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Robert M. Hoffmann
Christoph Günther
Helmut M. Diepolder
Reinhart Zachoval
Hans-Joachim Eissner
Helmut Forst
Matthias Anthuber
Gustav Paumgartner
Gerd R. Pape

Hepatitis C virus infection as a possible risk factor for ductopenic rejection (vanishing bile duct syndrome) after liver transplantation

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R.M. Hoffmann (✉) · C. Günther
R. Zachoval · G. Paumgartner · G.R. Pape
Department of Medicine II,
Klinikum Großhadern,
University of Munich,
Marchioninistrasse 15,
D-81366 Munich, Germany
Fax: + 49 89 7095 8879

M. Anthuber
Department of Surgery,
Klinikum Großhadern,
University of Munich,
Marchioninistrasse 15,
D-81366 Munich, Germany

H. Forst
Department of Anesthesiology,
Klinikum Großhadern,
University of Munich,
Marchioninistrasse 15,
D-81366 Munich, Germany

H.-J. Eissner
Department of Biometrics and
Epidemiology,
Klinikum Großhadern,
University of Munich,
Marchioninistrasse 15,
D-81366 Munich, Germany

H.M. Diepolder
Institute for Immunology,
Goethestrasse 31,
80366 Munich, Germany

Abstract Irreversible ductopenic rejection (DR) after orthotopic liver transplantation (OLT) is a major cause of late hepatic allograft failure. A variety of risk factors for DR have been postulated, but they are controversial. All transplant recipients at our institution with graft survival of more than 1 month ($n = 120$) were examined retrospectively with a view to possible risk factors for DR. These factors included age, sex, underlying liver disease, hepatitis B and C infections, donor-recipient CMV status, post-OLT CMV infections, immunosuppressive regimen, ABO blood type, and HLA class I and class II mismatches. Statistical analysis was performed with the univariate chi-square test or the two-tailed Fischer's exact test. Ten patients (8.3 %) developed DR. Seventeen patients had HCV infections after OLT. In this group, the incidence of DR was highest (4 of 17, or 23.5 %). This was significantly higher than for all other OLT groups (6 of 103 patients, or 5.8 %; $P < 0.03$). The other factors analyzed did not reach statistical significance, including those that other authors found important for the development of DR. It may well be that hepatitis C infection predisposes one to the development of DR after OLT.

Key words Liver transplantation, hepatitis C virus, vanishing bile duct syndrome · Hepatitis C virus, liver transplantation, vanishing bile duct syndrome · Vanishing bile duct syndrome, hepatitis C virus, liver transplantation

Introduction

Orthotopic liver transplantation (OLT) has become a widely accepted therapy for end-stage liver diseases. Up to 80 % of the transplant recipients undergo at least one episode of acute rejection that can usually be controlled by treatment with high doses of corticosteroids and/or OKT3 monoclonal antibody. However, 5 %–17 % of the transplant recipients develop irreversible ductopenic rejection (DR) [also referred to as vanishing bile duct syndrome (VBDS)] [19]. DR is a major cause of late hepatic allograft failure and retransplantation.

A variety of risk factors for DR have been discussed. Some investigators have found primary sclerosing cholangitis (PSC), as well as primary biliary cirrhosis (PBC), to be significant risk factors for DR [3, 7, 17]. Furthermore, the absence of azathioprine in the immunosuppressive regimen may also be a risk factor [16]. The results of studies about the role of donor/recipient HLA class I and II mismatches for the development of DR [2, 4] are controversial. Equally conflicting results of studies of the association of DR with cytomegalovirus (CMV) infection, with or without donor recipient HLA class II match, [1, 10, 12] have been reported. We therefore examined 152 patients who underwent liver transplantation at our institution for the risk factors mentioned and additional possible risk factors for DR.

Patients and methods

Study population

Between June 1985 and January 1993, 184 OLTs were performed in 152 patients at the University of Munich, Klinikum Grosshadern. The study group consisted of 120 patients in whom the allograft survived for more than 1 month. Of these patients, 115 received a first hepatic allograft, and 5 received a second transplant within 72 h after their first OLT. This was because of early graft failure due to preservation injury; there were no signs of allograft rejection. These 120 patients were examined for the following possible risk factors for DR: age, sex, underlying liver disease, donor recipient CMV status, post-OLT CMV infections, immunosuppressive regimen, ABO blood type, and HLA-class I and class II (DR,DQ) match and mismatch. Indications for OLT in these 120 patients were PBC ($n = 16$), PSC ($n = 4$), primary liver tumors ($n = 27$), cirrhosis due to viral hepatitis ($n = 33$); [hepatitis B, ($n = 12$); hepatitis D, ($n = 4$); hepatitis C, ($n = 16$), hepatitis C virus (HCV)-RNA-positive ($n = 13$), anti-HCV-positive, RNA-negative ($n = 3$)] unclassified cirrhosis ($n = 4$); alcoholic cirrhosis ($n = 20$); fulminant hepatic failure ($n = 7$), others (endocrine tumors, autoimmune liver disease, Budd-Chiari syndrome, alpha 1-antitrypsin deficiency, echinococcus alveolaris; $n = 10$).

Immunosuppression

Immunosuppressive therapy consisted of cyclosporin (2–4 mg/kg body weight per day), methylprednisolone (1000 mg intraoperatively, 250 mg on day 1, tapered down to 30 mg at 6 weeks), antilymphocyte globulin (4 mg/kg body weight per day for 7 days) and azathioprine (1–2 mg/kg body weight per day for 21 days). No azathioprine was given to 13 of the patients because of leukopenia or thrombocytopenia. Episodes of biopsy-proven acute rejection were treated with bolus injections of 500 mg methylprednisolone per day for 3–5 days. Resistant rejection was treated with 5 mg of the monoclonal anti-CD3 antibody OKT3 per day for 10–14 days.

Definition of DR

Irreversible DR was histologically defined as absence of interlobular and septal bile ducts from at least 50 % of the portal tracts in liver biopsies. Foam cell arteriopathy was considered a nonobligatory feature. Additional features included prominent cellular and canalicular cholestasis and a variable mononuclear cell infiltrate in portal tracts. Extrahepatic cholestasis, as well as bacterial cholangitis, had to be excluded.

The diagnosis was based on serial biopsies performed during the post-transplant course, and in nine of the ten patients who had died or had received a second transplant, the diagnosis was based on analysis of explanted livers. The mean number of biopsies performed before the diagnosis of DR was established was 4.2 (range 3–7). All biopsies were evaluated by the same experienced pathologist. In eight of the nine explanted grafts, foam cell arteriopathy was present.

Antiviral prophylaxis, CMV surveillance, and definition of CMV infection

All patients received acyclovir at a dosage of 5 mg/kg body weight three times a day for the first 21 days as prophylactic therapy against herpes simplex and herpes zoster. All patients with a positive CMV serology before OLT or who received CMV-positive organs were treated prophylactically with anti-CMV immunoglobulin (Cytoglobin® Tropon-Cutter) with 2 ml/kg as a first dose, followed by a dosage of 1 ml/kg body weight per week for 6 weeks.

Pretransplant sera were obtained from each patient to determine the CMV-specific antibody status. After OLT serologic testing was done in weekly intervals for 8 weeks and at follow-up visits thereafter. In parallel, samples of blood and urine were obtained for culture. The diagnosis of CMV infection was based on the isolation of CMV from urine or tissue and/or an increase in IgG-specific titer at least four times greater than of the baseline titer or a newly detected specific IgM titer without clinical signs of disease. All patients with CMV disease (fever, bone marrow suppression, pneumonitis, hepatitis, retinitis etc.) were treated with ganciclovir, 5 mg/kg body weight, twice a day, for 14 days. In addition, anti-CMV immunoglobulin (Cytoglobin®) was given at a dosage of 4 ml/kg body weight per week.

HLA phenotyping

HLA phenotyping for class I (A,B,C) and class II (DR,DQ) antigens was performed with a standard microlymphocytotoxicity-dye exclusion method.

Table 1 Univariate analysis of prognostic factors for ductopenic rejection following liver transplantation

	Odds ratio	*P* (Fischer's exact test)
Underlying liver disease		
Hepatitis B, C infections		
Hepatitis C infection	4.97	0.03
Hepatitis B infection	2.67	0.59
Primary biliary cirrhosis	0.70	1.00
Primary sclerosing cholangitis	1.13	1.00
Alcoholic cirrhosis	1.76	1.00
Immunosuppression without azathioprine	1.10	1.00
ABO mismatch	0.90	1.00
Surgical risk factors		
Use of Euro-Collins solution	0.61	1.00
Biliary complications	0.42	0.33
Use of prostacyclin	0.70	1.00
CMV infection	0.72	0.74
CMV disease	0.44	0.29
HLA mismatches		
Class I		
HLA-A	0.43	0.42
HLA-B	3.72	0.34
HLA-C	1.22	1.00
Class II		
HLA-DR	1.70	1.00
HLA-DQ	5.50	0.12

Diagnosis of HBV and HCV infection

Pre- and post-transplant sera of all patients were analyzed for HBV and HCV infection with commercial test kits (all Abbott Laboratories, Chicago, Ill., USA). In addition to the "routine" tests, polymerase chain reaction (PCR) was applied in all patients to detect HBV DNA (primers from the S-gene region) and HCV RNA after reverse transcription (primers from the 5' noncoding region of the HCV genome).

Statistics

Statistical significance was assessed with the univariate chi-square test or the two-tailed Fischer's exact test, where appropriate. A stepwise multivariate analysis of variance was planned, if multiple factors were found. A P value less than 0.05 was considered significant.

Results

Among the 120 patients whose allografts survived for longer than 1 months, 10 patients (8.3 %) developed histologically proven chronic DR.

Clinical course of the DR patients

Eight of the ten patients in whom DR developed received a second transplant. Seven of them are alive with normal graft function (six of the seven are receiving FK 506 therapy). One patient died before retransplantation, and one patient is on FK 506 therapy without a need for retransplantation.

DR and underlying liver disease, HBV and HCV infection

The incidence of DR was highest in the group of 17 patients whose serum was HCV RNA-positive after liver transplantation. This group consisted of the 16 patients receiving transplants because of HCV disease – 3 of the 16 were RNA-negative before transplantation but were classified as chronic HCV cases due to antibody status – and of 1 patient who received a transplant because of alcoholic cirrhosis but became HCV RNA-positive after transplantation. Four of these 17 patients (23.5 %) developed clinical and histological signs of DR. In all the groups investigated, this is the only significant association with the development of DR (other groups: 6 patients with DR in 103 patients; 5.8 %, $P < 0.03$). No correlation could be found for pre- or post-OLT hepatitis B virus infections or underlying liver diseases like PBC or PSC. Table 1 shows a summary of the univariate analysis of the prognostic factors investigated.

DR and immunosuppressive regimen

In our study, azathioprine was not given to 13 patients because of thrombocytopenia or leukopenia. Only one

Table 2 Association between pretransplantation CMV serology of recipient and donor and occurrence of ductopenic rejection *(DR)*. Nominator: Patients with Dr; Denominator: Total numbers in each group

	Donor CMV	
	positive	negative
Recipient CMV		
positive	3/33	2/39
negative	1/17	3/14

of these patients developed DR, which is statistically not significant.

DR and "surgical risk factors"

Table 1 also delineates other possible risk factors of biliary lesions (and possibly DR). No significant difference could be found either for cold preservation time (508 ± 109 min for the DR group; 547 ± 196 min for controls), or for preservation solution used, or for the complication of arteria hepatica thrombosis (no patient in the DR group appeared to be affected) or biliary complications (one biliary leak on day 18 postoperatively and one grade I stenosis in DR; 31 complications in non-DR). Prostacyclin was administered to all patients with suspected ischemia of the graft (based on duplex ultrasound examinations and/or arteriography). Thus, we looked for the significance of the therapeutic use of prostacyclin but could not define it as a risk factor.

DR and CMV infection

In Table 2, the pretransplant recipient and donor serologic CMV status is shown in association with the development of DR. DR occurred randomly in all groups of patients. We then correlated CMV infection and CMV disease with the occurrence of DR. Thirty-eight patients showed signs of post-OLT CMV infection. In 13 patients, CMV disease was diagnosed by the criteria mentioned. No statistically significant relationship to DR could be found (Table 1).

DR and donor recipient HLA class I and class II status

For this analysis, all the data for 84 patients were available. Table 3 summarizes the results for the three major HLA class I (HLA-A,-B,-C) and the two major HLA class II (HLA-DR,-DQ) matches of donors and recipients as potential risk factors for the development of DR. For seven of the ten patients with DR, complete data were available. Six of these seven patients showed an HLA-DQ mismatch, in contrast to only 40 of the 77 patients without DR. This difference, probably due to the small number of patients with DR, did not reach statistical significance (see Table 1).

As we had found only one significant factor in the univariate analysis of the risk factors investigated, a stepwise multivariate analysis was not performed.

Comparison of HCV with non-HCV patients

To exclude other factors that might influence the development of DR, we compared the HCV-infected patients with the noninfected group for treated rejection episodes and the use of immunosuppressive drugs. In the HCV group, 0.70 ± 0.98 (range 0–3) and in the noninfected group 0.86 ± 0.8 (range 0–3) rejection episodes were treated (P = NS). There was also no difference for the number of treated rejection episodes between the HCV-positive patients who developed DR and the HCV-positive patients who did not develop DR.

Steroids may increase the viral load and this may trigger DR. No difference could be demonstrated, how-

Table 3 Distribution of cases of vanishing bile duct syndrome *(VBDS)* by pretransplantation matches of HLA[a]

	Class I					
	HLA-A		HLA-B		HLA-C	
	VBDS	No VBDS	VBDS	No VBDS	VBDS	No VBDS
No match	3	49	7	62	6	64
HLA match[b]	4	28	0	15	1	13
	Class II					
	HLA-DR		HLA-DQ			
	VBDS	No VBDS	VBDS	No VBDS		
No match	6	60	6	40		
HLA match[b]	1	17	1	37		

[a] This analysis was based on 84 patients
[b] Match was partial or complete

ever, for the cumulative steroid dosage in the two groups (HCV-positive 4609 ± 2016 mg for 6 months; non-HCV 4692 ± 1845 mg). The doses of cyclosporin, ATG, and azathioprin (see DR and immunosuppressive regimen) were also similar in the two groups.

When we compared the time course for the development of DR between HCV and non-HCV patients, we found that DR was first diagnosed after 124 ± 61 days in the HCV group and after 113 ± 40 days in the noninfected group. This is not significantly different.

Clinical course of HCV patients after transplantation

Of the 17 patients serum-positive for HCV RNA after liver transplantation, 8 patients (47 %) showed near-normal liver histology and laboratory parameters and the histologies of 6 patients (35 %) were compatible with mild chronic hepatitis (one of these patients had an acute onset of hepatitis). Four patients (23 %) showed the typical picture of chronic active hepatitis. Two of these four patients developed DR later. None of the patients who developed DR had been treated with interferon.

Discussion

We found that HCV infection associated endstage liver disease may predispose one to the development of DR after liver transplantation. No correlation between DR and any of the previously suggested risk factors was observed in our series.

So far, four major risk groups have been postulated for the occurrence of DR. First, there is the underlying liver disease. The Mayo Clinic group points to PSC as a risk, and the Pittsburgh group to PBC [3, 17, 19]. In our series, the group of PSC patients was too small to confirm or refute this hypothesis. In accordance with the experience of the majority of liver transplant centers, PBC does not seem to be a risk factor for the development of DR [17, 19].

Second, CMV infection in the transplant recipient is probably the most controversial issue discussed. In one study [10], it was found that CMV infection predisposed one to DR; this was observed especially in cases with a concomitant HLA class II match (HLA DR). However, the diagnosis of CMV infection in that study was based exclusively on serological markers, whereas in our patients additional specimens for viral culture and for detection of viral antigens were taken from blood, urine, and tissues on a protocol basis. Our data agree with those of Paya et al. [12], who used a similar protocol for the diagnosis of CMV infection. Recently, Arnold et al. [1] of the King's group used in situ hybridization and found that, in a majority of patients with DR, CMV persists and can be detected in hepatocytes. In patients with uncomplicated CMV infection, the CMV occurred earlier and was eliminated more rapidly. One major difference between the studies from the King's group [1, 10] and those of Paya et al. [12] and ours is the use of antiviral therapy. All patients in our study received acyclovir during the first 21 days. In all patients positive for CMV before transplantation and who received organs from positive donors, CMV hyperimmunglobulin was administered prophylactically. Furthermore, all symptomatic CMV infections were treated with ganciclovir. Probably due to these measures, the infection rate in our study is lower than that published by O'Grady et al. (31.6 % vs 56.4 %) [10]. It is also possible that a shorter duration of CMV disease under treatment decreases the probability of CMV inducing uncontrolled rejection episodes. In kidney transplant recipients, it has been shown that high-dose hyperimmunglobulin can reduce CMV infection and kidney allograft rejection [15].

The third factor discussed with regard to the development of DR is the influence of MHC antigens and lymphocytotoxic crossmatch. A positive lymphocytotoxic crossmatch as a risk factor has been described by Batts et al. in a rather small series of 52 patients [2]. In a more detailed analysis published later by the same group, a positive crossmatch was found to be only of borderline significance [17]. In the largest study concerning this issue, no association of positive lymphocytotoxic crossmatch with decreased actuarial survival of patients or allografts could be demonstrated [6]. Early reports on HLA matching in liver transplantation had shown no influence of mismatches on allograft survival [13]. In subsequent studies from the same group, however, an association of DR with complete HLA class I mismatch and HLA class II match was postulated [4, 10]. The results of these studies were based on a limited number of antigens of the major histocompatibility complex. Like others in large studies [12, 17, 19], we could not demonstrate a significant association between HLA class I and class II matches or mismatches and the development of DR. It should be mentioned, however, that in our study, six of seven patients with DR showed a HLA-DQ mismatch. Thus, HLA-DQ mismatch may indeed represent an increased risk for DR though, due to the small number of DR patients, the risk factor HLA-DQ mismatch was not statistically significant.

Fourth, the immunosuppressive regimen used was considered a risk factor. Van Hoek et al. [16] found a significant risk for DR in those patients who did not receive azathioprine after transplantation. The lack of azathioprine in the immunosuppressive regimen was not a risk factor in our patients. As we usually use a quadruple immunosuppressive protocol including antilymphocyte globulin, the total or transient exclusion of aza-

thioprine may not have been so relevant in our patients. The role of FK 506 as a primary immunosuppressive agent for the prevention of DR has not been elucidated yet. In the Mayo Clinic series, 10 of the 11 patients who were retransplanted because of DR also developed DR in their second graft [17]. Eight of our ten patients with DR received second grafts; of these eight patients, seven received FK 506 therapy after retransplantation. Only one patient died (because of recurrence of hepatocellular carcinoma); seven are alive with normal graft function and no signs of DR. Two of the ten patients did not receive second grafts; one patient died while on the waiting list, and the other patient showed stable graft function while receiving FK 506 therapy. Thus, in our limited experience, FK 506 seems to be a useful immunosuppressive alternative for patients after retransplantation because of DR. The HCV-positive patients receiving second transplants because of DR demonstrated histological signs of mild chronic hepatitis without impairment of liver function under FK 506 therapy.

In this study, HCV infection as an underlying liver disease has been identified for the first time as a possible risk factor for the development of DR after OLT. The pathogenesis of DR has not been fully elucidated. Secondary ischemic lesions of small bile ducts as a consequence of an immune attack of T cells on vascular endothelium (mainly of small arteries) has been discussed [11]. In contrast, direct damage to small bile ducts by immunocompetent cells has been postulated [5, 18]. The mechanism that triggers this immune attack is unclear. Histologically, bile duct damage can be seen in 30 %–60 % of patients with HCV-induced chronic liver disease [8, 14]. By in situ hybridization techniques, it has been shown that HCV is located not only in hepatocytes and liver-infiltrating mononuclear cells, but also in bile duct epithelium of infected livers [9]. One can speculate that HCV peptides bound to HLA molecules and expressed on the surface of bile duct epithelia may trigger the cellular immune response against these bile ducts, leading to destruction and the development of DR. In situ hybridization with appropriate cDNA probes in these patients will be necessary to investigate this hypothesis further.

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