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Cholestatic syndromes in renal transplant recipients with HCV infection

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Abstract We present two distinct types of cholestatic syndrome identified in eight renal transplant (RTx) patients with HCV infection. Four patients developed fibrosing cholestatic hepatitis (FCH) and four, vanishing bile duct syndrome (VBDS). All patients with FCH were anti-HCV (–) at the time of Tx and developed a cholestatic profile 1–4 months post-Tx, with high HCV-RNA levels. Immunosuppressive therapy was drastically reduced. Two patients died of sepsis and liver failure 16 and 18 months post-Tx, and the other two showed marked improvement and seroconverted to anti-HCV. Regarding the patients with VBDS, three were anti-HCV (–) and one was anti-HCV (+)/HBsAg (+) at the time of RTx. Two patients became anti-HCV (+) 1 year, and one patient, 3 years post-Tx. Two patients developed progressive VBDS and died of liver failure 2 and 3 years after onset, and two showed marked improvement after withdrawal of immunosuppression. In two of the patients, the progression of the disease coincided with elevation in serum HCV RNA levels. We concluded that a progressive cholestatic syndrome acquiring features of FCH or VBDS may develop in HCV-infected RTx patients. The association with high viral load implicated the virus in the pathogenesis. Drastic reduction of immunosuppression may favourably affect the outcome.

Key words Fibrosing cholestatic hepatitis · Vanishing bile duct syndrome · Hepatitis C · Cholestatic syndrome · Renal transplantation · Immunosuppression

Introduction

Fibrosing cholestatic hepatitis (FCH) has initially been described as a specific manifestation of HBV infection in liver allograft recipients, characterized by a rapid progression to liver failure [4]. Subsequently, FCH has been reported in other immunocompromized groups of patients infected with HBV, as well as in a few transplant patients with HCV infection including renal transplant recipients [2, 3, 8, 12]. These observations indicate that FCH does not exclusively occur in liver allografts and that HCV is another hepatotropic virus involved in the pathogenesis of FCH.

Recently, it has been suggested that hepatitis B and C viruses may participate in the development of vanishing bile duct syndrome (VBDS) in liver grafts [9]. The assumption of a hepatotropic virus related VBDS derives from the setting of liver transplantation, and there is no reference, to the best of our knowledge, to the occurrence of a similar syndrome in other immunocompromized groups of patients infected by either HBV or HCV.

In the present study we report the development of a cholestatic syndrome in eight renal transplant recipients with HCV infection. In four patients the syndrome was associated with the histological entity of FCH and in the other four, with bile duct damage and loss.

Table 1 Liver disease profile in the patients with fibrosing cholestatic hepatitis (*LB* liver biopsy)

Pts	Time of 1st LB post-Tx (months)	γGT/ALP (IU/l)	ALT/AST (IU/l)	anti-HCV	bDNA (Eq/ml)	Genotype	Outcome
1	8	3040/650	250/100	–	58×10^6	3a	Sepsis, liver failure, death
2	2	2043/749	323/275	–	58×10^6	1b	Sepsis, liver failure, death
3	10	2250/500	100/70	+ (31 months post-Tx)	–	1b	Marked improvement in LFT's, myocardial infarction, death
4	9	100/190	57/40	+ (3 months post-Tx)	14.35×10^6	1b	Marked improvement in liver histology, graft failure

Patients and methods

The cases to be presented belong to a series of 78 HCV-infected renal transplant recipients who underwent transplantation between 1983 and 1995 at Laiko Hospital and were followed up biochemically, serologically and with consecutive liver biopsies. All eight patients were transfused perioperatively and received a graft from anti-HCV-negative donors.

Serial serum samples were tested for anti-HCV by ELISA-2, by a third generation ELISA (EIA-3; Ortho HCV 3.0 test System) and by an immunoblot assay with antigens representing core, E2/NS1, NS3, NS4 and NS5 proteins of HCV (INNO-LIA HCV Ab III; Innogenetics). Serum HCV RNA was determined by nested PCR in serum samples obtained on the day of the first liver biopsy. Serum HCV RNA levels were quantified by a branched DNA-enhanced label amplification assay [QUANTIPLEX™ HCV RNA 2.0 Assay (bDNA), Chiron Corporation Emeryville, Calif.]. This assay gives results in numbers of copies of HCV genome equivalents per millilitre, and the sensitivity threshold is about 0.2×10^6 equivalents per millilitre. The determination of HCV genotypes was done by a commercially available assay (INNOLIPA HCV, Innogenetics, N. V., Zvijwaarde, Belgium).

Liver biopsy specimens were fixed in neutral formalin solution and processed according to the routine protocol. Paraffin sections were stained with haematoxylin and eosin (H&E), PAS after diastase digestion, Van Gieson's collagen, Gomori's reticulin and Perl's Russian blue iron stain. Immunohistochemical staining was carried out for bile duct cytokeratin 19 and HBcAg. The ABC method of immunoperoxidase was applied using anti-CK 19 and anti-HBc antibodies (DAKO). The number of interlobular bile ducts was assessed in H&E sections and confirmed by CK 19 stain. Ductopenia was diagnosed when severe reduction of at least 50% of the interlobular bile ducts was observed or in the presence of a bile duct to portal tract ratio of less than 0.5.

Case reports

Patients with FCH

The diagnosis of FCH was made in four patients based on histogical findings that were characterized by a distinctive pattern of hepatocyte ballooning, ductular proliferation and liver fibrosis. Three of the patients were male and one was female; they were aged between 40 and 45 years. They had received a cadaveric renal graft and were on immunosuppressive therapy with a combination of methylprednisolone (MP), azathioprine (Aza) and cyclosporin A (CsA). All four patients were anti-HCV (–) at the time of renal transplantation, while two of them became anti-HCV (+) within an interval of 3–31 months post transplantation. Liver dysfunction appeared 1–4 months post transplantation and aquired features of a cholestatic syndrome in the patients. HCV RNA was detectable at the time of the first liver biopsy and reached high serum levels, $14–58 \times 10^6$ Eq/ml, when histological diagnosis was made, 3–11 months post transplantation. After histological diagnosis, immunosuppressive therapy was drastically reduced. Two patients died of sepsis and liver failure 16 and 18 months post transplantation, while the seroconverted patients showed marked biochemical and histological improvement.

Patients with VBDS

Four patients developed a cholestatic syndrome associated with bile duct damage and progressive bile duct loss on histological grounds. All patients were males, they were aged 34–60 years, three underwent live-related and one, cadaveric renal transplantation, and their immunosuppressive therapy included MP, Aza and CsA. At the time of renal transplantation, three patients were anti-HCV (–) and one, anti-HCV (+)/HBsAg (+). Two patients became anti-HCV (+) 2 years, and the third patient, 3 years post transplantation. A cholestatic biochemical profile appeared either simultaneously with serocorversion or 2–4 years later.

In all patients the biochemical appearance of cholestasis was histologically associated with lesions of the small-sized interlobular bile ducts. Early bile duct lesions were characterized by cytoplasmic vacuolar degeneration and nuclear irregularity of the epithelium. Late and more severe bile duct damage presented with bile duct remnants and bile duct loss.

Two patients (one with HBV co-infection) developed progressive VBDS and died of liver failure 2 and 3 years after biochemical onset of the cholestatic disease. Serum HBeAg, HBcAg IgM and tissue HBcAg remained negative. One patient, despite developing VBDS within a 10-month interval, showed marked improvement in his liver function after cessation of immunosuppression because of graft loss. In the fourth patient, who had mild biochemical and histological cholestatic changes, liver function tests almost normalized after withdrawel of Aza. In two of the patients the progression of disease coincided with elevation of serum HCV RNA levels, 36.24×10^6 and 13.5×10^6 Eq/ml. The liver disease profile for the patients with FCH, as well as for the patients with VBDS, are presented in Tables 1 and 2, respectively.

Table 2 Liver disease profile in the patients with vanishing bile duct syndrome (*IS* immunosuppression)

Pts	Time of 1st LB post-Tx (months)	γGT/ALP (IU/l)	ALT/AST (IU/l)	anti-HCV	bDNA (Eq/ml)	Genotype	Outcome
1	36	2000/1400	140/120	+ (12 months post-Tx)	36.24×10^6	1b	Improvement after IS withdrawal
2	9	3500/1400	54/42	+ (8 months post-Tx)			Liver failure, death
3	38	2500/450	80/55	+ (38 months post-Tx)	13.54×10^6	1b	Sepsis, liver failure, death
4	12	230/200	145/158	+ (12 months post-Tx)			Improvement after Aza withdrawal

Discussion

Cholestatic syndromes in association with viral infections, not uncommon in liver transplant recipients, have only rarely been reported in other immunocompromized groups of patients outside the setting of liver transplantation. In our series of renal transplant patients with HCV infection, a cholestatic syndrome developed in 10% of the cases, acquiring features either of FCH or of VBDS.

In the four patients with FCH the interval from transplantation to the appearance of liver dysfunction was 1–4 months, and to histological diagnosis, 3–11 months, while histological and biochemical findings and were consistent with the experience obtained to date. The biochemical profile in three patients manifested as a progressive cholestatic syndrome in the absence of marked aminotransferase increase. The fourth patient had only a small increase in liver enzymes, and was the only case with discordant biochemical and histological findings.

High HCV RNA levels in the serum coincided with disease progression indicating an enhanced replication of HCV leading to direct cell death. An unrestricted viral replication and transcription, as well as a defective export of viral proteins, are considered to be the effect of the underlying immunosuppression in HBV-related FCH in liver transplant recipients [12, 13, 15].

It should be noted that in all four patients no anti-HCV antibodies were detectable at the time of transplantation. Although data about the presence or absence of HCV RNA, at that time, are missing, it seems most probable that HCV infection was acquired shortly before or during the post-transplantation period. Thus, one may assume that in this group of patients, FCH represented a complication of a recently acquired HCV infection, fully developing at the time of maximal immunosuppression.

FCH leads to the development of liver failure within the first year post transplantation in HBV-infected liver transplant patients. An interval of 5 to 18 months is mentioned in liver graft recipients with HCV reinfection, while in two renal transplant recipients, one with HBV and one with HCV infection, the syndrome appeared years after transplantation and progressed rapidly to hepatic failure despite withdrawel of immunosuppression [7, 11, 17]. Information about therapeutic approaches [5, 11, 17] is very limited. In our patients immunosuppressive therapy was drastically reduced after histological diagnosis in order to decrease the viral load and to diminish drug liver toxicity. During the follow-up period, one patient showed marked biochemical improvement in his liver disease. Moreover, in a patient who was examined with consecutive liver biopsies, normalization of the liver function tests was followed by progressive regression of the histological lesions. The same patients seroconverted, the first one 11 months after reduction of immunosuppressive therapy and the second one during the early post-transplantation period. The other two patients died of sepsis and liver failure 4 ad 7 months after histological diagnosis and 18 and 16 months after transplantation, respectively. The both remained anti-HCV negative. It seems that the inability of the patients immune system to develop antibodies against HCV coincided with the development and destructive course of FCH, thus providing additional evidence of direct HCV-mediated liver damage.

VBDS in liver transplant recipients is associated with chronic rejection [16]. It is widely suggested that immunological pathways play a predominant role in this process [16]. However, statistical results raised the possibility of the participation of HBV and HCV in the pathogenesis. To the best of our knowledge, our four cases are the first reports of a group of immunocompromized patients with HCV infection outside the setting of liver

transplantation in which progressive cholestasis in association with bile duct damage developed.

At the time of transplantation, three of the patients were anti-HCV negative, while the fourth had HBV/HCV co-infection. In two of the former patients, anti-HCV antibodies were detected 1 year, and in one, 3 years after transplantation. Although data confirming the absence of HCV RNA in the serum before transplantation were available in only one case, it seems most probable that HCV infection was acquired during the post-transplantation period. All patients developed a cholestatic syndrome simultaneously or within an interval of 2–4 years after the detection of anti-HCV antibodies, and only in the patient with co-infection did elevation of the cholestatic enzymes appear in the early post-transplant period. The biochemical appearance of cholestasis was histologically associated with lesions of the small-sized interlobular bile ducts. Early bile duct lesions were of a mild degree and were characterized mainly by degenerative changes in the epithelium. Late and more severe bile duct damage was associated with the presence of bile duct remnants and with bile duct loss. In the follow-up period, two patients – one with HBV co-infection – developed progressive VBDS and died of liver failure 2 and 3 years after biochemical onset of the cholestatic disease. In both patients azathioprine (Aza) was gradually withdrawn after the appearance of cholestasis. Another patient, despite the establishment of ductopenia within a 10-month interval, showed markes improvement in his liver function after cessation of immunosuppression. The liver function tests of the fourth patient, who had mild biochemical and histological abnormalities, almost normalized after the withdrawel of azathioprine (Aza).

These cases showed similarities with HCV-infected liver transplant patients, combining the background of immunosuppression and HCV infection [9, 13]. In contrast to liver allograft patients, this group of patients provided a less confusing setting for the study of VBDS in relation to HCV because of the absence of the possible causative role of a HLA mismatch and, in particular, of chronic rejection [16]. This, therefore, reduces the possibility of an immune-mediated destructive process, and points to HCV as a more likely causative agent. In support of this hypothesis, there were certain observations that revealed a link between immonusuppression, HCV infection and the development of VBDS. The appearance of the cholestatic syndrome was related in three patients to the appearance of anti-HCV antibodies, while deterioration in the disease coincided with the elevation of HCV RNA levels. Moreover, with drawel or drastic reduction in immunosuppression resulted in cessation of the disease in two cases.

The only virus that has been pathogenetically associated with VBDS is CMV [1]. However, it has been documented that bile duct cells constitute an appropriate host for HBV and HCV, permitting replication of the genome and antigen expression [6, 14]. Other possible causative agents that have to be taken into consideration are immunosuppressive drugs, especially azathioprine (Aza), which has been reported to cause reversible bile duct injury [10]. Although this alternative pathogenetic pathway cannot be excluded, its significance is limited by indirect indications derived from epidemiological observations that revealed that none of the non-infected renal transplant recipients in or series ever developed a cholestatic syndrome.

The development of FCH and VBDS in HCV-infected renal transplant recipients provides a new disease setting of cholestatic syndromes that combine the pathogenetic background of immunosuppression and HCV infection. The association of disease progression with a high viral load pointed to the implication of the virus in the pathogenesis, while the susceptibility of the hepatic and biliary cells seemed to be higher when they were primarily infected under immunosuppression. There were indications that a drastic reduction or withdrawel of immunosuppressive therapy may favourably affect the outcome of the diseases.

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