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Duct-to-duct biliary reconstruction following liver transplantation for primary sclerosing cholangitis

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Abstract The biliary complications in patients undergoing biliary reconstruction by duct-to-duct (D-D) anastomosis or with a Roux-en-Y loop (RL) at the time of liver transplantation for primary sclerosing cholangitis (PSC, 16 D-D, 10 RL) or primary biliary cirrhosis (PBC, 31 D-D, 1 RL) were reviewed and compared. Patients were followed up for a mean period of 32 months. Extrahepatic biliary strictures occurred in 18.7 %, 10 % and 9.7 % of DD-PSC, RL-PSC and DD-PBC patients, respectively, leaks in 6.2 %, 20 % and 6.4 % DD-PSC, RL-PSC and DD-PBC patients, respectively (P = NS). Four intrahepatic biliary abnormalities developed in the PSC group. Duct-to-duct anastomosis did not significantly increase the risk of stricture formation or bile leaks in PSC patients compared to PBC patients. We conclude that duct-to-duct biliary reconstruction following liver transplantation for PSC is satisfactory unless the distal common bile duct is strictured.

Key words Liver transplantation, biliary reconstruction, sclerosing cholangitis · Biliary reconstruction, sclerosing cholangitis · Primary sclerosing cholangitis, biliary reconstruction

Introduction

Biliary complications following orthotopic liver transplantation (OLT) are a major cause of morbidity [1, 2, 3, 5, 7, 8, 16, 18, 24, 26]. Possible aetiological factors include technical errors, ischaemia, infection and chronic rejection. Biliary duct ischaemia and other aetiologic factors such as ABO incompatibility, ductopenic rejection and prolongation of cold ischaemia time may play a role in postoperative stricture formation and anastomotic leaks. Finally, speculation and some evidence suggest that the primary hepatic disease may play a role in the development of biliary complications after OLT [9, 12, 15]. Liver transplantation patients with primary sclerosing cholangitis (PSC) usually undergo biliary-enteric rather than duct-to-duct (D-D) anastomosis because of anxiety of possible disease progression in the recipient common bile duct (CBD), resulting in stricture formation or even cholangiocarcinoma. Most of these patients only have evidence of intrahepatic disease on radiological assessment [27].

Cholangiocarcinoma in the CBD remnant after transplantation has not been described. We report the results of direct D-D anastomosis in PSC patients undergoing OLT and compare complications to those with primary biliary cirrhosis (PBC).

Patients and methods

From November 1988 to January 1994, 207 liver transplants were performed, 28 of which were for PSC and 36 for PBC. The median age of the PSC and PBC patients was 36 (range 17–63) years and 55 (range 30–69) years, respectively. The ratios male/female were 19/9 and 4/32 in the two groups, respectively. The median cold ischaemic times were 10.8 (range 4.5–16.8) hours and 12.5 (range 7.2–20.6) hours, respectively.

Four patients from the PBC group and two patients from the PSC group were excluded from this study because they died in the

patients with serious infections compared with the other two groups, and that some parameters may be indicative of serious infections as early as during reperfusion, while others may detect patients who subsequently died.

Materials and methods

Patients

Between August 1993 and July 1994, 81 patients receiving 85 orthotopic liver transplants were prospectively monitored for various new parameters. Indications for liver transplantation included 5 patients with acute liver failure due to fulminant hepatitis B (HBV) and hepatitis C (HCV) infections, or intoxication, 23 patients with HBV and HCV cirrhosis, 16 patients with alcoholic cirrhosis, 11 with primary biliary cirrhosis, 8 with cryptogenic cirrhosis, 4 patients with autoimmune cirrhosis, and 15 patients with various other indications. Six patients who were undergoing retransplantation were also included: 1 initial non-function (INF), 1 refractory acute rejection, 1 chronic rejection, and 1 early graft failure following recombinant tissue-type plasminogen activator (rt-PA) lysis therapy and disseminated intravascular coagulopathy (DIC) due to lung artery embolism; 2 patients entered the study at the time of retransplantation: 1 patients with chronic rejection and 1 with HBV recurrence. The study was approved by the ethics commitee of the Humboldt University of Berlin and informed consent was received from each patient prior to participation in the study.

Immunosuppression

Immunosuppression was commenced as quadruple therapy comprizing cyclosporine A (CsA), azathioprine, prednisolone, and ALG (anti-lymphocyte immunoglobulin, Merrieur, France; $n = 24$) or interleukin (IL)-2 receptor antagonist BT563 (Biotest, Dreieich, Germany; $n = 47$) for the first 7 or 12 postoperative days, respectively, and was continued as triple therapy thereafter [8]. Twelve patients received no induction therapy. Two patients undergoing retransplantation who had been previously converted to FK506 because of steroid-resistant and chronic rejection received FK506 (Prograf, Fujisawa, Osaka, Japan) in conjunction with prednisolone.

Management of rejection

Diagnosis of acute rejection was based on clinical (fever, change of color, and amount of bile production) and laboratory (aspartate transaminase, alanine transaminase, bilirubin, γGT, and alkaline phosphatase) findings and was confirmed by histological evaluation of graft biopsies. Patients received methylprednisolone for treatment of acute rejection at a dosage of 500 mg/day for 3 days and FK506 or the combination of FK506 and OKT3 monoclonal antibody (Cilag, Sulzbach, Germany) for steroid-resistant or severe recurrent rejection [9].

Management of infection

Cytomegalovirus (CMV) infection was diagnosed by routinely performed PCR techniques and treated with gancyclovir. If pneumonia was suspected following clinical examination or chest X-ray, diagnostic bronchoscopy was performed to confirm diagnosis and allow appropriate antibiotic, antiviral, or antifungal treatment. Infections were defined as serious when more than two secondary organ failures, including acute renal failure (ARF), acute respiratory insufficiency (ARI), liver failure, DIC, sepsis or systemic inflammatory response syndrome (SIRS), requirement of cathecholamines, neurological disorders, or acute and chronic rejection, were present.

Clinical and experimental monitoring

Laboratory investigations and clinically adverse events were evaluated on a daily basis for the first month, and, subsequently, after 3, 6, 9, and 12 months. Experimental parameters were determined at the same time points, and additionally at predefined time points during transplantation and after reperfusion: 15 min, 2, 6, 12, 18, 24, 36, 48, and 72 h. Heparinized blood was immediately stored on ice and centrifuged at 4 °C for 10 min within 30 min of retrieval. Plasma was stored at – 70 °C until measured. Commercially available immunoassays with 96-well microtiter plates were used: neopterin (ELItest Neopterin, B.R.A.H.M.S. Diagnostica, Berlin, Germany); IL-1β, IL-6, and IL-10 (Biozol, Eching, Germany); sIL-2R, soluble tumor necrosis factor (sTNF)-RII, and soluble intercellular adhesion molecule-1 (sICAM-1) (DPC Biermann, Bad Nauheim, Germany). Hyaluronic acid was determined by radioimmunoassay (Kabi Pharmacia, Uppsala Sweden). The normal ranges ($n = 45$) of various plasma levels for healthy subjects were: 6.2 ± 0.3 nmol/l for neopterin; 0.0 pg/ml for IL-1β; 28.8 ± 0.9 pg/ml for IL-6; 4.7 ± 0.3 pg/ml for IL-10; 380 ± 20.6 U/l for sIL-2R; 1450 ± 52.7 pg/ml for sTNF-RII; 129 ± 24.1 ng/ml for sICAM-1; and 19.4 ± 2.5 µg/l for hyaluronic acid.

Statistical analysis

Kaplan Meier estimates, Wilcoxon, chi-square tests and analysis of variance were used as indicated. Results were expressed as means ± standard error of the mean.

Results

Survival

Actuarial 1-month and 1-year patient and graft survival was 97.5 % (79/81) and 88.9 % (72/81) for patients, and 94.1 % (80/85) and 84.7 % (72/81) for grafts, respectively. During the first year after transplantation, nine patients died; in five patients death was related to serious infections, two patients died because of fulminant HBV recurrence, one patient because of fulminant HCV, and one because of tumor recurrence.

Postoperative complications

Twenty-eight patients (34.7 %) developed acute rejection during the first month after transplantation; in 14 cases rejection was steroid-resistant and required treatment with FK506 (8 patients) or a combination of

Table 1 Serious infections after liver transplantation. (*LTX* Liver transplantation, *HCV* hepatitis C virus, *CR* chronic rejection, *NANB* non-A, non-B, *INF* initial non-function, *KTx* kidney transplantation, *CMV* cytomegalovirus, *ARF* acute renal failure, *ARI* acute respiratory insufficiency, *DIC* disseminated intravascular coagulopathy, *GI* gastrointestinal, *SIRS* systemic inflammatory response syndrome)

Patient number	Indication for LTX	Infection	Organ failure	Survival
1	ALV, fulminant hepatitis A	Atypical pneumonia, *Aspergillus* infection	ARF, ARI, cathecholamines, liver failure, coma	No
2	(1) ALV, unknown; (2) liver failure: re-LTX	*Enterococcus* sepsis, *Aspergillus* sepsis	Lung artery embolism, DIC, ARI, ARF, cathecholamines, coma, Guillam-Barre syndrome	No
3	(1) HCV cirrhosis; (2) CR: re-LTX	*Pseudomonas* sepsis	CR, ARF, ARI, cathecholamines, coma, GI bleeding	No
4	ALV, fulminant NANB hepatitis	*Aspergillus* pneumonia, *Staphylococcus aureus* sepsis	Steroid-resistant rejection, ARF, ARI, coma, extrapontine myelinolysis	No
5	Klatskin tumor, LTX + Whipple procedure	Necrotizing pancreatitis, peritonitis, CMV pneumonia	Septic liver failure, ARF, ARI, coma, cathecholamines, anastomotic leak	No
6	Alcoholic cirrhosis	*Aspergillus* pneumonia, unknown sepsis	CR, ARF, ARI, SIRS, cathecholamines, coma	Yes
7	(1) Autoimmune cirrhosis; (2) INF: re-LTX	*Enterococcus* pneumonia/sepsis	ARI, ARF, coma, acute rejection	Yes
8	Cryptogenic cirrhosis	Atypical pneumonia	ARI, ARF, cathecholamines	Yes
9	Alcoholic cirrhosis	*Pneumocystis carinii* pneumonia	ARI, ARF, cathecholamines, acute rejection	Yes
10	Oxalosis, LTX + KTX	*Legionella* pneumonia	ARI, cathecholamines, ARF	Yes

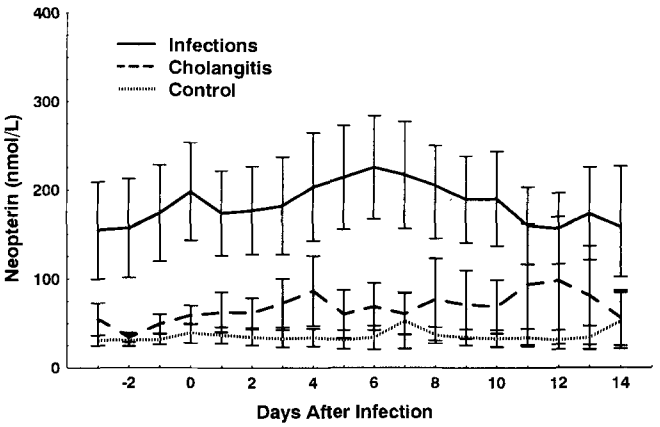

Fig. 1 Neopterin levels in patients with serious infection ($n = 10$), asymptomatic or mild cholangitis ($n = 11$), and an uneventful postoperative course ($n = 37$). $P \leq 0.01$ for serious infections versus cholangitis and uneventful postoperative course

FK506 and OKT3 (6 patients). Ten patients developed serious infections; five patients subsequently died (Table 1). Eleven patients with asymptomatic or mild cholangitis but no signs of concomitant acute allograft rejection were included in the control group together with the 37 patients with an uneventful postoperative course.

Neopterin

Mean neopterin levels were significantly increased in patients with serious infections (192 ± 48.2 nmol/l; $P \leq 0.01$) compared with patients with asymptomatic cholangitis (64.8 ± 27.1 nmol/l) and with patients with an uneventful postoperative course (43.1 ± 13.5 nmol/l) (Fig. 1). A further increase was observed in all five patients who subsequently died (245 ± 51.2 nmol/l versus 103 ± 50.8 nmol/l in surviving patients). In six of ten patients with serious infection, mean neopterin levels rose significantly during the early reperfusion period (149 ± 60.4 nmol/l versus 25.7 ± 6.9 nmol/l in patients with an uneventful postoperative course). There was no correlation, however, between neopterin levels and the extent of reperfusion injury.

Soluble TNF-RII

A significant increase in mean sTNF-RII occurred in patients with serious infections as early as 3 days prior to infection ($25\,522 \pm 6744$ pg/ml; $P \leq 0.01$) compared with patients with cholangitis ($14\,328 \pm 3087$ pg/ml) and with patients with an uneventful postoperative course (7352 ± 822 pg/ml (Fig. 2). Higher mean sTNF-RII levels were observed during the first week of infection in

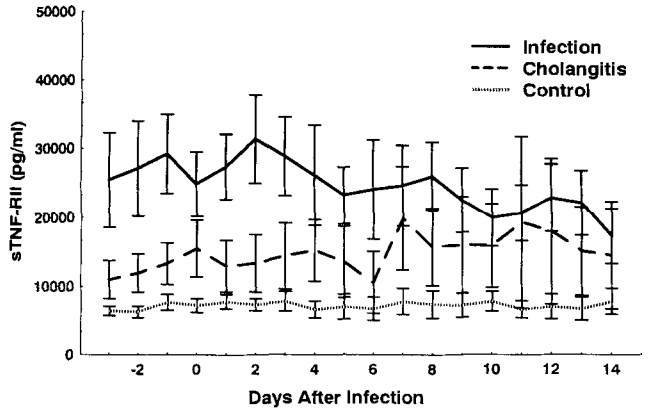

Fig. 2 Soluble tumor necrosis factor-RII *(sTNF-RII)* levels in patients with serious infection ($n = 10$), asymptomatic or mild cholangitis ($n = 11$), and an uneventful postoperative course ($n = 37$). $P \leq 0.01$ for serious infections versus cholangitis and uneventful postoperative course

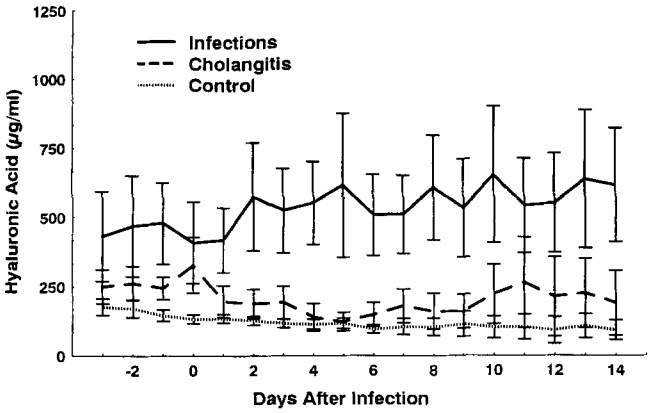

Fig. 3 Hyaluronic acid levels in patients with serious infection ($n = 10$), asymptomatic or mild cholangitis ($n = 11$), and an uneventful postoperative course ($n = 37$). $P \leq 0.01$ for serious infections versus cholangitis and uneventful postoperative course

four of five patients who died (26929 ± 6455 versus 20217 ± 5832 pg/ml in surviving patients). Soluble TNF-RII was significantly increased in all transplanted patients compared with healthy subjects.

IL-1β, IL-6, IL-10, and sIL-2R

IL-1β was virtually absent in all but four of five patients experiencing lethal infections (31.8 ± 19.2 pg/ml versus 2.1 ± 0.1 pg/ml in surviving patients; $P \leq 0.01$). IL-1β levels increased during the second week of infection and persisted until death. IL-6 was increased in patients with serious infections and cholangitis (191 ± 47.9 pg/ml and 220 ± 76.4 pg/ml, respectively) to a similar extent as that in patients with an uneventful postoperative course (35.1 ± 16.2 pg/ml). During the second and third week of infection, a progressive rise occurred in all five patients who died (384 ± 88.9 pg/ml versus 122 ± 31.3 pg/ml in surviving patients; $P \leq 0.01$). Mean levels of IL-10 were significantly elevated in patients with serious infections (114 ± 25.1 pg/ml; $P \leq 0.01$) compared with patients experiencing cholangitis (68.6 ± 24.8 pg/ml) and with an uneventful postoperative course (37.9 ± 9.7 pg/ml). A significant rise of IL-10 was observed during the entire infectious period in all five patients who died (152 ± 54.8 pg/ml versus 46.5 ± 12.9 pg/ml in surviving patients; $P \leq 0.01$). Soluble IL-2R was higher in patients with serious infections and cholangitis (3018 ± 698 U/l and 3829 ± 599 U/l, respectively) than in patients with an uneventful postoperative course (1460 ± 187 U/l). A further increase was observed in four of the five patients who died (3839 ± 897 U/l versus 2411 ± 743 U/l in surviving patients; $P \leq 0.01$).

Hyaluronic acid

Hyaluronic acid levels were significantly increased in all patients with serious infections (518 ± 94 µg/l; $P \leq 0.01$) compared with patients with cholangitis (227 ± 34 µg/l) and patients with an uneventful postoperative course (121 ± 26 µg/l) (Fig. 3). Differences between surviving and non-surviving patients with severe infections were minor (P not significant).

Soluble ICAM-1

Mean sICAM-1 levels were predominantly increased in patients with asymptomatic or mild cholangitis (1123 ± 189 ng/ml versus 908 ± 198 ng/ml in patients with severe infections and 690 ± 78.3 ng/ml in patients with an uneventful postoperative course). Slightly higher mean sICAM-1 levels were observed in patients with lethal infection.

Discussion

Within recent years, liver transplantation has become a highly successful treatment for end-stage liver disease and irreversible acute liver failure [8–11]. New approaches may now concentrate on patients at risk. Since atypical, fungal, and bacterial infections are still the predominant cause of death, in the present prospective study we attempted to analyze whether determination of new parameters, i.e., cytokines, adhesion molecules, and neopterin may be of value in improving the currently established monitoring of serious infections after liver transplantation.

Neopterin, an intermediate of tetrahydrobiopterin synthesis, is predominantly produced by interferon

(IFN)-γ-activated macrophages [12]. Neopterin increased more than 3 days prior to severe infection. Thus, neopterin may be considered as an early indicator of infectious complications after liver transplantation. The highest neopterin levels were observed in patients with a lethal outcome, while recovering patients showed a decrease in neopterin with recovery of clinical status. Besides retransplantation and, possibly, chronic rejection, which also led to a rise in neopterin levels, neopterin was highly specific for infection, which is in accordance with observations by others [13].

A variety of studies have shown an increase of the pro-inflammatory cytokines, IL-1β and TNF-α, in non-transplanted patients with serious infection, sepsis, and SIRS. In some studies, these cytokines and the counter-regulatory cytokine IL-6, correlated with severity of disease and ultimate outcome of infection [14, 15]. As a reflection of the activation of the pro-inflammatory cytokines, sTNF-RII increased significantly prior to serious infection. A further rise was observed in non-surviving patients, indicating a positive correlation of sTNF-RII increase with the severity of infection. Soluble TNF-RII has been shown to correlate with TNF-α production [16]. Because of its longer half-life, determination of sTNF-RII may more reliably reflect TNF-α production than determination of the cytokine itself. IL-1β was not detected except in four of five patients who died. This conforms to observations by others where, in non-transplanted, critically ill patients, although there was no increase in TNF-α and IL-1β levels during the early course of severe infection, high levels of the respective cytokine receptors were observed [17]. Vast production of endogen sIL-1Ra (receptor antagonist) may cope well with the early IL-1β production and may prohibit detection of IL-1β in these patients [18].

IL-6, which is known to increase after stimulation and secretion of endotoxin, TNF-α, IL-1β, and interferons, is produced by various cell types including endothelial cells and macrophages [19]. Although IL-6 levels failed to correlate with severity of infection, since it also increased during asymptomatic cholangitis, there was a marked IL-6 production during the late course of infection in all five patients who died. Increased sI-CAM-1 levels seemed to be more closely associated with cholangitis, although this group contained no patients with severe cholangitis. This may be explained by a high expression of ICAM-1 on bile ducts, as has been shown in liver biopsies during infection [20].

An increase of the downregulatory TH_2 cytokine, IL-10, was most pronounced in patients with lethal infection. IL-10, which is produced by monocytes and $CD4^+$ T-cell subsets [21, 22], is thought to have strong anti-inflammatory activity, by suppressing macrophage activity, as well as the release of reactive oxygen intermediates (ROI) and of reactive nitrogen intermediates (NO) [23, 24]. Furthermore, IL-10 has been shown to downregulate the activity of pro-inflammatory cytokines including TNF-α, IL-1β, and IL-8 [22, 25].

Next to stimulation of inflammatory cytokines, we also observed an increase in immunostimulatory TH_1 cytokines as reflected by an increase in sIL-2R in most patients with lethal infections. IL-1 and TNF have been shown to stimulate T- and B-lymphocytes and may thereby augment the specific immune response and IL-2 production [26]. The therapeutic dilemma of severe infection with signs of overimmunosuppression of the immunocompromized host and the stimulation of the specific immune response by the activated cytokine network may make selective inhibition of cytokines or their receptors desirable in the future.

Hyaluronic acid (HA), a high molecular weight protein, is located in the loose connective tissue at the inside of plasma membranes. It plays an important role in organisation of the extracellular matrix, cell-cell interactions, and inflammation [27–29]. HA increases upon stimulation with IL-1 [29]. Increased HA levels may most likely result from increased production by inflammatory cells such as fibroblasts [27, 28]. However, in patients with impaired septic liver dysfunction, a decreased catabolism of HA may also be possible, since liver endothelial cells are the predominant site of uptake and degradation of HA [27].

Neopterin and sTNF-RII are eliminated by the kidneys and acute renal failure may result in elevation of circulating plasma levels [3, 30]. There was indeed a positive correlation of both parameters with BUN and serum creatinine. However, there was no increase in neopterin and sTNF-RII levels in patients with ARF related to CsA toxicity or other origins than infection, indicating that the predominant source of neopterin and sTNF-RII during infection was an increased production.

Although most of these parameters are not specific for infections, we found a distinct timing and pattern of the respective parameters during infection and other diseases, including rejection. Furthermore, the extent of increase in neopterin and sTNF-RII levels detected patients at risk for lethal outcome as early as prior to, or with the onset of severe infection. In conclusion, we think that determination of cytokines, neopterin, and hyaluronic acid after liver transplantation is of great value and may influence postoperative management with respect to intensified infectious screening and earlier therapeutic approaches in high risk patients.

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