

Pro-atherosclerotic markers and cardiovascular risk factors one year after liver transplantation

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Abstract

AIM: To investigate pro-atherosclerotic markers (en-

dothelial dysfunction and inflammation) in patients one year after liver transplantation.

METHODS: Forty-four consecutive liver transplant (LT) outpatients who were admitted between August 2009 and July 2010, were followed-up by for 1 year, exhibited no evidences of infection or rejection, all of them underwent tacrolimus-based immunosuppressive regimens were consecutively enrolled. Inflammatory cytokines (TNF α , IFN γ , IL-8, and IL-10), endothelial biomarkers (sVCAM-1, sICAM-1, MPO, adiponectin, PAI-1, SAP, SAA, E-selectin, and MMP-9), high sensitive C-reactive protein, and Framingham risk score (FRS) were assessed. The anthropometric data, aminotransferases, metabolic syndrome features, glucose and lipid profiles, and insulin resistance data were also collected. The LT recipients were compared to 22 biopsy-proven non-alcoholic steatohepatitis (NASH) patients and 20 healthy controls (non-obese, non-diabetics, and non-dyslipidemic).

RESULTS: The LT recipients had significantly younger ages and lower body mass indices, aminotransferases, fasting glucose and insulin levels, glucose homeostasis model and metabolic syndrome features than the NASH patients. Classic cardiovascular risk markers, such as Hs-CRP and FRS [2.0 (1.0-8.75)], were lower in the LT patients compared to those observed in the NASH patients ($P = 0.009$). In contrast, the LT recipients and NASH patients had similar inflammatory and endothelial serum markers compared to the controls (pg/mL): lower IL-10 levels (32.3 and 32.3 vs 62.5, respectively, $P = 0.019$) and higher IFN γ (626.1 and 411.9 vs 67.9, respectively, $P < 0.001$), E-selectin (48.5 and 90.03 vs 35.7, respectively, $P < 0.001$), sVCAM-1 (1820.6 and 1692.4 vs 1167.2, respectively, $P < 0.001$), and sICAM-1 (230.3 and 259.7 vs 152.9, respectively, $P = 0.015$) levels.

CONCLUSION: Non-obese LT recipients have similar pro-atherosclerotic serum profiles after a short 1-year follow-up period compared to NASH patients, suggesting a high risk of atherosclerosis in this population.

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Key words: Cell adhesion molecules; Endothelial biomarkers; Cardiovascular disease; Nonalcoholic steatohepatitis; Metabolic syndrome

Core tip: Liver transplant (LT) patients have a high risk of long-term development of cardiovascular disease (CVD), which is currently recognized as an important cause of death 5 to 10 years after transplant in this population. Atherosclerosis is a hallmark of CVD, with both disorders involving a prolonged asymptomatic phase and often leading to morbidity and mortality upon initial clinical presentation. Regardless, endothelial dysfunction is the first step in developing early atherosclerosis. In the present study, we evaluated inflammatory and endothelial markers one year after transplantation in asymptomatic LT recipients in comparison to high-CVD-risk biopsy-proven nonalcoholic steatohepatitis (NASH) patients and healthy controls. We found that LT recipients had pro-inflammatory profiles and endothelial dysfunction similar to those of NASH patients, both of which were higher than those in the compared controls. These findings suggest that LT recipients, even in a short 1-year follow-up period, display a high atherosclerotic risk and should be carefully monitored to effectively prevent CVD.

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INTRODUCTION

Liver transplantation is the standard treatment for acute and chronic end-stage liver disease. Advances in medical therapy and surgical techniques have increased the life span of liver transplant (LT) recipients. As a result, medical complications that accompany long-term survival, including atherosclerotic cardiovascular disease (CVD), metabolic bone disease, and *de novo* malignancy, have accounted for an increasing proportion of late morbidity in these patients. CVD, which is responsible for 19% to 42% of all non-liver related mortality, is a major cause of morbidity and mortality after LT^[1-6].

Atherosclerosis is the hallmark of CVD and remains an important health issue in the modern world despite research aimed at understanding its underlying pathogenesis. This condition involves a prolonged asymptomatic

phase; symptoms only develop when blood flow is insufficient to ensure tissue vitality. The first clinical presentation often leads to morbidity and mortality^[7]. Arterial plaque with no symptoms is called subclinical atherosclerosis, and chronic inflammation is a risk factor for plaque rupture. High sensitivity C-reactive protein (Hs-CRP) is an inflammatory marker that predicts CVD in healthy individuals^[8,9]. Endothelial dysfunction is the first step in developing early atherosclerosis. Several studies confirm that elevated plasma levels of endothelial markers, such as von Willebrand factor, and soluble vascular cell adhesion molecule-1 (sVCAM-1) may serve as molecular markers for atherosclerosis and are independent risk factors for the development of coronary heart disease^[9]. Risk estimation for atherosclerotic and cardiovascular events that is based only on the presence of classical risk factors is often insufficient. Therefore, efforts have been made to identify blood markers that indicate the presence of preclinical disease.

This study was designed to investigate pro-atherosclerotic markers (endothelial dysfunction and inflammation) in patients one year after LT.

MATERIALS AND METHODS

Population

Between August 2009 and July 2010, 44 consecutive adult (older than 18 years old) outpatients who underwent orthotopic LT at the LT Unit of the University of São Paulo School of Medicine, Brazil, were followed for 1 year. The results were compared to 20 age-matched (10-year age classes) controls [body mass index (BMI) < 30 kg/m², non-diabetics, and non-dyslipidemic]. Additionally, because NASH is an important risk factor for CVD, 22 patients with biopsy-proven NASH were also compared with the LT recipients and controls. One experienced pathologist graded the liver biopsies from the NASH patients, according to the NAFLD activity score (NAS)^[10]. The LT recipients had no evidence of infection or rejection and were evaluated during regular outpatient clinic visits.

The transplant data were reviewed from the patient's charts. At the 1-year follow-up, features of MS, glucose and lipid profiles, HOMA-IR, inflammatory cytokines, and endothelial biomarkers were determined. MS was defined using the American Diabetes Association criteria^[11].

Framingham risk scoring system and physical activity

Framingham risk scoring system (FRS) was calculated by assigning gender-specific points for age, smoking, diabetes, blood pressure, low-density lipoprotein cholesterol (LDL-cholesterol), and high-density lipoprotein cholesterol (HDL-cholesterol). The gender-specific FRS equations were then used to calculate the risk of developing cardiovascular events over the next 10 years^[11]. The patients were graded as low risk (< 10%), intermediate risk (10%-20%), and high risk (> 20%), according to the National Cholesterol Education Program Adult Treat-

ment Panel III guidelines^[12].

The International Physical Activity Questionnaire assessed physical activity. Sedentary lifestyle was described as less than 10 min/wk of continuous exercises^[13].

Laboratory evaluation

The laboratory evaluation in all patients included a blood cell count and the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol and fractions, triglycerides, and fasting glucose and insulin levels. These parameters were measured using the standard techniques of clinical chemistry laboratories (Modular P800, Hitachi, Roche Applied Science, Indianapolis, IN, United States). Insulin resistance was measured using the glucose homeostasis model (HOMA-IR): the product of fasting plasma glucose level (mg/dL) and insulin concentration (mIU/L), divided by 405.

Serum cytokine measurements

For the cytokine and chemokine measurements, the serum was stored at -80 °C until use. The serum cytokine levels (TNF- α , IL-8, IFN γ , and IL-10) were then measured using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) kit (RD System Inc., Minneapolis, MN, United States). All measurements were performed in duplicate, and the average values were used in the statistical analyses.

Inflammation and endothelial dysfunction markers

To detect changes in inflammation and endothelial dysfunction markers, we analyzed the levels of high-sensitive C-reactive protein (Hs-CRP), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), E-selectin, adiponectin, plasminogen activator inhibitor 1 (PAI-1), serum amyloid P (SAP), serum amyloid A (SAA); matrix metalloproteinase 9 (MMP-9), and myeloperoxidase (MPO). Each measurement was performed in pg/mL in a multiplex assay on the Luminex platform, as described by the manufacturer (Milliplex CVD Panel 1, Millipore, Copenhagen, Denmark). The readers of the index tests and reference standard were blinded to the other test results.

Ethical concerns

The study was performed in accordance with the ethical standards of the Helsinki Declaration. An institutional ethics review board approved the protocol, and written informed consent was obtained from each patient. The Department of Gastroenterology (LIM-37/LIM07), School of Medicine, University of São Paulo, Brazil, supported this work. The authors have no conflicts of interest to disclose.

Statistical analysis

Sample estimation was not performed, as there was no similar study on which to base the calculation. The data are expressed as mean \pm standard deviation (SD) for variables with normal distribution and compared using one-

Table 1 Demographic and clinical data from liver transplant recipients included *n* (%)

Variable	Results (<i>n</i> = 44)
Recipient age (yr) ¹	50.8 \pm 14.3
Donor age (yr) ¹	41.9 \pm 17.6
Gender	
Male	29 (65.9)
Female	15 (34.1)
Etiology of liver disease ¹	
HCV-related	16 (36.4)
Non-HCV-related	28 (63.6)
Patients with NAFLD	4 (9.1)
Patients with hepatocellular carcinoma	15 (34.1)
Pre-transplant MELD ¹	19.3 \pm 9.4
Donor liver weight (g) ¹	1374.3 \pm 311.3
Recipient liver weight (g) ¹	1399 \pm 725.9
Cold ischemia time (min) ¹	400.9 \pm 137.9
Warm ischemia time (min) ¹	47.2 \pm 8.9
Intraoperative blood requirements ¹	
Packed red cells (Units)	1.09 \pm 1.6
Plasma (Units)	0.71 \pm 1.8
Platelets (Units)	1.03 \pm 3.53
Intraoperative albumin infusion (10 g bottles) ¹	4.32 \pm 4.8
Tacrolimus-based immunosuppression	44 (100)
Steroids withdrawal (d) ¹	208.5 \pm 106.8
Pre-transplant diabetes mellitus	16 (36.4)
Diabetes mellitus 1 yr after transplant	17 (38.6)
Pre-transplant BMI ¹	25.1 \pm 5.3
BMI 1 yr after transplant ¹	24.3 \pm 4.3
Abdominal circumference 1 yr after transplant (cm) ¹	89.4 \pm 8.7
Tobacco consumption	8 (18.2)
Arterial hypertension	16 (36.4)
Sedentarism	28 (66.3)
Metabolic syndrome	10 (22.7)
Framingham risk score (10-yr) ²	2.0 (1.0-8.75)

¹Mean and standard deviation; ²Median and 25th-75th percentiles. BMI: Body mass index.

way analysis of variance (ANOVA). The median and 25th and 75th percentiles were used for variables with skewed distribution, which were compared using the Mann-Whitney *U*-test. Multiple comparisons were conducted, and a significance level of 5% was established.

RESULTS

Table 1 describes the demographic and clinical data from the LT recipients and donors. Most of the patients were males, with non-hepatitis C virus (HCV)-related liver disease. Only 4 (9.1%) patients were transplanted for NAFLD cirrhosis. In addition, most of the included patients had no hepatocellular carcinoma diagnosed during the pre-transplant period or at the explant analysis. The mean age of the donors was young (41.9 years), and the intraoperative data (ischemia times, intraoperative blood requirements, and intraoperative albumin infusion) demonstrated that undergoing the procedure was uneventful.

All included LT recipients underwent tacrolimus-based immunosuppressive treatment, and steroids were withdrawn in 208.5-106.8 d. During the 1-year follow-up, obesity was not a prevalent comorbidity, with a mean BMI of 24.3-4.3 kg/m². The mean abdominal circumfer-

Table 2 Demographic and clinical characteristics of the studied population

Variable (mean \pm SEM) otherwise indicated	NASH (<i>n</i> = 22)	LT (<i>n</i> = 44)	Controls (<i>n</i> = 20)	<i>P</i> ¹
Age	58.5 \pm 6.51 ¹	50.8 \pm 14.3	51.2 \pm 9.31	< 0.001
Sex %male/female	36.3/63.7	65.9/34.1	53.4/46.7	
BMI	31.7 \pm 4.35 ¹	24.3 \pm 4.3	25.1 \pm 2.7	< 0.001
Fasting glucose	139.7 \pm 60.6	118.1 \pm 46.8	NA	< 0.001
Fasting insulin	17.6 \pm 8.68	12.15 \pm 7.7	NA	0.03
HOMA-IR	6.173 \pm 6.68	3.43 \pm 2.4	NA	< 0.001
AST	45.5 \pm 28.5	23 \pm 18.1	NA	0.002
ALT	58.8 \pm 45.5	23 \pm 15.3	NA	< 0.001
Total cholesterol	199.5 \pm 44.3	171.27 \pm 26.8	NA	NS
HDL cholesterol	50.1 \pm 12.3	49.6 \pm 15.9	NA	NS

¹LT vs NASH. SEM: Standard error of the mean; NS: Non-significant; NA: Not applied; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; HDL: High-density lipoprotein.

Table 3 Inflammatory cytokines in liver transplant recipients, non-alcoholic steatohepatitis and controls

Variable (median - 25 th -75 th percentile)	NASH (<i>n</i> = 22)	LT (<i>n</i> = 44)	¹ Controls (<i>n</i> = 20)	<i>P</i>
TNF α (pg/mL)	13.4 (8.85-22.2)	12.1 (8.58-26.6)	10.1 (5.0-13.6)	0.121
IFN γ (pg/mL)	411.9 (192.3-1361.7)	626.1 (286.9-1572.3)	67.9 (42.2-100.6)	< 0.001
IL-8 (pg/mL)	57.8 (43.8-70.2)	36.5 (31.2-44.9)	40.7 (36.5-53.3)	< 0.001
IL-10 (pg/mL)	32.3 (22.5-49.8)	32.3 (25.8-62.5)	62.5 (34.4-85.3)	0.019

¹Comparisons: IFN γ (NASH = LT-*P* = 0.3; NASH and LT > controls - *P* < 0.001); IL-8 (NASH > LT - *P* < 0.001; NASH > controls - *P* = 0.04; LT = controls - *P* = 0.11); IL-10 (NASH = LT - *P* = 0.84; NASH < controls - *P* = 0.02; LT < controls - *P* = 0.04).

ence 1 year post-transplant was 89.4-8.7 cm. MS features were present in only 22.7% of the LT recipients. While 38.7% of the patients had DM, and high blood pressure was identified in 36.4%. Only 18.2% of the patients smoked tobacco. Conversely, sedentarism was present in 66.3% of the studied sample. The mean FRS was low (2.0), consistent with the low MS prevalence.

Table 2 shows the laboratory results 1 year post-LT. The hepatic profiles were approximately normal and significantly lower than those of the NASH patients. The fasting glucose, insulin, and HOMA-IR levels were also lower than those in the NASH patients. Although NASH patients had higher total cholesterol levels, their HDL and LDL-cholesterol and triglyceride levels were not significantly different from those of the LT patients, likely because the NASH patients were taking medications. MS was observed more frequently in the NASH patients (100%) than in the LT recipients.

Regarding inflammation, the IFN γ level was comparable in the NASH patients and LT recipients (*P* = 0.3); the levels in both groups were higher than that in the controls (*P* < 0.001). The anti-inflammatory IL-10 was similar in the LT and NASH patients (*P* = 0.84), and significantly lower in both groups compared to the controls (*P* \leq 0.05), as shown in Table 3. There were no between-group differences in the TNF- α level, and the IL-8 levels were similar between the LT recipients and controls (*P* > 0.05).

The CVD risk (*i.e.*, Hs-CRP) was similar in the LT recipients and controls (*P* = 0.41) but significantly lower in the LT patients compared to the NASH (*P* = 0.007) patients (Table 4).

Regarding endothelial biomarkers, the liver recipients and NASH patients were comparable considering sVCAM-1 and sICAM-1 (*P* = 0.5), and these levels in both groups were higher than in the controls; sVCAM-1 was significantly higher in the LT recipients than in the controls (*P* < 0.001), and sICAM-1 exhibited an insignificant tendency to be higher between these groups (*P* = 0.05). The E-selectin level was higher in the NASH patients than in the transplanted patients, but it was also higher in the transplanted patients compared to the controls (*P* = 0.04). MPO and PAI-1 were significantly lower in the LT recipients than in the other groups, while the SAP and SAA levels were significantly lower only when comparing the LT recipients with the NASH patients. The serum adiponectin levels were higher in the transplanted patients than in NASH patients (*P* = 0.007).

Only one patient (a patient with post-transplant myocardial infarction) developed cardiovascular events during the follow-up period. No patients presented with recurrent hepatocellular carcinoma within the 1-year follow-up.

DISCUSSION

CVD is a major cause of morbimortality after LT, and identifying those candidates who are at the greatest risk of postoperative complications is a cornerstone strategy for optimizing outcomes^[14,15]. The present study demonstrated that at 1 year post-transplant, LT recipients have similar pro-atherosclerotic profiles, as measured by endothelial biomarkers and inflammatory cytokines, as patients with NASH, even when conventional cardiovas-

Table 4 Comparison among liver transplant recipients, non-alcoholic steatohepatitis and controls regarding endothelial biomarkers

Variable (pg/mL)	NASH (n = 22)	LTR (n = 44)	Controls (n = 10)	P ³
sVCAM-1 ¹	1692.4 ± 457.4	1820.6 ± 443.9	1167.2 ± 121.8	< 0.001
sICAM-1 ¹	259.7 ± 101	230.3 ± 96.3	152.9 ± 33.9	0.015
MPO ¹	198.3 ± 116.2	93.7 ± 60.9	409.2 ± 204.9	< 0.001
Adiponectin ¹	23789.1 ± 12040.4	47965.3 ± 33140.8	32683.2 ± 25065.4	0.008
PAI-1 ¹	149.2 ± 63.1	40.4 ± 28.7	132.3 ± 58.4	< 0.001
SAP ¹	59031.5 ± 17024.2	29174.5 ± 20175.2	40452.8 ± 18557.44	< 0.001
SAA ²	21303 (8723.6-30583.2)	5390.9 (2567.4-18562.04)	9008.6 (3230.7-13977.4)	< 0.001
E-selectin ²	90.03 (69.5-137.1)	48.5 (36.04-70.9)	35.7 (28.4-47.04)	< 0.001
MMP-9 ²	289.3 (107.6-410.4)	50.5 (35.2-99.5)	411.5 (241.2-587.4)	0.002
HsCRP ²	1.78 (0.70-3.51)	0.53 (0.21-1.13)	0.29 (0.16-0.55)	0.009

¹Mean ± SD; ²Median (25th-75th percentiles); ³Comparisons: sVCAM-1 (LTR = NASH - *P* = 0.5; LTR > controls - *P* < 0.001); s-ICAM-1 (LTR = NASH - *P* = 0.48; LTR > controls - *P* = 0.05; MPO (LTR < NASH - *P* = 0.02; LTR < controls - *P* < 0.001); Adiponectin (LTR > NASH - *P* = 0.007; LTR = controls - *P* = 0.27); PAI-1 (LTR < NASH and controls - *P* < 0.001); SAP (LTR < NASH - *P* < 0.001; LTR = controls - *P* = 0.22); SAA (LTR < NASH - *P* = 0.006; LTR = controls - *P* = 0.91); E-selectin (LTR < NASH - *P* = 0.001; LTR > controls - *P* = 0.04); MMP-9 (LTR < NASH and controls - *P* < 0.001); CRP (LTR < NASH - *P* = 0.007; LTR = controls - *P* = 0.41). sVCAM-1: Soluble vascular cell adhesion molecule-1; sICAM-1: Soluble intracellular cell adhesion molecule-1; PAI-1: Plasminogen activator inhibitor 1; SAP: Serum amyloid P; SAA: Serum amyloid A; MMP-9: Matrix metalloproteinase 9; HsCRP: High-sensitivity C-reactive protein; LTR: Liver transplant recipients; NASH: Non-alcoholic steatohepatitis.

cular risk factors, such as obesity or elevated Hs-CRP or/and high FRS, are not observed. In liver disease, NASH patients represent the major leading intersection between metabolic syndrome and CVD; therefore, they represent a good comparison group when considering CVD risk in a given population.

Post-transplant MS is an important risk factor for CVD, and it should be monitored^[16,17]. In our study, patients presented relatively low BMIs pre-transplant, and diabetes was uncommon. Moreover, after LT, the prevalence of diabetes, hypertension, and MS remained low. This finding contrasts with several other studies that demonstrated relatively higher post-LT MS prevalence^[6,16]. Additionally, BMI did not increase at the end of the 1-year follow-up. Correspondingly, in the present study, the LT recipients displayed a normal lipid profile, and FRS was not elevated; the mean FRS was 2%, which indicates low-risk. Hs-CRP is a well-established predictive marker of risk of coronary events. CRP can induce endothelial lectin-like oxidized low-density lipoprotein (ox-LDL) receptor-1, which is the primary endothelial receptor for ox-LDL and may lead to the activation of pro-inflammatory genes, including IL-8, sICAM-1, and sVCAM-1^[17]. Here, Hs-CRP was higher in the LT recipients than in the controls, but the difference was not significant. Indeed, this result is consistent with the aforementioned LT recipient characteristics and highlights the strength of our main results. Moreover, differences regarding age or BMI most likely have not influenced the results, as the LT recipients and controls had quite similar characteristics.

Increased levels of selectins and adhesion molecules are considered to be important indicators of atherosclerosis^[18]. One of the key initial events in the development of atherosclerosis is the adhesion of monocytes to the endothelial cells, with subsequent transmigration into the vascular intima. Soluble leukocyte and vascular cell adhesion molecules (CAM), such as selectins, integrins, sVCAM-1, and sICAM-1, play critical roles in the adhe-

sion of monocytes to endothelial cells^[17]. In the present study, the sVCAM-1 and sICAM-1 levels were higher in the LT recipients than in the controls. Remarkably, the CAM levels were comparable between the LT recipients and NASH patients. In addition, the E-selectin levels in the LT recipients were significantly higher than in the controls. These results could indicate the initiation of an atherosclerotic disease process.

In contrast, the LT recipients displayed lower MPO, MMP-9, and PAI-1 levels than the controls and NASH patients. It is unknown why these markers were low in our study; although tacrolimus itself can negatively impact MMP-9^[19], and PAI-1 can be low in association with thrombocytopenia, as approximately 90% of blood PAI-1 is found in the platelet compartment^[20]. Regarding MPO, which promotes atherosclerosis *via* oxidative stress^[21], levels under < 115 ng/mL were recently correlated with a longer event-free period in a high-risk population suffering from peripheral arterial disease^[22]. In our study, only the LT recipients had mean MPO levels ≤ 115.

Inflammation plays a leading role in atherosclerosis. Most of the studies on cytokines in LT patients indicate immediate complications, such as ischemia-reperfusion injury or rejection. In the current study, the TNFα levels were similar among the LT recipients, NASH patients, and controls, but a previous study from our group demonstrated that TNFα does not increase in NAFLD^[23]. IFNγ levels were lower in the controls than in the LT recipients, suggesting that LT recipients have more inflammation than normal. This result is confirmed by anti-inflammatory IL-10, a pivotal anti-inflammatory cytokine that showed higher levels in the controls compared to the LT patients. Down-regulation of IL-10 has also been recently demonstrated in NASH patients^[24], and low circulating levels have been demonstrated in obese patients. The role of IL-8 is not well documented, even in NAFLD. It has been suggested that cirrhosis itself (with hepatic shunts and liver dysfunction) can partially explain

the higher systemic levels of pro-inflammatory cytokines^[25]. We hypothesized that hepatic clearance is not the cause of the cytokine profile in our LT population, as we only included outpatients who had no significant liver function damage. HCV was also recently associated with the pro-inflammatory profile^[26]; thus, this variable must be considered when analyzing our results, as 36.4% of the patients were HCV-positive.

Adiponectin, an anti-inflammatory adipokine that acts as an anti-obesity hormone, is usually down-regulated in NAFLD^[27]. Few studies have investigated adiponectin in LT patients. In the present study, the LT recipients had higher adiponectin levels than the NASH patients, and there were no differences between the patients and controls.

Immunosuppressive therapy should be considered, as steroids and calcineurin inhibitors are related to a high risk of metabolic syndrome^[28]. Calcineurin inhibitors are also linked to renal injury and are prone to increase oxidative stress and lipid peroxidation. Thus, immunosuppressive agents might be somehow associated with our main results.

Most LT studies focused on CVD after a long-term follow-up. Longer follow-ups are associated with an increased likelihood that a patient will suffer from MS, and it is presumably difficult to alter this path. Studies predicting cardiac complications based on short follow-ups are scarce and do not focus on atherosclerotic disease^[29-32]. The present study was not designed to assess long-term prognosis but rather to evaluate the risk of LT recipients within 1-year post-transplant.

This study has several strengths that should be emphasized. The sample selection was adequate, as only outpatients without clinically evident inflammatory complications, such as rejection or infection, were included. Inclusion at the end of the first year post-LT enabled the authors to evaluate cardiac risk factors late enough to avoid specific LT complication biases and early enough to allow to prevent disease progression. The LT recipients were not obese; the MS prevalence was low, and the LT recipients were compared to both controls and NASH patients. Several limitations should also be noted. The sample size was small, and the study was conducted at a single-center. Liver biopsies were not performed following a protocol schedule; baseline endothelial function and inflammatory profile information was not available for the LT recipients; and no cardiovascular imaging studies were performed. Finally, although unlikely, it is impossible for us to determine whether the inflammatory cytokines and endothelial marker profiles were related to some inherent transplant issues, such as a continuous rejection stimulus.

In conclusion, we confirmed our hypothesis that LT recipients, even after a short follow-up period of 1-year post-transplant, are a population with a high atherosclerotic risk, as demonstrated by their inflammatory profiles and endothelial biomarkers. These results suggest that LT recipients should be carefully followed to prevent future CVD.

COMMENTS

Background

Advances in medical therapy and surgical techniques made common the long-term survival of liver transplant recipients. Indeed, liver transplant recipients have a high risk of long-term development of cardiovascular disease.

Research frontiers

Most post-transplantation studies are focused on noticeable cardiovascular after a long-term follow-up. Studies predicting cardiac complications based on short follow-ups are scarce and do not focus on atherosclerotic disease. The present study was not designed to assess long-term prognosis but rather to evaluate the risk of liver transplant recipients within 1-year post-transplant.

Innovations and breakthroughs

This study demonstrated a high pro-inflammatory profile and endothelial dysfunction in low-risk liver transplant recipients one year post-transplantation.

Applications

Liver transplant recipients should be evaluated for cardiovascular early after transplantation to allow for effective preventative strategies.

Terminology

Endothelial dysfunction is the first step and inflammation plays an important role in developing early atherosclerosis. One of the key initial events is the adhesion of monocytes to the endothelial cells, with subsequent transmigration into the vascular intima. Soluble leukocyte and vascular cell adhesion molecules (CAM), such as selectins, integrins, sVCAM-1, and sICAM-1, play critical roles in the adhesion of monocytes to endothelial cells.

Peer review

The manuscript provides information about pro-atherosclerotic markers and cardiovascular risk factors one year after liver transplantation. This subject would certainly contribute to improve the knowledge about liver transplantation.

REFERENCES

- Desai S, Hong JC, Saab S. Cardiovascular risk factors following orthotopic liver transplantation: predisposing factors, incidence and management. *Liver Int* 2010; **30**: 948-957 [PMID: 20500807 DOI: 10.1111/j.1478-3231.2010.02274.x]
- Stravitz RT, Carl DE, Biskobing DM. Medical management of the liver transplant recipient. *Clin Liver Dis* 2011; **15**: 821-843 [PMID: 22032531 DOI: 10.1016/j.cld.2011.08.007]
- Hanounieh IA, Feldstein AE, McCullough AJ, Miller C, Aucejo F, Yerian L, Lopez R, Zein NN. The significance of metabolic syndrome in the setting of recurrent hepatitis C after liver transplantation. *Liver Transpl* 2008; **14**: 1287-1293 [PMID: 18756451 DOI: 10.1002/lt.21524]
- Albeldawi M, Aggarwal A, Madhwal S, Cywinski J, Lopez R, Eghtesad B, Zein NN. Cumulative risk of cardiovascular events after orthotopic liver transplantation. *Liver Transpl* 2012; **18**: 370-375 [PMID: 22140067 DOI: 10.1002/lt.22468]
- Raval Z, Harinstein ME, Skaro AI, Erdogan A, DeWolf AM, Shah SJ, Fix OK, Kay N, Abecassis MI, Gheorghiadu M, Flaherty JD. Cardiovascular risk assessment of the liver transplant candidate. *J Am Coll Cardiol* 2011; **58**: 223-231 [PMID: 21737011 DOI: 10.1016/j.jacc.2011.03.026]
- Watt KD, Charlton MR. Metabolic syndrome and liver transplantation: a review and guide to management. *J Hepatol* 2010; **53**: 199-206 [PMID: 20451282 DOI: 10.1016/j.jhep.2010.01.040]
- Lauer MS. Screening asymptomatic subjects for subclinical atherosclerosis: not so obvious. *J Am Coll Cardiol* 2010; **56**: 106-108 [PMID: 20620725]
- Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. *J Am Coll Cardiol* 2007; **49**: 2129-2138 [PMID: 17531663]
- Kannel WB. Overview of hemostatic factors involved in atherosclerotic cardiovascular disease. *Lipids* 2005; **40**: 1215-1220 [PMID: 16477805]
- Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and vali-

- dation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461]
- 11 **Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.** Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; **285**: 2486-2497 [PMID: 11368702]
 - 12 **Grundey SM, Hansen B, Smith SC, Cleeman JI, Kahn RA.** Clinical management of metabolic syndrome: report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. *Arterioscler Thromb Vasc Biol* 2004; **24**: e19-e24 [PMID: 14766740]
 - 13 **Matsudo SM, Matsudo VR, Araujo TL, Andrade DR, Andrade EL, de Oliveira LC, Braggion GF.** The Agita São Paulo Program as a model for using physical activity to promote health. *Rev Panam Salud Publica* 2003; **14**: 265-272 [PMID: 14662077]
 - 14 **Pagadala M, Dasarathy S, Egtesad B, McCullough AJ.** Post-transplant metabolic syndrome: an epidemic waiting to happen. *Liver Transpl* 2009; **15**: 1662-1670 [PMID: 19938136 DOI: 10.1002/lt.21952]
 - 15 **Coss E, Watt KD, Pedersen R, Dierkhising R, Heimbach JK, Charlton MR.** Predictors of cardiovascular events after liver transplantation: a role for pretransplant serum troponin levels. *Liver Transpl* 2011; **17**: 23-31 [PMID: 21254341 DOI: 10.1002/lt.22140]
 - 16 **Calan M, Calan O, Gonen MS, Bilgir F, Kebapcilar L, Kulac E, Cinali T, Bilgir O.** Examination of adhesion molecules, homocysteine and hs-CRP in patients with polygenic hypercholesterolemia and isolated hypertriglyceridemia. *Intern Med* 2011; **50**: 1529-1535 [PMID: 21804277]
 - 17 **Yajnik CS, Joglekar CV, Chinchwadkar MC, Sayyad MG, Deshpande SS, Naik SS, Bhat DS, Ganpule A, Shetty P, Yudkin JS.** Conventional and novel cardiovascular risk factors and markers of vascular damage in rural and urban Indian men. *Int J Cardiol* 2013; **165**: 255-259 [PMID: 21925749 DOI: 10.1016/j.ijcard.2011.08.053]
 - 18 **Jarrar MH, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, Fang Y, Elariny H, Goodman Z, Chandhoke V, Younossi ZM.** Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **27**: 412-421 [PMID: 18081738]
 - 19 **Aharinejad S, Krenn K, Zuckermann A, Schäfer R, Paulus P, Seebacher G, Wolner E, Grimm M.** Matrix metalloproteases and their tissue inhibitor in cardiac transplantation. *Eur J Cardiothorac Surg* 2007; **32**: 48-51 [PMID: 17482473]
 - 20 **Migita K, Maeda Y, Abiru S, Nakamura M, Komori A, Yokoyama T, Takii Y, Mori T, Yatsuhashi H, Eguchi K, Ishibashi H.** Immunosuppressant FK506 inhibits matrix metalloproteinase-9 induction in TNF-alpha-stimulated human hepatic stellate cells. *Life Sci* 2006; **78**: 2510-2515 [PMID: 16303143]
 - 21 **Mueller AR, Platz KP, Haak M, Undi H, Müller C, Kötting E, Weidemann H, Neuhaus P.** The release of cytokines, adhesion molecules, and extracellular matrix parameters during and after reperfusion in human liver transplantation. *Transplantation* 1996; **62**: 1118-1126 [PMID: 8900313]
 - 22 **Elias-Miro M, Massip-Salcedo M, Jimenez-Castro M, Peralta C.** Does adiponectin benefit steatotic liver transplantation? *Liver Transpl* 2011; **17**: 993-1004 [PMID: 21671349 DOI: 10.1002/lt.22358]
 - 23 **Rabelo F, Oliveira CP, Faintuch J, Mazo DF, Lima VM, Stefano JT, Barbeiro HV, Soriano FG, Alves VA, Carrilho FJ.** Pro- and anti-inflammatory cytokines in steatosis and steatohepatitis. *Obes Surg* 2010; **20**: 906-912 [PMID: 20454933]
 - 24 **Brogren H, Wallmark K, Deinum J, Karlsson L, Jern S.** Platelets retain high levels of active plasminogen activator inhibitor 1. *PLoS One* 2011; **6**: e26762 [PMID: 22069469 DOI: 10.1371/journal.pone.0026762]
 - 25 **Wiest R, Weigert J, Wanninger J, Neumeier M, Bauer S, Schmidhofer S, Farkas S, Scherer MN, Schäffler A, Schölmacher J, Buechler C.** Impaired hepatic removal of interleukin-6 in patients with liver cirrhosis. *Cytokine* 2011; **53**: 178-183 [PMID: 20637651 DOI: 10.1016/j.cyt.2010.06.013]
 - 26 **Oliveira CP, Kappel CR, Siqueira ER, Lima VM, Stefano JT, Michalczuk MT, Marini SS, Barbeiro HV, Soriano FG, Carrilho FJ, Pereira LM, Alvares-da-Silva MR.** Effects of hepatitis C virus on cardiovascular risk in infected patients: a comparative study. *Int J Cardiol* 2013; **164**: 221-226 [PMID: 21784542 DOI: 10.1016/j.ijcard.2011.07.016]
 - 27 **Buechler C, Wanninger J, Neumeier M.** Adiponectin, a key adipokine in obesity related liver diseases. *World J Gastroenterol* 2011; **17**: 2801-2811 [PMID: 21734787 DOI: 10.3748/wjg.v17.i23.2801]
 - 28 **Oliveira CP, Stefano JT, Alvares-da-Silva MR.** Cardiovascular risk, atherosclerosis and metabolic syndrome after liver transplantation: a mini review. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 361-364 [PMID: 23639094 DOI: 10.1586/egh.13.19]
 - 29 **Estep JM, Baranova A, Hossain N, Elariny H, Ankrah K, Afendy A, Chandhoke V, Younossi ZM.** Expression of cytokine signaling genes in morbidly obese patients with non-alcoholic steatohepatitis and hepatic fibrosis. *Obes Surg* 2009; **19**: 617-624 [PMID: 19280268 DOI: 10.1007/s11695-009-9814-x]
 - 30 **Laish I, Braun M, Mor E, Sulkes J, Harif Y, Ben Ari Z.** Metabolic syndrome in liver transplant recipients: prevalence, risk factors, and association with cardiovascular events. *Liver Transpl* 2011; **17**: 15-22 [PMID: 21254340 DOI: 10.1002/lt.22198]
 - 31 **Dec GW, Kondo N, Farrell ML, Dienstag J, Cosimi AB, Semigran MJ.** Cardiovascular complications following liver transplantation. *Clin Transplant* 1995; **9**: 463-471 [PMID: 8645890]
 - 32 **Fouad TR, Abdel-Razek WM, Burak KW, Bain VG, Lee SS.** Prediction of cardiac complications after liver transplantation. *Transplantation* 2009; **87**: 763-770 [PMID: 19295324 DOI: 10.1097/TP.0b013e318198d734]

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