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High prevalence of early atherosclerotic and cardiac damage in patients undergoing liver transplantation: Preliminary results

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ABSTRACT

Liver transplanted patients are at high risk of metabolic syndrome and its complications. We aimed to prospectively evaluate the early onset of cardiovascular alterations in patients submitted to the transplant waiting list. From January 2014 to January 2016, 54 out of 79 patients on the waiting list with decompensated cirrhosis or hepatocellular-carcinoma received the transplant, 50 were followed for 24 months, 2 died post-surgery and 2 were lost to follow-up. A significantly increased prevalence of visceral adiposity (epicardial adipose tissue thickness ($p = 0.001$) and worsening of carotid damage ($p = 0.003$) and diastolic dysfunction (E/A $p = 0.001$) was observed at 6 months after transplant and remained stable at 24 months, corresponding to an increased prevalence of diabetes, metabolic syndrome, hypertension and dyslipidemia. The duration of steroid therapy, withdrawn in the majority of patients at 3 months, did not influence cardiovascular damage. No significant difference in early progression of cardiovascular damage was observed between patients who did or did not receive a graft with steatosis.
Conclusion: The occurrence of early cardiovascular alterations in the first 6 months after OLT accounts for the reported cardiovascular events in the first years after transplant. In light of these results, new strategies aimed at preventing or delaying cardiovascular alterations should be provided, starting from the first weeks after transplant.

1. Introduction

Liver Transplantation (LT) represents the only therapy for end-stage liver diseases of any aetiology and, from 1968 to 2016, 132,466 patients have been transplanted in Europe, with about 7300 LTs per year in the last few years [1].

The improvement of surgical techniques, the use of effective anti-rejection drugs and the availability of direct antivirals to prevent hepatitis B virus (HBV) and hepatitis C virus (HCV) graft reinfection has significantly improved the survival of transplant recipients. However, an increased incidence of metabolic disorders [2], as well as cerebrovascular and cardiovascular (CV) diseases occurring 5–10 years after transplant [3–5], have been reported leading to a better investigation of pre- and post-transplant cardiovascular risk factors and related morbidity and mortality [6–10].

Interestingly, it was observed that cardiovascular events occur early after transplant in more than half of the patients, after a median time from transplant of 17.4 months [5], suggesting the occurrence of subclinical and clinical atherosclerotic alterations in the first months after orthotopic liver transplantation (OLT). How-

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ever, no prospective studies are available on the timing of the development of atherosclerotic damage in adults and only little data have been reported in the paediatric population [11].

In the last few years, ultrasonographic techniques able to detect subclinical cardiovascular atherosclerotic changes have been developed [12]. Carotid intima thickness (cIMT), recently associated with the Quality Intima-Media Thickness (QIMT) measurement by radiofrequency signal-based technology, is considered an early marker of atherosclerosis and a useful way to predict the risk of future CV events [13].

Arterial wall stiffness, which is evaluated by carotid pulse wave velocity (PWV) and beta stiffness, the index that reveals arterial compliance independent of blood pressure, is another parameter which illustrates early vascular damage and parallels vascular ageing [14], as it measures the elasticity of vessel and is able to predict cardiovascular risk in the general population as well as in patients with hypertension, diabetes and end-stage renal disease [15,16]. Finally, epicardial adipose tissue (EAT) thickness, which mirrors visceral obesity, has been reported to be related to vascular damage and to diastolic dysfunction, both in patients with non-alcoholic fatty liver disease (NAFLD) and in the general population [14,17]. Recently, EAT has also been considered a cardio-metabolic risk marker [18] and a potential therapeutic target [19].

The development of atherosclerosis in patients with liver cirrhosis is debated. Some Authors have reported an increased risk of carotid atherosclerosis in cirrhosis due to HCV infection or due to NAFLD and coexistent diabetes [20,21], while others have reported that patients with end-stage cirrhosis are protected from cardiovascular disease [22,23].

Other factors with a possible clinical impact on the metabolic changes and cardiovascular disease occurrence in LT recipients are the use of liver grafts with steatosis, [24,25], as well as de novo steatosis [26], however there are few available data.

In the present study, we aimed to prospectively investigate risk factors, incidence and timing of cardiovascular alterations in relation to metabolic changes in patients receiving a liver transplant.

2. Material and methods

2.1. Study design and patients

We studied 79 patients with decompensated cirrhosis or hepatocarcinoma, who were waitlisted for liver transplantation from January 2014 to January 2016, of which 54 (68%) were transplanted within 2–8 months. Fifty patients were followed for 24 months (2 died immediately post-surgery and 2 were lost to follow-up). The flow chart of patients included in the study is reported in Supplemental Fig. 1. All patients enrolled in the study had no history of previous cardiovascular events (myocardial infarction or stroke). All patients before transplantation performed a stress echocardiography, and those over the age of 55 or with multiple cardiac risk factors performed a coronary angiography to exclude the presence of ischaemic heart disease.

All transplanted patients started immunosuppressive therapy as follows: prednisone followed by a gradual tapering until 20 mg/day after one week and withdrawal after 3 months [27]; 6 patients were given prolonged steroid therapy until month 6, while 4 patients were given the same therapy until month 10 due to acute rejection episodes. All received calcineurin inhibitors (Tacrolimus or Cyclosporine), which were administered within 12 h post-transplant. Tacrolimus (blood levels between 6 and 8 ng/mL) was given in 39 patients, while 13 patients received Cyclosporine (including the 2 patients lost during follow up). In 18 patients, Mycophenolate mofetil (MMF) and in 4 Everolimus were added to reduce Calcineurin inhibitors dosage.

2.1.1. General assessment

Evaluation of anthropometric, clinical and biochemical parameters was done at patient enrollment and post-transplant at months 6, 12 and 24. Diabetes mellitus was defined using standard criteria (fasting glucose > 126 mg/dL, 120-min glucose > 200 during oral glucose tolerance test, or treatment with antidiabetic drugs) and the metabolic syndrome (MetS) was defined according to the ATP III criteria. The presence of arterial hypertension was defined as having systolic blood pressure over 140 mmHg or diastolic blood pressure over 90 mmHg more than twice or as taking anti-hypertensive medications. Cardiovascular risk was calculated using the European cardiovascular disease risk assessment model (SCORE risk charts) [28]. According to smoking habits, individuals were categorized into current, former and never-smokers.

All patients underwent ultrasonography at enrollment and during follow-up for the evaluation of steatosis. The presence of steatosis in the donor graft was evaluated by pathological examination before transplant.

2.1.2. Specific assessment

Echocardiography study: A single-experienced cardiologist performed a focused two-dimensional transthoracic echocardiography, using a commercially available device (Esaote My lab 30 gold, Italy). Data acquisition was performed with a 3.5-MHz transducer at a depth of 16 cm in the parasternal and apical views. All recordings were digitally stored for off-line analyses. Conventional echocardiographic parameters were measured according to the American Society of Echocardiography (ASE) guidelines [29]. M-mode echocardiograms of the left ventricle were recorded from the parasternal long-axis view, guided by a two-dimensional image. The following parameters were determined: interventricular septum thickness diastolic (IVSTd), posterior wall thickness and left atrium (LA) diameter were evaluated in M-mode; the ejection fraction (EF) was calculated at the apical four chamber view. Left ventricular mass (LVM) was calculated in grams using the following formula: $0.8 \times (1.04[(\mathrm{LVID} + \mathrm{IVS} + \mathrm{PWT})3 - \mathrm{LVID3}]) + 0.6$. LVM was normalized for height to the 2.7 power (LVM/H2.7). Pulsed-wave Doppler images at the level of the mitral valve tips from apical four-chambers two-dimensional views were obtained to measure flow velocities in the peak early diastolic (E-wave) and peak late diastolic (A-wave) phase, and to calculate their ratio (E/A) as measurement of diastolic filling. The most accurate evaluation of the epicardial fat of the right ventricle was obtained by measuring EAT in the parasternal long- and short-axis views, with optimal cursor beam orientation in each view. Maximum EAT (the mean of at least three measures) was obtained during end systole.

Carotid Atherosclerosis assessment: B-mode examinations were undertaken on the distal wall of the common carotid artery on the optimal image and IMT were obtained. The common carotid artery, carotid bulb and portions of the internal and external carotid arteries on the left side were scanned. The scan encompassed the region between 30 mm proximal to the beginning of the dilation of the bifurcation bulb and 15 mm distal to the common carotid artery flow divider. Mean carotid arteries intima-media thickness (cIMT), and carotid plaques were determined by high-resolution B-mode US with a 7.5-MHz transducer, as described previously [30]. QIMT, assessed by vascular ultrasound of ESAOTE, employs radiofrequency signal-based technology and includes Quality Intima-Media Thickness (QIMT) measurement and Quality Arterial Stiffness (QAS) measurement. A radiofrequency signal is a reflected ultrasound signal that is captured by the transducer and converted in an electric signal, preserving all the characteristics of the acoustic wave in terms of Amplitude and Phase [12]. Local arterial stiffness is estimated as systo-diastolic changes in

Table 1
Characteristics at enrollment, of the overall patients in the waiting list and of the 54 patients who underwent OLT including 50 followed for 24 months.

Variables	Overall ($n = 79$)	Patients transplanted ($n = 54$)	Patients non transplanted ($n = 25$)	p
Age at enrollment years	54.8 ± 10	52.6 ± 10	58 ± 5	0.004
Age at transplant years	54.2 ± 10	52.3 ± 11	58.4 ± 10	0.004
Sex M	56 (71)	38 (72)	18 (72)	0.9
Smokers (%)	43 (54)	33 (61)	10 (40)	0.8
CVD familiarity (%)	23 (29)	15 (28)	8 (32)	0.7
Causes of underlying liver disease				
HCV (%)	25 (30)	13 (24)	12 (48)	0.5
HCV + alcohol intake (%)	12 (15)	11 (20)	1 (4)	0.2
HBV HBV/DELTA (%)	23 (28)	13 (24)	10 (40)	0.06
NAFLD/NASH/metabolic cirrhosis (%)	8 (10)	6 (11)	2 (8)	0.7
Alcohol abuse (%)	7 (8)	5 (9)	3 (12)	0.8
Other[a] (%)	9 (11)	6 (11)	3 (12)	0.9
Child Pugh Turcotte score				
A5–6 (%)	30 (37)	22 (41)	8 (32)	0.49
B7–8 (%)	12 (15)	7 (12)	5 (20)	0.85
C9–14 (%)	37 (46)	25 (47)	12 (48)	0.75
MELD	12 ± 4.8	13.8 ± 5.1	13.5 ± 4.6	0.3
Anti viral therapy (%)	45 (56)	37 (68)	8 (32)	0.06
Hepatocellular carcinoma (%)	34 (43)	25 (46)	9 (36)	0.8
Metabolic complications				
MetS (%)	11 (14)	8 (15)	3 (12)	0.2
Diabetes (%)	18 (23)	13 (24)	5 (20)	0.9
Hypertension (%)	30 (38)	20 (37)	10 (40)	0.2
Dyslipidemia (%)	14 (18)	9 (16)	5 (20)	0.8
Therapy for metabolic complications				
Anti hypertensive	24 (30)	19 (35)	5 (20)	0.8
Hypoglycaemic treatment	13 (16)	9 (17)	4 (16)	0.9
Statin	5 (6)	4 (8)	1 (4)	0.7

Mean ± SD, n (%).
CVD, cardiovascular disease; MetS, metabolic syndrome.
[a] Others = 4 primary sclerosing cholangitis, 1 autoimmune hepatitis, 1 liver adenomatosis.

arterial diameter/area over systo-diastolic changes in distending pressure (pulse pressure). Due to its high frame rate and radiofrequency signal resolution, QAS is able to follow the movement of the arterial wall throughout the cardiac cycle with great accuracy. From arterial distension and local pressure, the number of stiffness parameters is automatically calculated, including local carotid pulse-wave velocity (PWV), arterial distensibility [31] and β stiffness index (βSI) [32]. All carotid evaluations were performed with the high-resolution model ultrasonographic device, My Lab70 6–18 MHz B-mode (Esaote, Genoa, Italy).

2.2. Statistical analysis

To compare pre-OLT variables in transplanted and non-transplanted patients, we used the chi-squared (for categorical variables) and the Mann–Whitney test (for quantitative variables). To evaluate time trends while taking into account within-subject correlations, we used two types of random-intercept regression models: linear (for quantitative variables) and Poisson with robust standard error (for dichotomous variables). In these models, we calculated a single global Wald test on 6-, 12-, and 24-month regression coefficients together (vs baseline) and also a p-value for linear trend. The potential modifying effect of other variables on time trends was analyzed by fitting models containing interaction (product) terms and then by evaluating product terms by global Wald tests. Statistical analyses were performed with Stata 15 (Stata Corp. 2017, Stata: Release 15, Statistical Software, College Station, TX: StataCorp LP).

The study project was approved by the institutional review board, entitled "Comitato Etico Milano Area B, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico," with protocol number 2763. All patients gave informed, written consent to participate in the study, according to a protocol conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

3. Results

3.1. Pre-transplant

3.1.1. Characteristics, at enrolment, of the patients studied

The baseline characteristics of the overall patients in the waiting list (79 cases), and of the 54 patients who underwent OLT are shown in Table 1. The mean age of patients transplanted was 52.6 ± 10. Twenty-five patients (46%) had hepatocellular carcinoma, of whom 8 also had decompensated cirrhosis. HCV infection was present in 24 (44%) patients (11 with and 13 without coexistent alcohol intake); HBV in 13 (24%); alcohol alone in 5 (9%); NAFLD-related cirrhosis in 6 (11%); primary sclerosing cholangitis in 4 (5%); autoimmune hepatitis in 1; liver adenomatosis in 1. No differences, except for age, of epidemiological, anthropometric, metabolic and clinical characteristics were observed at enrollment among patients on the waiting list (79 cases), patients who were transplanted (54 cases) and those non-transplanted (25 cases) (see Table 1).

Overall smoking habit was 61% and more frequent in male patients (71%); while a family history of cardiovascular disease was present in 28%. Two patients died perioperatively.

3.1.2. Cardiovascular disease

Of the 50 patients who were transplanted and followed, 27 (55%) had carotid plaques before transplant: The highest prevalence was observed in patients with HCV (59%), followed by NAFLD (19%), alcohol-related cirrhosis (11%) compared to HBV (7%) and autoimmune/primary biliary cholangitis (4%) ($p = 0.002$). No differ-

Table 2
Vascular and echo-cardiac parameters of the 50 transplanted and followed at 6, 12 and 24 months after OLT.

Variables	Pre OLT $n=50$	6 months $n=50$	12 months $n=50$	24 months $n=50$	p 6, 12 and 24 months vs pre OLT	p-Trend over time
QIMT (μm)	607.1 ± 101	669.9 ± 112	668.1 ± 122	685.2 ± 118	<0.0001	<0.0001
cIMT (mm)	0.78 ± 0.16	0.82 ± 0.19	0.87 ± 0.19	0.83 ± 0.16	0.002	0.005
QAS						
βSI	10.4 ± 4.6	10.2 ± 4.6	10.8 ± 7.2	10.5 ± 3.7	0.97	0.97
PWV (m/s)	7.4 ± 1.7	7.7 ± 1.6	7.8 ± 2.0	7.7 ± 1.4	0.59	0.34
cplaques (%)	26 (52)	25 (51)	27 (54)	33 (67)	0.02	0.008
E/A	1.10 ± 0.31	0.86 ± 0.27	0.90 ± 0.32	0.86 ± 0.20	<0.0001	<0.001
EF (%)	74.4 ± 9.3	74.6 ± 9.8	74.1 ± 11.1	73.2 ± 11.5	0.86	0.44
IVSTd (mm)	10.2 ± 2.1	10.5 ± 2.0	10.2 ± 2.5	11.9 ± 1.9	0.46	0.001
LVM (g)	220.4 ± 76	221.8 ± 81	205.9 ± 76	227.9 ± 71	0.07	0.5
LA (mm)	39.3 ± 6.7	39.2 ± 6.3	37.4 ± 7.0	37.6 ± 8.8	0.07	0.03
EAT (mm)	5.9 ± 2.6	8.1 ± 2.8	7.7 ± 1.9	8.1 ± 2.3	<0.001	<0.0001

QIMT, intima media thickness (IMT) evaluated by radiofrequency; cIMT, carotid intima media thickness; QAS, carotid stiffness parameters: β Stiffness Index (βSI) and pulse wave velocity (PWV); cplaques, carotid plaques; E/A, early diastolic (E-wave)/late diastolic (A-wave), ratio; EF, ejection fraction; IVSd, interventricular septum thickness diastolic; LVM, left ventricular mass; LA, left atrium; EAT, epicardial adipose tissue thickness.

ence was observed in the frequency of carotid plaques in patients with or without HCC at enrolment in the study.

3.2. Post-transplant

3.2.1. Changes of cardiovascular, metabolic and clinical parameters

Vascular and echo-cardiac parameters are shown in Table 2 and Fig. 1 (panels A–C). A worsening of indices of early damage of carotid (IMT), diastolic dysfunction and echocardiographic alterations, including a significant increase in epicardial adipose tissue (EAT), was already evident at 6 months of observation and maintained the significance over time. The prevalence of carotid plaques, stable during follow-up, markedly increased at 24 months (52%, 51%, 54% at 0, 6, 12 months and 67% at 24 months) ($p=0.008$, trend over time).

The major changes in metabolic and clinical parameters were present at 6 months post liver transplant and considering coefficients of each parameter at 6, 12 and 24 months together compared to the one of pre-OLT, a statistically significant difference was maintained (Table 3). In addition, out of the 7 patients with MetS at enrollment, 4 maintained MetS, 3 reverted and 13 patients had de novo occurrence of MetS at 6 months. Diabetes was present in 13 patients at enrollment, 2 reverted, while 13 patients developed it de novo, with a significant increase at 6 months (26% vs 48%, $p=0.006$). Prevalence of hypertension and hypertriglyceridemia increased significantly at 6 months post-OLT (hypertension 40% vs 68%, $p=0.004$, hypertriglyceridemia 4% vs 17%, $p=0.05$), while systolic and diastolic blood pressure, level of lipids and creatinine significantly increased throughout the follow-up period.

3.2.2. Relationship between fatty liver after transplant and metabolic and CV parameters

Eleven patients received a liver graft with histological evidence of steatosis ≥20% (microvacular in 5, macrovacular in 3 and mixed in 3), in 6/11 steatosis disappeared during follow up. De novo steatosis developed in 13 (26%), with a total number of 21 cases of fatty liver observed during the follow-up period.

A trend of a higher prevalence of diabetes, hypertension and early progression of cardiovascular damage (documented by increased value of QIMT, PWV, carotid plaques and E/A) was observed in patients showing liver steatosis after transplant (de novo, recurrence or maintained steatosis in fatty graft) compared to those without steatosis, however these differences did not reach statistical significance. Only the prevalence of MeS was significantly different in patients without steatosis than in those with it (21% vs 47%, $p=0.05$).

3.2.3. Effect of immunosuppressive therapy on cardiovascular parameters

To evaluate if the type of immunosuppressive regimen was significantly associated with carotid and cardiac alterations, we performed the test for interaction. We observed a significantly lower progression in IMT (at baseline pre-OLT, 6 and 12 month post-OLT) in patients who were treated with Tacrolimus (cIMT 0.78–0.80–0.84 mm, QIMT 610 ± 99; 663 ± 121; 670 ± 119 μm) compared to those receiving CyA (cIMT 0.76–0.87–0.98 mm QIMT 596 ± 111; 687 ± 107; 737 ± 132 μm), p for interaction $p=0.02$ and $p=0.01$. By contrast, the duration of steroid therapy did not influence vascular and cardiac parameters.

4. Discussion

In the present prospective study, we demonstrate that atherosclerotic vascular alterations occur very early in patients transplanted for liver disease of any aetiology.

CVD represents a major cause of morbidity and mortality after OLT [33]. Madhwal et al. [33] reported that the pooled estimated 10-year risk of developing CV events among transplant recipients was 13.6% among patients with MetS, who were four-fold more likely to have CV events. Interestingly, in a large study including more than 775 post-OLT recipients, the median time of cardiovascular events occurrence from transplant was less than 18 months [4], and patients who developed CV events were more likely to have MetS. These data are supported by evidence reporting that metabolic alterations may occur in the first months after OLT [34], while information on the timing of subclinical and clinical atherosclerosis has never been described.

The results in our study series are in line with these data, with MetS present at enrollment in 15% of the patients and doubling after 6 months. Thus, the early worsening of cardiovascular parameters (QIMT, E/A) of our study series is supported by the concomitant metabolic alterations.

The cardiovascular parameters that significantly worsened after OLT were carotid thickness, E/A, which reflects diastolic dysfunction, and EAT – all of which are features that have previously been reported to be associated with the presence of steatosis [35,36] and with atherosclerosis development. Interestingly, after a very early progression (6 months), we observed a relative stability of cardiovascular parameters, as well as occurrence of diabetes, hypertriglyceridemia and MetS. These data parallel the results by Fernandez Miranda et al. [37], who reported an increased incidence of metabolic cardiovascular risk factors in the first year after transplant, followed by a decrease after 5 years, possibly related to lifestyle modification and change of immunosuppressive therapy schedule. Only the increase of the prevalence of carotid plaques

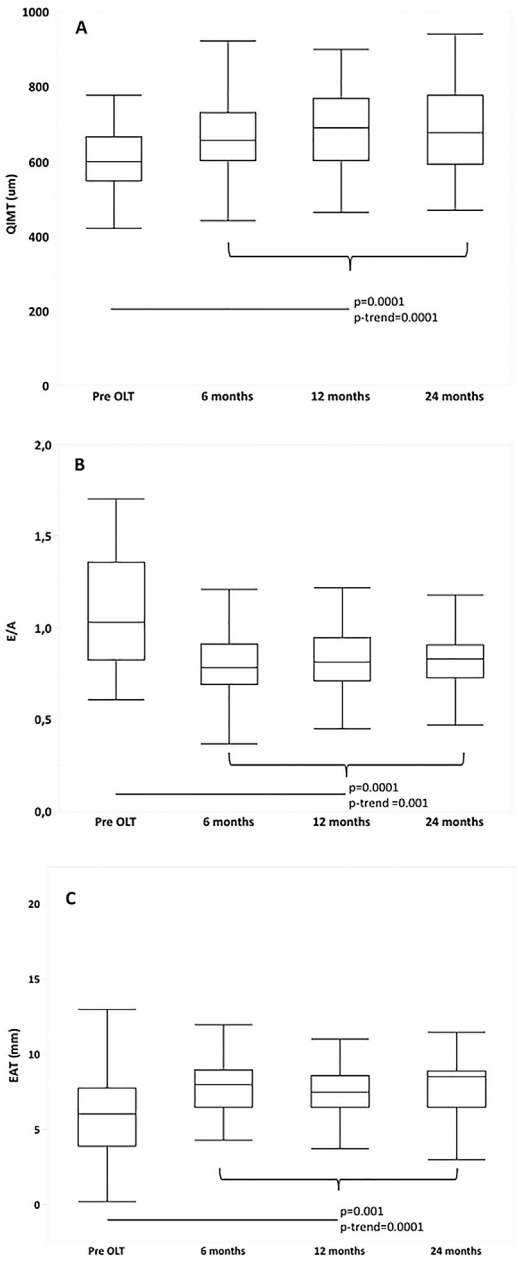

Fig. 1. Cardiovascular variables of the 50 patients who underwent OLT at enrollment (Pre OLT) and at 6, 12 and 24 months after OLT. Significant differences between pre OLT and 6, 12 and 24 months follow-up of QIMT (Quality Intima-media thickness) (Panel A), diastolic dysfunction (E/A) (Panel B) and epicardial adipose tissue (EAT) thickness (Panel C) ($p < 0.001$ for all variables, p-trend 0.0001).

was evident later, at 24 months. It is intriguing that, although at enrolment all patients had cirrhosis with nutritional changes (low cholesterol, etc.) that are potentially protective for cardiovascular damage, more than 50% of them had carotid plaques, with a prevalence higher than that of the same-age general population [38,39]. Plaques, as expected, were more frequent in older patients and in subjects with HCV infection [20,39]. Compared to the other CV parameters, the later onset of carotid damage is possibly due to the increase of cholesterol after OLT and to the longer process of plaque formation.

The role of immunosuppressive therapy in the development of metabolic alterations after transplant is well reported [40]. Different studies indicate that metabolic alterations, including arterial hypertension, hyperglycemia and hyperlipemia, are differently affected by steroid, cyclosporine and tacrolimus [41] after transplant, but few and contrasting data have been reported on the role of immunosuppressive therapy on early CV damage. Using the test for interaction, we could demonstrate a lower progression of carotid damage, as shown by the value of IMT, in patients in Tacrolimus therapy, confirming the reduced trend of cardiovascular risk score in patients on this therapeutic regimen, as recently reported by Cuervas-Mons et al. [42]. However, given that the large majority of our patients were on Tacrolimus, results need to be confirmed in larger series of patients taking different immunosuppressive therapies. Interestingly, the duration of steroid therapy did not influence the timing of CV alterations.

We studied lipid deposition around the heart, since it has been reported that high EAT plays an important role in the pathogenesis of cardiovascular disease, likely for the inflammatory process which takes place in the adipose tissue [43]. In addition, several reports indicate that overweight/obesity, either present before transplantation or developed de novo (in 30–70% after 1–5 years), may facilitate cardiovascular damage occurrence [39–41]. However, we did not observe significant weight gain after OLT in our cohort, since obesity developed in less than 10%, probably due to the strict follow-up and nutritional counselling to which our patients were submitted after OLT. Nevertheless, EAT, which represents a valid parameter of visceral adiposity (abdominal circumference cannot be used for the possible presence of ascites), increased at 6 months, thus confirming our preliminary data [40]. Our results suggest that visceral obesity, mainly around the heart and independent from BMI, plays an important role in cardiovascular disease development of transplanted patients. These results are also in agreement with what we previously observed in patients with NAFLD, in whom the distribution of fat, but not the weight gain, was related to vascular damage [44].

Interestingly, even if the transplanted patients had not undergone a precise aerobic and resistance physical exercises protocol, those who had performed regular physical activity had a significantly lower value of visceral adiposity, by EAT, over time ($p = 0.007$), as well as a lower prevalence of carotid plaques and lower diastolic dysfunction at 24 months ($p = 0.03$ and 0.04 respectively) (data not shown).

One recent emerging problem is the presence of steatosis in liver grafts, due to the large prevalence of obesity and advanced age in liver donors. To avoid the negative impacts of using donor liver grafts with steatosis, such as graft function and patient survival, it has been suggested to use only grafts with microvacuolar steatosis lower than 60% [25,45,46], since this variety of steatosis seems to be irrelevant on graft survival [24,47]. Recurrence of hepatic steatosis is common after OLT, occurring in 30%–80% of patients transplanted for metabolic liver diseases [47–49] and in up to 25% of patients with other indications [26]. In our series, about half of patients had steatosis (11 recipients received a graft with histological evidence of steatosis ≥20%), however we did not find significant differences of cardiovascular parameters in patients with or without steatosis, although the presence of steatosis has been reported to be associated with proatherogenic lipoproteins [26], development of cardiovascular risk factors such as diabetes, hypertension [47] and increased cardiovascular events [45]. However, the small sample size does not allow for any sound conclusion on the relation between steatosis and early atherosclerosis development.

The strength of this study is the finding that cardiovascular modifications occur at a very short time (6 months) after transplantation, highlighting the very early onset of vascular damage. Even if the main limitation is the small number of patients enrolled, by a methodological point of view, the timing of patient's enrollment, which began during waiting list and included at least three consecutive evaluations at different time intervals, provides unique and original data. If these preliminary findings will be confirmed in

Table 3
Anthropometric, biochemical, and clinical variables of the 50 patients transplanted and followed at 6, 12 and 24 months after OLT.

Variables	Pre OLT $n=50$	6 months $n=50$	12 months $n=50$	24 months $n=50$	p 6, 12 and 24 months vs pre OLT	p-Trend over time
BMI (kg/m^2)	25.5 ± 3.6	25.2 ± 3.0	25.4 ± 3.4	25.7 ± 3.8	0.32	0.39
SBP (mmHg)	119 ± 18	129 ± 13	132 ± 16	132 ± 15	<0.0001	<0.001
DBP (mmHg)	70 ± 9	78 ± 7	78 ± 11	76 ± 10	<0.001	<0.003
Fasting glucose (mg/dL)	109.7 ± 38	100.535	95.8 ± 19	102.5 ± 29	0.01	0.03
Glycated haemoglobin (%)	4.9 ± 0.7	5.4 ± 0.9	5.3 ± 1.0	5.4 ± 0.8	0.6	0.9
Total cholesterol (mg/dL)	128.7 ± 45	179 ± 44	173.0 ± 38	160.1 ± 39	<0.0001	<0.001
HDL (mg/dL)	43.7 ± 22	58.5 ± 19	54.1 ± 15	51.0 ± 17	<0.001	0.07
M	42 ± 18	51 ± 12	49 ± 12	46 ± 1	0.05	0.03
F	46 ± 30	75 ± 23	64 ± 15	62 ± 20	0.01	0.02
LDL (mg/dL)	66 ± 31	99 ± 38	97 ± 29	87 ± 23	<0.001	<0.001
Triglycerides (mg/dL)	82.6 ± 38	109.0 ± 41	109.5 ± 50	115.7 ± 82	<0.0001	<0.001
Serum creatinine (mg/dL)	0.81 ± 0.25	1.06 ± 0.27	1.1 ± 0.46	1.2 ± 0.4	<0.0001	<0.0001.
ALT (U/L)	48.2 ± 35	33.1 ± 49	17.5 ± 8	20.1 ± 13	0.0001	<0.0001
GGT (U/L)	93.2 ± 94	64.6 ± 89	37.7 ± 55	43.6 ± 56	0.006	<0.0001
AP (U/L)	157.5 ± 97	101.3 ± 46	102.8 ± 32	82.7 ± 25	<0.0001	<0.0001
Serum albumin (g/L)	3.5 ± 0.5	4.3 ± 0.4	4.4 ± 0.3	4.5 ± 0.3	<0.001	<0.001
Platelets ($\times 10^9$)	89.7 ± 58	148.5 ± 60	143.0 ± 58	160.8 ± 54	<0.001	<0.001
Cardiovascular risk score	1.6 ± 1.6	2.1 ± 2.3	2.2 ± 2.2	2.9 ± 2.0	0.001	<0.0001
MetS, n (%)	7 (14)	17 (34)	18 (36)	19 (38)	0.04	0.001
Diabetes, n (%)	13 (26)	24 (48)	26 (52)	18 (37)	0.006	0.08
Hypertriglyceridemia, n (%)	2 (4)	8 (17)	7 (16)	9 (18)	0.08	0.04
Hypertension, n (%)	20 (40)	34 (68)	27 (64)	31 (66)	0.01	0.03
Therapy						
Anti hypertensive, n (%)	18 (36)	27 (54)	29 (58)	32 (65)	0.007	0.001
Hypoglycaemic, n (%)	9 (18)	22 (44)	22 (44)	15 (31)	0.003	0.07
Statin, n (%)	4 (8)	2 (4)	1 (2)	4 (8)	0.33	0.9

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine-aminotransferase; GGT, gammaglutamyl transferase; AP, alkaline phosphatase; MetS, metabolic syndrome; HDL, high density lipoprotein.

larger multicenter studies, specific therapeutic approaches should be designed to prevent later cardiovascular events.

In conclusion, unexpectedly, early cardiovascular alterations are already evident after a few months following liver transplantation. The use of liver grafts with steatosis likely does not affect early cardiovascular damage. Therapeutic and behaviour strategies aimed at preventing or delaying cardiovascular alterations, which are eventually responsible for later cardiovascular events, should be provided in the first weeks after liver transplant. Future studies evaluating the role of endothelial biomarkers and inflammatory cytokines, which have been associated with transplant and NAFLD, could help in understanding these very early atherosclerosis alterations.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2019.07.007.

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