Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Retrograde reperfusion via vena cava lowers the risk of initial nonfunction but increases the risk of ischemic-type biliary lesions in liver transplantation – a randomized clinical trial

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Keywords
bile duct complication, ischemia and reperfusion injury, retransplantation.

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Received: 17 January 2006
Revision requested: 10 February 2006
Accepted: 1 May 2006

doi:10.1111/j.1432-2277.2006.00347.x

Summary

Initial nonfunction (INF) and biliary complications such as ischemic-type biliary lesion (ITBL) remain two major complications in clinical orthotopic liver transplantation (OLT). The influence of ischemia and reperfusion injury (I/R) as a significant risk factor for both complications is widely unquestioned. A new reperfusion technique that reduces I/R injury should lead to a reduction in both INF and ITBL. One hundred and thirty two OLT patients were included in this study and randomized into two groups. Group A underwent standard reperfusion with anterograde simultaneous arterial and portal reperfusion and group B received retrograde reperfusion via the vena cava before sequential anterograde reperfusion of portal vein and hepatic artery. Serum transaminase level as a surrogate parameter for I/R injury and serum bilirubin level as a parameter for graft function were significantly reduced during the first week after OLT in group B. INF rate was 7.7% in group A and 0% in group B ($P = 0.058$). ITBL incidence was 4.55% in group A versus 12.3% in group B ($P = 0.053$). Retrograde reperfusion seemed to be beneficial for hepatocytes, but was detrimental for the biliary epithelium. The unexplained increased incidence of ITBL after retrograde reperfusion will be focus of further investigation.

Introduction

Orthotopic Liver transplantation (OLT) is to date the most effective treatment of end stage liver disease being routinely carried out in specialized centers around the world. Nevertheless, a variety of complications can occur after initial successful transplantation. Although whole organ transplantations will always be confronted with ischemia and reperfusion injury (I/R) [1,2], there are also other complications to be considered such as initial nonfunction (INF) of the graft or ischemic-type biliary lesion (ITBL), where I/R is suspected to be a major risk factor [3–5]. The exact pathomechanisms for both of these complications remain, however, unclear.

Initial nonfunction occurs in 4.2–8.4% of patients after OLT, leads to graft loss and is an indication for immediate retransplantation [6–9]. Biliary complications have been reported to occur in approximately 10–20% with a sub-entity of ITBL occurring in 4–10% [10–13]. ITBL is the third most common reason for retransplantation. Both complications encounter major morbidity and mortality, create high costs and aggravate organ shortage [14,15].

In organ transplantation I/R is a well-studied phenomenon, and as mentioned above, is known as a significant risk factor for both complications [6,7,16,17]. Ischemia alone causes much less damage to the graft than the complex pathomechanisms during and after reperfusion. Oxy-

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gen free radicals are the center of detrimental factors. By minimizing the generation of oxygen free radicals, reperfusion injury was reduced in various experimental and clinical set ups [18–20].

To minimize graft damage during cold and warm ischemia, it is standard to re-establish hepatic blood flow as quickly and as forcefully as possible [21]. In the early phase of reperfusion, this leads to a massive production of oxygen free radicals [22]. Consecutively, a pronounced inflammation of the endothelium occurs, which can lead to an occlusion of small vessels and a decrease in perfusion called reperfusion paradox [23,24]. Simplified, a high flow of blood leads to a hypoperfusion of the graft via the detrimental effects of oxygen free radicals.

Various studies have reported that a reversed reperfusion might lead to a diminished production of oxygen free radicals. A retrograde reperfusion with low oxygenated blood after cardiac bypass operation leads to improved myocardial recovery time and a decreased myocardial cellular injury [25,26]. A retrograde reperfusion via the vena cava prior to anterograde reperfusion of hepatic artery and portal vein could also lead to a reduction of I/R in clinical liver transplantation. Slow elution of perfusion solution, slow re-warming and slow re-oxygenization with low-oxygenated blood might lead to a reduced production of oxygen free radicals. Tscheliessnigg presented data [27] with this new reperfusion technique in a retrospective study of 42 consecutive liver transplants. His group showed, that in contrast to the literature [28], circulatory problems or electrolyte imbalances after declamping of the anastomosis were very uncommon in their series. Postreperfusion syndrome occurred only in 3.6% following retrograde reperfusion compared with approximately 20% reported in the literature [29–31]. They reported no INF in their series.

In our study our hypothesis was, that a reduced I/R would reduce the incidence of complications like INF and ITBL where I/R represents a major risk factor.

In order to test the hypothesis we designed a randomized clinical trial with patients either receiving the standard reperfusion procedures with simultaneous anterograde reperfusion of the hepatic artery and portal vein, or retrograde reperfusion with venous blood via the hepatic veins followed by sequential anterograde reperfusion of the portal vein and the hepatic artery.

Patients and methods

Patients and clinical design

Between December 2001 and January 2004, 244 consecutive OLT were performed at the Charité, Campus Virchow-Clinic, Berlin (OLT-numbers 1385–1629). One hundred and thirty one adult patients were randomized and accepted into the controlled clinical trial. Patients receiving re-transplantations ($n = 29$), living related liver transplantation ($n = 33$), multivisceral ($n = 1$), acute liver failure ($n = 16$), pediatric transplantation ($n = 16$), combined liver/kidney transplantation ($n = 9$) and patients who denied informed consent ($n = 9$), were excluded from the study. Patients were randomized into either group A or B by drawing an envelope before transplantation, after having given their written informed consent. Investigators were not blinded for the randomization. The study was conducted in accordance to the Declaration of Helsinki and approved by the local ethics committee.

Group A underwent OLT as standard procedure with supra and infra hepatic end-to-end cava anastomosis before simultaneous reperfusion via the hepatic artery and portal vein with flushing autologous blood through the infra-hepatic vena cava. Simultaneous reperfusion is the standard procedure at our institution. As described later, simultaneous anterograde reperfusion seems superior to initial portal reperfusion according to the literature and our own experimental data [21,32]. Group B underwent retrograde low-pressure reperfusion with low oxygenated blood from the vena cava as previously described [27]. After establishing the caval anastomosis, the infrahepatic and suprahepatic clamps were removed. While completing the portal anastomosis the graft was reperfused via the hepatic veins with low oxygenated venous blood. Initial portal reperfusion of the graft was started to prevent stasis and clotting of blood in the graft followed by arterial reperfusion. During the anastomosis of the hepatic artery the graft was only perfused via the portal vein, keeping in mind that group A and B differ in two variables, anterograde versus retrograde and sequential versus simultaneous reperfusion. With regard to the patients, and as there are no data available on simultaneous reperfusion combined with retrograde reperfusion (with stasis of blood during one of the anastomosis) we adopted the published safe technique.

Donor characteristics like gender, age, causes of brain death, length of stay on the intensive care unit (ICU), perfusion solution, serum sodium, bilirubin, serum alanine aminotrasferase (ALT), gamma glutamyltransferase (γGT) and gluamate dehydrogenase (GLDH) and own versus shipped organs were evaluated. All organs procured by a local team underwent arterial pressure perfusion and thorough rinsing of the biliary tract with preservation solution.

Patient characteristics such as age, gender and indication for transplantation were assessed. Duration of transplantation procedure, warm and cold ischemia, use of extra corporeal bypass and mean blood loss were compared between groups. In Group A warm ischemic time

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(WIT) was defined as the duration from the start of the suprahepatic anastomosis until simultaneous reperfusion via hepatic artery and portal vein. In Group B WIT was defined as time from start of the caval anastomosis until declamping of the infrahepatic and suprahepatic vena cava. The duration of retrograde reperfusion and the time until declamping of portal vein or the time until declamping of hepatic artery was not evaluated. In other words, WIT was defined in both groups from time the organ was placed in the recipient until the time of any kind of reperfusion.

Immunologic factors like rejection episodes, grade of rejection and use of anti rejection therapy such as prednisolone or anti-CD3 monoclonal antibody (OKT-III) were compared between groups.

Reperfusion injury was assessed by serum levels of ALT, GLDH and bilirubin. Postoperative graft function was represented by plasmatic coagulation (prothrombin time, PT) and bile flow via a T-tube. PT was affected by administration of frozen fresh plasma products. Postoperative complications like hepatic artery thrombosis, portal vein thrombosis, rates of INF and initial poor function (IPF) were examined. INF was defined as cryptogenic liver failure, which required immediate retransplantation after exclusion of any vascular thrombosis or technical complication. Immunological reasons for suspected INF were ruled out by postexplantation pathology. For the more complex definition of IPF we used the one by Deschenes *et al.* [33]: the presence of at least one of the following between day 2 and 7 after liver transplantation: serum bilirubin >10 mg/ml (in noncholestatic liver diseases), plasmatic coagulation of <50% or hepatic encephalopathy. In all IPF cases patients had a prolonged hepatic dysfunction because of cryptogenic reasons and a prolonged convalescence.

Ischemic-type biliary lesion was defined as nonanastomotic intra or extra hepatic biliary strictures without any history of hepatic artery complications, ABO-incompatibility or other known causes of bile duct damages. Diagnosis of ITBL was always established by endoscopic retrograde cholangiography or percutaneous transhepatic cholangiography. All patients developing INF or ITBL were analyzed regardless of anterograde or retrograde reperfusion. Donor criteria and recipient data were evaluated. Pre-transplant and post-transplant laboratory values were assessed.

Liver transplantation

Orthotopic liver transplantation was performed using a standardized surgical technique with veno-venous bypass followed by reperfusion according to the study group, and by side-to-side choledochocholedochostomy for biliary reconstruction with insertion of a T-tube [11,34]. Intraoperatively, aprotinin was administered as a bolus of 500 000 KiU and subsequently as continuous infusion at 100 000 KiU/h to avoid the reperfusion fibrinolysis, and thereby reducing the necessity of a perioperative blood transfusion [35,36]. In patients with a normal graft function, the T-tube was closed after control cholangiography on postoperative day (POD) 5 and removed after 6 weeks.

Immunosuppressive regimen and concomitant treatment

Patients received steroids and a tacrolimus based immunosuppression with IL-2 antibody (20 mg basiliximab) induction therapy on POD 0 and POD 5. From POD 0 to POD 5 the infection prophylaxis consisted of perioperative systemic IV antibiotics (3×500 mg metronidazole plus 4×1 g ceftriaxone). For prophylaxis of herpes or cytomegalovirus (CMV) oral aciclovir (3×200 mg) was administered. Prophylaxis of pneumocysitis carinii pneumonia consisted of oral cotrimoxazol, 960 mg, $3 \times$ /week for 6 weeks. Ursodesoxycholic acid, vitamins and minerals were given to all patients for 4–6 weeks postoperatively. Patients with hepatitis B-related liver disease were treated during the anhepatic time with 10 000 IU of anti-HBs hyperimmunoglobuline (HBIG) and 2000 IU daily until seroconversion was detectable. Thereafter, HBIG was administered to ensure plasma levels of more than 150 U/ml.

Management of rejection

Acute rejection was suspected with fever, leukocytosis, change in color and amount of bile, and when rise of serum transaminases and rise of serum bilirubin was observed. Rejections were confirmed by percutaneous liver biopsy. Clinical and histological findings determined the diagnosis of acute rejection. All graft biopsies were performed as core biopsies with a 1.6-mm menghini needle, processed routinely, stained with hematoxylin and eosin, Masson–Goldner, periodic acid-Schiff, and iron staining and evaluated by the pathologist blinded to clinical information. Histological findings were divided into four grades: aR 0, no evidence of rejection; aR I, mild periportal mononuclear infiltrate with minimal endothelitis and minimal bile duct injury without hepatocyte necrosis; aR II, moderate periportal mononuclear infiltrate extending beyond the portal triad, marked endothelitis, marked bile duct injury and single cell hepatocyte necrosis; aR III, the same alterations as described in II plus severe injuries and massive hepatocyte necrosis [37,38].

First episodes of any grade of rejection were treated with steroids (3×500 mg methylprednisolone). Ongoing

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biopsy-proven steroid-resistant rejections were treated with OKT III (5 mg for 5–7 days).

Clinical monitoring and follow-up

Routine intra- and postoperative hemodynamic monitoring included central venous catheter and invasive arterial blood pressure control. Whenever possible, respirator treatment was withdrawn immediately after surgery. Tubes, drains and lines were removed between POD 3–7. Liver enzymes (ALT, GLDH, γGT, bilirubin, alkaline phosphatase), tacrolimus blood levels and other routine laboratory parameters were analyzed daily until POD 12 and then three times a week until discharge. After discharge all patients were admitted to our center 6 months later and at yearly intervals.

Statistical analysis

All statistical calculations were performed in SPSS 11.3© (SPSS Inc., Chicago, IL, USA). Data were given as mean values ± standard error of mean (SEM). Descriptive statistics were used to summarize the donor and recipients characteristics. For independent variables, cross tabulations, ANOVA and the chi-square test were performed. Nonparametric variables were evaluated with Mann–Whitney *U*-test and asymptotic significance was calculated.

All tests performed were two-sided. *P*-values of $P < 0.05$ were considered as statistically significant. All calculations were performed in association with the Department of Biometrical Medicine of the Humboldt University of Berlin.

Results

Patient characteristics

A total of 131 patients were randomized of whom 66 patients received an anterograde reperfusion procedure (group A) and 65 a retrograde reperfusion (group B). Patient follow-up was 12–36 months (mean 27 months). No patient was lost to follow-up. Indications for OLT are given in Table 1, with both groups having comparable characteristics. In group A there were more patients with primary biliary cirrhosis (PBC, $n = 6$) than in group B ($n = 3$). There was no higher incidence of ITBL in PBC patients. Mean patient age was 51 ± 9 years in group A and 52 ± 10 years in group B. Mean duration of operation was 303 ± 68 min in the anterograde group and 286 ± 65 min in the retrograde group. Mean WIT (as defined earlier) was 49 ± 12 min in group A and 32 ± 8 min in group B ($P < 0.001$). Mean blood loss was comparable in both groups with <1.5 l. In the standard group the use of extracorporeal bypass was 100% and in the retrograde group only 91% ($P = 0.013$). Details of surgical procedures are depicted in Table 1.

Table 1. Recipient characteristics.

Variables	Anterograde reperfusion	Retrograde reperfusion	*P*-value
n	66	65	
Recipients gender			
Male	44 (67%)	41 (64%)	0.559
Female	22 (33%)	24 (36%)	
Recipients age	51 ± 9	52 ± 10	0.737
Underlying disease			
Alcoholic liver disease	22 (33%)	25 (38%)	0.123
Hepatitis B related cirrhosis	7 (11%)	7 (11%)	
Hepatitis C related cirrhosis (incl. HCV + HBV)	11 (17%)	10 (15%)	
Hepatocellular carcinoma	9 (13%)	8 (12%)	
Primary sclerosing cholangitis	6 (9%)	3 (5%)	
Primary biliary cirrhosis	4 (6%)	0	
Polycystic liver disease	1	1	
Budd–Chiari disease	0	1	
Cryptic liver disease	3 (5%)	2 (3%)	
Others	3 (5%)	8 (12%)	
Duration of operation (min)	303 ± 68	286 ± 65	0.174
Use of extracorporeal bypass (%)	100%	91%	**0.013**
Cold ischemia (min)	557 ± 147	511 ± 146	0.094
Warm ischemia (min)	49 ± 12	32 ± 8	**<0.001**
Blood loss (ml)	1450 ± 1290	1300 ± 1010	0.798

There were no major differences in patient demographics. Extracorporeal veno-venous bypass was used signicantly less frequent in the retrograde group ($P = $ **0.013**). Warm ischemic time was significantly shorter in the retrograde group, but this was also due to definition (see text) ($P <$ **0.001**).

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Patient and graft survival

Actual patient survival 1 and 3 years after OLT was 95.5% and 92.4% in group A, and 93.8% and 89.2% in group B. Actual graft survival 1 and 3 years after OLT was 87.9% and 84.4% in group A, and 89.2% and 86.2% in group B.

Twelve patients died during this study, five in group A and seven in group B. This difference did not reach statistical significance. Causes of death were recurrent hepatocellular carcinoma 8, 18, 24 and 27 months after OLT, recurrent hepatitis C (HCV) infection 9 months after OLT, ITBL 36 month after OLT, myocardial infarction in two cases 7 and 16 months after OLT and multi organ failure (MOV) with bacterial infection 4 and 6 months after OLT and MOV following retransplantation on POD 1 and 25 after re-OLT. There were no significant differences between groups.

Rejection episodes

Biopsy proven rejection occurred in 27.5% of the patients in group A and in 24.5% in group B. Thirty-four liver biopsies with suspicion of acute rejection were performed in group A and 27 in group B without any further complications. There were no differences in grade of rejection or treatment of rejection between groups, except for two OKT-III treatments in group A because of ongoing rejection and four OKT-III treatments in group B. All patients recovered from the rejection episodes without any graft loss (Table 2).

None of the patients from both groups developed chronic rejection within the observation time and vanishing bile duct syndrome was neither suspected nor diagnosed.

Table 2. Rejection and anti-rejection therapy.

Variables	Anterograde reperfusion	Retrograde reperfusion	*P*-value
n	66	65	
No rejection	45 (68%)	46 (70%)	0.765
Rejection grade I	11 (17%)	10 (15%)	
Rejection grade II	6 (9%)	5 (8%)	
Rejection grade III	1 (1.5%)	1 (1.5%)	
1 × liver biopsy	20 (30%)	17 (26%)	0.863
2 × liver biopsies	7 (11%)	5 (8%)	
Prednisolone 3 × 500 mg	18	18	1.000
OKT-III	2	4	0.653

Rejection episodes were classified according to the Banff criteria (see also text)OKT-III; anti-CD-3 monoclonal antibody therapy. ns, nonsignificant; $P > 0.05$.

Donor and graft characteristics

Donor age was 53 ± 15 years in group A and 49 ± 17 years in group B. In group A 48% of the donors were male and 60% in group B. Donors of group A had a longer stay on the ICU prior to organ harvest with 5.7 ± 6 days vs. 4 ± 5.5 days in group B. Donor serum sodium, ALT, total and direct bilirubin and alkaline phosphatase were similar prior to organ harvest as shown in Table 3. There were no differences in the causes of brain death between both groups.

In group A 56% of the organs were harvested by a local team compared with group B where only 38% of the organs were harvested locally ($P = 0.055$). There were no graft damages reported during back table preparation of shipped organs (i.e. a cut artery). In both groups University of Wisconsin (UW) solution was used in more than 70% of patients (Table 3).

Graft function and complications

Early postoperative graft function was excellent in both groups. Bile production, serum bilirubin and post-transplant PT showed no statistical differences between groups (Figs 1, 2 and 3). Mean bile flow was 230 ± 157 ml on POD 1 in group A and 229 ± 142 ml in group B, and

Table 3. Donor characteristics.

Variables	Anterograde reperfusion	Retrograde reperfusion	*P*-value
n	66	65	
Donors age	53.5 ± 15.1	49.2 ± 17.4	0.130
Donors gender (m/f)	32/34	39/26	0.227
Shipped organs (yes/no)	37/29	25/40	0.055
Perfusion solution (HTK/UW)	18/48	17/48	1.000
Stay on ICU prior to organ harvest (days)	5.7 ± 6.1	4.0 ± 5.5	0.104
Cause of death			
Subarachnoiale bleeding	36 (54%)	36 (54%)	0.641
Brain trauma	14 (21%)	13 (21%)	
Hypoxia	6 (9%)	6 (9%)	
Cerebral infarction	8 (12%)	8 (12%)	
Myocardial infarction	2 (3%)	2 (3%)	
Donors laboratory before organ harvest			
Bilirubine (total) (mg/dl)	3.0 ± 5.0	3.9 ± 7.7	0.548
Bilirubine (direct) (mg/dl)	2.1 ± 2.8	1.33 ± 1.7	0.265
AST (IU/ml)	45.5 ± 39.4	41.1 ± 39.4	0.661
γGT (IU/ml)	64.4 ± 117.6	73.8 ± 159.3	0.358
AP (IU/ml)	121.3 ± 90.5	106.8 ± 113.8	0.259
Na^+ (mmol/l)	143.8 ± 20.0	149.1 ± 16.2	0.389

There were no statistical differences between anterograde and retrograde group in regard of donor and graft criteria. HTK, histidine-tryptophane-ketoglutarate solution; ICU, intensive care unit; ns, nonsignificant; $P > 0.05$; γGT, gamma glutamyltransferase; AP, alkaline phosphatase.

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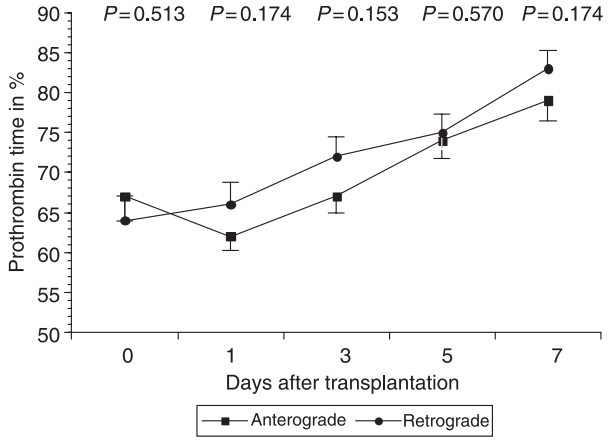

Figure 1 Plasmatic coagulation (PT) after transplantation. There are no statistical significant differences between groups. PT is influenced by use of fresh frozen plasma, which was not examined. Expressed as mean ± SEM, ANOVA test.

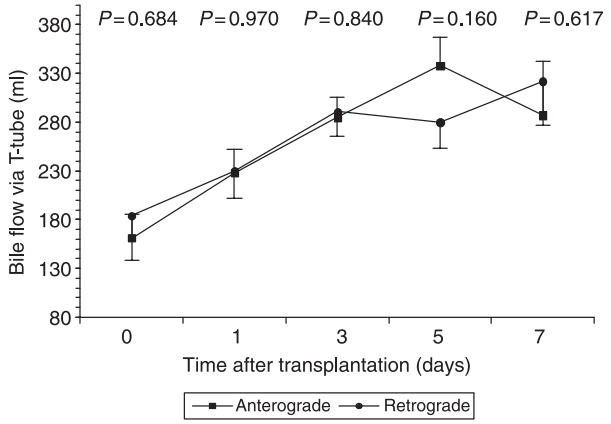

Figure 2 Postoperative measurements of bile flow via T-tube as a parameter for hepatic graft function. There were no significant differences between groups. Expressed as mean ± SEM, ANOVA test.

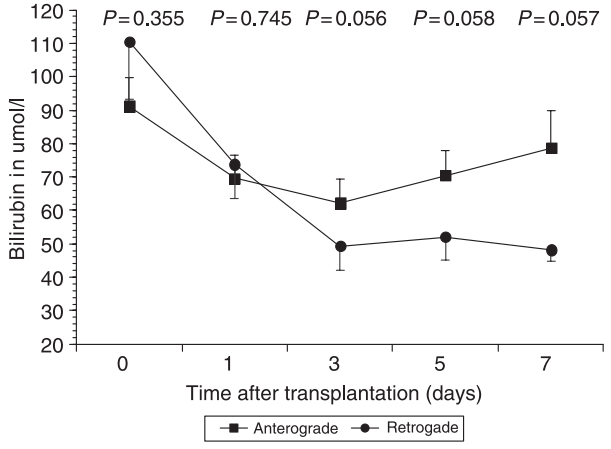

Figure 3 Serum bilirubin as a marker for hepatic graft function. There were no significant differences between groups. Expressed as mean ± SEM, ANOVA test.

322 ± 197 ml vs. 287 ± 223 ml on POD 7 (Fig. 2). As surrogate marker for I/R alanine aminotransferase (ALT) was significantly elevated in group A on POD 0, 1, 3 and 5 compared with group B ($P < 0.05$) (Fig. 4). The difference of GLDH was only significant on POD 5 ($P = 0.024$) (Fig. 5).

Initial nonfunction occurred exclusively in the anterograde reperfusion group A with an incidence of 7.7% of patients. However, in all instances immediate retransplantation was successful. Retransplantation rate of group A was 9.1% (one case of portal thrombosis and five INF-patients) and 4.6% in group B (one retransplantation due to ITBL, two due to hepatic artery thrombosis). Both, incidence of INF and rate of retransplantation were not statistically significantly different between groups ($P =$

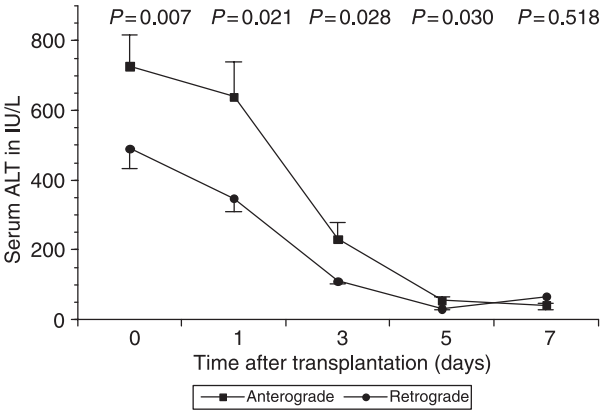

Figure 4 Postoperative serum ALT from postoperative day (POD) 0 until POD 7. ALT was significantly increased as a sign of hepatocellular damage because of ischemia and reperfusion injury (I/R) in the anterograde group within the first 5 days after orthotopic liver transplantation. Retrograde reperfusion decreases I/R. Expressed as mean ± SEM, ANOVA test.

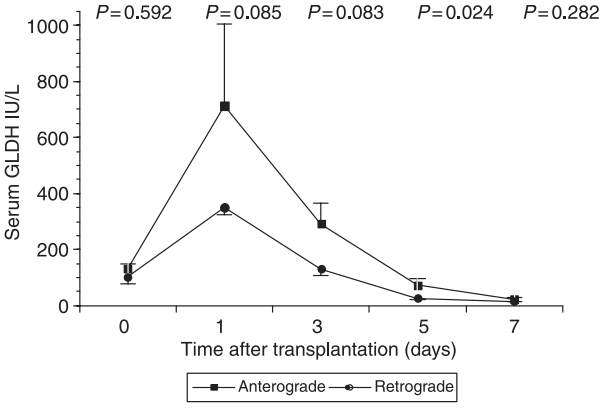

Figure 5 Serum glutamate dehydrogenase after transplantation. Only difference on postoperative day 5 was statistical significant gluamate dehydrogenase is a marker for hepatocellular damage due to ischemia and reperfusion injury. Expressed as mean ± SEM, ANOVA test.

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0.058 and $P = 0.208$, respectively). In group A IPF was evident in 21 cases (31.3%), in group B it was however only evident in nine cases (13.8%) ($P = 0.022$) (Table 4).

Table 4. Complications after transplantation.

Variables	Anterograde reperfusion	Retrograde reperfusion	*P*-value
n	66	65	
ITBL	2 (3%)	8 (12.3%)	0.053
Hepatic artery thrombosis	2 (3.0%)	2 (3%)	1.000
Portal vein thrombosis	1 (1.5%)	0	1.000
INF	5 (7.6%)	0	0.058
IPF	21 (31%)	9 (13.4%)	**0.022**
Re-transplantation	6 (9.1%)	3 (4.6%)	0.115
1-year patient survival	95.5%	93.8%	1.000

The incidence of ITBL in the retrograde reperfusion group was 12.3% vs. 3% in the retrograde group. INF was only present in the anterograde group. INF, initial non function, ITBL, ischemic-type biliary lesion; IPF, initial poor function. IPF occurred significantly more often in the anterograde group ($P = \mathbf{0.022}$).

Table 5. Complications: (a) INF patients versus non-INF patients, (b) ITBL patients versus non-ITBL patients.

Variables	INF Patients	Non–INF Patients	*P*-value
(a)			
n	5	126	
Donors age	60.4 ± 17.3	51.1 ± 16.3	0.569
Warm ischemia (min)	60 ± 22	41 ± 13	0.090
Cold ischemia (min)	592 ± 174	532 ± 147	0.221
Duration of operation (min)	345 ± 87	293 ± 66	0.125
Na^+ (mmol/l)	145 ± 11.5	146 ± 18.5	0.982
Recipients gender (m/f)	2/3	83/46	1.000
Shipped organs (yes/no)	4/1	69/60	0.106
Perfusion solution (HTK/UW)	1/4	34/92	0.575
Stay on ICU (days)	7.5 ± 7.6	4.7 ± 5.7	0.444
	ITBL Patients	Non–ITBL Patients	
(b)			
n	11	120	
Donors age	52.0 ± 17.1	51.3 ± 16.4	0.886
Warm ischemia (min)	37 ± 12	42 ± 14	0.152
Cold ischemia (min)	569 ± 187	531 ± 144	0.198
Duration of operation (min)	275 ± 66	296 ± 67	0.353
Na^+ (mmol/l)	145 ± 9.4	147 ± 18.7	0.447
Recipients gender (m/f)	5/6	80/40	0.327
Shipped organs (yes/no)	3/8	59/61	0.100
Perfusion solution (HTK/UW)	2/9	33/87	0.463
Stay on ICU (days)	2 ± 1.4	5.0 ± 6.0	**0.050**

Patients that developed initial nonfunction (INF) or ischemic-type biliary lesion (ITBL) were evaluated separately versus patients that were free of these complications. INF patients had a longer WIT, but this difference was not significant. Organs that developed ITBL had a significant shorter stay on ICU compared with organs without ITBL. ICU, intensive care unit; ns; not significant; $P > 0.05$. Grafts that developed ITBL had a significantly shorter length of stay on ICU ($P = \mathbf{0.050}$).

Patients who developed INF were compared with those without INF. INF patients had a mean WIT of 60 ± 22 min compared with 41 ± 13 min of those without ($P = 0.090$). Their donors stayed longer in the ICU with 7.5 ± 7.6 days vs. 4.7 ± 5.7 days ($P > 0.05$). Data are shown in Table 5.

Ischemic-type biliary lesion rate of group A was 3% compared with 12.3% of group B. This difference was not significant ($P = 0.053$). One patient with ITBL died of recurrent cholangitis and sepsis with MOV 35.6 months after OLT. One patient with ITBL was placed on the waiting list for a retransplantation. The remaining patients are receiving individual endoscopic therapy with elective or emergency interventions.

Patients with ITBL showed no significant differences in donor or recipient characteristics compared with the remaining patients in this study, except for the donors stay in ICU which was shorter in the ITBL patients compared with the other patients. There were less shipped organs in the ITBL group (27%) than in the non-ITBL group (50%) ($P = 0.214$). Data are shown in Table 5.

Discussion

In this present study a strong correlation was found between the recently introduced retrograde reperfusion technique and the absence of INF. Serum transaminases, being indicators for hepatocellular damage and I/R, were significantly reduced after retrograde reperfusion. There were no differences observed in the two groups with regard to patient survival, graft survival and allograft rejection. In contrast, we documented an increased incidence of ITBL of 12.3% after retrograde reperfusion ($P = 0.053$). Both groups had comparable donor and recipients characteristics.

Experimental studies have shown, that the complex pathomechanisms involved during reperfusion are mainly responsible for cellular injury after liver transplantation compared with ischemic injury alone [2]. Cellular injury is mediated by cytokines, interleukins, adhesion molecules and cellular systems (Kupffer's stern cells, lymphocytes) [39–41]. Oxygen free radicals play a crucial role during the early phase of reperfusion. Research has focused on minimizing cellular damage caused by oxygen free radicals by either interfering with their production or by promoting a variety of salvage systems. Many strategies have been described but up until now, no accepted routine protocol has been established yet.

The question of the ideal technique of reperfusion in liver transplantation is still a matter of debate. It remains unknown which sequence of reperfusion could possibly diminish reperfusion injury best: arterial before portal, portal before arterial or simultaneous reperfusion? It was

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hypothesized that the oxygenized arterial blood of the hepatic artery may contribute to the generation of oxygen free radicals [42–44]. While some investigators could not show any differences between sequential and simultaneous hepatic reperfusions [45] others demonstrated that simultaneous hepatic artery and portal vein reperfusion showed better perioperative and postoperative results [46]. When grafts were reperfused simultaneously, both nonperfused acini and nonperfused sinusoides were significantly reduced by 71–78%. Leukocyte accumulation in simultaneous reperfused sinusoids and postsinusoidal venuoles was decreased by 17% and 64%, respectively [21]. On the other hand, several clinical studies have shown an advantage for liver allografts being re-arterialized before portal reperfusion [47–50]. One experimental study showed that the major part of reperfusion injury is constituted during the portal venous reperfusion and that this injury can be, at least partially, attenuated by initial arterial reperfusion [51]. Other authors tested a prereperfusion with either dextrose 5% or Ringer's lactate solution prior to reperfusion, but could not show a significant benefit [52,53]. Simultaneous reperfusion is the standard procedure in our institution.

In 2003, Tscheliessnigg and colleagues [27] reported a new reperfusion concept. Instead of putting emphasis on the sequence of anterograde reperfusion, they described a technique of an initial reversion of graft reperfusion via the vena cava, followed by a anterograde sequential reperfusion of portal vein and hepatic artery. They hypothesized that low-pressure perfusion with low oxygenated blood reduces the production of oxygen free radicals. They showed in their series which is in contrast to the literature [28], that circulatory problems or electrolyte imbalances after declamping were very uncommon. Post reperfusion syndrome occurred only in 3.6% of patients after retrograde reperfusion [29] compared with approximately 20% reported in the literature [30,31]. They also reported no cases of INF in their series. This safe technique of retrograde reperfusion was used in our study. We were conscious about the fact, that group A and B differed in two variables, direction and timing of reperfusion. However, as there are no data on simultaneous retrograde reperfusion, safety for the patient was put first.

The pathophysiology of INF is still not clearly understood. Incidence of INF has been studied in many series and varies between 4.2% and 8.4% [6–9]. Cold ischemic time (CIT), steatosis hepatis, cause of brain death, donor sodium and renal insufficiency were described as risk factors [6–8,54]. We did not evaluate steatosis hepatis in this study and all other described factors were statistically not relevant.

Initial poor function after OLT is an ubiquitously observed phenomenon, which is; however, poorly defined in the literature [33,55]. IPF occurred significantly more frequently in the anterograde reperfusion group with 31% vs. 13.4% in the retrograde group. The reported incidence of IPF lies between 20% and 30% [54,56]. IPF can be criticized as a poor marker because of its difficult definition. On the other hand, it is a ubiquitously observed phenomenon and the incidence of IPF in the anterograde group was very consistent with the literature. Thus, the definition somehow fulfilled its purpose. Markers of hepatocellular injury (ALT and GLDH) were significantly decreased in the retrograde reperfusion group. The hypothesis, that warm and low oxygenated blood reduces the reperfusion injury by reducing oxygen free radicals is strongly supported by the presented study.

However, retrograde reperfusion seemed to have a detrimental effect on the biliary epithelium or other cells of the biliary tract. Incidence of ITBL varies in the literature between 2% and 20% [4,17,57,58]. Ischemia after hepatic artery thrombosis leads to similar pathomorphological findings [59]. The incidence of ITBL in our own patient cohort (1800 consecutive OLT) was 4.2%. The incidence in the anterograde reperfusion group was very comparable with 3%. However, in the retrograde reperfusion group we however documented an incidence of 12.3% ($P = 0.053$). In one series the rate of ITBL was elevated if sequential portovenous reperfusion was started prior to arterial reperfusion [32]. There were 34.6% biliary complications after initial portal revascularization documented versus only 2% after simultaneous portal and arterial revascularization [32]. The sequence of initial portovenous reperfusion also occurred in the retrograde reperfusion group to prevent stasis and thrombosis of blood after retrograde reperfusion. It is hypothesized that thrombosis of small arterioles of the biliary tract may be the cause of ITBL. It is possible, that low-pressure retrograde reperfusion leads to thrombosis of small arterioles of the peri-biliary plexus. The bile ducts are exclusively perfused by the hepatic artery, which leads to an extensive WIT of the biliary tract during retrograde reperfusion and portal reperfusion. However, we documented no statistical differences of hepatic artery thrombosis between groups. CIT has been described in many other studies as a relevant risk factor for the development of ITBL. The probability for the development of ITBL significantly increased in UW perfused grafts after 13 h of preservation ($P < 0.01$) [15,60]. If cold, warm and total ischemic time of patients with or without ITBL were compared in one clinical study, all parameters demonstrated significant differences [5]. A CIT of more than 12 h was a risk factor for ITBL [10]. We therefore examined donor and recipient factors of all patients who developed ITBL in their post transplant course and compared these data with the

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rest of the patients in this study. We could see no significant difference in any of the proposed risk factors, which could mean that the observed differences between groups are due to the new reperfusion technique.

The results of this study underline the fact that the outcome after liver transplantation is influenced by multifactorial events. We demonstrated in our study that reperfusion injury and subsequently the incidence of INF could be reduced by the introduction of retrograde reperfusion via the vena cava. The unexplained increased risk of ITBL will be subject to further investigations. As all patients with INF received successful high urgency retransplantation, however all patients with ITBL experienced major morbidity or even death, simultaneous anterograde reperfusion still remains the standard procedure at our institution.

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