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HABILITATIONSSCHRIFT

New Extrahepatic Manifestations of Hepatitis C Infection after Kidney and Liver Transplantation

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Contents

ABBREVIATIONS

ACA	Anticardiolipin Antibodies
CHR	Chronic Humoral Rejection
CNIT	Calcineurin Inhibitor Toxicity
DM	Diabetes Mellitus
HCV	Hepatitis C Virus
HOMA	Homeostatis Model Assessment
HUS	Hemolytic Uremic Syndrome
IR	Insulin Resistance
ivGTT	intravenous Glucose Tolerance Test
MPGN	Membranoproliferative Glomerulonephritis
PBMCs	Peripheral Blood Mononuclear Cells
PTDM	Posttransplant diabetes mellitus
RTMA	Renal Thrombotic Microangiopathy
S_I	Insulin Sensitivity
TAC	Tacrolimus
TG	Transplant Glomerulopathy
TMA	Thrombotic Microangiopathy

1. INTRODUCTION

1.1 Background and aim of present work

Since the advent of its molecular characterization and cloning in 1989 by Choo et al,[1] hepatitis C virus (HCV) has been identified as a major cause of parenterally transmitted non-A non-B hepatitis. It has been recognized as an important global public health problem affecting an estimated 170 million (2%) of the world's population.[2] Although the incidence of HCV infection has dropped sharply since the early 1990s because of improved blood-supply screening, the infections that were acquired before 1990 are likely to dramatically increase the morbidity, mortality and costs of HCV-associated liver disease over the next two decades.[3]

The disease spectrum associated with HCV infection varies greatly. In addition to chronic hepatic disease, a number of complicating diseases of the organs and tissues other than the liver, referred to as extrahepatic manifestations, have been associated with HCV infection over the last two decades. The extrahepatic manifestations are especially common, with 40-76% of patients presenting with at least one symptom thereof.[4] The extrahepatic manifestations are often the first and the only clinical signs of a chronic hepatitis C. The most prevalent extrahepatic manifestation of HCV infection is mixed cryoglobulinemia with or without membranoproliferative glomerulonephritis (MPGN).[5-7] Recent epidemiological studies have added another clinical condition, type 2 diabetes mellitus (DM), to the spectrum of HCV-associated diseases.[8-10] However, the precise pathogenetic mechanism/s for the link between HCV and extrahepatic manifestations remain unclear, although they are obviously caused by the chronic viral infection. Most extrahepatic manifestations of HCV infection appear to be immunological, and the chronic infection seems to be necessary for their development.

Recent reports including ours indicate that similar extrahepatic syndromes associated with HCV, including DM, can also occur after kidney and liver transplantation and contribute to the increased morbidity and mortality in these transplant populations.[11-16] This fact is especially relevant as the prevalence of HCV in dialysis and kidney transplant patients is significantly higher than in general population. It is associated with an increased risk of death, an inferior graft survival and is the leading cause of posttransplant liver disease. The lower graft survival in HCV-positive kidney patients may reflect lower patient survival and the presence of HCV-associated renal disease in the allograft. The presence of HCV-associated renal disease in the allograft has been shown

to result in accelerated loss of the graft. Nevertheless, kidney transplantation still remains the best option for HCV-positive patients with end-stage renal disease on the waiting list, because survival would be substantially lower due to high cardiovascular mortality on dialysis. Similar to kidney transplantation, HCV infection is associated with increased morbidity and mortality also following liver transplantation, mainly due to recurrent allograft hepatitis and other extrahepatic complications. Currently, HCV-associated end-stage liver disease is the most frequent indication for orthotopic liver transplantation worldwide. The presence of extrahepatic complications like HCV-associated renal disease and posttransplant diabetes mellitus (PTDM) are important causes of chronic renal failure in this population and markedly impair the patient survival.[17, 18] Thus, overall prognosis and quality of life after kidney and liver transplantation are affected not only by hepatic but also by extrahepatic manifestations of HCV infection, many of which are not well understood.

This manuscript constitutes a part of the ongoing attempt to identify other newer extrahepatic manifestations of HCV infection in kidney and liver transplant recipients and to determine the prevalence, pathogenesis and clinical relevance of these complications. The recognition of novel extrahepatic manifestations associated with HCV infection and an insight into their pathogenic mechanisms will help to develop further reasonable therapeutic strategies aimed at minimizing the detrimental effects on graft and patient survival after transplantation.

1.2 Hepatitis C infection: Epidemiology

HCV is a small, single stranded flavilike RNA virus of 30-36 nm in diameter with a lipoid envelope. The genome consists of a large open-reading frame of 9378 to 9481 nucleotides. There are at least six main genotypes, numbered from 1 to 6, corresponding to the main branches in the phylogenetic tree, and several subtypes a, b and c. Distinction of genotypes is important, because it has predictive value in terms of the response to antiviral therapy, with better responses associated with genotypes 2 and 3 than with genotype 1.

HCV infection is relatively common, affecting approximately 2% of the world's population, but there are marked geographical differences (Europe 0.6-2.2 %, North America 0.8-1.8%, Egypt 6-28%, Japan 1.5-2.3%, China 3.2%).[2, 19] It occurs among people of all ages, but the prevalence is highest among men 20 to 39 years old. The main route of transmission of HCV is parenteral, i.e. risk factors are intravenous drug abuse, transfusions until 1989 (when the first anti-HCV screening tests became available),

needle stick injuries or organ transplantation. Heterosexual transmission is rare with a risk of 2-5% in 20 partnership-years but is high in homosexual relationships. However, at least 40% of transmission routes remain unclear and nosocomial transmission in households, endoscopy and cardiac, vascular and obstetric surgery units has been documented.

Hemodialysis and kidney transplant patients have also been identified as a high-risk group for HCV infection. The prevalence of HCV infection among patients on dialysis is higher than in the general population ranging from 5 to 50% depending on the assays used and the patient population studied.[20] The incidence of a *de novo* HCV infection in the hemodialysis population fluctuates between 0.7% and 3% per year. Given the markedly low incidence in the peritoneal dialysis group and the time-dependent increase in the incidence of HCV infection in the hemodialysis group, it has been concluded that the nosocomial spread under hemodialysis is an important epidemiological factor. Consequently, HCV infection is also a significant health problem in kidney transplant population, occurring in between 10 to 40% of the recipients.[20] The great majority of kidney transplant recipients become infected while they are on dialysis. A larger historical cohort analysis of kidney transplant patients based on the USRDS data showed that in addition to the universal epidemiological risk factors, positivity for HCV is epidemiologically linked to the following characteristics: African-American race, male gender, age, alcohol abuse and repeat transplantation.[21] HCV infection is also a primary cause of orthotopic liver transplantation and the prevalence in liver transplantation population lies between 20-60%.

1.3 Natural history of hepatitis C

In general population

The disease spectrum associated with HCV infection varies greatly. It accounts for approximately 20% of cases of acute and 70% of chronic hepatitis. HCV infection persists in about 80% of cases. Chronic hepatitis C is a major cause of cirrhosis and hepatocellular carcinoma. Over 20-30 years, approximately 20-30% of chronically HCV-infected individuals are expected to develop liver cirrhosis. Patients with HCV-induced liver cirrhosis run an annual risk of 1-4% for developing hepatocellular carcinoma.[19] HCV-related liver disease accounts for 40-50% of all liver transplantation.[22]

Since 1990, it has become clear that infection with HCV causes not only acute and chronic liver disease, but also various extrahepatic manifestations, mainly related to chronic stimulation of the immune system and to virus-induced autoimmunity. Of these,

“essential mixed cryoglobulinemia” was the first in which chronic HCV infection was demonstrated.[23, 24] Subsequently, MPGN, a Sjogren’s-like syndrome, porphyria cutanea tarda, and chronic corneal ulcerations have also been associated with chronic HCV infection.[5, 25]

Mixed cryoglobulinemia is the main extrahepatic biological manifestation and is present in about 40% of patients with chronic hepatitis C. It is a multi-system disorder with clinical manifestations including vasculitis with purpura, leg ulcers, arthralgias and/or arthritis, peripheral neuropathy, and glomerulonephritis in about half of the patients.[5-7] Despite the high frequency of cryoglobulin in patients with HCV, severe symptomatic mixed cryoglobulinemia with vasculitis is rare- noted in 2-3% of patients who are cryoglobulin positive. HCV infection has also been linked to distinct histologic patterns of immune-complex glomerulonephritis. The MPGN is the most common pattern encountered.[25] Less frequently, HCV infection has been reported in association with membranous nephropathy, and recently, a possible relationship between HCV infection and fibrillary glomerulonephritis has been suggested. It is important to emphasize that most patients with HCV-associated MPGN have mixed cryoglobulins in serum (type II or III) suggesting that the cryoglobulins play an essential role in the development of the renal disease.[26] The pathogenesis of HCV-associated glomerular disease likely involves the *in situ* formation or deposition of immune complexes (cryoglobulins) within the glomeruli. Support for this hypothesis is provided by the demonstration of HCV antigens in kidney deposits of patients with HCV-associated MPGN.[27]

Interestingly, several studies in recent years have found DM to be a new extrahepatic manifestation of HCV infection. A high prevalence of DM among patients with HCV-associated liver cirrhosis (50 to 62%) was first reported in 1994 by Allison et al. and has since been corroborated by multiple studies even in absence of cirrhosis.[8, 28, 29] A large scale epidemiologic survey showed an adjusted odds ratio of 3.8 for type 2 DM in HCV-positive individual older that 40 years of age.[10]

After kidney transplantation

HCV infection has been recognized as an important health problem in end-stage renal failure, and consequently, in the kidney transplant population. Several studies have shown that the prevalence of anti-HCV antibody among patients on dialysis is consistently higher than in the general population.[20] As a result, the presence of HCV infection is commonly detected in a subset of patients being evaluated for kidney

transplantation. Furthermore, it has been demonstrated that HCV infection can be transmitted by organs from HCV-positive donors.[30]

The long-term clinical impact of HCV infection in kidney allograft recipients is still under study, but accumulating evidence indicates that these patients are at higher risk of liver disease as well as death due to sepsis.[20] Graft survival is also lower in HCV-positive kidney recipients.[20] This seems to be due in part to the occurrence of *de novo* and recurrent glomerulopathy. As in native kidneys, the association of HCV infection with *de novo* and recurrent MPGN, with or without cryoglobulinemia, has been extensively documented in recipients of kidney transplants.[31, 32] It can occur in up to 6% of HCV-positive transplant recipients. [33] Although MPGN is the most common lesion described in this population with HCV infection, other lesions such as membranous nephropathy, acute and chronic transplant glomerulopathy have also been reported.[34-36] More recently, a few reports including ours have also suggested an association between HCV infection and new onset posttransplant diabetes mellitus (PTDM) after kidney transplantation.[12-16]

After liver transplantation

As hepatitis C leads to liver cirrhosis in up to 30% of cases, it is a leading indication for liver transplantation accounting for 40-50% of all liver transplants currently performed.[22] After liver transplantation, recurrence of HCV infection is virtually universal, and can range from an asymptomatic carrier state to chronic active hepatitis or cirrhosis. It has been established that more than 95% of HCV-positive recipients have persistent viremia using sensitive RT-PCR assays, and about 90% develop recurrent graft hepatitis within the first five years posttransplant, 20% of whom have HCV cirrhosis. [21, 22] In contrast, only 20% of HCV-negative patients develop chronic graft hepatitis in the same time-period. Aggressive immunosuppressive therapy, in particular corticosteroids and OKT3, leads to a dramatic rise of the viral load resulting in progression of the hepatitis C or recurrence of the disease with a more severe and fulminant course. Retransplantation in HCV-positive liver graft cirrhosis has a worse prognosis than with primary transplantation.

In recent years, a number of reports of HCV-associated glomerular disease with nephrotic syndrome and of HCV-associated mixed cryoglobulinemia and/or MPGN after liver transplantation have suggested that the increase in HCV viremia has the potential to exacerbate renal disease and/or cryoglobulinemia.[35, 37-41] Similar to the general and kidney transplant populations, an association between HCV infection and new onset PTDM after liver transplantation has also been suggested by several investigations including us.[11, 42-44]

1.4 Diagnostic tests

Two categories of assays may be used in the diagnosis of HCV infection: 1) serologic tests, detecting antibodies specifically directed against HCV antigens, and 2) molecular assays detecting and quantifying HCV antigens, HCV genomes and analyzing their sequence.[4] Serologic assays include screening tests based on enzyme immunoassays, supplemental immunoblots, and serologic assays detecting genotype-specific antibodies. Molecular assays include qualitative tests detecting HCV-RNA, quantitative assays measuring the HCV viral load as an index of HCV replication, and tests analyzing the nucleotide sequence of the HCV genome.

The diagnosis of HCV is made by detecting either anti-HCV antibody or HCV-RNA. Screening tests as the anti-HCV antibody test by a third generation ELISA are helpful in depicting a HCV infection with a sensitivity and specificity of 95%. Through use of the antibody testing, the infection can be diagnosed within the first 4-10 weeks from disease onset, although one should be aware of false positive results in conditions such as paraproteinemias or diseases with autoantibody formation. This diagnostic uncertainty can be removed by the use of an immune blot (recombinant immunoblots assay, RIBA). On the other hand, a negative test result from the immunoassay does not always exclude the presence of an acute or chronic infection. The so-called false negative results (up to 20%) may be observed in immunocompromised individuals such as dialysis or transplant patients who have a decreased cellular and humoral immunity, as well as in patients with cryoglobulinemia. All these patients are viremic by positive PCR result despite a negative anti-HCV antibody result. It is for this reason that the direct detection of viral RNA together with the enzyme immunoassays is now a part of the standard diagnostic repertoire. Anti-HCV antibody is recommended for routine testing in patients with suspected HCV infection, and PCR for confirmation of the presence of active infection. Genotyping and viral load are only helpful to evaluate the efficacy of a planned virostatic treatment. Liver enzymes are not helpful surrogate markers for chronic HCV infection in hemodialysis and transplant patient populations, as they remain within normal limits despite HCV infection in 80% of infected patients. Consequently, liver biopsy remains the only reliable method of evaluating the grade of fibrosis and stage of histological activity, where the precise involvement and the degree of liver damage can be assessed. In addition, the severity of pretransplant liver disease has been shown to be an important predictor of adverse posttransplant outcome including patient and graft survival in kidney recipients.[45]

1.5 Treatment

Chronic hepatitis C has historically been a difficult-to-treat disease. Treatment options have evolved significantly over the last few years. Until last decade, interferon-alfa was the only therapy available for the treatment of patients with chronic hepatitis C. After 48 weeks of treatment (3 MU subcutaneously three times a week), an initial response is seen in about half the patients, but a sustained biochemical and virologic response with histologic improvement occurs only in 15 to 20% of treated patients.[46] The introduction of ribavirin, a synthetic guanosine nucleoside analogue with in vitro antiviral activity against a range of RNA and DNA viruses, has dramatically improved HCV therapy. It is administered orally, at doses $\geq$ 10.6 mg/kg. Several studies have demonstrated that the efficacy of combined interferon and ribavirin therapy doubles the response rate.[47, 48] The main severe adverse events related to interferon are influenza-like symptoms, alopecia, severe depression, suicidal ideation, and sustained hypothyroidism. The major side-effect of ribavirin is severe hemolytic anemia limiting its use in many patients, especially hemodialysis patients.

Another advance in therapy for HCV infection was the introduction of pegylated interferons, which allow a once-weekly subcutaneous administration and show more favorable pharmacokinetics and greater efficacy. Two forms are available: pegylated interferon-alfa-2b (1.5 microgram/kg) and pegylated interferon-alfa-2a (fixed dosage of 180 microgram). Significantly greater sustained virological responses are attained with a combination therapy of pegylated interferon and ribavirin compared to the combination with non-pegylated formulations. Current therapy with a combination of pegylated interferon and ribavirin is effective in 50% to 60% of patients with previously untreated infection, showing even better results in genotype 2 and 3 patients.[49] Although there is some encouraging progress in new antiviral drug development for hepatitis C, e.g. protease and polymerase inhibitors, it will be several years before any of these novel compounds are available in clinical practice. In the interim, pegylated interferon and ribavirin remain the cornerstone of therapy.

In the past, patients with mixed cryoglobulinemia and aggressive glomerular disease were treated by plasma exchange to remove circulating cryoglobulins from the plasma and, consequently, to diminish the deposition of immune complexes in the kidney. In addition, immunomodulatory agents such as steroid pulses and cyclophosphamide were also used in the acute phase of the disease. However, the flare up of HCV viral load observed during immunosuppressive therapy may be harmful for HCV-related liver disease. The current understanding of the association between HCV infection, mixed

cryoglobulinemia and glomerular disease has prompted the use of antiviral agents as the first-line therapy for patients with these conditions. In non-transplant patients with HCV-associated mixed cryoglobulinemia and glomerulonephritis, combined treatment with interferon-alfa (standard or PEG) with/without ribavirin has been shown to reduce HCV viremia and cryoglobulin levels, decrease proteinuria, and stabilize renal function.[50-53] Of interest, a good clinical and biochemical response correlated with disappearance of HCV RNA from the serum during treatment, indicating that the beneficial effect of the combination therapy paralleled its antiviral action. Ribavirin alone has also been used successfully to treat mixed cryoglobulinemia (with and without MPGN) in isolated cases.[54, 55]

The recommended treatment strategies, however, cannot be simply applied to kidney transplant patients as interferon has been associated with triggering acute allograft rejection.[20, 56, 57] In addition to rejection, acute renal dysfunction after interferon therapy can also be the result of other causes such as acute tubular necrosis, with or without diffuse interstitial edema. However, in some reports, interferon-alfa (+/- ribavirin) has been used successfully to treat chronic hepatitis caused by HCV or to manage HCV-associated glomerulonephritis after kidney transplantation, without inducing renal dysfunction.[58-60] Since ribavirin is not associated with acute rejection, monotherapy with ribavirin has been evaluated in kidney transplant recipients with HCV-associated liver or renal disease [61, 62]. However, only improvement in transaminases without any significant reduction in the rate of fibrosis progression or clearance of HCV RNA was found. The risk of ribavirin-induced hemolysis is also increased in kidney transplant recipients because of frequent suboptimal renal function. Consequently, at present, the best option is to treat all HCV-positive dialysis patients with pegylated interferon-alfa with low-dose ribavirin while waiting for kidney transplantation.[20] Several observations suggest that antiviral treatment before transplantation may be beneficial not only to prevent posttransplant liver disease, but also to prevent posttransplant HCV-related glomerular disease and new onset PTDM, and should therefore be considered for all HCV-infected kidney transplant candidates.[63-65]

In contrast to kidney transplant patients, treatment with interferon-alfa alone or in combination with ribavirin can be recommended in HCV-infected liver transplant patients. Only sporadically acute and chronic rejections have been described under this treatment in liver recipients. The first retrieved data from a randomized trial on the efficiency and side effect profile of pegylated interferon-alfa therapy with/without ribavirin do not show an increased rejection among the treated liver transplant patients.[21, 66] After liver

transplantation, combination therapy with interferon-alfa and ribavirin has been used successfully to treat HCV-associated glomerulonephritis.[67]

Clearly, safer and more cost-effective drugs are required to treat transplant recipients with chronic HCV infection in view of the increased risk of rejection with interferon-alfa. The evaluation of efficient therapeutic strategies for kidney transplant recipients requires further clinical studies and intensive research.

2. OWN CONTRIBUTIONS

2.1 Acute renal thrombotic microangiopathy and HCV infection

Thrombotic microangiopathy (TMA) is a rare but well-recognized and serious complication of kidney transplantation. The term TMA describes a syndrome characterized by microangiopathic hemolytic anemia, thrombocytopenia and variable signs of organ damage due to platelet thrombi in the microcirculation. The clinical presentation of posttransplantation TMA is variable. Often, TMA will manifest systemically as hemolytic uremic syndrome (HUS), with classic findings of renal failure, hemolytic anemia with schistoctyes, and thrombocytopenia. Conversely, cases of TMA localized to the allograft present with worsening renal function or delayed graft function, but few or no systemic manifestations of HUS.

The diagnosis of localized renal thrombotic microangiopathy (RTMA) is confirmed by renal biopsy. Histopathologically, it is characterized by subendothelial accumulation of amorphous material in glomeruli, with narrowing or occlusion of capillaries, fibrinoid or mucoid change in the intima of small arteries, glomerular and/or arterial fibrin thrombi, and fragmented red blood cells in the vascular wall, glomeruli and interstitium.

The rates of recurrent HUS in kidney transplant recipients have varied considerably, ranging from 33% to 56% in adults. However, rates of *de novo* RTMA have been cited between 1% to 14%.[68] In the past, occurrence of *de novo* RTMA after kidney transplantation was linked to the use of calcineurin inhibitors, suggesting that these agents may directly damage the vascular endothelium and possible also induce platelet aggregation.[69] Despite the widespread use of cyclosporine, however, *de novo* RTMA occurs rarely after transplantation. Therefore, it is likely that other factors play a role in triggering this syndrome. Interestingly, we encountered a renal allograft recipient with chronic HCV infection who presented with RTMA and renal artery thrombosis after transplantation. A recent report then also noted a high prevalence of IgG isotype ACA in patients with chronic HCV infection, and their presence was associated with an increased incidence of thrombotic microangiopathy.

Related publications:

2.1.1 Acute *de novo* renal thrombotic microangiopathy associated with HCV infection in transplanted kidneys.

Baid S, Pascual M, Williams WW, Tolkoff-Rubin N, Johnson S, Collins AB, Chung RT, Delmonico FL, Cosimi AB, Colvin RB: Renal thrombotic microangiopathy associated with anticardiolipin antibodies in hepatitis C-positive renal allograft recipients. *J Am Soc Nephrol* 1999; 10: 146-153

In this clinico-pathologic study, we sought the association between ACA and *de novo* RTMA in HCV-positive renal allograft recipients.

Refer to:

J Am Soc Nephrol 1999; 10: 146-153.

Renal thrombotic microangiopathy associated with anticardiolipin antibodies in hepatitis C-positive renal allograft recipients.

Baid S, Pascual M, Williams WW, Tolkoff-Rubin N, Johnson S, Collins AB, Chung RT, Delmonico FL, Cosimi AB, Colvin RB.

Hepatitis C virus (HCV) infection has been associated with de novo or recurrent membranoproliferative glomerulonephritis and acute transplant glomerulopathy in transplanted kidneys. Recently, anticardiolipin antibodies (ACAs) have been linked with chronic HCV infection. A few reports have suggested an association between ACAs and renal allograft thrombosis. In this study, we reviewed the clinical and pathological features of HCV positive renal allograft recipients at our institution. From 1990 to 1996, 379 kidney transplants were performed. We identified 18 recipients (4.8%) with HCV positive serology pretransplant. Determination of IgG and IgM ACAs was performed by ELISA using pretransplant serum. Among the 18 patients, 5 patients presented with biopsy-proven de novo renal thrombotic microangiopathy (RTMA), occurring 5 to 120 days (median 14 days) after transplant. No differences in pretransplant characteristics were observed between patients with (n=5) or without (n=13) RTMA. All 5 patients had a positive ACA test (either IgG or IgM titer > 2 SD above normal), as compared to only 1/13 patients without RTMA. The mean value for IgG ACA was significantly higher in the RTMA patients than in patients without RTMA (22.9±14.1 versus 6.9±4.9 GPL units, p=0.02); however, there were no significant differences in IgM ACA titers. Rheumatoid factor and complement C4 levels were normal in pretransplant sera of patients with RTMA. Patients with RTMA had their cyclosporine withdrawn (4/5) or the dose was decreased (1/5), and 1/5 underwent plasmapheresis. 4/5 patients died within 5 years after transplant, as compared to no deaths in the other 13 patients Finally, as a control group, 7 HCV negative renal allograft recipients who presented with RTMA/HUS during the same time period were found to have normal ACA values (IgG or IgM).

Conclusion: RTMA associated with ACAs in HCV positive renal allograft recipients may represent a new clinical entity. The occurrence of this syndrome may have deleterious consequences for patient and graft survival.

PMID: 9890320

2.1.2 Acute thrombotic microangiopathy associated with HCV infection in native kidneys

Baid S, Pascual M, Cosimi AB, Chung RT, Colvin RB, Tolkoff-Rubin N: Viruses and thrombotic microangiopathy. *Transplantation* 1999; 68: 710-711.

In further support of our observation in transplant kidneys, we assessed the role of ACA and HCV infection in two patients who presented with RTMA in native kidneys.

Refer to:

Transplantation 1999; 68: 710-711.

Viruses and thrombotic microangiopathy.

Baid S, Pascual M, Cosimi AB, Chung RT, Colvin RB, Tolkoff-Rubin N.

In this letter to the editor, we reported two patients who presented with renal thrombotic microangiopathy in their native kidney in association with hepatitis C virus (HCV) infection and high titres of anticardiolipin antibodies (ACA).

PMID: 10507496

2.2 Transplant glomerulopathy and HCV infection

Transplant glomerulopathy (TG) is a glomerular lesion common in long-standing kidney allografts, and recently has received much attention as a manifestation of chronic humoral (antibody-mediated) rejection (CHR). However, many cases lack C4d deposition and/or circulating donor-specific antibodies, and the contribution of other potential causes has not been fully addressed. TG is clinically manifested by low-grade to nephrotic-range proteinuria, and has extremely poor prognosis with eventual graft loss in 40% to 70% of the affected patients.[70-75] The prevalence of TG has been shown to be approximately 5% to 10% in all allograft biopsies performed for clinical indications.[70-75] The precise pathogenesis of TG remains unclear, theories include alloreactivity to the donor or chronic infection. Interestingly, the histopathologic features of TG, i.e., glomerular basement membrane duplication and increase in mesangial matrix, are reminiscent of membranoproliferative glomerulonephritis (MPGN), although immune complexes are typically not a feature of TG.[70, 76, 77] However, immune complexes may be scant or absent after transplantation, thus rendering a clear distinction between TG and MPGN difficult in most cases.

In 1995, a possible association between HCV and TG was suggested in two kidney allograft recipients at 3 and 7 years after transplantation.[36] Subsequently, another group also reported an increased prevalence (33%) of HCV infection in 27 kidney allograft recipients with TG.[78] In this study, however, the clinicopathologic differences were not analyzed, as immunofluorescence and electronmicroscopy findings were available only in a minority of patients.

Related publication:

2.2.1 Association between transplant glomerulopathy and HCV infection in kidney transplant recipients

Baid-Agrawal S, Farris AB, Pascual M, Mauiyyedi S, Collins AB, Farrell ML, Tolkoff-Rubin N, Frei U, Colvin RB: Overlapping pathways to transplant glomerulopathy: chronic humoral rejection, hepatitis C infection and thrombotic microangiopathy. *Kidney International* 2011, 80: 879-885.

In this clinico-pathologic study with a long-term follow-up, we aimed to delineate the pathogenesis of TG after kidney transplantation and sought clinical and pathological evidence for additional potential causes of TG apart from CHR. We also wanted to determine whether the pathology or clinical features differed in HCV (+) and HCV (-) patients with TG.

Refer to:

Kidney International 2011, 80: 879-885.

Overlapping pathways to transplant glomerulopathy: chronic humoral rejection, hepatitis C infection and thrombotic microangiopathy.

Baid-Agrawal S, Farris AB, Pascual M, Mauiyyedi S, Collins AB, Farrell ML, Tolkoff-Rubin N, Frei U, Colvin RB.

Transplant glomerulopathy (TG) has received much attention in recent years as a manifestation of chronic humoral rejection (CHR). However, many cases lack C4d deposition and/or circulating donor-specific antibodies (DSA), and the contribution of other potential causes has not been fully addressed. Of 209 consecutive renal allograft indication biopsies for chronic allograft dysfunction, 25 that met pathologic criteria of TG (>10% duplication of the GBM without immune complex deposition) were examined for various etiologies, including hepatitis C infection (HCV), thrombotic microangiopathy (TMA), and CHR. Three partially overlapping categories accounted for 84% of the cases: $C4d^{+}$TG (48%), HCV^{+}TG (36%) and TMA^{+}TG (32%). The majority of TMA^{+} cases were HCV^{+} (63%) and the majority of HCV^{+} cases had TMA (56%). Donor specific antibodies were associated with $C4d^{+}$TG (7/8 vs. 1/4 $C4d^{-}$TG; $P<0.02$), but not with HCV^{+}TG. The prevalence of HCV was higher in the TG group than in 29 control patients (36% vs. 7%, $P<0.01$). HCV^{+}TG patients developed allograft failure earlier than HCV^{-}TG patients (71.1 ± 52.7 mo versus 153.7 ± 120.5 mo, $P=0.03$). We conclude that TG is not a specific diagnosis, but a pattern of pathologic injury with 3 major overlapping pathways involving CHR, HCV infection and TMA. It is important to distinguish these mechanisms, as they may have different prognostic and therapeutic implications.

Comment in: Kidney Int. 2011; 80: 801-3.

PMID: 21697808

2.3 New-onset posttransplant diabetes mellitus (PTDM) and HCV infection

Recent studies suggest an association between HCV infection and DM in the general population. In 1994, Allison et al first reported an increased prevalence of DM in patients with HCV-associated liver cirrhosis as compared to patients with cirrhosis due to hepatitis B virus, alcohol, cholestatic liver disease, or autoimmune hepatitis. [28] Subsequently, various reports have confirmed the association between DM and HCV infection, even in absence of cirrhosis.[8-10, 29] For example, in a recent retrospective review of 1117 patients with chronic viral hepatitis, only HCV infection and age, but not cirrhosis, were found to be independent predictors of DM by multivariate analysis.[8] In these studies, the prevalence of DM in patients with liver disease secondary to HCV infection has been reported to vary from 20% to 50%, in contrast to the 2.5% to 25% prevalence found in patients with non-HCV related liver disease. More recently, several preliminary reports have also suggested an association between HCV infection and DM after orthotopic liver transplantation.[42, 43] These investigators noted the prevalence of PTDM to vary from 40% to 60% in HCV-infected liver transplant recipients, which was significantly higher than that found in recipients with other causes of liver failure. In one of these studies, the majority of patients with PTDM required insulin; by multivariate analysis, HCV-related liver failure was found to be an independent risk factor for PTDM one year after transplantation.[43]

New-onset PTDM is a common complication after organ transplantation, and its overall incidence has been reported to vary between 5% and 30% in kidney transplant recipients. It has been associated with significant deleterious effects on long-term patient and graft survival. In addition to the usual risk factors for DM in the general population such as age, body mass index, race and a family history of DM, immunosuppressive agents, particularly steroids and calcineurin inhibitors (cyclosporine or tacrolimus) are important risk factors for the development of PTDM in allograft recipients.[79, 80] Tacrolimus (TAC) has been shown to be slightly more diabetogenic than cyclosporine, particularly in the initial clinical trials.[79, 81, 82] TAC-induced PTDM has been associated with steroid use, TAC level and the recipient race.[81, 82] However, the role of HCV infection in the development of PTDM after kidney transplantation remains unclear.

Despite the current epidemiologic evidence linking DM with HCV, the pathogenic basis of this association has not been elucidated. Possible pathophysiologic mechanisms involved in the association between HCV infection and DM include a secondary effect of liver cirrhosis, iron overload, an autoimmune reaction against β-cells induced by chronic HCV, a direct effect of HCV on islet-cells inducing β-cell dysfunction, or the induction of peripheral insulin resistance (IR).

Related publications:

2.3.1 New Onset PTDM and HCV in liver transplant recipients

Baid S, Cosimi AB, Farrell ML, Schoenfeld DA, Feng S, Chung RT, Tolkoff-Rubin N, Pascual M: Post-transplant diabetes mellitus in liver transplant recipients: risk factors, temporal relationship with hepatitis C virus allograft hepatitis, and impact on mortality. *Transplantation* 2001; 72: 1066-1072.

Our study aimed to determine: 1) the prevalence and determinants of new onset PTDM in HCV-positive liver transplant recipients, 2) the temporal relationship between recurrent allograft hepatitis and the onset of PTDM, and 3) the effects of antiviral therapy on glycemic control.

Refer to:

Transplantation 2001; 72: 1066-1072.

Post-transplant diabetes mellitus in liver transplant recipients: risk factors, temporal relationship with hepatitis C virus allograft hepatitis, and impact on mortality.

Baid S, Cosimi AB, Farrell ML, Schoenfeld DA, Feng S, Chung RT, Tolkoff-Rubin N, Pascual M.

Background: Recent studies suggest an association between diabetes mellitus and hepatitis C virus (HCV) infection. Our aim was to determine: 1) the prevalence and determinants of new onset posttransplant diabetes mellitus (PTDM) in HCV (+) liver transplant (OLT) recipients, 2) the temporal relationship between recurrent allograft hepatitis and the onset of PTDM, and 3) the effects of antiviral therapy on glycemic control.

Methods: Between 1/91-12/98, of 185 OLT performed in 176 adult patients, 47 HCV (+) cases and 111 HCV (-) controls were analyzed. We reviewed and analyzed the demographics, etiology of liver failure, pretransplant alcohol abuse, prevalence of diabetes mellitus, and clinical characteristics of both groups. In HCV (+) patients, the development of recurrent allograft hepatitis and its therapy were also studied in detail.

Results: The prevalence of pretransplant diabetes was similar in the two groups, whereas the prevalence of PTDM was significantly higher in HCV (+) than in HCV (-) patients (64% vs. 28%, P=0.0001). By multivariate analysis, HCV infection (hazard ratio 2.5, P=0.001) and methylprednisolone boluses (hazard ratio 1.09 per bolus, P=0.02) were found to be independent risk factors for the development of PTDM. Development of PTDM was found to be an independent risk factor for mortality (hazard ratio 3.67, P<0.0001). The cumulative mortality in HCV (+) PTDM (+) vs HCV (+) PTDM (-) patients was 56% vs 14% (P=0.001). In HCV (+) patients with PTDM, we could identify 2 groups based on the temporal relationship between the allograft hepatitis and the onset of PTDM: 13 patients developed PTDM either before or in the absence of hepatitis (gr. A), and 12 concurrently with the diagnosis of hepatitis (gr. B). In gr. B, 11/12 patients received antiviral therapy. Normalization of liver function tests with improvement in viremia was achieved in 4/11 patients, who also demonstrated a marked improvement in their glycemic control.

Conclusion: We found a high prevalence of PTDM in HCV (+) recipients. PTDM after OLT was associated with significantly increased mortality. HCV infection and methylprednisolone boluses were found to be independent risk factors for the development of PTDM. In about half of the HCV (+) patients with PTDM, the onset of PTDM was related to the recurrence of allograft hepatitis. Improvement in glycemic control was achieved in the patients who responded to antiviral therapy.

PMID: 11579302

2.3.2 New Onset PTDM and HCV in kidney transplant recipients

Baid S, Tolkoff-Rubin N, Farrell ML, Delmonico FL, Williams WW, Hayden D, Ko D, Cosimi AB, Pascual M: Tacrolimus-associated posttransplant diabetes mellitus in renal transplant recipients: Role of hepatitis C infection. *Transplant Proc* 2002; 34:1771-1773.

In this study, we assessed the incidence of TAC-associated new onset PTDM in kidney transplant recipients and analyzed possible risk factors including HCV infection in patients receiving TAC for three different clinical indications.

Refer to:

Transplant Proc 2002; 34:1771-1773.

Tacrolimus-associated posttransplant diabetes mellitus in renal transplant recipients: Role of hepatitis C infection.

Baid S, Tolkoff-Rubin N, Farrell ML, Delmonico FL, Williams WW, Hayden D, Ko D, Cosimi AB, Pascual M.

Introduction: In renal transplantation (Tx), Tacrolimus (TAC)-associated posttransplant diabetes mellitus (PTDM) has been linked with steroid use, TAC level and recipient race. In liver Tx, hepatitis C (HCV) infection has been recently found to be an independent risk factor for PTDM as well. We studied a cohort of renal Tx recipients at our institution who received TAC for different indications and analyzed the role of HCV infection in TAC-associated PTDM.

Methods: Between 7/94-7/00, 114 non-diabetic renal recipients received TAC at our institution. TAC was used only for special indications as divided into the following 3 groups: 1) Prophylactic (P group): for immunologically mediated glomerular disease, highly sensitized patients, and recipients of repeat transplants (target levels: 10-12 ng/ml), 2) Rescue group (R group): as rescue treatment for steroid-resistant rejection requiring antilymphocyte antibody therapy (target levels: 10-15 ng/ml), and 3) Conversion (C group): conversion in stable patients greater than three months posttransplant for hirsutism, gingival hyperplasia or hyperlipidemia (target levels: 6-8 ng/ml). A multivariate analysis using the Cox Proportional-Hazards model was performed to determine the risk factors for the development of PTDM.

Results: The overall incidence of TAC-associated PTDM was 11.4%. The mean time from Tx to the onset of PTDM was 84 ± 105 days (median: 49 days). The overall prevalence of HCV infection was 15.8% (18/114). The incidence of TAC-associated PTDM was significantly higher in HCV (+) patients (7/18, 38.9%) than in HCV (-) patients (6/96, 6.3%) (*P*=0.0001). The incidence of PTDM was significantly greater in the P and R groups (17.2 % and 14.6%, respectively) than in the C group (0%). The prevalence of HCV infection was not significantly different between the 3 groups. By multivariate analysis, HCV infection and TAC serum levels were found to be independent risk factors for the development of PTDM. Patients with HCV infection were found to have an 8.8-fold higher risk of becoming diabetic posttransplant (95% CI, 2.6-29.3, P=0.0004), and a patient's risk for developing PTDM increased by a factor of 12% for each ng/ml increase in serum TAC level (95% CI, 4 to 20%, *P*=0.002).

Conclusions: HCV may play a pathogenic role in TAC-associated PTDM after renal Tx.

PMID: 12176569

2.3.3 Mechanism of association between HCV and PTDM in liver transplant recipients

Delgado-Borrego A, Casson D, Schoenfeld D, Somsouk M, Terella A , Jordan SH, Bhan A, **Baid S ,** Cosimi AB, Pascual M, Chung RT: Hepatitis C Virus is independently associated with increased insulin resistance after liver transplantation. *Transplantation* 2004; 77: 703-710.Increased IR as a possible pathogenic basis for the association of DM with HCV in liver transplant recipients.

The aim of this investigation was to evaluate the relationship of IR and β-cell function with HCV infection in a post liver transplant cohort in order to assess the mechanism of association between HCV infection and PTDM in liver transplant recipients. We hypothesized that HCV infection is associated with increased IR.

Refer to:

Transplantation 2004; 77: 703-710.

Hepatitis C Virus is independently associated with increased insulin resistance after liver transplantation.

Delgado-Borrego A, Casson D, Schoenfeld D, Somsouk M, Terella A , Jordan SH, Bhan A, Baid S , Cosimi AB, Pascual M, Chung RT.

Background and Aims: There is a strong epidemiologic association between diabetes mellitus (DM) and hepatitis C virus (HCV) infection. However, the pathogenetic basis for this association has not been established. We sought to evaluate the association between insulin resistance (IR), β-cell dysfunction, and HCV among orthotopic liver transplant (OLT) recipients.

Method: We performed a cross sectional analysis comparing 39 HCV(+) with 60 HCV(-) OLT recipients. IR and β-cell function were calculated using validated measures and were correlated with clinical variables.

Results: By multivariate analysis of the entire cohort, HCV infection and body mass index (BMI) were independent predictors of IR (*P*=0.04 and 0.0006, respectively). HCV infection was associated with 35% increase in IR. Because the model used to calculate IR was derived from nondiabetic subjects, we performed additional analysis of patients who did not meet criteria for diabetes at the time of their study evaluation. In this analysis, HCV(+) subjects had greater fasting insulin and homeostasis model assessment (HOMA) IR (15.3 μU/mL and 3.8) compared with HCV(-) patients (10.7 μU/mL and 2.5) (*P*=0.03, 0.03). There was no difference in β-cell function or hepatic insulin extraction between the HCV (+) and (-) groups. HCV (*P*=0.0005), BMI (*P*<0.0001), and high high-density lipoprotein (*P*=0.039) were the only independent predictors of IR. The presence of HCV infection and a 10-fold increase in HCV RNA *were associated with a 62% and 8% increase in IR, respectively.*

Conclusions: HCV is independently associated with increased IR after OLT. These findings provide a possible pathogenetic basis for the association of DM with HCV.

PMID: 15527696

2.3.4 Mechanism of association between HCV infection and PTDM in kidney transplant recipients

Baid-Agrawal S, Frei U, Reinke P, Schindler R, Kopp MA, Martus P, Berg T, Juergensen JS, Anker SD, Doehner W: Impaired insulin sensitivity as underlying mechanism linking hepatitis C infection and posttransplant diabetes mellitus in kidney transplant recipients. *American Journal of Transplantation* 2009, 9: 2777-2784.

The purpose of our current study was to extend our findings from liver transplant recipients and investigate the underlying mechanism of association between HCV infection and PTDM in kidney transplant recipients by means of sophisticated 3 hour-ivGTT.

Refer to:

American Journal of Transplantation 2009, 9: 2777-2784.

Impaired insulin sensitivity as underlying mechanism linking hepatitis C infection and posttransplant diabetes mellitus in kidney transplant recipients.

Baid-Agrawal S, Frei U, Reinke P, Schindler R, Kopp MA, Martus M, Berg T, Juergensen JS, Anker SD, Doehner W.

Background: A significant association has been found between hepatitis C virus (HCV) infection and posttransplant diabetes mellitus, both in kidney and liver transplant recipients. The precise underlying mechanism for the link remains unclear. We sought to investigate the possible mechanism/s in kidney recipients.

Methods: In this cross-sectional study, 20 non-diabetic HCV-positive kidney recipients were studied in comparison to 22 non-diabetic HCV-negative kidney recipients and 24 healthy subjects. A 3-hour intravenous glucose tolerance test was performed; peripheral insulin sensitivity (S_I) and hepatic insulin uptake were assessed by minimal modelling. Pancreatic insulin secretion and pancreatic autoantibodies were also assessed. Serum levels of proinflammatory cytokines (tumor necrosis factor-α, intereukin-6 and high-sensitive C-reactive protein) were measured. A linear regression model was used to determine the factors associated with impaired S_I.

Results: HCV-positive recipients were found to have a significantly lower S_I as compared to HCV-negative recipients (3.0 ± 2.1 vs. 4.9 ± 3.0 (mu/l)^-1.min^-1, P= 0.02). Insulin secretion and hepatic insulin uptake were not statistically different between the two groups. Pancreatic antibodies were negative in all kidney recipients. By multivariate analysis, HCV status (P=0.004) and age at transplantation (P=0.015) were independent predictors of S_I. No significant correlation was found between any of the measured cytokines and S_I.

Conclusion: Our results suggest that impairment of peripheral insulin sensitivity, or in other words, induction of peripheral insulin resistance, but not a deficit in insulin secretion, is the most likely pathogenic mechanism involved in the development of PTDM associated with HCV infection in kidney recipients.

PMID: 19845589

2.4 Acute humoral rejection after antiviral therapy for HCV

In non-transplant patients, combined antiviral treatment with interferon and ribavirin is currently the best available combination for treatment of HCV infection, yielding a high rate of viral clearance and improvement of liver histology. Antiviral therapy with interferon-alfa in HCV-infected kidney transplant recipients remains controversial. In most patients interferon is not used, as it has been associated with triggering severe acute allograft rejection.[35, 57] An incidence of acute rejection varying from 15% to 64% has been reported after starting therapy with interferon-alfa. After liver transplantation, interferon-alfa has been used successfully in treating symptomatic cryoglobulinemia with or without glomerular involvement and for recurrence of allograft hepatitis. In contrast to its use in kidney transplant recipients, the use of interferon after liver transplantation does not appear to carry a significant risk of acute rejection.[35, 66]

The exact mechanism of acute rejection triggered by interferon in kidney recipients is not clear. Suggested inciting pathways include increased cell surface expression of HLA alloantigens and induction of cytokine gene expression by interferon, or enhancement of antibody production by B cells,[83, 84] but evaluation of humoral responses by repeat crossmatches or staining of biopsies for C4d was not undertaken. Despite its recognized risks, anti-HCV therapy with interferon may be required in a subset of kidney recipients suffering from chronic hepatitis as increasing evidence suggests that untreated chronic hepatitis results in increased morbidity and mortality in these patients.[20, 85]

Related publication:

2.4.1 Acute humoral rejection after antiviral therapy in HCV-infected kidney transplant recipients

Baid S, Tolkoff-Rubin N, Saidman S, Chung R, Williams WW, Auchincloss H, Colvin RB, Delmonico FL, Cosimi AB, Pascual M: Acute humoral rejection in hepatitis C-infected renal transplant recipients receiving antiviral therapy. *American Journal of Transplantation* 2003; 3: 74-78.

The aim of this study was to assess the incidence and characterize the type of acute rejection after interferon-alfa therapy in 12 HCV-infected kidney transplant recipients with biopsy-proven chronic hepatitis C.

Refer to:

American Journal of Transplantation 2003; 3: 74-78.

Acute humoral rejection in hepatitis C-infected renal transplant recipients receiving antiviral therapy.

Baid S, Tolkoff-Rubin N, Saidman S, Chung R, Williams WW, Auchincloss H, Colvin RB, Delmonico FL, Cosimi AB, Pascual M.

Background: The use of interferon-alfa (IFN) in hepatitis C (HCV)-infected renal recipients has been associated with acute rejection and graft loss. We reviewed our recent experience in HCV(+) renal recipients treated with antiviral therapy for biopsy-proven chronic hepatitis C.
Methods: Twelve HCV (+) recipients who recently received antiviral therapy were analyzed. Post-treatment sera were tested for donor-specific HLA antibodies (DSA).
Results: Within 6 months of initiating antiviral therapy, two of 12 patients (17%) developed acute rejection, which was characterized as acute humoral rejection (de novo DSA in serum and C4d deposits in peritubular capillaries). Both progressed to graft failure. Nine of the remaining 10 patients tested did not have DSA.
Conclusion: The use of IFN was associated with severe acute humoral rejection (C4d +, DSA +). The recognition of IFN-associated acute humoral rejection in this series may explain the high rate of graft loss reported previously in renal recipients receiving IFN.

PMID: 12492714

3. DISCUSSION

From the foregoing review of the results of our studies and the literature, it becomes obvious that chronic HCV infection is a distinct systemic disease with a varying spectrum of extrahepatic manifestations in the general population as well as kidney and liver transplant recipients. Moreover, the high prevalence of HCV infection in kidney and liver allograft recipients puts these populations at special risk for developing a spectrum of extrahepatic manifestations associated directly or indirectly with the viral infection, including MPGN with or without cryoglobulinemia, membranous nephropathy, acute renal thrombotic microangiopathy, transplant glomerulopathy and last but not the least, *de novo* PTDM.

3.1 Acute renal thrombotic microangiopathy and HCV infection in transplanted and native kidneys

We described a novel syndrome of *de novo* RTMA occurring in both native and transplanted kidneys associated with ACA in HCV-infected patients. HUS or TMA occurring after solid organ or bone marrow transplantation has been associated with the use of cyclosporine or tacrolimus.[69] However, HUS/TMA is relatively rare after transplantation, indicating that other cofactors are likely to be important in triggering this syndrome. Our reports suggest that ACA may be implicated in the pathogenesis of RTMA/HUS in a subset of HCV-positive patients. Indeed ACA, particularly of the IgG isotype have been strongly associated with the development of both arterial and venous thrombosis, as well as RTMA in primary antiphospholipid antibody syndrome, in lupus patients, or during pregnancy.[86]

The precise underlying mechanisms linking ACA to RTMA remain to be determined. They could include antiendothelial or antiplatelet activity of some ACA.[86, 87] The possible role of HCV, in association with ACA also remains to be clarified; one possibility could be related to exaggerated endothelial cell activation, particularly after transplantation, in patients receiving cyclosporine or tacrolimus. Interestingly, ACA are frequently found both in chronic HCV and HIV infected patients, and TMA can be a complication of both viral infections.[88, 89] Procoagulant properties of these viruses may also play an important role.[90] HCV infection has also been associated with the development of several autoantibodies, some of which have the potential to be pathogenic, e.g. cryoglobulins with rheumatoid factor activity, and recently ACA.[24, 88, 91] Of particular interest is the recent description of antibodies to von Willebrand factor-cleaving protease in patients with acute thrombocytopenic purpura, a syndrome that closely resembles HUS.[92, 93] Whether HCV

infection induces production of antibodies to von Willebrand-cleaving protease in some patients with TMA will be important to determine in the future. Whatever the precise mechanisms involved, it seems that HCV infection may be implicated in pathogenesis of HUS/TMA both in allografted organs and in native kidneys. Prompt search for an ongoing HCV infection and ACA is warranted in such instances because early initiation of antiviral therapy in addition to the conventional therapeutic approaches may improve the outcome in these patients.

3.2 Transplant glomerulopathy and HCV infection

In this study, we found a striking prevalence of HCV infection in kidney transplant recipients with TG. The prevalence of HCV infection observed (36%) in patients with TG was five-fold higher than the prevalence seen in the CNIT control group (7%) or in our overall kidney transplant population (4.8%).[94] These results extend and confirm previous observations[36, 95] and, suggest a strong causal association between HCV infection and TG. Furthermore, HCV^{+}TG recipients differed from HCV^{-}TG patients clinically by a significantly more rapid progression to graft failure after transplantation and increased frequency of abnormal liver function tests.

We found C4d deposition in peritubular capillaries in approximately half of the patients with TG, supporting an antibody-mediated (humoral) alloreactive mechanism of tissue injury in TG in a subset of patients, as suggested by previous studies. The second possible pathogenetic pathway that is strongly suggested by our observations is that induced by chronic HCV infection. Last but not the least, approximately one third of patients with TG had evidence of TMA in their biopsies, which may be postulated as the third potential overlapping pathway for development of TG.

Whatever may be the precise mechanism underlying TG, the recognition of association between HCV infection and lesions of TG is potentially important, as the high prevalence of HCV infection among patients on dialysis and consequently in kidney transplant recipients puts this population at an increased risk for developing HCV-associated TG. Screening for HCV infection is therefore warranted in all patients who develop lesions of TG after kidney transplantation as it might have significant therapeutic and prognostic implications.

Overall, it appears that a number of above described pathogenetic mechanisms (i.e. chronic antibody-mediated rejection, HCV-associated *de novo* glomerular lesions and TMA) are involved in the pathogenesis of TG and may operate simultaneously producing an overlap, making it difficult to recognize the relative role of a single factor. In the future,

more work will be needed to find specific pathologic markers that may differentiate these mechanisms. Further larger, prospective studies utilizing protocol biopsies including EM, C4d staining, endothelial gene expression and DSA analysis, carefully looking at the association between TG, TMA and HCV infection are required to confirm our findings. Early diagnosis of TG and better understanding of its pathogenesis may improve management and outcome of this deleterious posttransplant complication.

3.3 New-onset posttransplant diabetes mellitus (PTDM) and HCV infection

Our four studies presented here suggest an association between HCV infection and PTDM both in kidney and liver transplant recipients, and further attempt to delineate the underlying pathogenesis of this association. Our findings support recent studies that found an association between HCV infection and DM in the general population. In liver transplant recipients, ours was one of the pioneering studies that suggested an association between HCV infection and PTDM and showed that the development of PTDM was an independent risk factor for mortality. Further, an improvement in glycemic control was achieved in HCV-positive patients who responded to antiviral therapy. From our observations in kidney transplant patients, it can be hypothesized that the diabetogenic effects of TAC may be enhanced by HCV infection, particularly when higher levels of TAC are used in early posttransplant period. It can be speculated that lower dosage and levels of TAC may lower the incidence of PTDM in the subset of HCV-infected kidney transplant patients.

Multiple investigations in the recent years have noted an association between HCV infection and DM in general population as well as after transplantation. However, the pathogenesis of diabetes in HCV has not been systematically evaluated. Few studies have considered IR in patients with chronic HCV infection, and none has specifically evaluated a possible association between these two conditions nor included a comprehensive analysis of the various possible mechanistic links described above. Some studies have found elevated fasting insulin levels and greater insulin resistance in association with advanced fibrosis among patients with HCV infection. In a cross-sectional study Duong *et al*. found that HIV-HCV co-infected patients and HCV–mono-infected patients had significantly higher IR than HIV mono-infected patients.[96] Important impediments to dissecting the pathophysiologic association between HCV and DM include the lack of consistency in the selection of comparison groups, a lack of inclusion of possible confounders, and the intrinsic difficulty in controlling for severity of liver disease. In this regard, a post-liver transplant cohort that we evaluated has the advantage of being a reasonable model of *de novo* HCV liver disease.

We demonstrated an independent association between HCV infection and increased IR in posttransplant liver allograft recipients using HOMA. We could confirm this relationship in kidney transplant recipients using the more sophisticated ivGTT for the first time. Preserved insulin secretion and hepatic uptake, and absence of pancreatic autoantibodies in both our studies strongly suggest that HCV is likely associated with DM by means of IR rather than through impaired β-cell function or through an autoimmune process. It is possible that HCV could induce glucose intolerance indirectly through cirrhosis or advanced fibrosis by several mechanisms, including decreased glucose uptake as a result of splanchnic shunting as well as increased gluconeogenesis. This is a logical assumption given the observation that other forms of liver disease associated with cirrhosis lead to DM. Most studies evaluating the prevalence of DM in HCV-infected patients as compared with patients with other causes of liver disease did not control specifically for the level of fibrosis or cirrhosis. Indeed, a recent report suggests that patients chronically infected with HCV who have advanced fibrosis have increased IR when compared to patients with chronic HCV infection and no liver cirrhosis,[97] raising the possibility that cirrhosis itself is the driving force for insulin resistance. However, this study was not designed to assess a possible association between HCV and IR because it did not include an HCV-negative comparison group. Nevertheless, our data demonstrate an association between HCV and IR that is not explained merely by liver cirrhosis or advanced fibrosis.

The mechanisms by which HCV is linked to IR merit further investigation. The recent finding that HCV infection induces genes in the sterol regulatory element-binding protein signaling pathway,[98] a protein also central to insulin signaling provides a potentially unifying explanation for this association. Further elucidation of the association between HCV infection and IR should lead to preventive measures that may eventually reduce morbidity and mortality associated with post-transplant diabetes. While the cross-sectional nature of this study does not permit conclusive statements regarding the causal relationship between HCV infection and IR, longitudinal assessment of HCV infected cohorts will help to clarify this relationship.

3.4 Acute humoral rejection after antiviral therapy for HCV infection

In the current study, we describe our experience with the use of antiviral therapy (interferon-alfa and ribavirin) in kidney transplant recipients. Biochemical or complete response to therapy was observed in majority of patients. However, the use of interferon-alfa was associated with severe irreversible acute humoral rejection in 17% of kidney recipients resulting in graft loss. Histopathologically, both patients were found to have

acute humoral rejection (positive peritubular C4d deposits), a finding that was associated with concurrent detection of anti-HLA donor-specific antibodies in the recipient sera. In the past, 'acute vascular rejection' has been described in patients receiving interferon-alfa, but it could not be determined whether this represented acute humoral rejection as no testing for donor-specific antibodies in serum or immuno-staining for C4d deposits in biopsies were performed. In view of our findings, it can be speculated that these cases may also have been acute humoral rejection. The recognition of acute humoral rejection in this series may explain the high rate of allograft loss reported previously in recipients receiving interferon-alfa. The mechanism of interferon-induced acute humoral rejection remains unclear. Interferon-alfa may cause increased cell surface expression of HLA antigens and induction of cytokine gene expression with subsequent stimulation of antibody production.[83, 84] Alternatively, interferon may primarily enhance donor-specific antibody production. Interestingly, the use of interferon therapy for recurrent HCV infection in liver transplant recipients has not been found to be associated with an increased incidence of acute rejection. It is important to note that in contrast to the kidney, the liver is an organ that is less susceptible to antibody-mediated rejection. Donor-specific antibody production was not found to be enhanced in liver transplant recipients receiving interferon-alfa.[99] This may partially explain the safety of interferon-alfa reported in liver transplant recipients requiring antiviral therapy.

Our results further indicate that the potential benefits of antiviral therapy need to be weighed against the risk of allograft rejection in kidney transplant recipients. Safer and more effective strategies are required to treat kidney transplant patients with HCV infection.

3.5 Perspective

The basic pathogenetic mechanisms for extrahepatic manifestations of HCV infection have not been defined clearly, although they are associated with chronic viral infection. Most extrahepatic manifestations of HCV infection appear to be immunological, and the chronic infection seems to be necessary for their development. Several concurrent factors may account for the development of *de novo* immune-mediated glomerular disease in HCV-infected transplant recipients. After transplantation, HCV-infected patients show an increase of the viral titer, indicating HCV replication. Immunosuppression and HCV infection itself may also modify the lymphocyte response and antibody formation against HCV. Consequently, in combination, modulation of the lymphocyte activity and increased viral load may produce an antigen-antibody imbalance, which predisposes to the long-term development of *de novo* glomerular disease.

The precise mechanism linking HCV and PTDM remains unclear; however, our results in kidney and liver transplant recipients suggest that increased insulin resistance, but not a deficit in insulin secretion is the most likely pathogenic mechanism involved in the development of PTDM associated with HCV infection in these patients. In contrast to the non-transplant setting, increased insulin resistance does not seem to be mediated by proinflammatory cytokines after kidney transplantation. The cross-sectional nature of our studies does not permit conclusive statements regarding the causal and temporal relationship between HCV infection and PTDM. Longitudinal assessment of HCV infected cohorts is required to clarify this relationship further.

The detrimental effect of HCV infection on both patient and graft survival after transplantation could also be attributed to posttransplant renal disease and PTDM. This makes it necessary to define pretransplant risk markers of *de novo* renal disease and new onset PTDM among HCV-infected transplant recipients, to carefully follow the transplant recipients for early detection of proteinuria, worsening of liver disease and development of PTDM, to implement an active policy of renal biopsies in this population, and to develop reasonable therapeutic strategies aimed at minimizing the detrimental effects on graft and patient survival of HCV-associated extrahepatic manifestations. A renal biopsy should be performed in all HCV-infected kidney or liver transplant recipients with a *de novo* proteinuria, a *de novo* nephritic sediment, or acute or chronic renal dysfunction to rule out the various renal syndromes associated with HCV infection.

Management of HCV-related extrahepatic disease is difficult. Despite impressive advances in our knowledge about the epidemiology, clinical course and management of HCV infection, a number of important questions regarding the care of the HCV-infected kidney transplant recipient remain unanswered. The therapeutic approach to extrahepatic disease should concentrate on eradication of HCV. Antiviral therapy has shown to be effective in clearing HCV infection in a proportion of patients with HCV-associated glomerular disease, although mostly, features related to cryoglobulinemia have been studied in trials. Many of the extrahepatic manifestations can be severe and resistant to antiviral therapy. Immunosuppressive drugs plasmapheresis, and lately, anti-CD 20 monoclonal antibody, rituximab, are counted among therapies that can be applied complementarily to the antiviral therapy, especially during the acute fulminant phase of the disease. However, this should be done in conjunction with antiviral therapy, as immunosuppressive therapy alone may flare up the viral load and worsen the infection.

At present there is no precise therapeutic approach to treating HCV-related renal disease

after transplantation. The limited efficacy of interferon-alfa, together with its high cost, risk of acute rejection and side-effects have diminished the enthusiasm for its use in kidney transplant recipients with chronic HCV infection. The safety and efficacy of ribavirin in reduced dose and with close monitoring of ribavirin plasma concentrations and hemoglobin levels in patients with advanced impairment of renal function, including dialysis patients and transplant recipients, is still to be determined. In the absence of effective and safe antiviral treatment for HCV infection in kidney transplant recipients, the management of these patients remains a challenge. Antiviral treatment of transplant candidates while on dialysis remains the best option and may be beneficial not only to prevent posttransplant liver disease, but also to prevent HCV-associated kidney disease after transplantation and new onset PTDM.

In addition to the antiviral approach, several other measures taken after transplantation may minimize the consequences of HCV infection. It is possible that optimizing immunosuppression in HCV-infected transplant recipients may have the potential advantage of reducing viremia after transplantation, which could result in decreased complications of HCV infection. Our observations in liver transplant recipients suggest that high doses of steroids used may be an important factor in enhancing viral replication and associated complications.[11, 100] Antilymphocyte antibodies have also been shown to be associated with more rapid progression to cirrhosis after liver transplantation. [101, 102] Although no specific prospective studies directly comparing different immunosuppressive regimens have been performed in HCV-infected kidney recipients, it may be extrapolated from the observations in liver recipients that steroid-sparing protocols and avoidance of T-cell depleting antibodies may also be preferable in kidney recipients with HCV infection. Calcineurin inhibitors have not been shown to directly enhance HCV replication in non-transplant patients. However, from our observations in kidney transplant patients, it can be hypothesized that the diabetogenic effects of tacrolimus may be enhanced by HCV infection, particularly when higher dosage and levels are used in the early posttransplant period.[12] It can be speculated that lower dosage of tacrolimus or the use of cyclosporine may lower the incidence of PTDM in a subset of HCV-positive kidney transplant recipients. Conflicting data exist on the effect of other immunosuppressive drugs such as mycophenolate mofetil, mTOR inhibitors and anti-IL2-receptor monoclonal antibodies on HCV reactivation. Therefore, there is still an unmet need to define optimal immunosuppressive therapy for HCV-infected transplant recipients.

In future, newer antiviral therapy against HCV should allow safe and effective suppression of HCV in kidney transplant recipients. These are currently under study and

include antiproteases, immunomodulatory agents such as new interferons or therapeutic vaccines, and alternatives to ribavirin and several new polymerase inhibitors. Until these drugs are available, early diagnosis, antiviral treatment before transplantation and longitudinal monitoring are crucial for their management.

Finally, to date, prospective studies of extrahepatic replication are missing from the available literature. Retrospectives studies suggest several potential associations between extrahepatic replication of HCV and important clinical outcomes. For example, a few preliminary studies have found that HCV RNA may be present in peripheral blood mononuclear cells (PBMCs), but not the corresponding serum or liver, especially in immunocompromised patients.[103] Therefore, subpopulations within PBMCs may represent true reservoirs of HCV replication. Interestingly, patients with detectable negative-strand HCV RNA in PBMCs had lower sustained response rates to interferon-alfa therapy compared to those without detectable negative-strand HCV RNA in PBMCs. Thus, it may be speculated that low-level replication of HCV in PBMCs may lead to reactivation of HCV after termination of therapy and/or predict response to therapy. The focus of our future research is to prospectively investigate the prevalence and clinical consequences of such occult HCV infection in PBMCs of hemodialysis and kidney transplant patients, not detected by conventional methods. The findings of this study would be crucial to develop new screening strategies for both hepatitis B and C viral infections, and to develop therapeutic interventions that may lead to marked reduction in morbidity and mortality in hemodialysis and kidney transplant patients.

4. SUMMARY

Chronic HCV infection is an important health problem in kidney and liver transplant recipients with a deleterious impact on both patient and graft survival. In the last couple of decades, it has become evident that apart from hepatic manifestations, HCV infection is also associated with a variety of extrahepatic manifestations both in native kidneys as well as after organ transplantation. Of these, essential mixed cryoglobulinemia with or without membranoproliferative glomerulonephritis is the most extensively documented one. In the cumulative work presented here, we identified a few other novel extrahepatic manifestations associated with HCV infection in kidney and liver transplant recipients. Further, we attempted to determine the pathogenesis and clinical relevance of these complications. We showed that HCV infection may also affect graft and/or patient survival either by enhancing the risks of *de novo* glomerulopathies and posttransplant diabetes mellitus, or by virtue of the complications of antiviral therapy.

We described for the first time a unique lesion of *de novo* renal thrombotic microangiopathy occurring in transplant kidneys in association with HCV infection and anticardiolipin antibodies. These patients had a bad prognosis along with increased thrombotic complications. Our findings suggest that anticardiolipin antibodies may be implicated in the pathogenesis of renal thrombotic microangiopathy in a subset of HCV-positive patients. We also presented an evidence for the association between HCV infection and transplant glomerulopathy in kidney transplant recipients, providing further insight into the pathogenesis of transplant glomerulopathy. Furthermore, in one of the pioneering works, we reported an association between HCV infection and new onset posttransplant diabetes mellitus after liver transplantation and further confirmed this finding also in kidney transplant patients. We identified increased insulin resistance i.e. impaired insulin sensitivity as the underlying mechanism for HCV-associated posttransplant diabetes mellitus in both these populations. To our knowledge, ours was the first study evaluating this mechanism using sophisticated intravenous glucose tolerance test in kidney transplant recipients.

More work is needed to further elucidate the implicated mechanisms and identify the potential strategies to prevent some of these specific extrahepatic syndromes after kidney and liver transplantation. Major challenges to be addressed in future include delineation of optimal antiviral therapy for HCV before and after transplantation, ideal immunosuppressive therapy after transplantation, and appropriate handling of patients who develop graft dysfunction or posttransplant diabetes mellitus as a result of HCV

infection. Adequately powered prospective, controlled, long-term studies are required in HCV-infected hemodialysis patients on waiting list and kidney transplant recipients to answer these questions which would help to minimize the detrimental effects of HCV on graft and patient survival after transplantation.

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ERKLÄRUNG

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