ORIGINAL ARTICLE

Prospective assessment of subclinical cardiovascular damage and associated factors in liver transplant recipients

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SUMMARY

Cardiovascular (CV) disease plays a major role after liver transplantation (LT). This prospective study assessed subclinical CV damage after LT by measuring pulse wave velocity (PWV), intima-media thickness (IMT) and left-ventricular mass index (LVMI) and characterized associated risk factors. We included 112 patients with a median of 1.8 years after LT (q1–q3 0.9–9.2). Fifty-three percent ($n = 59$) of patients had ≥2 annual assessments (median follow-up 1.6 years, q1–q3 1.1–2.0), with a total of 195 assessments. We found increased PWV (indicating arteriosclerosis) in 16% ($n = 17$), elevated IMT in 5% ($n = 5$; indicating atherosclerosis) and increased LVMI in 25% ($n = 24$; indicating left-ventricular hypertrophy). A linear mixed model analysis using all 195 assessments revealed that higher age and systolic blood pressure (BP) were associated with higher PWV ($\beta = 0.069$, $P < 0.001$ and $\beta = 0.022$, $P = 0.005$) and higher IMT ($\beta = 0.005$, $P < 0.001$ and $\beta = 0.001$, $P = 0.029$), while higher body mass index was associated with higher IMT ($\beta = 0.004$, $P = 0.023$). Higher systolic BP ($\beta = 0.200$, $P = 0.034$), male sex ($\beta = 8.847$, $P = 0.031$) and lower glomerular filtration rate ($\beta = -0.288$, $P < 0.001$) were associated with higher LVMI. Our data highlight not only the rate of subclinical CV damage in LT patients, but also the impact of classical CV risk factors (such as BP and body mass index) which outweighed LT-related factors. These modifiable risk factors are suitable targets for interventions to reduce CV morbidity in LT patients.

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Introduction

Liver transplantation (LT) is the established therapy for end-stage liver disease. Overall survival after LT is excellent [1,2], shifting the focus of post-LT care to late complications and comorbidities. In the past, cardiovascular (CV) disease was thought to be less common in patients with chronic liver disease [3]. CV mortality after LT, however, is responsible for 11–21% of overall post-LT mortality [1,4]. It is the predominant

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nonhepatic cause of death and is likely to increase with the nonalcoholic fatty liver disease (NAFLD) epidemic and the growing number of NAFLD necessitating LT [5]. CV risk factors such as dyslipidemia, hypertension or diabetes have been shown to increase post-LT [6]. Apart from these classical CV risk factors, nonclassical risk factors, such as microinflammation [7] and anaemia [8], are known to exacerbate CV risk, both highly relevant for LT patients. Inflammation is causal for a variety of chronic liver diseases, such as viral, autoimmune or steatohepatitis. The liver is the main extrarenal erythropoietin producer [9], and inadequate erythropoietin synthesis was reported after LT [10]. Furthermore, anaemia can also be a result of chronic renal disease, which is a known issue after LT [11,12] and is a further CV risk factor itself [13,14]. Lastly, side effects of the immunosuppressive therapy further increase the CV burden [15].

Published data on the risk for major adverse CV events in LT patients are heterogeneous, due to differing definitions of endpoints and duration of follow-up [16]. CV events are preceded by subclinical CV damage such as athero- and arteriosclerotic processes and left-ventricular hypertrophy (LVH) [17]. These parameters can be quantified by assessment of pulse wave velocity (PWV, indicating arteriosclerosis), intima-media thickness (IMT, indicating atherosclerosis) and left-ventricular mass index (LVMI, a measure for LVH) and are established surrogate markers predictive of future CV events [18–20]. Few studies are available on subclinical CV damage in subjects with chronic liver disease. A higher rate of carotid atherosclerosis was described in patients with NAFLD [21], as well as hepatitis C patients and was shown to improve after hepatitis C virus eradication [22]. Elevated PWV was found in patients with NAFLD [23]. Pre-LT LVH was shown to increase mortality post-LT [24]. We recently described elevated PWV in 22%, elevated IMT in 57% and LVH in 11% of paediatric LT recipients [25]. Data on subclinical CV damage in adult LT recipients, however, are lacking [26]. In addition, there are no studies characterizing cardiac geometry after LT.

The aim of this prospective study was to characterize the prevalence of subclinical CV damage in a large cohort of patients after LT. As we followed a subgroup of our patients longitudinally, we were able to explore the persistence of subclinical CV damage over time using linear mixed models and to determine factors associated with the markers for subclinical CV damage in LT patients.

Patients and methods

Study design

Between April 2014 and February 2018, 298 patients were identified in the LT outpatient clinic and approached. The inclusion criteria were age >18 years and having undergone LT. The only exclusion criterion was severe limb malformations that would have made measurements impossible and was not applicable to any patient. Of the screened patients, a total of 112 patients (38%) agreed to participate. Median time since LT was 1.8 years (first quartile – third quartile, q1–q3 0.9–9.2). Of these patients, the majority were followed longitudinally and investigated annually, leading to 59 patients (53%) completing a second assessment; 23 of those patients (21%) completing a third, and one patient a fourth post-LT assessment resulting in a total of 195 observations available for analysis. At each visit, we assessed CV risk factors and measured the markers for subclinical CV damage as described below, including the collection of blood and urine samples. Data on medical history and medication were obtained from patient interviews and medical records. Written informed consent was obtained. The study was approved by the institutional review board (#504) and complies with the Declaration of Helsinki.

CV examination

Blood pressure (BP) was measured in a standardized fashion using the Dinamap v100 (GE Healthcare, Chicago, IL, USA). Three readings were taken in a seated position after 10 minutes of rest and averaged. Carotid-femoral PWV was measured oscillometrically using the Vicorder device (Skidmore Medical Ltd., Bristol, UK) [27]. Carotid IMT measurement was conducted according to the Mannheim consensus [28] with a 3–12 MHz linear array transducer on the Philips CX50 device (Philips Healthcare, Amsterdam, the Netherlands). Five measurements were taken from the common carotid artery on every side, 1–2 cm proximal of the carotid bulb, and averaged. Echocardiography was performed using 1–5 MHz sector array transducer with the aforementioned device using the American Society of Echocardiography guidelines [29]. Measurements were taken on a perfectly aligned parasternal M-Mode view, by a single-blinded technician. LVMI was determined using the Devereux formula [30] and divided by the body surface area [29]. Relative wall thickness (RWT) was calculated using the formula (2× posterior wall

thickness)/(left-ventricular internal diameter in end-diastole) [29]. All measurements were performed by two experienced investigators who were uniformly trained based on standard operating procedures developed prior to the start of the study.

Definitions

BMI was classified according to the World Health Organization definition (25–<30 kg/m^2 as overweight, ≥30 kg/m^2 as obese) [31]. Waist circumference >88 cm for women and >102 cm for men was considered elevated and classified as central obesity [32]. Arterial hypertension was defined as systolic or diastolic BP values ≥140/90 mmHg or receiving antihypertensive therapy [33]. Lipids were classified according to current guidelines [32]: total cholesterol ≥200 mg/dl and low-density lipoprotein cholesterol ≥130 mg/dl as elevated; high-density lipoprotein cholesterol <40 mg/dl as low for men and <50 mg/dl for women; and triglycerides ≥150 mg/dl as elevated. Dyslipidemia was defined as any pathological lipid finding. Diabetes was defined as physician-diagnosed or if on antidiabetic drugs. eGFR was calculated using the cystatin C-based CKD-EPI formula and classified according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines [34]. Microinflammation was defined as high-sensitive C-reactive protein between 1 and 10 mg/l. Anaemia was defined as haemoglobin <12 g/dl for women and <13 g/dl for men [35]. PWV ≥ 10m/s was considered elevated [36]. IMT results were normalized for age and sex, using appropriate reference values and expressed as *z*-scores [37]; a *z*-score of 0 representing the 50th percentile. IMT with a *z*-score >1.645 (i.e. the 95th percentile) was considered elevated. LVH was defined as LVMI >95 g/m^2 for women and >115 g/m^2 for men. LVH was further divided as either concentric hypertrophy (RWT >0.42) or eccentric hypertrophy (RWT ≤0.42). Normal LVMI with RWT >0.42 was classified as concentric remodelling [29].

Statistical analysis

Calculations were performed using SAS EG 7.1 (Statistical Analysis Software, Cary, NC, USA). Continuous values are given as mean with standard deviation, or as median with first and third quartiles in case of non-normally distributed variables, as well as the range. Categorical values are given as numbers and percentages. A value of $P < 0.05$ was considered statistically significant. For the analysis of factors independently associated with subclinical CV damage, a separate multivariable linear mixed model was employed for every endpoint. All observations ($n = 195$) from all patients ($n = 112$) entered the model, with the exception of one patient after heart transplantation who was not included in the model for LVMI. Repeated measurements were accounted for in the model. The variance components structure was selected as the covariance matrix. Time since LT was used as the time variable, and age and sex were forced into the model. Furthermore, underlying disease, use of steroids, use of mammalian target of rapamycin inhibitor, serum albumin and aspartate aminotransferase as LT-related factors were entered into the model; and BMI, systolic BP, presence of diabetes, low-density lipoprotein cholesterol and eGFR as CV risk factors. All covariates were entered as fixed effects. Backward selection was employed for the final model building, with a $P > 0.05$ as threshold for removal. From the final models, standardized β regression coefficients and 95% confidence intervals were calculated and shown as Forest plots, in order to make them comparable between different covariates. Standardized β signifies the change in the dependent variable (PWV, IMT or LVMI) per 1 standard deviation change (or presence/absence in case of female sex) of the independent variable.

Results

Patient characteristics

A total of 112 patients (63% male, $n = 71$) aged 52.3 ± 12.0 years were enrolled (age range 19.3–76.9 years). Viral hepatitis was the most common underlying disease, followed by autoimmune liver disease. Furthermore, 21% ($n = 23$) of all patients had a diagnosis for hepatocellular carcinoma. Of all, 53% ($n = 59$) had more than 1 and 21% ($n = 23$) more than 2 assessments during the study period. Table 1 summarizes the patient characteristics at inclusion. Median time after LT was 1.8 years (q1–q3 0.9–9.2 years), with median age at LT at 49.6 years (q1–q3 39.9–58.0 years). Five patients had undergone liver retransplantation, and one patient had undergone a heart transplant before LT due to dilatative cardiomyopathy (and was excluded from the LVMI analysis). The most common immunosuppressive regimen consisted of tacrolimus, mycophenolate mofetil and prednisone ($n = 38$, 34%).

CV risk factors

CV risk factors at inclusion are summarized in Table 2 and Fig. 1. Thirty-three per cent ($n = 37$) of all patients

Table 1. Patient characteristics (n = 112).

Sex (male/female)	71/41 (63%/37%)
Age (years)	52.3 ± 12.0 (19.3–76.9)
Weight (kg)	75.2 ± 16.3 (42.0–129.0)
Height (m)	1.74 ± 0.11 (1.48–1.98)
Underlying disease	
Viral hepatitis	25/112 (22%)
Autoimmune*	23/112 (21%)
Toxic†	21/112 (19%)
Hepatitis C and toxic liver failure	2/112 (2%)
Cryptogenic cirrhosis	19/112 (17%)
Metabolic disease‡	9/112 (8%)
Other§	13/112 (12%)
Hepatocellular carcinoma	23/112 (21%)
Transplant information	
Age at liver transplantation (years)	49.6 [39.9–58.0] (0.3–67.8)
Time since liver transplantation (years)	1.8 [0.9–9.2] (0.0–35.1)
Time on waiting list (years)	0.4 [0.02–0.9] (0.0–13.3)
Retransplantation	5/112 (5%)
Combined transplantation (heart transplantation)	1/112 (1%)
Immunosuppression	
Use of tacrolimus	81/112 (72%)
Tacrolimus trough level (μg/l)	6.4 ± 2.8 (0.9–13.6)
Use of cyclosporin A	27/112 (24%)
Cyclosporin A trough level (μg/l)	63.4 ± 32.5 (14.9–117.0)
Use of mycophenolate mofetil	82/112 (73%)
Use of mammalian target of rapamycin inhibitor	15/112 (13%)
Use of steroids	72/112 (64%)
Most common immunosuppressive regimen	
Tacrolimus – mycophenolate – steroid	38/112 (34%)
Tacrolimus – mycophenolate	19/112 (17%)
Cyclosporin A – mycophenolate – steroid	13/112 (12%)

Continuous values are given as mean ± standard deviation, or as median [with first and third quartile in square brackets] for non-normally distributed variables, with the range in round brackets. Categorical values are given as numbers and percentages. The denominator shows the number of available observations for each parameter, and the numerator indicates the number of patients who fulfil that measure.

*Includes autoimmune hepatitis, primary sclerosing cholangitis, secondary sclerosing cholangitis and primary biliary cholangitis.

†Includes alcohol, amanita phalloides, paracetamol and other drug intoxications.

§Includes autosomal dominant polycystic kidney disease, primary hyperoxaluria, liver damage of unknown aetiology, Osler disease, biliary atresia, nodular regenerative hyperplasia and multiple focal nodular hyperplasia.

‡Includes non-alcoholic fatty liver disease, hemochromatosis, α1-antitrypsin deficiency, cystic fibrosis, Wilson's disease, glycogenosis.

were overweight, and 13% (n = 14) were obese. Diabetes was present in 14% (n = 16) of patients, and 15% (n = 15) were active smokers. Of all patients, 76% (n = 84) showed one or more findings indicative of dyslipidemia, with hypercholesterolaemia (n = 70; 63%) and hypertriglyceridemia (n = 50; 45%) as the most common factors (Table 2). Arterial hypertension was found in 71% (n = 79) with more than half of those patients undertreated (i.e. elevated BP in the presence of treatment) or even untreated (i.e. elevated BP without treatment, Table S1). Mean eGFR was 53.5 ± 22.4 ml/min/1.73 m^2. Thirty-seven per cent (n = 41) of all patients after LT showed severe renal impairment with eGFR < 45 ml/min/1.73 m^2, and 36% (n = 35) displayed moderate or severe albuminuria. According to KDIGO guidelines, 73% of all patients (n = 72) exhibited moderate or high risk for chronic kidney disease (Table S2). Anaemia was present in 45% (n = 50) of the cohort, and microinflammation could be found in 70% (n = 69).

Table 2. Cardiovascular risk factors at inclusion.

BMI (kg/m^2)	24.6 [21.1–26.9] (17.4–43.1)
Overweight (BMI ≥25 kg/m^2)	37/112 (33%)
Obese (BMI ≥30 kg/m^2)	14/112 (13%)
Waist circumference (cm)	97.2 ± 14.3 (73.0–131.0)
Central obesity [waist circumference ≥102 cm (male)/≥88 cm (female)]	50/106 (47%)
Active smoking	15/97 (15%)
Arterial hypertension*	79/112 (71%)
Elevated systolic BP (≥140 mmHg)	39/110 (36%)
Elevated diastolic BP (≥80 mmHg)	16/110 (15%)
Diabetes	16/112 (14%)
Dyslipidemia†	84/111 (76%)
Elevated triglycerides (≥150 mg/dl)	50/112 (45%)
Elevated cholesterol (≥200 mg/dl)	70/112 (63%)
Elevated LDL cholesterol (≥130 mg/dl)	33/111 (29%)
Reduced HDL cholesterol [<40 mg/dl (male)/<50 mg/dl (female)]	17/111 (15%)
eGFR (ml/min/1.73 m^2)	53.5 ± 22.4 (10.8–110.0)
Severe renal impairment (eGFR < 45 ml/min/1.73 m^2)	41/111 (37%)
Albuminuria	35/98 (36%)
Moderate albuminuria (ACR 30–300 mg/g)	24/98 (25%)
Severe albuminuria (ACR >300 mg/g)	11/98 (11%)
Anaemia [<13 g/dl (male)/<12 g/dl (female)]	50/112 (45%)
Microinflammation‡ (hsCRP 1−10 mg/l)	69/99 (70%)

ACR, albumin/creatinine ratio; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitive C-reactive protein; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.

Continuous values are given as mean ± standard deviation, or as median [with first and third quartile in square brackets] for non-normally distributed variables, with the range in round brackets. Categorical values are given as numbers and percentages. The denominator shows the number of available observations for each parameter; the numerator indicates the number of patients who fulfil that measure.

*Arterial hypertension is defined as high BP (≥140/90 mmHg) and/or use of antihypertensive treatment. BP was not available for $n = 2$ patients. Both patients were on antihypertensive treatment and were therefore classified as hypertensive.

†Dyslipidemia is defined as any of the following: hypercholesterolaemia, hypertriglyceridemia, elevated LDL cholesterol or low HDL cholesterol.

‡Twelve patients showed hsCRP >10 mg/dl and were excluded.

Subclinical CV damage after LT at inclusion

The results on the measures for subclinical CV damage at inclusion are summarized in Table 3 and Fig. 2. Mean PWV was at 8.2 ± 2.0 m/s. Sixteen per cent ($n = 17$) of all patients exceeded the threshold of 10 m/s (Fig. 2a).

Mean IMT was 0.60 ± 0.10 mm, and mean IMT z-score was 0.09 ± 0.82 (Table 3). When compared to age-appropriate reference values, 5% of all patients ($n = 5$) exhibited a z-score >1.645 (reflecting the 95th percentile, Fig. 2b).

Left-ventricular hypertrophy was present in 25% ($n = 24$) of all patients. When taking RWT into account, 12% ($n = 12$) of all patients exhibited eccentric hypertrophy, 12% ($n = 12$) concentric hypertrophy and 21% ($n = 21$) concentric remodelling (Table 3, Fig. 2c, d). Female sex was associated with lower LVMI (Table 4); female patients, however, showed a tendency for a higher rate of abnormal cardiac geometry in female LT patients [$n = 20/35$ (57%) vs. $n = 25/63$ (40%) in female and male patients, respectively, $P = 0.097$].

Longitudinal analysis of subclinical CV damage

To longitudinally assess intra-individual changes, a linear mixed model was employed. All observations ($n = 195$) from all visits from all patients ($n = 112$) entered the model, with the time elapsed since LT as the time variable, age and sex to correct for interindividual variations. BMI, underlying disease, time since LT, use of steroids, use of mammalian target of rapamycin inhibitor, serum albumin, aspartate aminotransferase, systolic BP, presence of diabetes, low-density lipoprotein cholesterol and eGFR were chosen as

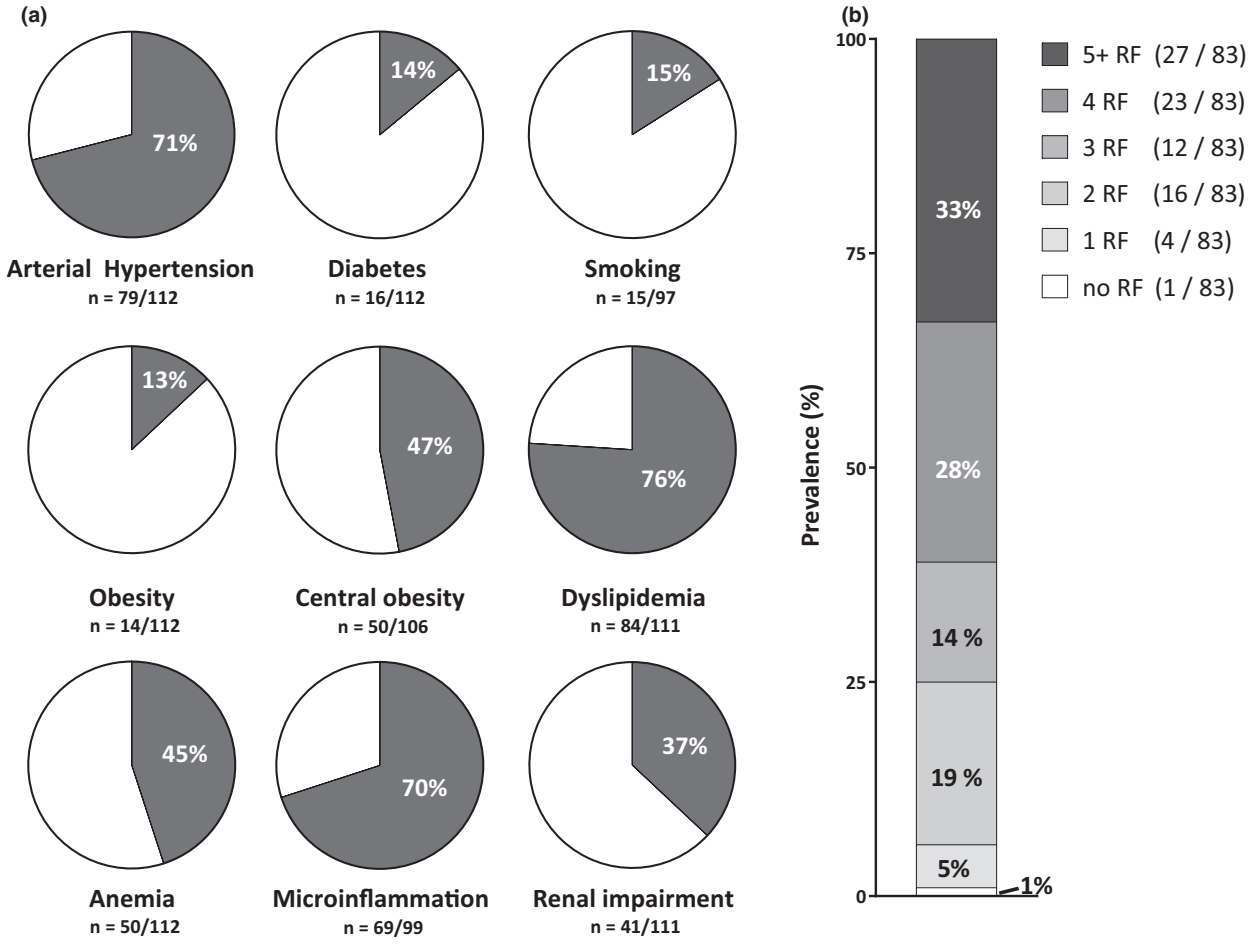

Figure 1 (a) Prevalence of CV risk factors (RF); (b) Accumulation of CV risk factors. The graph shows the proportion of patients with respect to the number of CV risk factors they exhibit (analysis of $n = 83$ patients with no missing values in the parameters of interest). CV, cardiovascular.

covariates. The results of the longitudinal multivariable model are presented in Table 4; Fig. 3 shows the standardized β regression coefficients.

For PWV, the multivariable longitudinal model revealed older age and higher systolic BP to be independently associated with higher PWV (Table 4, Fig. 3a).

For IMT, the final multivariable longitudinal model age, systolic BP and BMI were shown to be independently associated with IMT (Table 4, Fig. 3b).

For LVMI, male sex, higher systolic BP and lower eGFR could be found to be independently associated with higher LVMI in the final multivariable linear mixed model (Table 4, Fig. 3c).

Time elapsed since LT was not associated with either PWV, IMT or LVMI in the multivariable analysis, indicating that no significant change in any of the parameters occurred during the study period. Furthermore, neither use of steroids nor use of mammalian target of rapamycin inhibitor, nor underlying disease was significant in the model.

Discussion

The current study is the first to characterize the prevalence of subclinical CV damage utilizing established vascular and cardiac parameters, as well as describing cardiac geometry, and identifying associated risk factors in a large cohort of LT recipients in a prospective observational study.

LVH could be found in a fourth of our cohort, comparable to the Framingham Heart Study. Our results also reflect the tendency for higher rates in women aged 50 and older seen in the Framingham Heart study [38]. LVH is a known predictor for CV mortality in individuals with no clinically apparent CV disease [20], as well as in patients with different underlying conditions [39,40], including renal transplantation [41]. Considering patients with liver disease, Batra et al. reported an early increase in post-LT mortality for patients with pre-LT LVH, independent of classical risk factors [24]. No data exist on the effect of post-LT LHV on the risk

Table 3. Subclinical cardiovascular damage at inclusion.

Pulse wave velocity (PWV)	
PWV (m/s)	8.2 ± 2.0 (3.4–16.0)
Elevated PWV (>10 m/s)	17/106 (16%)
Intima-media thickness (IMT)	
IMT (mm)	0.60 ± 0.10 (0.42–0.84)
IMT *z*-score	0.09 ± 0.82 (–0.50 to 2.38)
Elevated IMT (>95th percentile)	5/109 (5%)
Left-ventricular mass index (LVMI)	
Left-ventricular mass (g)	184.6 ± 48.8 (97.0–320.8)
LVMI (g/m^2 BSA)	93.0 [79.3–108.1] (60.6–178.6)
LVH [>115 g/m^2 BSA (male)/>95 g/m^2 BSA (female)]	24/98 (25%)
Cardiac geometry	
Normal cardiac geometry (no LVH, RWT ≤0.42 cm)	53/98 (54%)
Pathological cardiac remodelling	45/98 (46%)
Eccentric hypertrophy (LVH, RWT ≤0.42 cm)	12/98 (12%)
Concentric hypertrophy (LVH, RWT >0.42 cm)	12/98 (12%)
Concentric remodelling (no LVH, RWT >0.42 cm)	21/98 (21%)

BSA, body surface area; IMT, intima-media thickness; LVH, left-ventricular hypertrophy; LVMI, left-ventricular mass index; PWV, pulse wave velocity; RWT, relative wall thickness.

Continuous values are given as mean ± standard deviation, or as median [with first and third quartile in square brackets] for non-normally distributed variables, with the range in round brackets. Categorical values are given as numbers and percentages. The denominator shows the number of available observations for each parameter; the numerator indicates the number of patients who fulfil that measure.

of CV mortality, but a similar negative effect can be hypothesized for the post-LT period. Abnormalities in cardiac geometry, found in almost half of our cohort, can adversely affect cardiac function. Patients exhibiting concentric hypertrophy, present in 12% (n = 12) of our cohort, have been reported to exhibit more severe diastolic dysfunction than patients with eccentric hypertrophy [42]. Pre-LT diastolic dysfunction is associated with late mortality in LT patients [43]. It is therefore possible for post-LT diastolic dysfunction to have a negative effect as well. Higher systolic BP and lower eGFR were independently associated with LVMI, which was also found in patients with chronic kidney disease [40]. This is of particular importance for LT patients, since more than a third of our patients showed a severely impaired kidney function with eGFR <45 ml/min/1.73 m^2. Every 5-unit decrease in eGFR is associated with a 5% higher hazard for CV mortality in LT recipients [14], and comorbidities associated with chronic kidney disease are risk factors themselves, such as hypertension and anaemia [8], both highly prevalent in our cohort [71% (n = 79) and 45% (n = 50), respectively, also occurring independently of chronic kidney disease].

Elevated PWV values above 10 m/s were present in 16% (n = 17) of our cohort. Higher age was independently associated with higher PWV in our cohort. This relationship had not been found in a smaller study in LT patients [44], possibly due to sample size and methodological issues. The increase of PWV with age, shown in large studies of the general population [27,45], is especially important since every 1 m/s increase in PWV leads to a 15% increase in CV mortality [18]. In fact, the majority of our cohort (56%, n = 59) exhibited values at the high-normal range between 7 and 10 m/s. It therefore has to be assumed that although these values are below the reference cut-off of 10 m/s [36], patients still face an increased risk for CV mortality. The strong association between BP and PWV is comparable to previous findings from our group (in patients after paediatric LT [25], and paediatric renal transplantation [46]) and other investigators [47,48]. In light of the high prevalence of arterial hypertension in our cohort, the finding that higher systolic BP was independently associated with higher PWV is especially relevant as arterial hypertension is a modifiable risk factor.

Elevated IMT values were present in 5% of patients (n = 5) reflecting the distribution in healthy age-matched individuals [37]. This is in contrast to our results in paediatric LT patients, where 57% of all patients exhibited elevated IMT >95th percentile [25]. The mean time elapsed since LT was actually higher in the paediatric cohort than the adult cohort, arguing against a potential reduction in IMT over time.

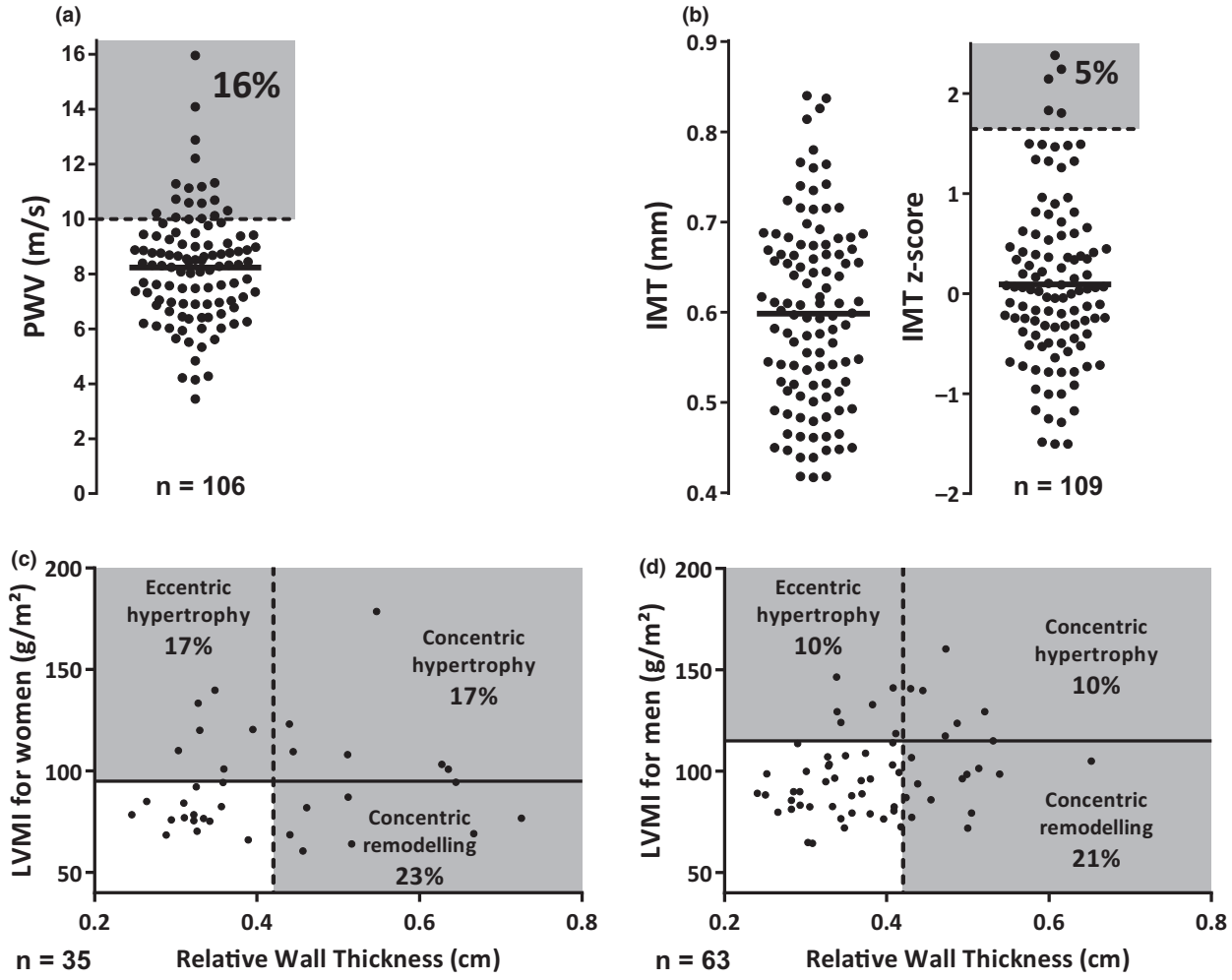

Figure 2 (a) Pulse wave velocity (PWV). The grey area indicates elevated PWV >10 m/s; (b) intima-media thickness (IMT) (left) and IMT*z*-score (right), grey area indicates values above 95th percentile; (c) cardiac geometry for women and (d) for men. Left-ventricular mass index (LVMI) is blotted on the*y*-axis, relative wall thickness (RWT) on the*x*-axis. The broken line indicates RWT of 0.42 cm, and the solid line shows the LVMI threshold (>95 g/m^{2}for women, >115 g/m^{2}for men). The grey area indicates pathological cardiac geometry.

Naturally, paediatric patients had been transplanted at a younger age. Interestingly, younger age at LT was independently associated with higher IMT in the paediatric group [25]. It is therefore conceivable that the younger vasculature is more susceptible to CV damage in the paediatric cohort, where almost half of all patients had been transplanted in infancy. Higher systolic BP was associated with higher IMT, in line with literature [49]. Higher age was associated with higher IMT after LT, with an increase of 5 μm per year. This is in accordance to data on healthy individuals, where an increase in IMT of 5.2 μm/year for men and 5.0 μm/year for women has been described [37]. The association of higher BMI with higher IMT is consistent with literature [37] and of special significance, since 46% ($n = 51$) of our LT patients were overweight or obese. The relationship between IMT and chronic liver disease is best described for NAFLD, which is associated with increased IMT when compared to controls [21]. Data on the presence of current NAFLD were not available for our patients. However, only one of our patients had undergone LT due to NAFLD, potentially explaining the similarity of our cohort with results from a healthy population with regard to IMT.

Time elapsed since LT was not independently associated with either PWV, IMT or LVMI, indicating no significant change during the study period. This suggests that these subclinical CV injuries are persistent findings and not temporary phenomena.

CV risk factors contributing to the described subclinical changes were very common in our cohort. Of all

Table 4. Multivariable linear mixed model (n = 112 patients*, 195 observations).

	β	SE	*P*
PWV			
Age	0.069	0.011	**<0.001**
Female sex	–0.101	0.282	0.721
Time since LT	0.003	0.023	0.887
Systolic BP	0.022	0.008	**0.005**
IMT			
Age	0.005	0.001	**<0.001**
Female sex	–0.000	0.015	0.989
Time since LT	0.002	0.001	0.207
Systolic BP	0.001	0.000	**0.029**
BMI	0.004	0.002	**0.023**
LVMI			
Age	0.117	0.164	0.478
Female sex	–8.847	3.992	**0.031**
Time since LT	–0.205	0.312	0.515
Systolic BP	0.200	0.092	**0.034**
eGFR	–0.288	0.082	**<0.001**

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; IMT, intima-media thickness; LT, liver transplantation; LVMI, left-ventricular mass index; PWV, pulse wave velocity; SE, standard error.

P-values ≤ 0.05 are bolded.

*One patient had undergone heart transplantation and was not included in the analysis for LVMI.

patients, 60% (n = 50) exhibited four or more CV risk factors at the same time (Fig. 1b). Prevalence of dyslipidemia and hypertension at 76% (n = 84) and 71% (n = 79), respectively, were higher than previously described for patients that were 5–7 years after LT [6,50]. Prevalence of diabetes and obesity were lower, while active smoking and renal impairment were comparable [6,50]. The high rate of microinflammation was surprising and more than double the rate of paediatric LT recipients of 33% [25]. Low-level viral infections or bacterial biliary infections could be speculated as origin for this inflammatory activity, indicating towards potential over-immunosuppression; or this could on the other hand reflect subclinical alloreactivity in the graft as a result of under-immunosuppression.

While the association of BP with all parameters for subclinical CV damage is not novel, it clearly highlights the importance of this potent CV risk factor. The relevance is further underlined by its high prevalence of 71% and the fact that more than half (n = 43) of hypertensive patients were insufficiently controlled showing opportunity for therapeutic intervention.

The strengths of this study are the prospective design ensuring a high level of data quality and completeness as well as the possibility of longitudinally analysing the majority of the patients. Furthermore, we analysed vascular and cardiac parameters for subclinical damage by specifically trained personnel in pre-determined study visits. The study, however, also has several limitations. Pre-LT measurements were not obtainable for the majority of patients which would have helped to better discern the impact of the underlying disease from the effect of LT. CV disease can be a slow process that develops over many years. Therefore, the study duration may have been too short to detect changes over time.

In conclusion, our study comprehensively describes a high prevalence of CV risk factors and a substantial amount of subclinical alterations of the heart and vasculature in a large cohort of LT recipients. Our prospective approach allowed deciphering the contribution of certain risk factors. Interestingly, classical risk factors such as arterial hypertension and higher BMI as well as impairment of renal function turned out to be the main associated factors of the investigated markers. BP was independently associated with all three measures of subclinical CV damage and may therefore be the ideal target for preventive strategies. Future interventional studies that target BP control should therefore take PWV, IMT and LVMI into consideration. These noninvasively obtainable measures of subclinical CV damage may serve to enable early detection of CV damage and risk assessment for future CV events and therefore allow for individualized therapeutic measures to prevent clinical CV disease.

Authorship

AM, BMWS and EJ: involved in conception and design. AM and BMWS: involved in procurement of funding and ethics approval. NM, CK, EB and AW: patient recruitment. NM, CK, BB-M, AW, EB and JB: collected and managed the data. NM, CK, RIS, BMWS and AM: analysed and interpreted the data. BB-M, EJ, BM, FWRV, BMWS and AM: provided professional expertise. NM and CK: drafted the manuscript. NM, CK, B-B-M, AW, EB, EJ, BM, FWRV, RIS, JB, BMWS and AM: revised the manuscript.

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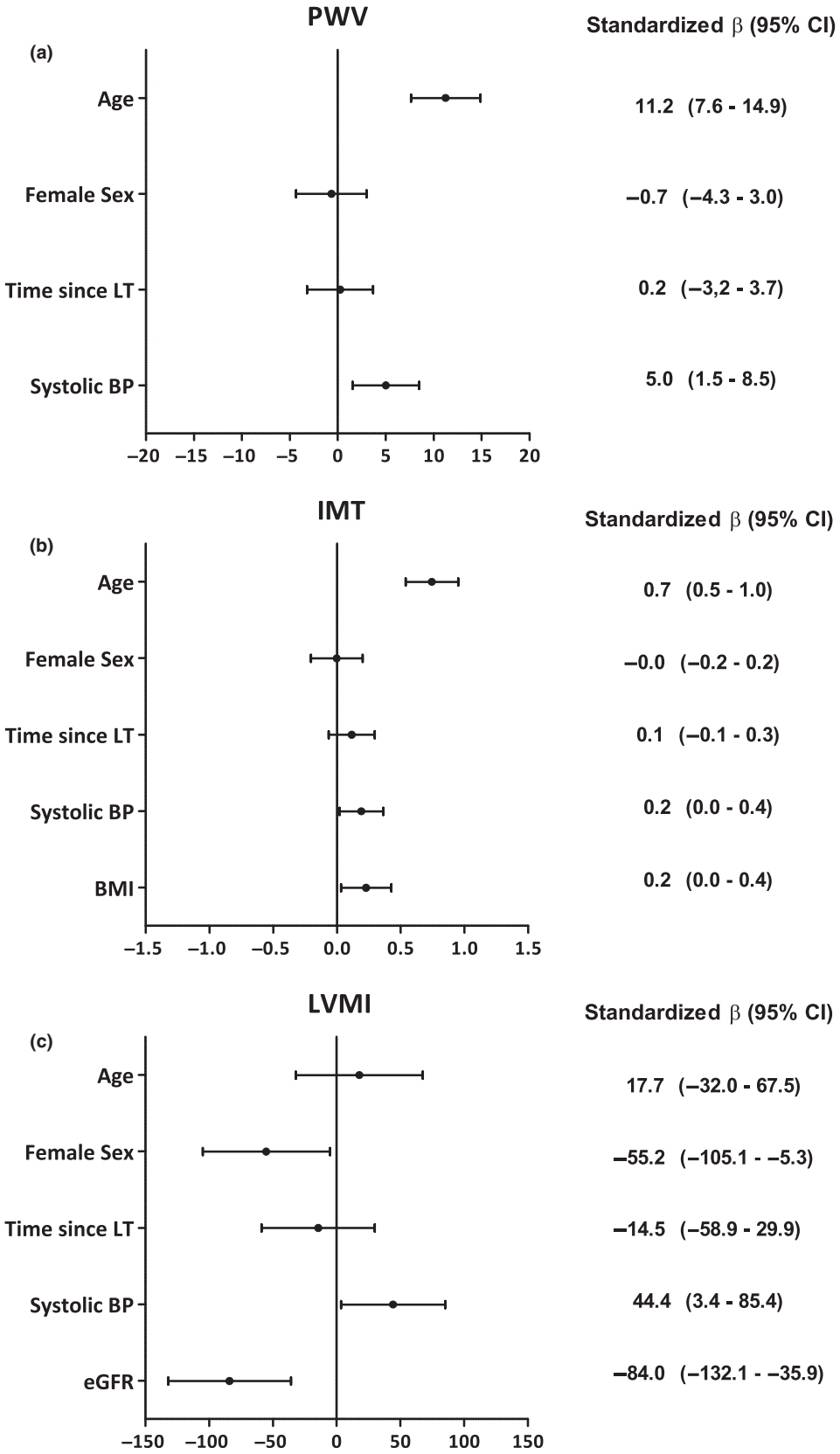

Figure 3 Forest plots of standardized β coefficient and 95% confidence intervals (CI) from the longitudinal linear mixed model of pulse wave velocity (a), intima-media thickness (b) and left-ventricular mass index (c).

Conflict of interest

The authors have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Classification of arterial hypertension according to treatment status.

Table S2. Risk of chronic kidney disease.

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