated with an increased risk of death, but the data were not as easy to interpret as in the previous study, since mortality after subsequent transplantation was also included (127).

Biochemical markers such as elevated liver enzymes often define liver disease. However, several investigators have found that liver enzyme levels are lower in HCV-infected dialysis patients compared with patients with normal renal function. A number of factors have been suggested to explain these findings, which are related to uraemia and dialysis as such (118, 128-130). Hepatocyte growth factor (HGF) a mitogen for hepatocytes has been shown to stimulate liver regeneration in mice. Twenty-one patients with a known duration of HCV infection of 4 years, 11 dialysis patients and 10 without renal disease were examined with measurements of HGF, viral load and liver biopsy. HGF was induced by haemodialysis treatment and HGF levels were still higher 24 hours after dialysis compared to non-renal patients. Histology showed a markedly lower grading (a necroinflammatory index), as well as staging (a fibrosis index), than in non-renal patients and liver enzymes, measured as ALT and AST were significantly lower (p<0.0001 and 0.0025 respectively). Viral load was also significantly higher in patients who were not on dialysis (p<0.05) (131). Recently IFN-alpha was suggested to

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be induced by the dialysis procedure. Eleven haemodialysis patients were studied by HCV RNA quantification as well as IFN-alpha measurements before and after one dialysis session and again before the following session. The procedure was carried out during two consecutive weeks, first by using a hemophan filter and subsequently a dialysis filter with a high biocompatibility was used. IFN-alpha was in these patients found to be induced by dialysis, in some patients up to 50 % of the levels observed after therapeutic administration, and the viral load was also found to decrease concurrently (132).

Cryoglobulinemia has been described in dialysis patients. In a Greek study cryoglobulins were detected in about one-third of 73 HCV-infected dialysis patients and approximately the same proportion was found in 87 patients with normal renal function. The mean level of cryoglobulins, RF and C4 component were however significantly lower in the dialysis patients as were HCV viral load and liver enzymes (133).

To summarize, even though HCV manifestations such as cirrhosis, HCC and cryoglobulinemic vasculitis may be of clinical importance in dialysis HCV seems in most dialysis patients to be a relatively mild condition, often with a low viral load, low liver enzymes and mildly to-moderately affected liver histology. The duration of the infection is likely to be of importance as well as known co-factors such as alcohol. Measurement of liver enzymes alone is not a reliable tool to assess liver disease activity and liver damage.

4.2   TRANSPLANTATION: SHORT-TERM VS LONG-TERM

Kidney transplantation has repeatedly been shown to be a superior treatment compared to remaining on dialysis for patients on the transplant waiting list (134, 135). Therefore considerable attention has been given to the influence of HCV on outcome after kidney transplantation. Initial studies in anti-HCV positive patients could demonstrate good short-term outcome (< 5 years), at least in non-cirrhotic patients compared to non-infected patients (136, 137). A small retrospective study also compared 33 anti-HCV positive kidney transplant recipients with 25 anti-HCV positive patients remaining on the transplant waiting list. Survival was lower in patients remaining on dialysis during a 4-year follow-up (p=0.043), however, no histology data were available for this cohort of HCV patients (138).

More recent studies have reported the long-term survival (>10 years) to be significantly lower for anti-HCV-positive patients compared to anti-HCV-negative patients (139-141). A single centre study from Hôpital Necker, Paris followed 499 patients; 387 HCV negative and 112 HCV positive, with a mean follow-up of 81 (HCV-) versus 79 months (HCV+). Time spent on dialysis was longer in the HCV-infected patients. Patient and graft survival was significantly shorter in HCV positive individuals (p< 0.01 and p< 0.0001 respectively). Increased mortality was mainly caused by liver disease and sepsis (139). These findings have been corroborated in a retrospective Japanese study and another French study, the latter also showing an even more dismal outcome for cirrhotic patients in particular (140, 141). All three studies have relied on the presence

or absence of anti-HCV-antibodies for the diagnosis of HCV-infection. After transplantation a 1.8- to 30.3-fold increase of HCV viral titers has been observed (137, 142). Immunosuppression in general and the use of anti-lymphocyte/thymocyte globulin in particular, as well as azathioprine have been identified as negative factors possibly contributing to a more severe liver disease (31).

Factors other than liver disease could also contribute to the increased risk of mortality seen in HCV-infected patients. Pereira et al have confirmed the increased risk of death from septicaemia found by Legendre et al (139), which could possibly be linked to enhanced immunodeficiency due to advanced liver disease (143). In a small Greek study of 12 anti-HCV-positive and 23 anti-HCV-negative kidney transplant recipients it was suggested that even in patients with a histologically mild chronic hepatitis the immune status of kidney transplant recipients with HCV was altered compared to non-infected patients. The HCV–positive compared to the HCV–negative ESRD patients had significantly lower B lymphocytes and naive T helper cells and higher serum levels of soluble CD8 protein prior to transplantation. The differences in peripheral blood lymphocyte subsets were however found before and not after transplantation. This could suggest an additional burden of HCV-induced immunodeficiency, which may offer an alternative explanation to the enhanced risk of death by infection in HCV-positive transplant recipients (144). In this study only 1 of 12 patients suffered an acute rejection in the HCV-positive group compared to 7 of 23 in the HCV-negative group, but this difference between groups did not reach significance.

As previously mentioned, the increased risk of post transplant diabetes has been shown to be significantly higher in tacrolimus treated HCV-infected patients than in non-infected patients (68). The long-term effects on these patients are not known, but in a study by Cosio et al, *de novo* diabetes mellitus after kidney transplantation, without adjusting for HCV, patient survival was significantly reduced after an average follow-up of 8.3 years (145).

The development of recurrent or *de novo* HCV-related glomerulonephritis with or without cryoglobulinemia may result in a premature graft loss (146-148). In the most recent study all renal biopsies from 96 kidney transplant patients with proteinuria were examined. Most biopsies were performed with a mean of 5–6 years after transplantation. Out of the 96 patients 44 were HCV positive (46 %). MPGN and MGN were found in 63.2 % of the HCV-positive biopsies in contrast to 13,5 % of the HCV-negative. By multivariate analysis, HCV infection was the only independent predictor of graft loss (RR 2.64; 95% CI, 1.35-5.17; $p = 0.005$).

Fibrosing cholestatic hepatitis, a serious and often deadly complication in viral hepatitis after transplantation has also been reported in HCV-infected kidney transplant recipients (149).

4.3   TREATMENT OF HCV IN DIALYSIS AND TRANSPLANTATION

Due to the unfavourable disease progression after kidney transplantation in many HCV-infected patients there is an increasing interest in the use of anti-viral therapy. A small number of studies have attempted to evaluate the use of interferon in already transplanted patients, but the results are rarely encouraging. Very few SVR are achieved and an unacceptably high incidence of acute rejection varying from 15 – 64 % has been reported (150, 151).
In a recent small un-controlled study, combination therapy with interferon-alpha and low-dose ribavirin (400 mg) was used. SVR was achieved in only 3 out of 12 patients. Two patients developed acute rejection with detection of de novo donor specific antibodies and C4d deposits in peritubular capillaries and in spite of anti-rejection therapy the grafts were lost (152). Interestingly, similar problems with rejection have not been a major concern in liver transplantation, where treatment of recurrent HCV is fairly frequent (153).

A number of small and un-controlled studies, ranging from case-studies to pilot-studies, have been published using interferon monotherapy in dialysis patients, with end of treatment results of 16 to 64 % SVR (154). In one controlled study from Saudi-Arabia 4 out of 11 patients who received 3 million units (MU) interferon-alpha 3 times a week for 12 months remained HCV-RNA-PCR negative. Interestingly, even patients who did not achieve SVR still had a significantly better histology activity index scores compared to untreated patients after subsequent transplantation (155). In this study interferon was reasonably well tolerated, but in general interferon seems to be less well tolerated by dialysis patients (156). Interferon-alpha is not dialysed and pharmacokinetic analyses have demonstrated that clearance of interferon-alpha is reduced by approximately 50 % in dialysis patients compared to non-uraemic patients. This could in part explain the poorer tolerance as well as the slightly better outcome in successfully treated patients (157, 158).

In accordance with current European and U.S guidelines many centres today offer interferon monotherapy prior to kidney transplantation for HCV infected dialysis patients. Interferon in combination with ribavirin has not been recommended due to the contraindication for ribavirin therapy in renal insufficiency. Anti-viral therapy after kidney transplantation is recommended only for severe disease manifestations such as fibrosing cholestatic hepatitis. In case of established cirrhosis of the liver, diagnosed by a liver biopsy, a combined kidney-liver transplantation rather than a single kidney transplant should be considered due to the increased risk of death from liver failure (159,160).

5 AIMS OF THE STUDY

The aim of the study was to investigate the following:

1. Does chronic HCV infection affect long-term patient and graft survival after kidney transplantation patients in a Swedish cohort? In this cohort what is the relation between time in renal replacement therapy, HCV infection and outcome after transplantation?

2. Can a method for pharmacological monitoring of ribavirin be developed to enable its use in patients with renal insufficiency or end-stage renal disease, a special population in which ribavirin has been considered to be contraindicated?

3. Can a combination of interferon-alpha and ribavirin safely be used in dialysis patients?

4. Can a combination of interferon-alpha and ribavirin safely be used in HCV-related renal disease and renal insufficiency?

5. What is the role of renal function for the pharmacokinetics of ribavirin and can a dosing schedule be based on these results?

6 PATIENTS

Study I) A cohort consisting of 520 HCV-negative and 51 HCV-positive patients, all HBsAg negative. All transplanted at the Transplant Surgery Department at Huddinge University Hospital, Stockholm from January 1, 1989 to December 31, 1997 with a follow up to December 31, 2002.

Study II) Ten randomly selected patients with normal renal function from the Infectious Diseases Department, Huddinge University Hospital, all treated with interferon-alpha and ribavirin for chronic HCV infection and sampled for ribavirin concentrations. Like-wise two dialysis patients were sampled after having received a single-dose ribavirin.

Study III) Six dialysis patients, 5 undergoing haemodialysis and 1 peritoneal dialysis, all patients at the Division of Renal Medicine at Huddinge University Hospital were sampled for ribavirin plasma concentrations during interferon-alpha and ribavirin therapy for HCV.

Study IV) Seven patients with HCV related renal disease with or without cryoglobulinemia and renal insufficiency were sampled for ribavirin plasma concentrations during interferon-alpha and ribavirin therapy.

Study V) The sampling of ribavirin plasma concentrations for the pharmacokinetic analysis included the following 63 patients all with active HCV infection:
44 patients with normal serum creatinine:
10 control patients from study II undergoing interferon-alpha and ribavirin treatment.
34 patients from the Infectious Diseases Department treated with interferon-alpha in combination with ribavirin and consecutively entered.

19 patients with impaired renal function including:
6 dialysis patients from study III and one additional haemodialysis patient not included in the study, all treated with interferon and ribavirin.
4 patients with renal insufficiency from study IV, one treated with ribavirin monotherapy.
4 liver transplant and 2 kidney transplant patients, mainly treated with ribavirin monotherapy.
Finally, a single kidney-patient and one patient with an HIV-HCV co-infection with renal involvement both treated with interferon-alpha and ribavirin.

6.1 STUDY I)

Background: Chronic hepatitis C infection (HCV) is common among kidney transplant recipients and is known to affect both long-term graft and patient survival (139-141). Time in renal replacement therapy (RRT) has also been shown to be a risk factor for death and graft loss after transplantation (161-163). HCV-infected kidney transplant patients have repeatedly been shown to have a significantly longer exposure to dialysis than non-infected patients (139-141). The aim of the study was to investigate HCV in relation to time in RRT and its impact on patient and graft survival after kidney transplantation in a Swedish cohort.

Patients and Methods: A follow-up cohort study using univariate Kaplan-Meier analysis and the multivariate Cox proportional hazards model was performed in 545 kidney and 26 kidney-pancreas transplant recipients transplanted 1989-97 with last follow-up on December 31, 2002. HCV status at transplantation and time in RRT were analysed. HCV status was not available in 40 subjects excluding them from further analysis. Three patients chronically infected with Hepatitis B being HBsAg positive were also excluded. Patients characteristics are summarized in **Table 1**

	HCV negative n = 520	HCV positive n = 51	p-value
Age at tx (years) mean ±SD[a]	45.5±13.8	45.4±13.7	n.s
Sex F/M (% Female)	180/340(34.6%)	24/27(47.1%)	0.08
RRT prior to tx (yrs)mean ±SD	2.3± 3.6	5.7±6.4	<0.0001
Type of tx (CD[b]/LD[c]/CD+P[d])	331/164/25	39/11/1	n.s
Diabetes Yes/No (%)	98/422 (23.2 %)	7/44 (16%)	n.s
Previous transplants Yes/No (%)	53/467 (10.2%)	12/39 (23.5%)	0.04

Table 1 Patients characteristics including age at tx, gender, RRT prior to tx, type of tx, diabetes, previous transplants
[a]Standard deviation [b]cadaveric kidney donation [c]living kidney donation
[d]cadaveric kidney and pancreas

Main findings: Time in RRT was significantly longer (p<0.0001) and previous transplantations were more common (p= 0.04) in the HCV-group (**Table 1**). By Kaplan-Meier survival analysis HCV significantly reduced patient (p = 0.0012) and graft survival (p = 0.0003) after transplantation with a 5 and 10 year patient survival of 69% and 49 % for HCV + and 85% and 68% for HCV - respectively, whereas 5 and 10 year graft survival was 52 % and 31% versus 72% and 49%. **Figure 4** showing patient survival during follow-up after kidney transplantation and **Figure 5** showing graft survival during the same time period.
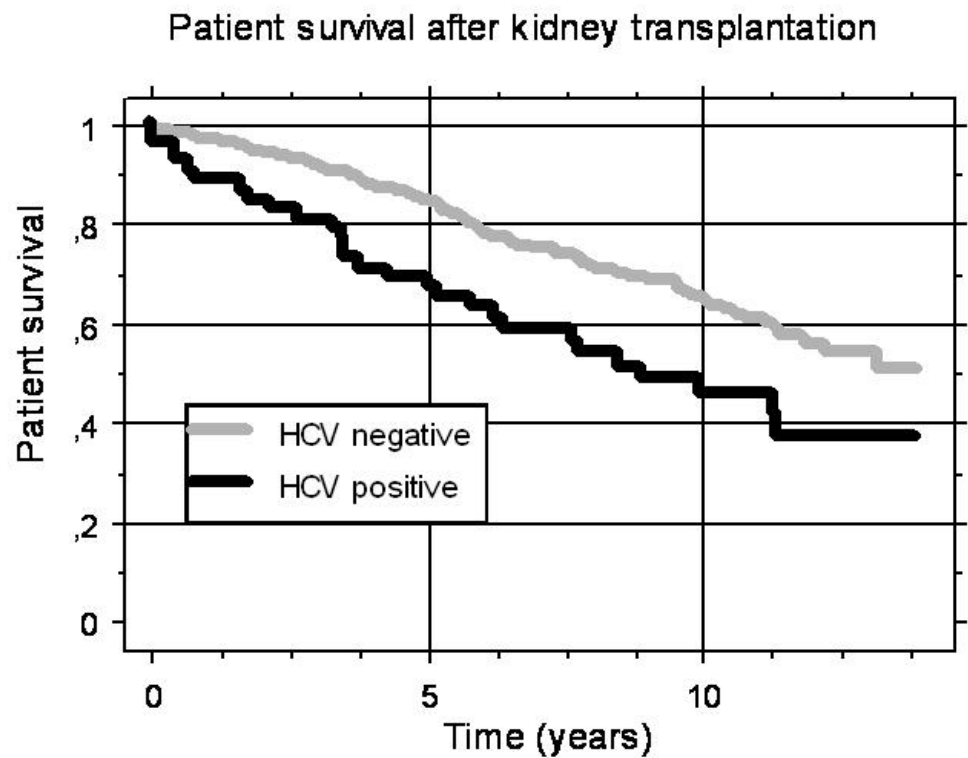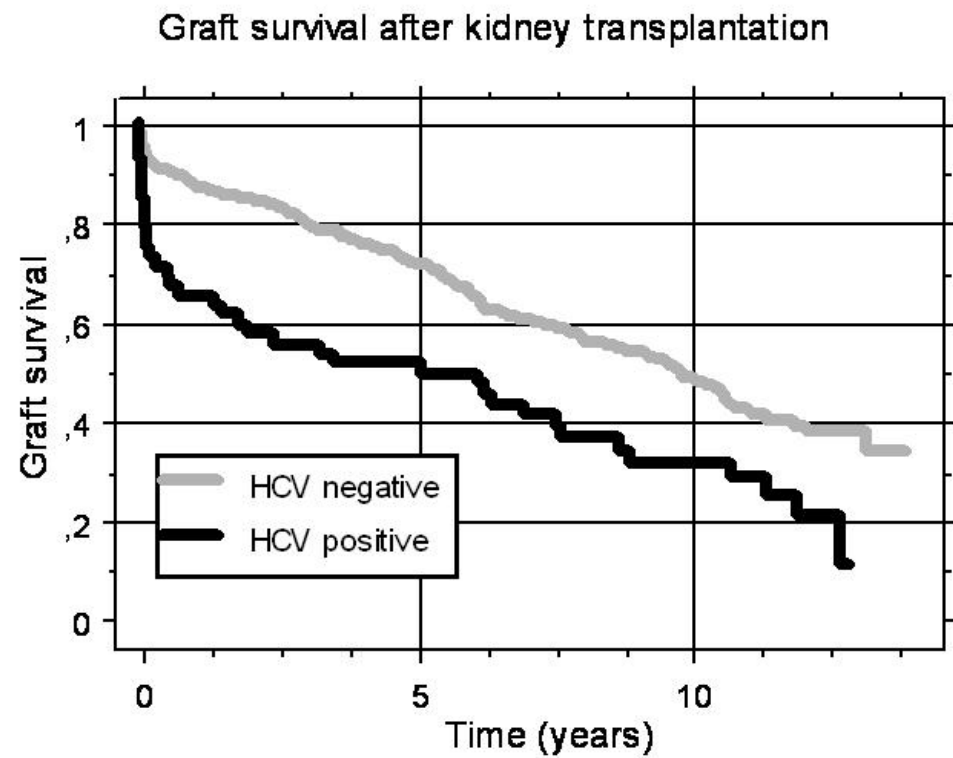
Figure 4

Patient survival after kidney transplantation

Figure 5

Graft survival after kidney transplantation

Adjustment for age, gender, diabetes, previous transplantations, type of transplant and time in RRT by Cox proportional hazards model resulted in a relative risk (RR) for death of 2.23, 1.92 and 1.07 (CI 95%) for HCV, diabetes and age respectively. The RR for graft loss was 1.96 and 1.03 for HCV and age. Gender, previous transplants and time in RRT did not affect HCV as an independent risk factor for patient death or graft loss. The leading cause of death was cardiovascular disease in both groups.

An additional way to examine the survival data is to plot the logarithm of cumulative hazard over time. **Figure 6** displays the cumulative hazard for HCV positive and negative patients. The natural logarithm Ln time=0 is equal to 1 year after transplantation. As shown in the figure the hazard lines are almost parallel until the latter part of the time-period, when the lines tend to converge. The lines do however not cross, which can be interpreted as a reduced but still remaining risk at the end of the time-period. The figure thus shows that the mortality risk is increased among HCV-positive patients as compared to HCV-negative ones throughout the follow-up.

Kaplan-Meier Ln Cumulative Survival Hazard Plot
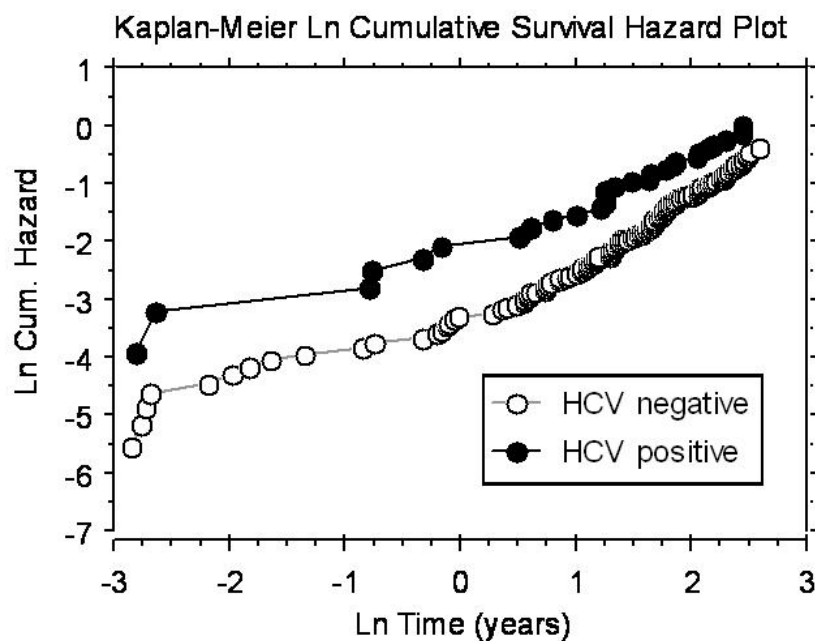
Conclusions: In our series we found that HCV is an independent risk factor for death and graft loss after kidney transplantation whereas time in RRT does not influence this risk. The results could possibly lend support to the suggestion that successful anti-viral HCV therapy prior to transplantation may be preferable to an early transplant for long-term patient and graft survival after kidney transplantation. However, this remains to be confirmed in prospective studies.

6.2   STUDY II)

Background: Ribavirin is a nucleoside analogue mainly used in combination with interferon for treatment of HCV infection. Its use has been limited in renal patients due to a contraindication below a creatinine clearance of 50 ml/min. The aim of the current project between the Divisions of Renal Medicine, Infectious Diseases and Clinical Pharmacology was to set up a clinically applicable and inexpensive ribavirin monitoring method for routine use primarily in renal patients with HCV, using commercially available components.

Method and main findings: In a first step solid-phase phenylboronic acid columns are used to bind ribose-containing structures at a high pH. When pH is lowered ribavirin is released and eluted with a high degree of purification and close to 100 % recovery and is subsequently detected through HPLC (high-performance liquid chromatography) and ultraviolet (UV)-detection.

10 interferon-alpha and ribavirin treated patients without renal dysfunction were sampled at steady-state in order to find a through level interval to be used clinically in patients with renal insufficiency. The ribavirin dose in the reference population was 1000 or 1200 mg daily.
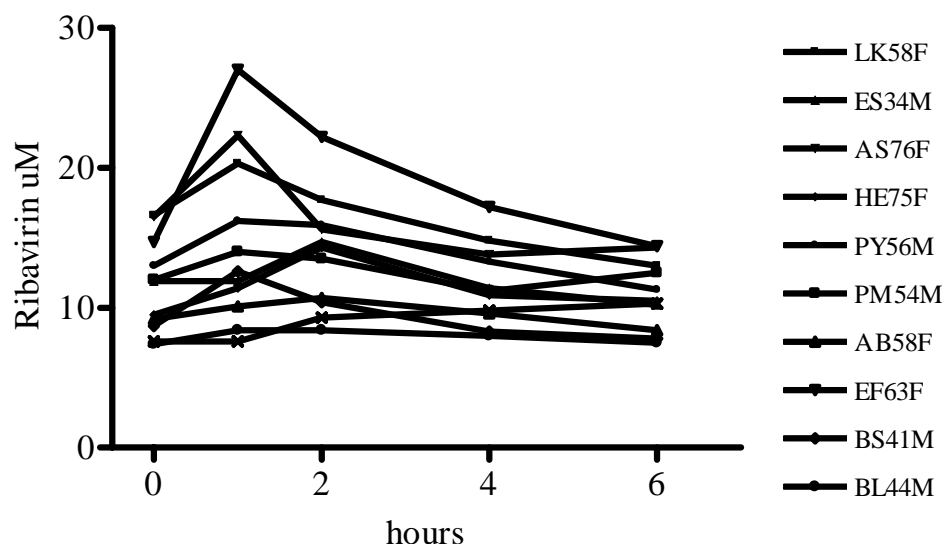The patients are shown in **Figure 7.**

Figure 7 Patients with normal GFR sampled for ribavirin concentrations in plasma at 0, 1, 2, 4 and 6 hours

Samples from patients with ESRD were also analysed after giving single-dose ribavirin. Interference of compounds, endogenous or immunosuppressive drugs as well as interferon were not found. Renal failure did not influence the performance of the assay.

Conclusion: Ribavirin trough level plasma concentration at steady-state varied between 7 – 17 µmol/L in the reference population. The ribavirin concentration method developed by us is fairly simple and fast and is thereby useful for routine therapeutic drug monitoring in clinical use.

6.3 STUDY III)

Background: The aim of the study was to apply ribavirin concentration measurements during a pilot study, to evaluate the feasibility of treating dialysis patients with a combination of interferon-alpha and ribavirin.

Patients and methods: Five haemodialysis and 1 peritoneal dialysis patients were included in the study. Five of the patients were infected with genotype 1, one with genotype 4. A target concentration of 10-15 µmol/L was selected aiming at the central part of the plasma concentration range found in the reference population in study **II** (**Figure 7**). All patients underwent a liver biopsy, pre-treatment HCV-RNA quantification and HCV genotyping. A simplified scheme (**Table 2**) for histological grading and staging of chronic hepatitis was used as in Wejstål et al (36). Background patient data are given in **Table 3.**

Score	Portal inflammation	Interface hepatitis	Lobular inflammation with necrosis/apoptosis	Fibrosis
0	None	None	None	None
1	Minimal	Minimal, patchy	Only inflammation	Portal fibrosis
2	Mild	Mild, involving most portal areas	Mild, little hepatocyte damage	Periportal fibrosis
3	Moderate	Moderate, involving all portal areas	Moderate, with noticeable hepatocyte damage	Septal fibrosis
4	Severe	Severe, involving all portal areas	Severe, with prominent hepatocyte damage	Cirrhosis with nodules

Table 2. Histological grading and staging for chronic hepatitis. Reprinted with permission from (36)

Ribavirin was monitored weekly for the first 12 weeks, later in most patients every second week or when required. Initial treatment was interferon-alfa-2-b 3 MU thrice weekly adding ribavirin at a dose of 200-400 mg after 4 weeks, for a total intended treatment of 28 weeks, in order to better separate side-effects. Full blood count and haemoglobin was monitored weekly during the first 8 weeks, Hb was thereafter monitored on a weekly basis.

Patient	Age	HCV genotype	S-ALT pre-treatment (μkat/L)	Liver histology[a]
1	54	1a	0.4	Grade 1/Stage 1
2	35	1b	0.6	Grade1/Stage 3
3	54	1a	0.9	Grade 1/Stage 2
4	43	1a	1.5	Grade 1/Stage 1
5	50	1a	0.8	Grade 1/Stage 3
6	40	4	0.9	Grade 0/Stage 0-1

Table 3 Background data in 6 dialysis patients with HCV treated with interferon-alpha-2b and ribavirin [a]Grade 0 – 4 (0 = no inflammation and 4 = severe inflammation) Stage 0 – 4 (0 = no fibrosis and 4 = cirrhosis).

Main findings: Target concentration was reached with average daily doses of 170-300 mg ribavirin within 2-6 weeks. All patients had initial flu-like side effects of interferon, but the major side effect was anaemia, which was managed by low-average doses of i.v iron and high-dose erythropoietin (20000-30000 IU/week) maintaining haemoglobin levels between 95 and 110 g/L. Five of six patients became HCV-RNA negative during treatment after 4 (pat no 5 and 6), 8 (pat no 3 and 4) and 16 (pat no 1) weeks of treatment respectively, i.e. 2 patients during interferon monotherapy and 3 patients during combination therapy. Patient no 2 did not become HCV-RNA negative but the HCV RNA levels fell from 1,5 million copies/ml pretreatment to < 200.000 copies/ml (cut-off level) after 14 weeks of treatment. Four patients completed the study but only one patient maintained SVR after end-of-treatment. An example of the treatment course is given in **Figure 8.**
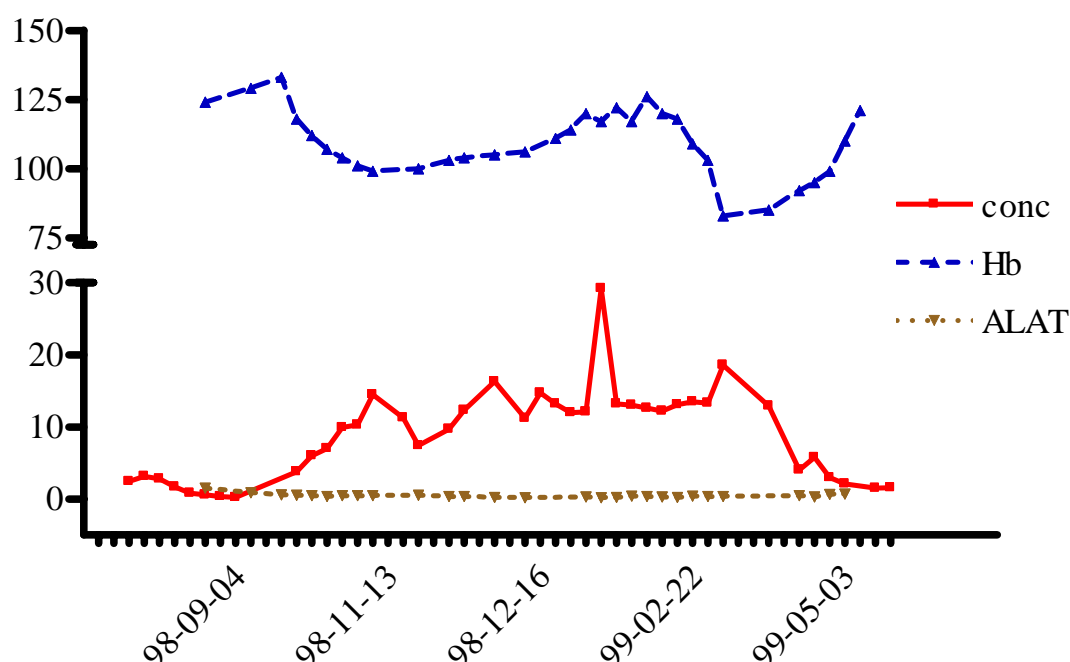

Figure 8 Treatment course in one dialysis patient treated with 4 weeks of interferon-alpha monotherapy followed by 24 weeks of interferon-alpha and ribavirin therapy.

One patient suffered marked side effects from interferon with severe weight loss and malaise and therapy was terminated at 16 weeks of combination therapy. Patient 2 developed heart failure after an upper respiratory infection and died after 14 weeks. Autopsy showed a grossly enlarged heart, in particular the left ventricle, but no signs of infarction, myocarditis or storage disease in the myocardium. The interpretation of the findings was that the heart failure was related to a long-standing and periodically severe hypertension and to dialysis treatment for more than 20 years, and that the death was probably not related to the antiviral treatment. Our overall impression was that apart from anaemia in all patients and possibly pruritus in patient 3 most side effects were related to interferon therapy. Viral response, ribavirin dose and side effects are summarized in **Table 4.**

Patient	Viral response/ sustained viral response	Ribavirin dose	Side effects	Side effects other
1	Yes/No	400 mg t.i.w [c]	Anaemia	Initial fever, malaise
2[a]	No/No	200 mg/day	_ " _	Initial fever, malaise
3	Yes/No	400 mg t.i.w	_ " _	Initial fever, malaise pruritus
4	Yes/No	400 mg t.i.w 200 mg q.i.w [d]	_ " _	Initial fever, malaise, substantial weight loss
5[b]	Yes/No	200 mg/day	_ " _	Initial fever, malaise
6	Yes/Yes	200 mg/day	_ " _	Initial fever, malaise, nausea

Table 4 Viral response, ribavirin dose and side effects. Combination therapy stopped at [a]14 weeks [b] 16 weeks. [c] t.i.w thrice weekly, [d]q.i.w four times weekly.

Conclusion: This study shows that treatment with interferon-alpha in combination with ribavirin is feasible and relatively safe in dialysis patients. However this requires ribavirin dosing modification as well as close ribavirin plasma concentration and Hb monitoring which are strongly recommended. Due to unfavourable HCV genotypes present in the treated patients longer duration of therapy, in accordance with current recommendations, could have increased the SVR.

6.4   STUDY IV)

Background: HCV is associated with glomerular disease such as MPGN with or without cryoglobulinemia, MGN and FSGS. Very few patients have achieved SVR with interferon monotherapy only and ribavirin has been withheld in most renal patients due to a contraindication for renal insufficiency (31). The aim was to retrospectively analyse the outcome, regarding viral and renal response as well as tolerability, of seven patients treated with interferon-alpha-2b or pegylated interferon-alpha-2b in combination with ribavirin for HCV-related renal disease and renal insufficiency

Patients and methods: Seven patients with renal disease and a glomerular filtration rate (GFR) between 10 - 65 ml/min were treated for HCV-related renal disease. Two patients had symptomatic cryoglobulinemia. One patient was infected with HCV genotype 1, the remainder with genotype 2 and 3. Background data are given in **Table 5.**

Patient	Sex	Age	Clinical manifestations	Auto anti-bodies	Complement
1	M	45	GN[a], NS[b]	Negative	C4 low
2	M	70	GN, NS	Negative	Normal
3	F	47	Fever, purpura, arthralgia, pleuritis, cerebral vasculitis, peripheral neuropathy, GN	RF, ANCA, cryo	C4 borderline
4	F	50	GN	Negative	Normal
5	M	43	GN, NS	Negative	Normal
6	F	50	Fever, oral and skin ulcers, GN	ANA, anti-DNA, ANCA, cryo	C4 low
7	M	43	GN, NS	Negative	Normal

Table 5. Background data, clinical manifestations and immunological profile
[a]GN (glomerulonephritis), [b]NS (nephrotic syndrome)

Five patients were given interferon-alpha-2b and ribavirin, whereas two patients were given pegylated interferon-alpha-2b and ribavirin. Ribavirin trough level monitoring was determined on average three times during the initial 2-3 months of therapy, later occasionally. Duration of therapy was according to genotype (6-12 months).

Main findings: Six of seven patients became HCV-RNA-PCR negative and four of seven (MPGN, FSGS and one vasculitis patient) have maintained both virological and renal remission. One of seven (MPGN) has maintained virological and partial renal remission. These data are summarized in **Table 6.**

Pt	Viral response/ Sustained viral response[a]	GFR at entry mL/min	GFR at follow-up mL/min	Hematuria at entry/follow-up	Albuminuria/ 24 hours at entry/follow-up
1	Yes/Yes	52	95	Yes/No	6 g/ 0,3 g
2[b]	No/No	10	38	Yes/No	3 g/0,07g
3	Yes/No	65	69	Yes/Yes	0,8g/0,4g
4	Yes/Yes	58	56	Yes/No	1,2g/0,25g
5	Yes/Yes	64	64	Yes/No	4,5g/0,13g
6	Yes/Yes	62	89	No/No	1,6g/0,11g
7	Yes/Yes	25	23	Yes/No	10g/4,6g

Table 6. Viral and renal response to antiviral therapy
[a]Viral response: HCV-RNA-PCR negative during treatment; sustained viral response: HCV-RNA-PCR negative at follow-up.
[b]Pat 2 intolerant to interferon-alpha-2b.

One MPGN patient did not tolerate interferon, but is in renal remission with low-dose ribavirin. One vasculitis patient responded with complete remission, but relapsed virologically and had a minor vasculitic flare after 9 months. Only one vasculitis patient had low-dose immunosuppression, prednisone, in addition to anti-viral therapy. Average daily ribavirin dose was 200-800 mg. Ribavirin-induced anaemia was managed in five of seven patients with adequate iron stores and erythropoietin up to 20 000 IU/week. Initial interferon-related side-effects were common, but long-term interferon was fairly well tolerated. The use of pegylated interferon was in our experience a valuable novelty. An additional problem was severe hyperuricemia in the first two cases, which led to pre-emptive prophylactic treatment with either allopurinol or sodium bicarbonate and probenicide in the following patients.

Conclusion: In HCV related glomerulonephritis and vasculitis interferon-alpha in combination with ribavirin can be used with reasonable safety irrespective of renal function. As in the previous study in dialysis patients this however requires a reduction in ribavirin doses as well as monitoring of ribavirin plasma concentrations and surveillance of side-effects, mainly anaemia.

6.5   STUDY V)

Background: Interferon and ribavirin is standard therapy for HCV infection. At present ribavirin dosing recommendations are based on body weight. Based on our previous experience in treating patients with reduced renal function with ribavirin we carried out a population pharmacokinetic analysis with the primary aim to evaluate the rationale of current dosing recommendations. A secondary aim was to suggest a ribavirin dosing schedule for patients with renal insufficiency based on this analysis.

Patients and methods: 383 ribavirin samples from a total of 63 patients were collected, 44 patients of which had normal serum creatinine and 19 with renal impairment with an estimated GFR of 5 – 57 ml/min. GFR was estimated by means of a modified version of the Cockroft-Gault equation. For dialysis patients, GFR was set to 5 ml/min. Ribavirin levels were measured by solid phase extraction, followed by HPLC (high-performance liquid chromatography) and ultraviolet (UV)-detection. A two-compartment linear model with first order absorption was used and a population pharmacokinetic analysis was performed using non-linear mixed effect modelling (NONMEM) in order to evaluate population factors such as age, gender, body weight, serum creatinine and GFR.

Main findings: Ribavirin clearance was found to be linearly dependant on renal function with a small non-renal clearance component dependant on body-weight and age **Figure 9**.
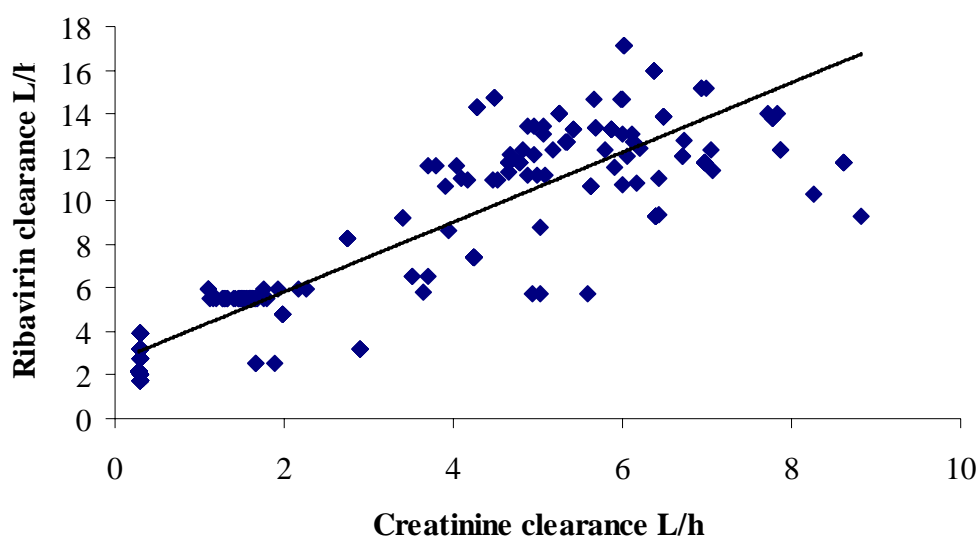

Figure 9 Plot of ribavirin clearance (L/h) as a function of creatinine clearance (L/h)

Renal function, defined as estimated GFR, was a significantly better predictor of ribavirin clearance than body-weight alone. The volume of distribution was large and proportional to body weight (V=44.3 x body-weight), which resulted in a long half-life (100-500 hours, depending on GFR) and a long time to steady state (3-12 weeks). There remained a 40 % inter-individual variability in ribavirin total clearance not explained by estimated GFR and body weight.

Conclusion: Ribavirin initial dosing should mainly be based on renal function and not on body weight alone. A dosing schedule for ribavirin based on GFR and body-weight is proposed in **Table 7**

CrCL mL/min	120	100	80	60	40	20
Target Css						
6 μmol/L	600	600	400	400	200	200
10 μmol/L	1000	1000	800	600	400	400+200
14 μmol/L	1600	1200	1000	800	600	400

Table 7 Suggested starting daily doses for different target concentration levels (Css) and creatinine clearance (Cr CL) (20-120 ml/min). The patient´s weight is set to 70 kg. 400 + 200 denotes one 400 mg capsule every other day and 200 mg every other day. For considerable deviation from 70 kg and creatinine clearance < 20 ml/min equations given in manuscript **V** are recommended

Time to steady state increases with reduced renal function (t½= VSS ln2/CL) and steady state was considered to be attained after 4 · t½. (VSS = apparent volume of distribution and CL = apparent clearance). Even if the initial ribavirin dose, resulting in a correct target concentration, is correctly chosen a patient with renal insufficiency is likely to have a low ribavirin concentration for a substantial amount of time. Hereby the patient may be subjected to a suboptimal treatment until steady state is reached. This problem can be solved by using one or more loading doses and subsequent ribavirin plasma monitoring with a readiness for ribavirin dose adjustments.

Given the remaining inter-individual variability in ribavirin clearance one should consider ribavirin monitoring in clinical studies also in patients with normal renal function. This could clarify the relationship between ribavirin dose and ribavirin concentration for each patient and make it possible to evaluate a possible therapeutic target concentration for HCV infection.

7 GENERAL DISCUSSION

HCV infection is a clinical problem leading in the long-term to decreased patient and graft survival in infected kidney transplant recipients. It is also emerging as a recognized cause of renal disease, which is an element of the HCV epidemic affecting 170 million people world-wide. However, the prevalence of renal disease in HCV infection has not yet been completely characterized. In spite of recent advances in the treatment of HCV infection, primarily combining the nucleoside analogue ribavirin with interferon and more recently pegylated interferon, therapy in renal disease and renal insufficiency has been limited to interferon monotherapy due to a contraindication to ribavirin for patients with a creatinine clearance below 50 ml/min.

The aim of these studies could be described as follows:

1) To focus on the importance of HCV for patient and graft-survival in relation to time on dialysis in a Swedish kidney transplant cohort.

2) To develop therapeutic alternatives for HCV infection in renal patients by

a) the development of a method to measure the concentration of ribavirin for clinical use

b) applying ribavirin concentrations measurements in dialysis patients and patients with HCV-related renal disease and renal insufficiency, in order to optimize therapy while handling side effects.

c) the evaluation of current ribavirin dosing recommendations by a population-based pharmacokinetic analysis and specifically analyse the importance of renal function for ribavirin clearance.

1) We confirmed the finding of previous studies (139-141) that HCV is a negative factor for long-term patient and graft-survival after kidney transplantation. We were also able to show that chronic HCV infection was significantly more important than time in RRT for outcome after kidney transplantation. Time in RRT was not associated with an increased risk in our series. This relationship between HCV infection, time in RRT and patient and graft survival after kidney transplantation has not previously been well characterized. Recent data show that successful pre-transplant interferon-alpha therapy leading to a SVR can be maintained after kidney transplantation (164-165). Our results in the present study could possibly lend support to those favouring the use of anti-viral therapy prior to kidney transplantation, the suggestion being that successful anti-viral HCV therapy pre-transplant may be preferable to an early transplant for long-term patient and graft survival after kidney transplantation. Treatment could thus be worthwhile in many patients even if transplantation would be postponed for up to one year depending on viral genotype. However, the long-term benefits of anti-viral therapy prior to transplantation need to be confirmed in prospective studies with regard to graft

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and patient survival in HCV infected kidney transplant patients.

In our series hepatitis C was associated with a significantly longer duration of dialysis as is also described in previous studies (139-141). It is possible that HCV infection may confound the results of studies (161-163) which show that prolonged time in RRT is a negative factor for post-transplant patient and graft survival. However, when HCV was excluded in the proportional hazards model, we were unable to find an increased risk for poor post-transplant patient and graft survival with regard to time in RRT. It is possible that the size of the present cohort was to small to detect any significant negative influence of time on dialysis. It would therefore be of considerable interest to analyse larger kidney transplant cohorts in order to see if similar results can be observed.

Factors other than HCV, time in RRT, type and source of the transplanted organ as well as comorbidities such as diabetes could influence long-term outcome after kidney transplantation. Reduced GFR in the donor and poor haplotype match are known to affect graft function in kidney transplant recipients (166, 167). Due to the relatively small size of the cohort the number of covariates studied had to be limited. It is however reasonable to assume that reduced donor GFR and haplotype match was not differently distributed in the HCV positive and HCV negative group in any major way and that the strong main findings of the study remain valid.

2a + 2b) The ribavirin monitoring method developed by us for use in ESRD and patients with renal insufficiency has enabled the clinical use of ribavirin in combination with interferon-alpha in these patients. We believe that treatment by these means has been comparable to non-renal patients. However, one should keep in mind that a fixed optimal therapeutic target concentration for ribavirin remains to be established.

In study **III** a total of 6 dialysis patients and in study **IV** 7 patients with renal insufficiency were treated with interferon-alpha in combination with ribavirin. A summary of the viral response rate during interferon-alpha and ribavirin therapy in study **III** and **IV** is given in **Figure 10.**

In study **III** 2 patients became HCV RNA negative after 4 weeks, another 2 after 8 weeks and finally 1 after 16 weeks of treatment. One patient, who never completed the study, remained HCV RNA positive at 14 weeks. The HCV RNA quantitative test at that time was however negative reflecting a significant reduction in HCV viral load. Only one of the four patients completing the study remained HCV-RNA-PCR negative after long-term follow-up. Given the unfavourable HCV genotypes found in the dialysis cohort longer duration of therapy, in accordance with current recommendations, might have increased the SVR

In study **IV** 2 patients became HCV RNA negative at 4 weeks, 1 patient at 8 weeks and finally 3 patients at 12 weeks. One patient did not tolerate interferon-alfa and thus did not become HCV RNA negative. Of the 6 patients completing interferon and ribavirin therapy, 4 have maintained SVR and renal remission and 1 has maintained SVR and partial renal remission, all with extended follow-up.
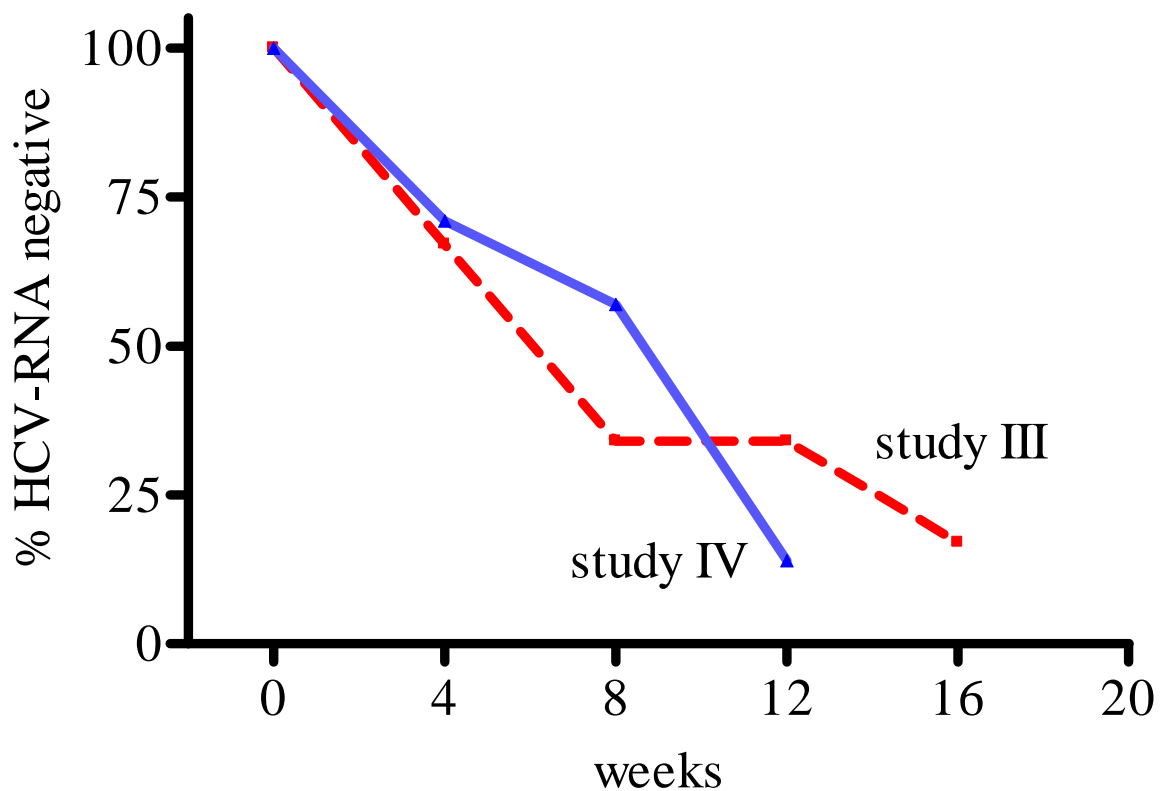
Figure 10 Time point at which patients reached HCV-RNA-PCR negativity during study **III** and **IV**

Side effects due to interferon in dialysis patients as well as in patients with renal insufficiency were similar to those found in non-renal patients. Dialysis patients, however, seemed to have slightly more pronounced symptoms, which is in accordance with previous interferon monotherapy studies in ESRD showing reduced tolerance during treatment (154). This is likely to be related to the pharmacokinetic properties of interferon in ESRD and possibly to the induction of interferon production during haemodialysis treatment (132, 154).

Nevertheless our major concern was how to manage ribavirin-associated anaemia. This was achieved by supporting the patients throughout treatment with high doses of subcutaneous erythropoietin as well as low-dose iron i.v, thereby avoiding blood transfusions. Anaemia was also a major problem in a Dutch dialysis pilot study also published in 2001 (168). Five patients were given interferon-alpha at a dose of 3 million units, 3 times weekly with an initial starting dose ribavirin of 200 mg daily. Three out of 5 patients had to permanently discontinue ribavirin treatment due to severe anaemia and blood transfusions were frequent. No data on the erythropoietin dose was available. A retrospective analysis of ribavirin concentrations was performed showing

slightly lower levels than our target concentration. Interestingly, the initial virological response during treatment was high, which is in accordance with our study. A German study group led by Dr Renders in Kiel, has also investigated interferon-alpha and ribavirin therapy in dialysis patients. The daily ribavirin dose was 200 mg, HPLC was used to monitor ribavirin concentrations and the levels were similar to those found in our patients. To date, six out of nine patients, all genotype 1, have achieved a viral response and five patients have maintained SVR (Renders: personal communication 2003, unpublished data).

In summary we have shown that, in contrast to the current contraindication for the use of ribavirin in patients with a creatinine clearance below 50 ml/min, ribavirin in addition to interferon-alpha is possible to use in ESRD and renal insufficient patients. However, this requires reduced ribavirin doses adjusted for renal function and close monitoring of ribavirin plasma concentrations as well as side effects. Although the studies are small and un-controlled pilot studies it is likely that the use of ribavirin combined with interferon has synergistic effects in ESRD and patients with renal insufficiency and in this respect, they do not differ from patients with normal renal function.

2 c) In study **V,** ribavirin clearance was found to be linearly dependant on renal function, with a small non-renal clearance dependant on body weight and age. Renal function, defined as estimated GFR, was a significantly better predictor of ribavirin clearance than body weight alone. The volume of distribution was large and proportional to body weight (V=44.3 x body-weight), which resulted in a long half-life (100-500 hours depending on renal function) and a long time to reach steady state (3-12 weeks). There was a 40 % inter-individual variability in ribavirin total clearance not explained by estimated GFR and body weight. We therefore concluded that the initial dosing of ribavirin should mainly be based on renal function and not on body weight alone. A dosing schedule based on GFR and body weight is proposed. Given the remaining inter-individual variability in ribavirin clearance, one should consider clinical studies including ribavirin monitoring in patients with normal renal function as well, in order to identify a possible optimal ribavirin therapeutic target concentration for HCV infection.

Ribavirin-associated anaemia is the major and dose limiting side effect not only in patients with renal insufficiency patients but also in HCV infected patients with normal renal function. We have recently shown that ribavirin concentration is a more important predictor of anaemia than ribavirin dose per kilogram body weight in a study of 108 consecutive HCV infected patients with normal GFR (169). In another study from Pittsburgh including 72 HCV-infected liver transplant patients ribavirin dose modification based on renal function was proposed to reduce haemolysis associated with ribavirin therapy (170). In this study a significant correlation between anaemia and renal function was found. Ribavirin concentration monitoring was, however, not available. The results from this study and our own pharmacokinetic analysis results (Study **V**) again emphasises the importance of renal function for ribavirin clearance.

The quality of the population pharmacokinetic model depends on several factors. The number of patients in the pharmacokinetic study was relatively small. The precision of

the parameter averages improves with the square root of the number of patients while the variances are much less precise. In the present study, the equations for CL and V are considered to be fairly accurate and so is the precision of the intra-individual variance of the plasma concentrations. The inter-individual variances remaining, after estimated GFR was included in the model, are less precise but are on the other hand small. With regard to GFR it is neither necessary nor an advantage to have the same distribution of GFR in the study population as in the HCV population. To estimate the relationship between GFR and ribavirin clearance, which was the aim of the study, it is necessary to study a wide range of GFR.

The ribavirin dosing schedule developed by us in paper **V** is currently used in clinical practice for renal patients at Huddinge University Hospital. We also recommend ribavirin monitoring as a tool for safety purposes in ESRD and renal insufficiency as well as in patients with pronounced side effects. The fairly long time to steady state and the large inter-individual variability in ribavirin clearance found in the pharmacokinetic study and (study **V**) also supports the use of ribavirin concentration measurements.

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8   CONCLUSIONS

I. We confirmed that HCV is an independent risk factor for patient ($p<0.0012$) and graft-survival ($p<0.0003$) after kidney transplantation found in previous studies. After adjusting for age, gender, diabetes, previous transplants, type of transplant and time in RRT we found that HCV was significantly more important than time in RRT for patient and graft survival after kidney transplantation. This has not been well characterized before. The results lend support to the suggestion that successful anti-viral HCV therapy prior to transplantation may be preferable to an early transplant for long-term patient and graft survival after kidney transplantation. However, this remains to be confirmed in prospective studies.

II. A high-performance liquid chromatography (HPLC) method for ribavirin plasma concentration measurement was developed. A trough level interval of 7-17µmol/L was found in a reference population with normal renal function treated with interferon-alpha and ribavirin. The method was found to be fairly simple and fast and is therefore useful for routine therapeutic monitoring.

III and IV. The ribavirin monitoring method enabled clinical use of ribavirin in combination with interferon-alpha with fair tolerability and viral response in 6 HCV infected dialysis patients and in 7 patients with reduced renal function with HCV-related renal disease. The use of ribavirin is likely to have had an effect in addition to that of interferon in these patients and in that respect they would not differ from patients with normal renal function. Side effects, mainly ribavirin-associated anaemia, were handled by means of medium-high doses of erythropoietin as well as low-dose iron therapy. The use of interferon-alpha and ribavirin in HCV infection is thus feasible and relatively safe in dialysis and in renal insufficient patients. However this requires ribavirin dosing modification as well as ribavirin plasma concentration and monitoring of side effects.

V. Ribavirin is currently dosed according to body weight. A population pharmacokinetic analysis was performed. Estimated GFR was found to be a significantly better predictor of ribavirin clearance, being linearly dependant on renal function, than body weight alone. In addition, significant interindividual 40 % variability in ribavirin total clearance was found which could not be explained by GFR and body weight. We conclude that ribavirin should be dosed primarily on renal function and not on body weight alone. A dosing schedule is proposed. Therapeutic drug monitoring is not an established standard in ribavirin therapy. However with regard to the importance of renal function and the interindividual variability in ribavirin clearance found here clinical studies with ribavirin monitoring in patients with normal renal function in order to identify a possible therapeutic ribavirin target concentration for HCV infection should be considered.

9 RECOMMENDATIONS AND FUTURE ASPECTS

An issue of decisive importance is whether the use of anti-viral therapy for HCV prior to transplantation is worthwhile in the long-term. The benefit of clearing HCV-related renal disease is apparent. Nevertheless neither short nor long-term outcome after kidney transplantation has been clear in dialysis patients achieving a SVR prior to transplantation. Recent studies, however, have shown that pre-transplant interferon therapy can prevent *de novo* HCV-associated glomerulonephritis in successfully interferon-treated HCV infected dialysis patients. SVR has been found to be maintained after kidney transplantation with extended follow-up, the upper limit being 88 months (164, 165, 171). The evidence that anti-viral therapy prior to kidney transplantation is of benefit is supported by these encouraging results.

We have shown that ribavirin in addition to interferon-alpha is, with reasonable safety, possible to use in ESRD and patients with reduced renal function. In light of our results it should therefore be possible to modify the current guidelines for HCV-infected kidney transplant candidates (160, 161). An updated algorithm is thus given in **Figure 11.**

Clinical investigations prior to anti-viral therapy should include a thorough cardiac assessment. Many kidney transplant candidates have an increased cardiovascular risk, which should be addressed before transplantation (124,125). Interferon and ribavirin treatment should therefore in some cases not be recommended due to an increased risk of side effects, which could worsen the underlying cardiac problems.

The HCV RNA positive patient, even with normal transaminase levels, should undergo a liver biopsy in order to grade the liver disease by histological means. Patients with chronic hepatitis may then proceed to interferon and ribavirin therapy for 6-12 months depending on HCV genotype.

The question-marks in the scheme represent clinical dilemmas. Should anti-viral therapy be recommended if an HCV-infected dialysis patient has a completely normal liver biopsy? At the moment it is difficult to predict the long-term consequences of HCV infection in the individual patient, but if it is successfully treated, a number of known complications may well be avoided including premature graft loss, progressive liver disease, cryoglobulinemic vasculitis and death. One could argue that anti-viral therapy could be postponed until progressive liver disease or other complications occur. Treatment at that point, however, is likely to reduce the chance of SVR compared to treatment prior to transplantation and would also be associated with the risk of acute rejection. It is also well known that therapy is less successful in case of advanced liver disease. With the current treatment possibilities and likely use of pegylated interferon in the near future I would favour anti-viral therapy for dialysis patients who are eligible for kidney transplantation even with minimal liver damage.

The second question-mark represents the case of failure to reach SVR with anti-viral therapy. It is difficult not to accept these patients for kidney transplantation, since they

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can do fairly well at least in the short or medium term (< 5 years). The study by Huraib et al (155) showed a beneficial effect on liver histology up to a year after transplantation, even if SVR was not achieved in interferon treated patients.
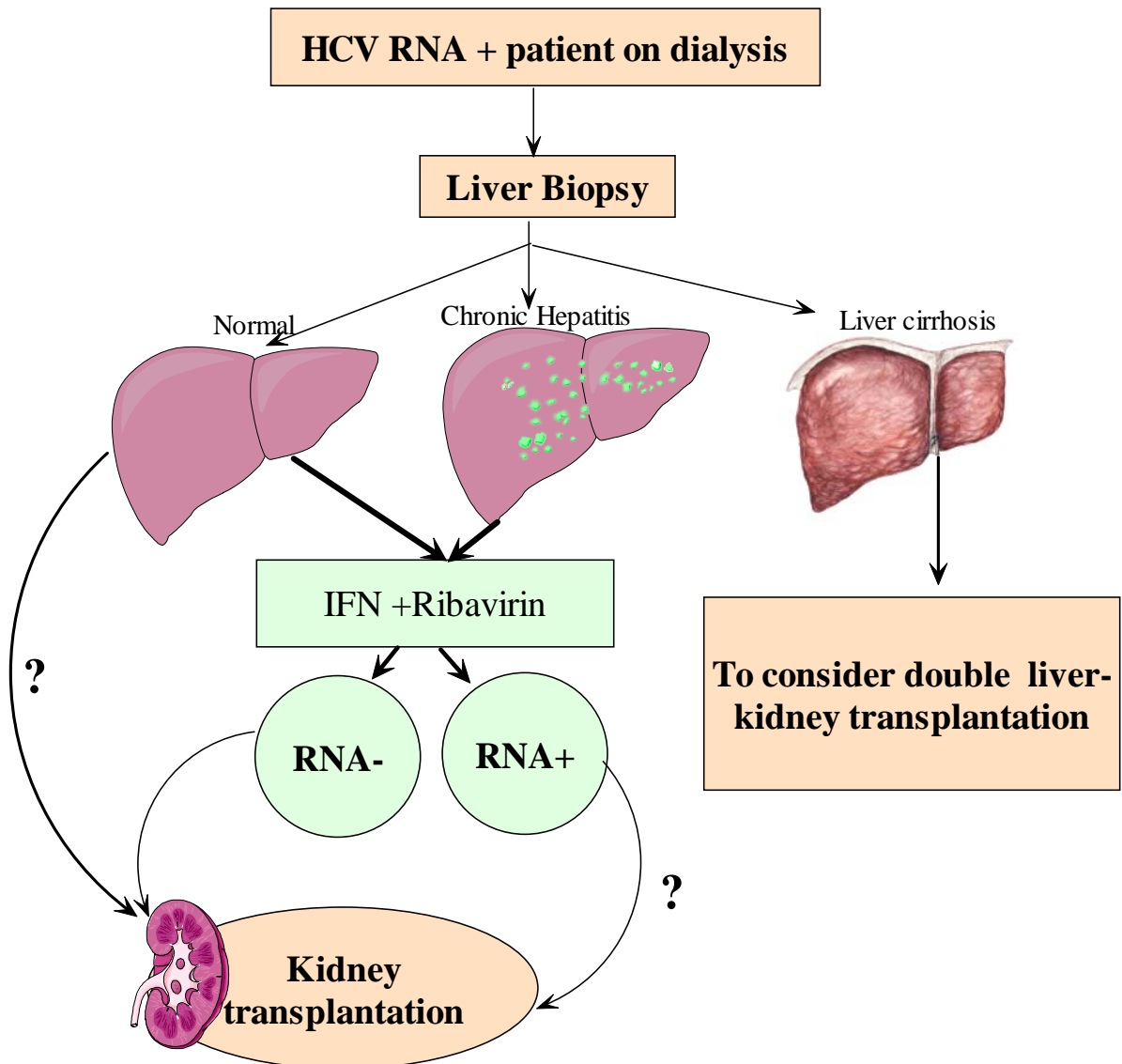

Figure 11 Updated algorithm for HCV-RNA-PCR positive patient on dialysis

A number of issues remain to be addressed in already transplanted HCV-infected patients. Immunosuppression, in particular avoidance of anti-lymphocyte/thymocyte globulin as well as azathioprine, should be chosen and adjusted to minimize the negative effect of HCV. Patients should be regularly monitored, preferably by liver biopsy, as well as by regular ultrasonography and alpha-fetoprotein to detect progressive liver disease and HCC. HCV-related renal disease should also be assessed. Drugs with possible anti-fibrotic effects such as ACE-inhibitors and A II-blockers, which may attenuate proteinuria in HCV-related renal disease should be considered.

Ribavirin monotherapy could be an option in HCV-related renal disease after transplantation. Ribavirin therapy resulted in complete remission of *de novo* HCV-related glomerulonephritis in 4 liver transplant patients (172). A recent study by Kamar et al compared ribavirin monotherapy during 12 months in 16 kidney transplant patients with historical controls (173). Twelve patients completed the study and the main side-effect was anaemia. Ribavirin monotherapy was associated with a decrease in liver enzymes, serum creatinine and proteinuria in those completing the study. There was, however, an increase in liver fibrosis score compared to untreated historical controls in 7 patients with a similar follow-up. It is possible that long-term ribavirin therapy with continuous haemolysis could be a negative factor in this setting especially for patients with reduced renal function. On the other hand the patients in the study who received ribavirin had signs of a more active disease prior to treatment having significantly elevated liver enzymes compared to historical controls. Ribavirin may thus have been insufficient in controlling progressive liver disease in these patients. The use of ribavirin as a time-saver for HCV-related renal disease after kidney transplantation remains an option, but in selected cases with progressive liver disease one should consider interferon as well even if there is an increased risk of an acute rejection.

Some dialysis patients are not eligible for kidney transplantation for various reasons. It is reasonable to consider therapy if the patient has symptomatic cryoglobulinemia or progressive liver disease with a fair life expectancy.

In cases of glomerular disease and vasculitis, HCV infection should be considered as a possible cause and should be thoroughly investigated, also in patients with a history of autoimmune disease. The fairly encouraging results in study **IV** indicate that interferon and ribavirin may be the first-line-therapy in HCV-associated renal disease and vasculitis.

The use of pegylated interferon in combination with ribavirin is today standard therapy for HCV-infected patients with normal renal function. The compilations of treatment outcome shows that HCV is increasingly becoming a curable disease, especially in the cases with easy-to-treat genotypes (11, 12). Several studies are ongoing to evaluate the use of pegylated interferon alfa-2a and 2b in renal patients and studies combining pegylated interferon and ribavirin are also in the pipeline. Our own experience with pegylated interferon-alfa-2b in HCV-associated renal disease with renal insufficiency has been encouraging. Preliminary data (unpublished results) from an ongoing open pilot study at Huddinge University Hospital with pegylated interferon-alfa-2b and ribavirin shows a 100 % viral response during treatment in 4 dialysis patients.

Development and early-phase studies of ribavirin-analogues, with less influence on erythrocytes thereby diminishing the side effects, are presently in progress. A possible maintenance therapy for HCV-infected transplant patients and a development towards SVR in most patients will hopefully also be aided by the introduction of protease inhibitors currently developed for HCV infection.

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