TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Similar outcome after transplantation of moderate macrovesicular steatotic and nonsteatotic livers when the cold ischemia time is kept very short

Andrie C. Westerkamp,[1] Marieke T. de Boer,[1] Aad P. van den Berg,[2] Annette S. H. Gouw[3] and Robert J. Porte[1]

1 Section of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
2 Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
3 Department of Pathology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Keywords
cold ischemia time, donor, liver transplantation, steatosis, survival.

Correspondence
Robert J. Porte MD, PhD, Section of Hepatobiliary Surgery and Liver Transplantation, Department of Surgery, University of Groningen, University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands.
Tel.: 31 50 3612896;
Fax: 31 50 3614873;
e-mail: r.j.porte@umcg.nl

Conflicts of interest
The authors have declared no conflicts of interest.

Received: 5 September 2014
Revision requested: 9 October 2014
Accepted: 5 December 2014
Published online: 29 December 2014

doi:10.1111/tri.12504

Summary

Background: Livers with moderate (30–60%) macrovesicular steatosis have been associated with poor outcome after transplantation. Aim of this study was to examine the outcome after transplantation of livers with moderate macrovesicular steatosis when the cold ischemia time (CIT) is kept very short.
Methods: Postoperative outcome of 19 recipients of a moderate steatotic liver were compared with a matched control group of 95 recipients of a nonsteatotic liver graft (1:5 ratio). We studied graft/patient survival rates, incidences of primary nonfunction, postoperative complications (classified according to the Clavien–Dindo classification), first-week postoperative hepatic injury serum markers (AST/ALT), and liver function tests (PT time/bilirubin/lactate). In addition, we studied reversal of graft steatosis in follow-up biopsies.
Results: Median CIT in livers with moderate steatosis and in controls was below 8 h in both groups. Although short- and long-term patient/graft survival rates and results of liver function tests were similar, serum markers of hepatic injury and postoperative complications (especially grade IVa) were significantly higher in recipients of a moderate steatotic liver. Reversal of steatosis was seen in 9 of the 11 (82%) recipients with follow-up liver biopsies.
Conclusion: Despite the association with severe postoperative complications, moderate macrovesicular steatotic livers can be used successfully for transplantation if the CIT is kept very short.

Introduction

The success of orthotopic liver transplantation (OLT) has resulted in an expanding demand of liver grafts, which is not balanced by an increase in donation of livers. The annual report of the Eurotransplant International Foundation 2013 shows a growing difference between available livers, and the number of patients awaiting a new liver [1]. To reduce the present imbalance between organ demand and supply, many centers have been using extended criteria donor (ECD) livers. ECD livers are livers associated with a higher postoperative risk of graft failure, compared with livers from optimal or reference donors [2,3].

Hepatic steatosis has been identified as an important risk factor for graft failure after transplantation because of a higher susceptibility for ischemia/reperfusion (I/R) injury [4–6] Therefore, steatosis has been considered as one of the most important criteria to classify a donor liver as an ECD liver [7]. Based on histology, steatosis can be divided into macrovesicular and microvesicular steatosis. Macrovesicular steatosis can be subcategorized as mild (>10–30%), moderate (between 30% and 60%), or severe (>60%),

depending on the number of hepatocytes with fat accumulation [8]. Macrovesicular steatosis is considered to be a more important risk factor for graft failure than microvesicular steatosis. In particular, severe macrovesicular steatosis has been associated with a higher prevalence of primary nonfunction (PNF) and unfavorable patient and graft survival rates [9–15]. Because of these poor results, livers with a severe degree of macrovesicular steatosis are usually not accepted for transplantation. On the other hand, livers with moderate macrovesicular steatosis seem to be suitable for transplantation if no other risk factors for poor postoperative outcome are present [16–21]. But, many centers will not accept moderately steatotic livers for transplantation because of the greater susceptibility to I/R injury and the perceived negative impact on postoperative outcome [22].

We hypothesized that donor livers with moderate macrovesicular steatosis may have similar outcomes as nonsteatotic livers, if the degree of I/R is minimized by keeping the cold ischemia (CIT) as short as possible. The aim of this study was to examine the effect of moderate steatotic liver grafts on outcome after OLT when all possible efforts are taken to keep the CIT below 8 h.

Postoperative outcome was assessed by determining patient and graft survival rates, as well as surgical complications according to the Clavien–Dindo classification [23], and by studying first-week postoperative hepatic injury serum markers (AST/ALT), and liver function tests (prothrombin time/bilirubin/lactate). In addition, we have investigated the possible reversal of steatosis on follow-up liver biopsies after transplantation.

Patients and methods

Study population

Between the first of January 2000 and 31 of December 2012, our center received 2468 donor liver offers, of which 657 liver grafts were accepted for transplantation. Seven percent of the unaccepted livers were turned down because of the combination of suspected severe graft steatosis in combination with other donor risk factors. After excluding pediatric transplants, retransplantations, and combined organ transplantations, 373 adult patients undergoing a first OLT formed the first basis of this study. Follow-up was until the first of July 2013, allowing a minimal follow-up period of half a year in all patients.

Among the 373 patients, biopsies were taken from the donor liver at the time of transplantation in our center in 319 (86%) cases. One hundred and twenty-six patients received a liver with a certain degree of steatosis. These 126 patients were categorized according to the percentage of macrovesicular steatosis and included in one of the following subgroups; low (<10%; $n = 86$), mild (10–30%; $n = 21$), and moderate (30–60%; $n = 19$) macrovesicular steatosis. During the study period, no grafts were transplanted with more than 60% macrovesicular steatosis.

To examine the effect of moderate macrovesicular steatosis (30–60%) on outcome after transplantation, patients who received a graft with moderate macrovesicular steatosis were matched at random in a 1:5 ratio with control patients, who received a liver without any degree of steatosis in the same time period. The two groups were matched for type of donation [donation after circulatory death (DCD) versus donation after brain death (DBD)], laboratory model for end-stage liver disease (MELD) score, CIT, recipient age, body mass index (BMI), and status on the waiting list of Eurotransplant (high urgent versus elective). The laboratory MELD score was calculated on the day of OLT, disregarding extra points for standard or nonstandard exceptions. According to national legislation, this type of retrospective analysis using anonymous data is allowed in the Netherlands and does not require informed consent from the individual patients.

Surgical procedure and logistics

Our institutional transplantation protocol contains guidelines to keep the CIT as short as possible. It is the institutional policy to start with the surgical procedure in the recipient as soon as we have discussed quality aspects of the graft with the surgeon performing the donor procedure, resulting in parallel procedures. In many cases, the recipient operation has advanced that far, that the donor liver can be implanted immediately upon the arrival after the back table procedure. We do not routinely request for a frozen section liver biopsy to quantitate steatosis because of its inherent low sensitivity and specificity [24]. Moreover, frozen section analysis, ultrasound or computer tomography (CT) scanning for evaluation of liver steatosis are not common practice in the Eurotransplant countries. Therefore, only the judgment of the donor surgeon and the clinical conditions with laboratory tests of the donor will help us in the decision process of accepting an organ. Furthermore, the cava-sparing piggyback technique is our preferred method for graft implantation which results in a shorter anastomosis time in comparison with the classical implantation technique.

Histological assessment of steatosis

Liver biopsies were routinely performed at the bench in the recipient center. Biopsies were obtained with a 1.6-mm Menghini needle located deep parenchymal from the gallbladder fossa in the direction of Cantlie's line to include enough portal triads for clear and representative pathology diagnosis. For examination of steatosis reversal, percutaneous liver biopsies were taken routinely after 1 week until

2005 and thereafter only when clinically indicated (e.g., suspicion of acute rejection or unexplained rise in serum liver enzymes). Biopsy specimens were fixed in formalin, embedded in paraffin, and subsequently stained with hematoxylin–eosin, periodic acid Schiff's reagent (after diastase digestion), Masson trichrome, Gomori's reticulin, Perls' iron staining, and rhodamine for copper accumulation. All histological slides were evaluated by an experienced hepatopathologist (ASHG), who was unaware of the clinical assessment of steatosis. The semiquantitative assessment of steatosis was determined by estimating the percentage of hepatocytes containing lipid droplets (both micro- and macrosteatotic droplets) in 10 consecutive fields (magnification 25×), independently from lobular distribution. Macrovesicular steatosis was defined as fat vesicles larger than the cell nucleus, often displacing the nucleus. Microvesicular steatosis was defined as fat vesicles with similar size or smaller than the liver cell nucleus. The average size of a liver cell nucleus is about 5 μm. The amounts of micro- and macrovesicular steatosis were not taken together to achieve a higher degree of steatosis. Depending on the degree of macrovesicular steatosis, liver biopsies were graded in mild (10–30%), moderate (between 30% and 60%), or severe (>60%) steatotic infiltration according to the histological scoring system for nonalcoholic fatty liver disease designed by the NASH Clinical Research Network [8]. If follow-up biopsies were acquired, the same pathologist (ASHG) performed the histological assessment.

Donor, recipient characteristics and surgical variables

The donor risk index (DRI) was calculated according to Feng *et al.* [25], with minor adjustments as described by Braat *et al.* [26] (considering all donors Caucasian and local). In addition, other donor-related risk factors were collected such as gender, BMI, type of donor (DBD versus DCD), first warm ischemia time in DCD donors, cause of donor death, graft type (full size versus partial grafts), stay at the intensive care (ICU), and type of organ perfusion fluid [histidine–tryptophane–ketoglutarate (HTK) versus university of Wisconsin (UW)]. Other donor variables were collected using the Eurotransplant donor data files with results of the medical history, medication administered at the intensive care (ICU), laboratory tests on the day of donation, and radiology/pathology data of the donor liver. Recipient variables collected and included were the following: age, gender, year of transplantation, BMI, indication for transplantation, laboratory MELD score, recipient status on the Eurotransplant waiting list (elective versus high urgency), and time on the waiting list.

Furthermore, the following surgical variables were examined: CIT, recipient warm ischemia time (WIT), type of venous and bile duct anastomosis, and total amount of blood loss. With respect to intra-operative transfusion requirements, the following variables were analyzed: the number of units of allogeneic red blood cells (RBC; 1 U contained 250 ml), units of fresh frozen plasma (FFP; 1 U contained 225 ml) and units of platelets concentrates (1 U contained approximately 150 ml and was obtained from five donors).

Liver graft I/R injury and function were assessed by laboratory parameters as serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, albumin, prothrombin time (PT), all measured on postoperative day 1, 3, and 7. The serum level of lactate was assessed on the first day postoperative for the first 12 h.

Outcome parameters

Postoperative outcome was studied by assessing PNF, patient and graft survival rates. PNF was defined as non-life-sustaining function of the liver requiring retransplantation or leading to death within 7 days after OLT. Graft and patient survival were assessed at 60 days, 1 year, and 3 year after transplantation. Graft survival was defined as the time period between transplantation and patient death or retransplantation. Patient survival was defined as the time period between transplantation and patient death.

To evaluate the surgical complications after OLT, the complications were graded according the Clavien–Dindo classification [23]. In addition, bile duct strictures were recorded and classified as either at the site of the anastomosis (anastomotic strictures) or at any location in the donor biliary system (nonanastomotic strictures), as diagnosed by endoscopic retrograde cholangiography, magnetic resonance imaging, or percutaneous transhepatic cholangiography.

Statistical analysis

Categorical variables were presented as numbers and percentages and compared using the Pearson chi-square test or the Fisher's exact test where approriate. Continuous variables were expressed as medians and interquartile ranges, and groups were compared using the Mann–Whitney *U*-test. Patient and graft survival rates were analyzed according to the Kaplan–Meier method, and differences between groups were investigated using the log-rank test. Statistical significance was indicated by *P*-values of less than 0.05. Statistical analyses were performed using the statistical software package SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Recipient and surgical characteristics of patients in the group with moderate steatosis and the nonsteatotic control

group are presented in Table 1. There were no statistically significant differences among the two groups. As expected, there were also no significant differences in the matching variables in the moderate steatosis group and the nonsteatotic control group, type of donation, laboratory MELD score, recipient age, status on the waiting list (high urgency versus elective), CIT, and BMI. The CIT was below 8 h in both groups; 7:29 h:min and 7:41 h:min, respectively. In addition, no differences were found in the intra-operative transfusion requirements. The median postoperative follow-up was 81 months (range 8–152 months). The percentage of missing variables was ≤6%.

Donor characteristics

A comparison of donor characteristics in the two groups is presented in Table 2. There were no differences in DRI or other donor-related risk factors and donor

Table 1. Recipient and surgical characteristics in patients who received a donor liver with moderate macrovesicular steatosis versus controls without steatosis.

Variable	Moderate steatosis (n = 19)	Control group (n = 95)	P-value
Age (years)*	52 (30–67)	54 (19–68)	0.86
Gender			
Male	10 (53%)	63 (66%)	0.30
Female	9 (47%)	32 (34%)	
BMI (kg/m^2)	28 (23–31)	26 (23–27)	0.09
Indication for OLT			
Postnecrotic cirrhosis	8 (42%)	36 (38%)	0.51
Biliary cirrhosis	6 (32%)	25 (26%)	
Metabolic disease	2 (10%)	4 (4%)	
Acute liver failure	0	7 (8%)	
Miscellaneous	3 (16%)	23 (24%)	
MELD score (laboratory MELD)	18 (15–27)	21 (14–25)	0.89
Serum creatinine before OLT (μmol/l)†	115 (66–353)	87 (69–127)	0.29
Serum total bilirubin before OLT (μmol/l)‡	52 (33–99)	70 (31–195)	0.22
INR before OLT	1.3 (1.1–2.1)	1.4 (1.3–2.0)	0.62
Status on waiting list			
Elective	18 (95%)	89 (94%)	1.00
High urgency	1 (5%)	6 (6%)	
Waiting time on list (days)	234 (32–589)	186 (38–336)	0.46
Surgical variables			
CIT§(hour:min)	7:29 (6:06–8:33)	7:41 (6:44–8:48)	0.49
WIT¶(hour:min)	0:43 (0:38–0:56)	0:45 (0:40–0:50)	0.88
Type of vena cava anastomosis			
Piggyback	16 (84%)	82 (86%)	0.53
Classical	3 (16%)	13 (14%)	
Type of bile duct anastomosis			
Duct-to-duct	18 (95%)	86 (90%)	0.75
Hepaticojejunostomy	1 (5%)	9 (10%)	
Blood loss (l)	3.0 (2.0–7.5)	2.5 (1.5–6.0)	0.51
RBC (units) (allogenic)	5 (0–9)	3 (0–8)	0.76
FFP (units)	0 (0–7)	3 (0–5)	0.74
Platelets (units)	0 (0–3)	3 (0–8)	0.88

Data represent median with interquartile ranges (IQR) for continuous variables or numbers (percentages) for categorical variables.
BMI, body mass index; CIT, cold ischemia time; FFP, fresh frozen plasma; INR, international normalized ratio; MELD, model of end-stage liver disease; OLT, orthotopic liver transplantation; RBC, red blood cell; WIT, warm ischemia time.
*Data presented as median and range.
†Normal <110 μmol/l, to convert the value for creatinine to mg/dl, divide by 88.4.
‡Normal 0–17 μmol/l, to convert the value for bilirubin to mg/dl, divide by 17.1.
§Time from *in situ* flushing of the donor organ until the liver is removed from ice for implantation.
¶Time from removal of liver from ice until reperfusion via portal vein, hepatic artery or both.

characteristics between the two groups. As expected, assessment of hepatic steatosis before or during organ procurement (i.e., by ultrasound, CT scanning or frozen section analysis) was performed in only a minority of the donors.

The impact of graft steatosis on patient and graft survival

When comparing the moderate macrovesicular steatosis group with the matched control group, we found no differences in the 60-day, 1-year or 3-year patient survival rates

Table 2. Donor characteristics in patients who received a donor liver with moderate macrovesicular steatosis versus controls without steatosis.

Variable	Moderate steatosis (n = 19)	Control group (n = 95)	P-value
Gender			
Male	14 (74%)	55 (58%)	0.304
Female	5 (26%)	40 (52%)	
BMI (kg/m^2)	25 (25–26)	24 (22–26)	0.12
Type of donor liver			
DBD	14 (74%)	70 (74%)	1.00
DCD	5 (26%)	25 (36%)	
Donor risk index	1.6 (1.5–2.0)	1.6 (1.4–2.0)	0.83
First WIT DCD donor (min)	19 (13–23)	14 (12–19)	0.23
Cause of donor death			
Cerebrovascular accident	11 (58%)	37 (39%)	0.57
Trauma	4 (21%)	22 (23%)	
Subdural hematoma	3 (16%)	26 (28%)	
Anoxia	1 (5%)	7 (7%)	
Other	0	3 (3%)	
Graft size			
Full size	19 (100%)	94 (99%)	0.99
Reduced size or split	0	1 (1%)	
Organ preservation fluid			
UW	16 (84%)	66 (69%)	0.27
HTK	3 (16%)	29 (31%)	
Total hospital stay (days)	2 (1–4)	2 (1–4)	0.97
Total stay in intensive care unit (days)	2 (1–2)	2 (1–3)	0.46
Total duration of mechanical ventilation (days)	2 (1–2)	2 (1–3)	0.46
Medical history donor			
Hypertension	5 (26%)	22 (23%)	0.77
Diabetes mellitus	0	4 (5%)	0.49
Alcohol abuse	2 (11%)	9 (10%)	0.58
Smoking	7 (37%)	40 (42%)	0.44
Medication at the intensive care unit			
Vasopressors or inotropes	16 (84%)	81 (87%)	0.49
Corticosteroids	4 (21%)	19 (20%)	0.58
Laboratory results			
Hemoglobin (mmol/l)*	7.0 (6–8)	7.4 (6.3–9.0)	0.37
WBC ($\times 10^9$/l)†	14 (9–17)	14 (10–18)	0.56
Platelet count ($\times 10^9$/l)‡	179 (121–247)	185 (148–285)	0.29
Sodium (mmol/l)§	143 (138–153)	146 (141–152)	0.36
Creatinine (μmol/l)¶	79 (67–104)	71 (59–97)	0.47
Urea (mmol/l)**	5 (3.4–5.9)	5.2 (3.4–7.6)	0.77
LDH (U/l)††	258 (183–310)	300 (112–340)	0.10
AST (U/l)‡‡	39 (20–71)	41 (25–69)	0.59
ALT (U/l)§§	37 (20–63)	26 (17–50)	0.30
Gamma-GT (U/l)¶¶	37 (28–47)	30 (18–63)	0.37
Total bilirubin (μmol/l)***	10 (8–17)	10 (7–16)	0.42
Alkaline phosphatase (U/l)†††	61 (43–69)	66 (51–88)	0.39
Albumin (g/l)‡‡‡	27 (20–33)	29 (24–31)	0.37
C-reactive Protein§§§	110 (14–164)	70 (16–152)	0.75
pH in blood gas¶¶¶	7.43 (7.39–7.48)	7.40 (7.28–7.44)	0.11

Table 2. continued

Variable	Moderate steatosis (n = 19)	Control group (n = 95)	P-value
Radiology and pathology results of donor			
Ultrasound or CT abdomen	5 (26%)	31 (33%)	0.80
Frozen section analysis liver	0	1 (1%)	0.83

Data represent median with interquartile ranges (IQR) for continuous variables or numbers (percentages) for categorical variables.
BMI, body mass index; CT, computer tomography; DCD, donation after cardiocirculatory death; DBD, donation after brain death; HTK, histidine–tryptophane–ketoglutarate; UW, university of Wisconsin; WBC, white blood count; WIT, warm ischemia time.
*Normal value 7–13 mmol/l.
†Normal value 4–11 $\times$ 10^9/l.
‡Normal value 150–400 $\times$ 10^9/l.
§Normal value 130–155 mmol/l.
¶Normal value 25–150 μmol/l, to convert the value for creatinine to mg/dL divide by 88.4
**Normal value 1–12 mmol/l, to convert the value urea to mg/dl divide by 0.357.
††LDH: lactate dehydrogenase, normal value 50–300 U/l.
‡‡AST: aspartate aminotransferase, normal value <48 U/l.
§§ALT: alanine aminotransferase, normal value <42 U/l.
¶¶Normal value <35 U/l.
***Normal value 0–30 μmol/l, to convert the value for bilirubin to mg/dl divide by 17.1
†††Normal vaule < 150 U/l.
‡‡‡Normal value 25–40 g/l.
§§§Normal value < 10 mg/l.
¶¶¶Normal value 7.35–7.45.

(Table 3). Similarly, we found no significant differences in 60-day, 1-year, and 3-year graft survival rates between the two groups.

Postoperative outcome and complications

Postoperative outcome and complications are presented in Table 4. The incidence of PNF was similar in the two groups. In the study group, the only patient with PNF was retransplanted, while four patients in the control group died because of PNF before retransplantation. The classification of surgical complications according the Clavien–Dindo grading [23] was significantly different in the steatotic study group, especially grade IVa. The majority of grade IVa complications include single organ dysfunction such as respiratory insufficiency and renal insufficiency. Organ dysfunction was, in most of the cases, responsible for a significantly longer significant ICU stay. There were no significant differences in the incidences of biliary complications between the two groups.

During the first week after transplantation, serum markers of hepatocellular injury, the transaminases AST and ALT, were significantly higher in the group with a steatotic liver than the controls (Fig. 1). To determine whether the length of the CIT influences the degree of hepatic injury as reflected by serum values of AST/ALT, we compared subgroups of steatotic livers with a CIT <8 h and ≥8 h with nonsteatotic livers (Fig. 2.) Postoperative day 1 serum values of AST and ALT in the recipients of a moderate steatotic liver graft and CIT < 8 h were almost comparable with the values in the control recipients of a nonsteatotic liver graft. However, recipients of a steatotic liver graft with a

Table 3. Comparison of patient and graft survival rates in patients who received a donor liver with moderate macrovesicular steatosis versus controls without steatosis.

Survival	Moderate steatosis (n = 19)	Control group (n = 95)	P-value
Patient survival			
60-day survival	95% (89–100%)	94% (89–99%)	0.86
1-year survival	90% (82–98%)	90% (84–96%)	0.93
3-year survival	79% (71–87%)	74% (68–84%)	0.66
Graft survival			
60-day survival	90% (80–100%)	92% (87–97%)	0.75
1-year survival	84% (73–94%)	85% (78–92%)	0.93
3-year survival	73% (63–83%)	73% (64–82%)	0.93

The values between brackets represent the 95% confidence interval of the survival rate.

Table 4. Postoperative outcome parameters in patients who received a donor liver with moderate macrovesicular steatosis versus controls without steatosis.

Postoperative outcomes	Moderate steatosis (n = 19)	Control group (n = 95)	P-value
Primary nonfunction	1 (5%)	5 (5%)	0.67
Surgical complications*			
Grade II	8 (42%)	63 (66%)	0.010
Grade IIIa	0	1 (1%)	
Grade IIIb	1 (5%)	13 (14%)	
Grade IVa	9 (48%)	8 (9%)	
Grade IVb	1 (5%)	5 (5%)	
Grade V	0	5 (5%)	
Nonanastomotic biliary strictures	2 (11%)	13 (14%)	0.90
Anastomotic biliary strictures	4 (21%)	8 (8%)	0.11
Length of stay in ICU (days)	7 (3–10)	3 (1–7)	0.006

Data represent median with interquartile ranges (IQR) for continuous variables or numbers (percentages) for categorical variables.
ICU: intensive care unit.
*Surgical complications were graded according the Clavien–Dindo classification [23]. In both groups, complication grade I was not allocated because of the therapeutic regimen with standard antibiotic care after orthotopic liver transplantation. Grade II: requiring pharmacological treatment with drugs other than such allowed for grade I complications, blood transfusions and total parenteral nutrition are also included. Grade III: requiring surgical, endoscopic or radiological intervention. Grade IIIa: intervention not under general anesthesia. Grade IIIb: intervention under general anesthesia. Grade IV: life-threatening complication (including CNS complications) requiring ICU management. Grade IVa: single organ dysfunction (including dialysis). Grade IVb: multiorgan dysfunction. Grade V: death of a patient.

CIT ≥8 h had significantly higher serum values of AST and ALT in comparison with recipients of a nonsteatotic liver. This is compatible with a greater susceptibility for I/R injury in steatotic livers in comparison with nonsteatotic livers.

Laboratory markers of liver function, such as serum bilirubin, albumin, and lactate, were not significantly different between the group recipients with moderate graft steatosis and their controls (Fig. 1). The only significant difference in liver function was a longer PT on day 1 and 3 in the recipients with a moderate steatotic liver. But, this difference had disappeared at day 7. Therefore, the almost similar results of liver function indicates that the hepatocellular function was not severely impaired in moderate steatotic livers and those moderate steatotic livers more can be seen as initial slow functional livers.

Reversal of graft steatosis

Eleven of the 19 patients who received a donor liver with moderate macrovesicular steatosis had a liver biopsy within 2 months after transplantation. The median time period between transplantation and the postoperative liver biopsy was 12 days (IQR 7—35 days). In nine of the 11 cases (82%), the total amount of macrovesicular steatosis decreased from 30–60% to ≤10% (Fig. 3). In one patient, the post-transplant biopsy still displayed mild amounts of steatosis (10–30% macrovesicular steatosis), and in one patient, the second biopsy (obtained at day 7 after OLT) demonstrated persistent moderate macrovesicular steatosis.

Discussion

Moderately steatotic liver grafts are more prone to I/R injury, and transplantation of these livers is associated with an increased risk of poor postoperative outcome compared with reference donors [22]. It was the aim of the current study to assess the effect of moderate graft steatosis on patient outcome when the effects of I/R injury are minimized using a strict policy to keep the CIT as short as possible. Our data show that the postoperative function of moderate steatotic livers is not impaired in comparison with reference donors. We report similar patient/graft survival rates and PNF incidences between recipients with a moderate steatotic liver and their nonsteatotic controls. Also, the first-week postoperative liver function parameters, such as serum bilirubin, albumin, and lactate were not different between the two groups. Nevertheless, our data show that moderate steatotic livers are more sensitive for I/R injury (reflected by significant higher serum levels of transaminases) and recipients of these livers suffer from higher rates of severe complications. In particular, respiratory and renal insufficiency are more frequently seen in recipients with a moderate steatotic graft.

The impact of macrovesicular moderate or severe graft steatosis on outcome after liver transplantation has been studied by other groups, and this has resulted in conflicting data [9–21]. Large registry studies revealed that moderate macrovesicular steatosis is an independent prognostic factor for poor postoperative outcomes [14,15]. However, single center reports with a small number of recipients

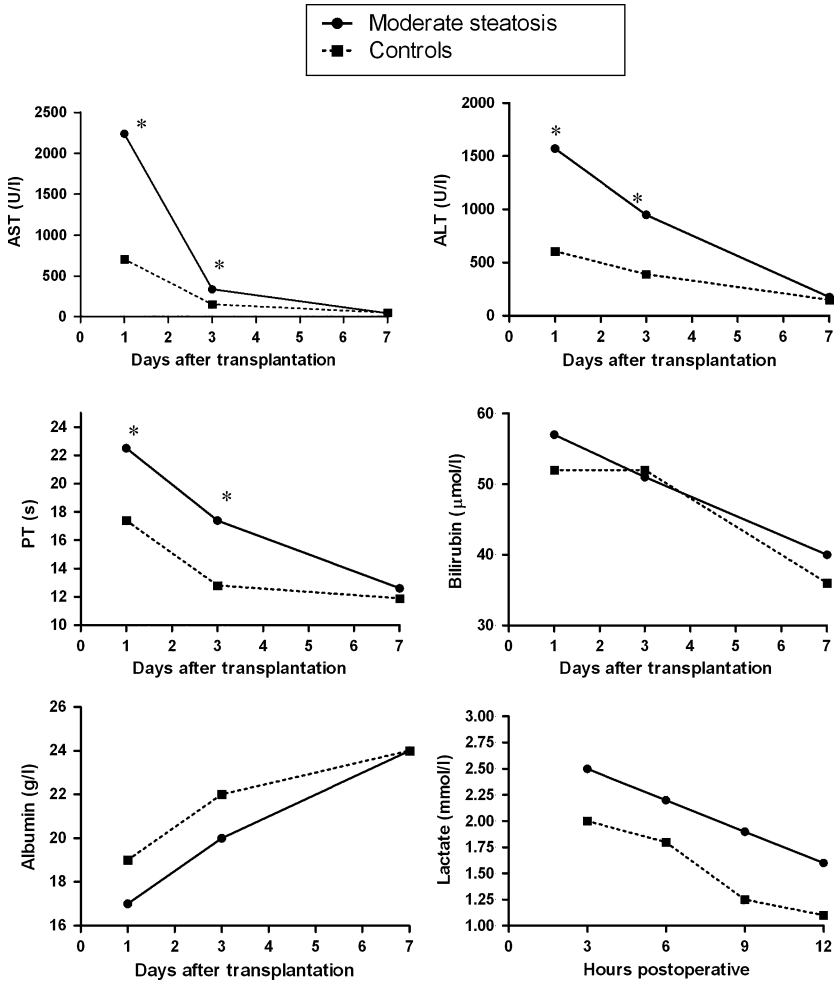

Figure 1 Comparison of laboratory parameters after transplantation of livers with moderate macrovesicular steatosis versus livers without steatosis. Median serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the prothrombin time (PT) were significantly higher in the group of steatotic livers on day 1 and day 3 (*); AST day 1 ($P = 0.001$), AST day 3 ($P = 0.048$), ALT day 1 ($P = 0.001$), ALT day 3 ($P = 0.007$), PT time day 1 ($P = 0.003$), and PT time day 3 ($P = 0.009$).

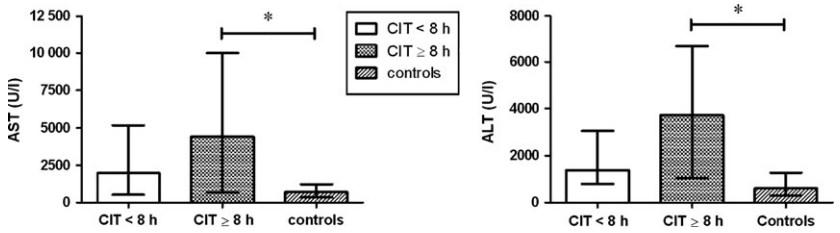

Figure 2 Comparison of median values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) on postoperative day 1 in recipients of a liver graft with moderate steatosis and a cold ischemia time (CIT) <8 h or ≥8 h, versus recipients of a nonsteatotic liver graft (controls). Errors bars represent the interquartile range. ALT and AST levels were significantly higher in the recipients of a moderate steatotic liver with CIT ≥8 h in comparison with the control recipients (*); AST ($P = 0.016$) and ALT day 1 ($P = 0.004$), respectively.

reported similar outcomes in postoperative graft function between moderate or severe steatotic livers and nonsteatotic liver grafts [16–21]. The differences in postoperative outcome in these studies could depend on the presence or absence of other donor-related risk factors for graft failure, such as DCD, prolonged CIT, and advanced donor age,

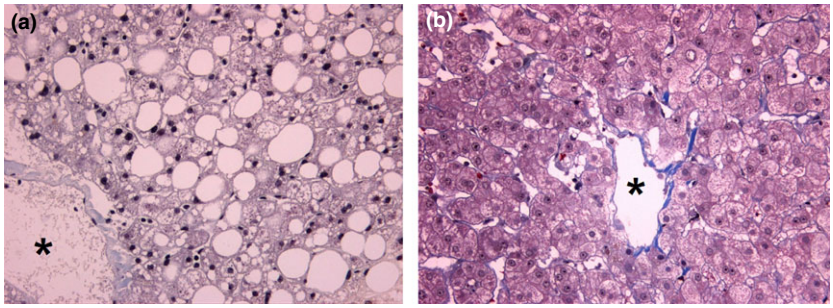

Figure 3 Example of complete reversal of moderate macrovesicular steatosis in a liver graft within 1 month after transplantation. Masson trichrome staining of donor liver biopsy at the time of transplantation (a) and follow-up biopsy at 30 days after liver transplantation (b); * reflects the central vein.

which are all reflected in the DRI score [25,26]. In studies with favorable postoperative outcomes after transplantation of moderate steatotic livers, the frequency of additional donor risk factors might have been relatively low [16,18–21]. In the current series, we observed a median DRI of 1.6 (IQR 1.5–2.0) in the group of moderate steatotic livers. This indicates that apart from steatosis, most livers have additional donor-related risk factors. Moreover, in our center, steatotic liver grafts are not preferentially allocated to low-risk recipients. The liver graft allocation system in the Netherlands is patient oriented (based on the MELD score) and not based on center allocation. Therefore, steatotic liver grafts are in general assigned to patients high on the national waiting list and not reserved for candidates in relatively good clinical condition. Our median laboratory MELD score is 18 (IQR 15–27), and this value is comparable or even higher in comparison with the study of McCormack and coworkers who have also reported successful postoperative outcome after transplantation of moderate steatotic livers (median laboratory MELD score of 12 (range 6–25) [17].

Steatotic livers are more susceptible for the effects of cold ischemia during organ preservation and subsequent warm reperfusion [4]. This I/R injury initiates a sequence of events that lead to cellular damage with early graft dysfunction as consequence [6]. In most studies, the definition of early graft dysfunction included laboratory markers of both liver injury (i.e., serum transaminases) and function (i.e., PT and serum bilirubin) [9,10,13,18–21]. In our opinion, this may cause confusion, because high serum markers of I/R injury such as serum AST and ALT are not always accompanied by a poor liver function. By separating markers of hepatocellular injury and function, we confirmed the high susceptibility of steatotic grafts to I/R injury, yet we also demonstrated that most livers may function well and survival rates are similar between the groups of moderate steatosis and nonsteatotic livers. When accepting a donor liver with moderate steatosis one can expect very high levels of serum transaminases postoperatively in the recipient; however, this does not necessarily mean that graft function is poor.

A very interesting finding in our study is the reversal of steatosis in 82% of the donor livers with a follow-up biopsy. In these livers, the degree of fatty infiltration decreased from 60–30% to 10% or less. Similar observations have been made by others, although very few studies have performed long-term follow-up examinations [10,17,20,27,28]. Nevertheless, the mechanism of steatosis reversal remains unknown. Disappearance of steatosis in follow-up biopsies may not necessarily mean elimination of the fat content of hepatocytes. It could also reflect necrosis and subsequent disappearance of fatty hepatocytes that are very susceptible to I/R injury, early after transplantation. Sampling error may also play a role as the location of the biopsy taken during OLT is different from the side of the liver where the percutaneous biopsy is taken from.

Macroscopic evaluation of steatosis by the surgeon during the donor procedure is subjective and susceptible for errors [29]. Nevertheless, in our region, liver grafts are not routinely investigated with liver frozen sections to evaluate the degree of steatosis because this method is not reliable [24]. Also the use of ultrasound or CT scanning is not routinely performed during the donor work-up. Only medical history and laboratory parameters of the donor in combination with the macroscopic judgment of the liver by the donor surgeon give us, in most cases, information on whether we have to accept the donor liver or not. Given these circumstances, in our study we declined 7% of the donor offers because of a suspicion of moderate and severe graft steatosis in combination with additional risk factors. We cannot assess whether this aspect has influenced our study results to some extent.

A limitation of our study is that we are not able to show that moderate steatotic donor livers in combination with prolonged cold ischemia times (≥8 h) are related to poorer

postoperative outcomes in comparison with moderate steatotic livers with short CIT (<8 h). Nevertheless, we show that recipients of a steatotic liver graft and longer CIT (≥8 h) have significantly higher values of AST/ALT for the first day postoperative than recipients of a nonsteatotic liver graft. However, recipients of a steatotic donor liver and short CIT (<8 h) have almost similar serum levels of AST and ALT compared with recipients of a nonsteatotic liver. This data are supportive for our hypothesis that short cold ischemia times (≥8 h) are necessary when moderate steatotic donor livers are used for OLT.

By the use of very strict logistics, we are able to keep the CIT below 8 h. We believe that this is currently the best strategy to deal with donor livers with significant steatosis. Better and more prolonged preservation of steatotic donor livers could come from more sophisticated techniques such as machine preservation. In this respect, machine preservation is a promising new method that could allow *ex vivo* assessment of liver viability and function prior to transplantation, especially when livers can be perfused while maintaining a physiological temperature during normothermic machine preservation [30]. In conclusion, it is known that livers with moderate macrovesicular steatosis are more prone to I/R injury and related to higher incidences of graft failure. Nevertheless, when using a strict policy where the CIT is kept as short as possible, moderate macrovesicular steatotic livers can be used successfully for liver transplantation despite a higher degree of postoperative complications. The use of moderate steatotic livers with a strict protocol to keep the CIT as short as possible is a safe way to expand the donor pool.

Authorship

ACW: designed and conducted the study, analyzed the data and wrote the manuscript. MTB: designed and conducted the study and wrote the manuscript. APB: designed the study and wrote the manuscript. ASHG: designed and conducted the study, analyzed the data and wrote the manuscript. RJP: designed and conducted the study, reviewed the analysis of the data and approved the final manuscript.

Funding

The authors have declared no funding.

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