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The Role of Arterial Stiffness in the Estimation of Cardiovascular Risk in Liver Transplant Recipients

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Background. Long-term cardiovascular (CV) events are a frequent cause of death and disability after liver transplant (LT). Although a more in-depth, risk-adapted control of CV risk factors may result in improved post-LT CV outcomes, an accurate stratification of the CV risk of LT recipients to better implement preventive strategies is lacking. Aortic pulse wave velocity (aPWV) is a surrogate of arterial stiffness that has been suggested as a biomarker of CV risk; it has never been evaluated in adult LT recipients.

Methods. In a single-center prospective study, we included 122 LT recipients at 12 (n=39), 60 (n=45), or 120 (n=38) mo after LT. aPWV estimation by oscillometry, clinical assessment of CV risk factors, and CV risk estimation by standard clinical scores (systematic coronary risk evaluation and pooled cohort equation) were performed. The incidence of CV events during prospective follow-up was registered. **Results.** aPWV was independently associated with age and the grade of control of blood pressure. After a median follow-up of 35 mo, 15 patients (12%) presented a CV event. Higher aPWV, diabetes, past or present smoking habit, previous CV events, lower eGFR, being in systematic coronary risk evaluation or pooled cohort equation high-risk groups, and higher levels of total cholesterol, LDL-cholesterol, creatinine, and triglycerides were associated with the incidence of CV events at univariate analysis; aPWV, past or present smoking habit, and triglycerides were independent predictors of CV events.

Conclusions. According to our results, aPWV mirrors CV risk in LT recipients and thus may be a useful CV risk biomarker in this population. Considering these preliminary results, its accuracy in stratifying risk requires confirmation in further studies.

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INTRODUCTION

Cardiovascular (CV) events are frequent causes of long-term morbimortality in liver transplant (LT) recipients.^{1,2} Although many clinical variables have been associated with the risk of post-LT CV events,³⁻⁶ the accuracy of such variables, or that of clinical risk scores like the Framingham Risk Score, the PROspective CARDiovascular Munster (PROCAM), or the Systematic COronary Risk Evaluation

(SCORE)^{7,8} to identify LT recipients at highest risk of CV events is not perfect; thus, a simple, accurate, and objective system to identify patients at higher risk of long-term CV events is lacking.⁶

The estimation of CV risk is clinically important, as it permits to match the efforts of preventive interventions with the risk of each individual. Although clinical risk scores are used to assess long-term CV risk and define the targets to consider controlled

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L.S. designed study, performed research, and did critical review of article.

R.G. and J.G.G. performed research, critical review of article. P.F.L., A.J.A., C.S., L.E., P.R., J.C., and E.O. did critical review of article. M.N. designed study, critical review of article. G.C. designed study, performed research, wrote the article.

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each CV condition, they are limited by their moderate accuracy. Thus, novel ways to characterize CV risk are continuously being evaluated.^{9,10} Among these, estimation of arterial stiffness by measuring aortic pulse wave velocity (aPWV) has been shown to be a sensitive biomarker of CV risk.¹¹ In a large meta-analysis, after adjustment for conventional CV risk factors, aPWV improved risk prediction, particularly in certain subgroups, such as younger individuals at intermediate risk.¹² Additionally, aPWV is useful to identify high-risk individuals in patients with type 1 diabetes.¹³ Although the gold standard to estimate aPWV is the measurement of carotid-femoral PWV by tonometry, oscillometric methods that measure brachial blood pressure (BP) have been validated as surrogates of aPWV and have also shown to predict CV events.^{14,15}

In solid organ transplant recipients, arterial stiffness has been mainly studied after kidney transplant, where it was shown to be associated with clinical risk factors and to independently predict mortality, CV events, and loss of renal function.¹⁶ In contrast, data regarding arterial stiffness in LT recipients are scarce and mainly derived from the pediatric LT population.^{17,18} Consequently, we designed this cross-sectional study with prospective follow-up to investigate the potential association between aPWV and CV risk in adult LT recipients.

MATERIALS AND METHODS

This is a prospective study performed in the Liver Transplant Unit of Hospital Clinic, Barcelona, including 2 different parts: a cross-sectional evaluation of aPWV and CV risk factors, followed by a prospective follow-up on CV events after cross-sectional evaluation.

Cross-sectional Assessment of aPWV and Cardiovascular Risk Factors

Between March 2017 and July 2018, consecutive adult LT recipients with a follow-up of 12 (± 3), 60 (± 3), and 120 (± 3) mo after LT seen in the Outpatient Clinic were considered to participate in the study, and the cross-sectional evaluation was performed. Retransplanted patients, those with multiple organ transplantation and patients infected with HIV were excluded. In addition, patients were only included if they were in the outpatient setting, without evidence of graft rejection or dysfunction, technical complications, or active infections.

Assessment of Cardiovascular Risk Factors

The presence of diabetes mellitus (DM), arterial hypertension (AHT), and dyslipidemia and their grade of control were prospectively assessed according to the following criteria¹⁹:

- AHT: office BP $\geq 140/90$ mm Hg in untreated individuals, or use of BP-lowering medication. Controlled BP was defined as an office BP $< 140/90$ mm Hg.
- DM: anytime glycemia ≥ 200 mg/dL, or fasting glycemia ≥ 126 mg/dL at least twice, or glycated hemoglobin (HbA1c) $\geq 6.5\%$, or use of antidiabetic drugs. Controlled DM was defined as HbA1c $< 7\%$.
- Dyslipidemia: total cholesterol > 200 mg/dL, or low-density lipoprotein (LDL) cholesterol (LDL-C) > 130 mg/dL, or triglyceride levels > 150 mg/dL, or use of lipid-lowering drugs. Controlled dyslipidemia was defined as an LDL-C < 100 mg/dL.

In addition, we prospectively evaluated smoking habit (classified as never, current, or former) and the total pack-year

consumption. A complete physical examination was performed including waist circumference measurement, and body mass index was calculated. Metabolic syndrome was defined when at least 3 of the following 5 risk factors were met²⁰: (1) waist circumference ≥ 102 cm for men and ≥ 88 cm for women; (2) triglycerides ≥ 150 mg/dL; (3) high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL for men and < 50 mg/dL for women; (4) systolic BP ≥ 130 mm Hg or diastolic BP ≥ 85 mm Hg; and (5) fasting serum glucose ≥ 100 mg/dL.

Ten-year CV risk assessment was done by calculating the pooled cohort equation (PCE)²¹ and the SCORE²² using the corresponding online calculators. According to the algorithms, SCORE is not calculated in patients with type 2 diabetes or previous CV events; in this latter group PCE is neither calculated as according to the guidelines those group of patients are already considered to be at high/very high CV risk.

A detailed anamnesis on previous CV disease was made, and a review of clinical history was also performed. We specifically registered the following:

- Previous episodes of acute coronary syndrome (myocardial infarction with or without ST elevation or unstable angina), stable angina, or coronary artery disease diagnosed by any means.
- Previous episodes of ischemic or hemorrhagic stroke or transient ischemic attack.
- Previous episodes of heart failure, based on signs and symptoms along with echocardiographic evidence of ventricular dysfunction.
- Previous diagnosis of peripheral artery disease, clinically diagnosed and confirmed with lower limb ultrasonography or ankle-brachial index (ABI < 0.9).
- Previous episodes of arrhythmia, including atrial or ventricular fibrillation and flutter, atrioventricular block, torsades de pointes, and sinus bradycardia requiring treatment.

Immunosuppression

Dose and trough levels of tacrolimus, cyclosporine A, or everolimus were registered when appropriate. Additionally, we also registered the dose of prednisone or mycophenolate mophetil when applicable.

Arterial Stiffness

We evaluated arterial stiffness through the estimation of aPWV using the validated Mobilograph device (IEM, Stolberg, Germany). Assessment was performed in a quiet, temperature-controlled examination room, in supine position and using an adequately sized cuff. Patients were in fasting status, and the assessment took place before the intake of immunosuppressants and antihypertensive drugs. Procedures were performed in triplicate; mean values were used for analyses. First, brachial systolic and diastolic BP are obtained. Then, the brachial cuff is inflated to the diastolic blood level and held for 10 s to record pulse waves, and a proprietary mathematical model (ARCSolver, Austrian Institute of Technology, Vienna, Austria) that combines several parameters from pulse wave and wave separation analysis estimates aPWV.¹⁴ An aPWV ≥ 10 m/s was considered as subclinical organ damage.²³

Blood Tests

The same day of arterial stiffness assessment, a blood sample was obtained in fasting status. Liver and kidney function tests, as well as a lipid profile including total, HDL- and LDL-C,

triglycerides and HbA1C levels were determined. A serum sample was frozen at -80°C and stored for further studies.

Prospective Assessment of CV Events

After the cross-sectional assessment, patients were prospectively followed until December 31, 2020, to evaluate the incidence of new-onset CV events. During this time, the incidence of the following CV events was recorded:

- Acute coronary syndrome (including myocardial infarction with or without ST elevation or unstable angina).
- Ischemic or hemorrhagic stroke, including transient ischemic attack.
- Peripheral artery disease based on the Fontaine classification and confirmed with lower limb ultrasonography or $\text{ABI} < 0.9$.
- Arrhythmias, that included atrial or ventricular fibrillation and flutter, atrioventricular block, torsades de pointes, and sinus bradycardia requiring treatment.
- Heart failure, diagnosed on signs and symptoms of volume overload with echocardiographic evidence of ventricular dysfunction.

Statistical Analysis

Continuous variables are expressed as medians and interquartile range, and categorical variables are expressed in absolute numbers and percentages. Patients were grouped according to the time of post-LT follow-up: 12, 60, or 120 mo (Groups A, B, and C, respectively). Nonparametric tests (Kruskal-Wallis, Fisher test) were used to study differences between groups. The association between aPWV and the rest of the variables was studied with uni- and multivariate linear regression analysis and graphically represented by dot plots or bar graphs. Variables with a $P < 0.1$ in the univariate analysis were included in the multivariate analysis. Time-dependent univariate and multivariate Cox-regression analysis were used

to evaluate baseline variables associated with the incidence of CV events during follow-up, considering the number of events only variables statistically significant in the univariate analysis were included in multivariate models. Since SCORE and PCE scoring are not performed in a significant proportion of patients as per definitions of the algorithms (SCORE is not calculated in type 2 diabetic patients and patients with previous CV events, whereas PCE is not calculated in patients with previous CV events), for Cox-regression analysis purposes these variables were categorized. SCORE was categorized as $\geq 15\%$ (including diabetic patients and those with previous CV events) versus $< 15\%$,²² and PCE was categorized as $\geq 20\%$ (including patients with previous CV events) versus $< 20\%$.²¹ A $P < 0.05$ was considered statistically significant for all tests.

Ethical Issues

The study was performed in accordance with the Declaration of Helsinki, the Declaration of Istanbul, and the E6 Good Clinical Practice Standards ICH. All the study data were treated anonymously with restricted access only by authorized personnel for the purposes of the study. The study was approved by the Clinical Research Ethical Committee of Hospital Clínic, Barcelona (approval number HCB/2016/490). All patients were properly informed about the study and provided written consent for inclusion.

RESULTS

During the study period, 168 patients were considered for the study, and finally 122 met inclusion criteria and were included. Among them, 39 formed group A (12 mo after LT), 45 group B (60 mo), and 38 group C (120 mo). The flowchart of the study is depicted in Figure 1, and the main characteristics of the whole cohort and according to the timing groups are shown in Table 1.

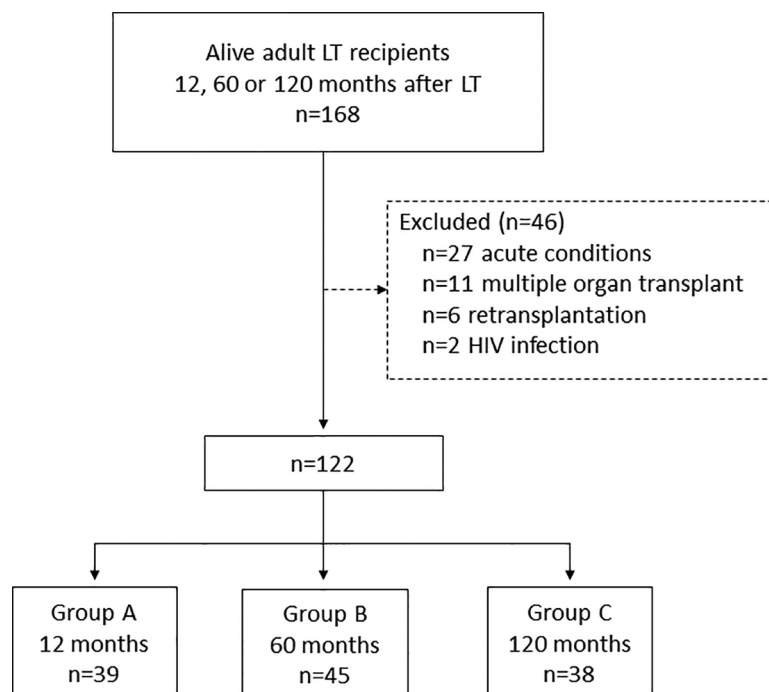


FIGURE 1. Flowchart of the study. LT, liver transplant.

TABLE 1.
Baseline characteristics of the whole cohort and according to the timing group

Variable	All n = 122	Group A n = 39	Group B n = 45	Group C n = 38	P
Sex (male)	87 (71)	27 (69)	30 (67)	30 (79)	0.351
Age (y)	61 (55–68)	58 (54–62)	62 (53–68)	67 (59–71)	0.001
Etiology of liver disease					0.132
HCV	44 (36)	13 (33)	18 (40)	13 (34)	
Alcohol	36 (29)	8 (20)	12 (27)	16 (42)	
NASH/cryptogenic	11 (9)	6 (16)	3 (7)	2 (5)	
Indication of LT (HCC)	47 (38)	22 (56)	18 (40)	7 (18)	0.006
Never smoker	49 (40)	12 (31)	22 (44)	17 (45)	0.211
Previous CV events	20 (16)	5 (13)	5 (11)	10 (26)	0.113
Arterial hypertension	81 (66)	20 (51)	30 (67)	31 (82)	0.005
Diabetes mellitus	61 (50)	22 (56)	21 (47)	18 (47)	0.427
Dyslipemia	58 (47)	18 (46)	19 (42)	21 (55)	0.430
SCORE (%) (n = 58)	4 (2–5)	3 (1–4)	3 (1–6)	5 (4–6)	0.01
PCE (%) (n = 102)	11.6 (6.4–21.3)	9 (3.2–21.8)	12.8 (6.2–20.4)	14.8 (9.4–23.8)	0.189
Metabolic syndrome	60 (50)	19 (50)	19 (42)	22 (58)	0.493
eGFR (mL/min/1.73m ²)	69 (52–88)	71 (61–95)	73 (60–89)	62 (50–75)	0.051
Creatinine (mg/dL)	1.06 (0.85–1.33)	1.09 (0.82–1.29)	0.99 (0.82–1.22)	1.19 (0.96–1.41)	0.089
Total cholesterol (mg/dL)	175 (153–198)	177 (166–210)	172 (153–197)	176 (144–190)	0.298
LDL-cholesterol (mg/dL)	102 (85–120)	110 (88–124)	102 (86–122)	95 (84–111)	0.218
HDL-cholesterol (mg/dL)	46 (37–60)	52 (28–60)	46 (37–63)	45 (34–55)	0.320
Triglycerides (mg/dL)	111 (85–150)	111 (86–158)	99 (78–133)	124 (97–161)	0.086
HbA1c (%)	5.6 (5.1–6.3)	5.6 (5.1–6.3)	5.7 (5.1–6.2)	5.6 (5.2–6.5)	0.748
BMI (kg/m ²)	27.1 (24.6–31.1)	26.9 (24.2–30.8)	26.8 (24.1–31.9)	27.8 (25.7–30)	0.677
CNI-free immunosuppression	21 (17)	2 (5)	4 (9)	15 (40)	<0.001
aPWV (m/s)	8.8 (7.6–9.9)	8.1 (7.3–9.1)	9.1 (7.8–10.1)	9.5 (8.2–10.4)	0.002
aPWV >10 (m/s)	30 (25)	3 (8)	12 (27)	15 (40)	0.001

Data are given as absolute count (%) or median (IQR). Bold indicates variables with statistically significant differences between groups.

aPWV, aortic pulse wave velocity; BMI, body mass index; CNI, calcineurin inhibitor; CV, cardiovascular; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; LT, liver transplant; NASH, nonalcoholic steatohepatitis; PCE, pooled cohort equation; SCORE, systematic coronary risk evaluation.

CV Risk Factors

The overall prevalence of DM, AHT, and dyslipidemia was 50%, 66%, and 47%, respectively (Table 1). Twenty patients (16%) had had a previous CV event, either before or after LT. As expected, patients in group C were older and presented more frequently AHT than the rest of the groups, whereas there were no statistically significant differences in the rest of clinical CV risk factors.

Aortic Pulse Wave Velocity

Median aPWV was 8.8 m/s. Thirty patients (25%) presented an aPWV >10 m/s (8% in group A, 27% in group B, and 40% in group C, $P=0.001$). Univariate and multivariate linear regression analyses of clinical variables associated with aPWV are shown in Table 2. In the univariate analysis, arterial stiffness was significantly and directly associated with age, timing group, hepatocellular carcinoma as the indication of LT, presence of AHT, lack of AHT control, DM, metabolic syndrome, body mass index, waist circumference, PCE, SCORE, and history of previous episodes of CV events, and arterial stiffness was inversely associated with estimated glomerular filtration rate (eGFR). Additionally, it was significantly associated with using a CNI-free immunosuppressive regimen, and, in the few patients with cyclosporine A, with lower trough levels of this immunosuppressant. In the multivariate analysis, the variables independently associated with aPWV were age and AHT, either as a diagnosis or when evaluated as controlled BP. Figure 2

shows the correlation between aPWV and age, time after LT, grade of control of BP, and eGFR.

Arterial Hypertension and aPWV

Overall, 64 patients (52%) were receiving BP-lowering medication. Most patients ($n=35$) were taking 1 single medication, although 20 and 9 patients were under 2 and 3 antihypertensive drugs, respectively. The most frequent group of antihypertensive drugs used were angiotensin II type 1 receptor blockers (28 patients, 23%), followed by calcium channel blockers ($n=23$, 18%) and angiotensin-converting-enzyme inhibitors ($n=14$, 11%).

aPWV increased in parallel with the number of BP-lowering medications used. However, for each category of number of medications used, aPWV tended to be lower if BP was within the predefined limits of control (<140/90 mm Hg) (Figure 3), with differences being statistically significant for the groups of 0 and 3 BP-lowering medications.

In the group of patients on a single antihypertensive drug, we did not find statistically significant differences in aPWV according to the type of antihypertensive drug taken. Similarly, in patients with 2 or 3 medications including calcium channel blockers, angiotensin II type 1 receptor blockers, or angiotensin-converting-enzyme inhibitors as mainstay of antihypertensive treatment together with any other drug (mainly betablockers or diuretics), there were no statistically significant differences in aPWV depending on the main antihypertensive taken.

TABLE 2.**Uni- and multivariate linear regression analysis of variables associated with aPWV**

Variable	Univariate			Multivariate		
	B	95% CI	P	B	95% CI	P
Age (y)	0.134	0.122, 0.146	<0.001	0.130	0.083, 0.177	<0.001
Gender (male)	-0.349	-1.00, 0.307	0.294			
Etiology of liver disease (NASH)	-0.433	-1.47, 0.604	0.363			
Never smoker	-0.257	-0.855, 0.341	0.396			
Tobacco pack-y	0.009	-0.004, 0.021	0.187			
Indication of LT (HCC)	0.777	0.178, 1.375	0.011			0.850
Timing (Group A/B/C)	0.661	0.306, 1.017	<0.001			0.195
SCORE (n = 58) ^a	0.314	0.107, 0.193	<0.001			
PCE (n = 102) ^a	0.089	0.07, 0.108	<0.001			
AHT ^a	1.691	1.143, 2.238	<0.001			
Controlled BP	-1.095	-1.674, -0.515	<0.001	-0.620	-0.813, -0.428	<0.001
Diabetes	0.652	0.068, 1.236	0.029			0.314
Controlled diabetes	-0.360	-1.148, 0.427	0.367			
Dyslipidemia	0.443	-0.148, 1.035	0.140			
Body mass index	0.086	0.024, 0.147	0.006			0.533
Waist circumference (cm)	0.044	0.022, 0.066	0.002			0.165
Metabolic syndrome ^a	1.003	0.445, 1.561	0.001			0.846
Creatinine(mg/dL)	0.254	-0.654, 1.162	0.581			
eGFR (mL/min/1.73 m ²)	-0.028	-0.042, -0.013	<0.001			0.065
Total cholesterol	0.005	-0.002, 0.012	0.177			
LDL-C	0.004	-0.006, 0.014	0.424			
HDL-C	0.006	-0.011, 0.022	0.479			
Triglycerides	0.003	-0.001, 0.008	0.143			
CNI-free IMS	0.864	0.105, 1.623	0.026			0.789
Cyclosporin A-based IMS	0.151	-0.116, 1.339	0.105			
Tacrolimus trough levels (ng/mL) (n = 77)	-0.147	-0.333, 0.040	0.121			
Cyclosporin A trough levels (ng/mL) (n = 23) ^a	-0.010	-0.018, -0.002	0.018			
Previous CVE	1.016	0.248, 1.785	0.01			0.559

Bold indicates variables with statistically significant differences between groups.

^aConsidering the low number of patients under immunosuppression with cyclosporine A, the variable "Cyclosporin A trough levels" was not included in multivariate analysis. Additionally, cardiovascular risk scores ("SCORE" and "PCE") and "Metabolic syndrome" were not included in multivariate analysis considering they include variables already studied in the model. Finally, the model was constructed including either "Controlled BP" (shown in the table) or "Arterial hypertension." In this latter case, independent variables remained age and arterial hypertension.

AHT, arterial hypertension; aPWV, aortic pulse wave velocity; BP, blood pressure; CNI, calcineurin inhibitor; CVE, cardiovascular events; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; HDL-C, high-density lipoprotein cholesterol; IMS, immunosuppression; LDL-C, low-density lipoprotein cholesterol; LT, liver transplantation; NASH, nonalcoholic steatohepatitis; PCE, pooled cohort equation; SCORE, systematic coronary risk evaluation.

Prediction of Cardiovascular Events

During a median follow-up of 35 mo (31–38) after assessment, 15 patients (12%) presented a new CV event. Characteristics of these patients and events are detailed in Table 3. In univariate Cox-regression analysis (Table 4), the variables associated with the incidence of new-onset CV events were higher aPWV, the presence of DM, the absence of present or past smoking habit (inversely), having presented previous CV events, lower eGFR, and higher serum levels of creatinine, total cholesterol, LDL-cholesterol, triglycerides, and being in the high-risk groups in SCORE or PCE algorithms. Considering the number of events, the number of variables statistically significant in the univariate analyses, and the fact that clinical algorithms include variables individually associated with CV events in the analysis, we approached multivariate analyses by performing different models. First, we performed multivariate analyses including aPWV and individual clinical variables (without risk algorithms). Smoking habit, previous CV events, aPWV, and diabetes were included in all these models with triglycerides, LDL-cholesterol, or total cholesterol and either eGFR or creatinine; second, we performed the multivariate analyses including aPWV and either SCORE or PCE, finally resulting in 8 models (Table S1, SDC, <http://links.lww.com/TXD/A396>).

In models with clinical variables, aPWV and absence of smoking habit (inversely) were independently associated with CV events; additionally, triglyceride levels were also independently associated with CV events when included in the model. In contrast, total cholesterol, LDL-cholesterol, diabetes, previous CV events, and creatinine/eGFR were not independently associated with the incidence of CV events in any model. Importantly, these results did not change when age or the grade of control of BP were forced into the models. In the models including clinical algorithms, aPWV was independently associated with CV events when confronted with SCORE, which was not an independent predictor of CV events. In the model including aPWV and PCE, none of the variables were independently associated with CV events.

DISCUSSION

In this study, we show for the first time that the assessment of arterial stiffness by estimating aPWV correlates with several CV risk factors in LT recipients, and more importantly, it seems to be associated with the risk of presenting new CV events during follow-up. Thus, it may be an accurate predictor of CV events in this population, showing the particular

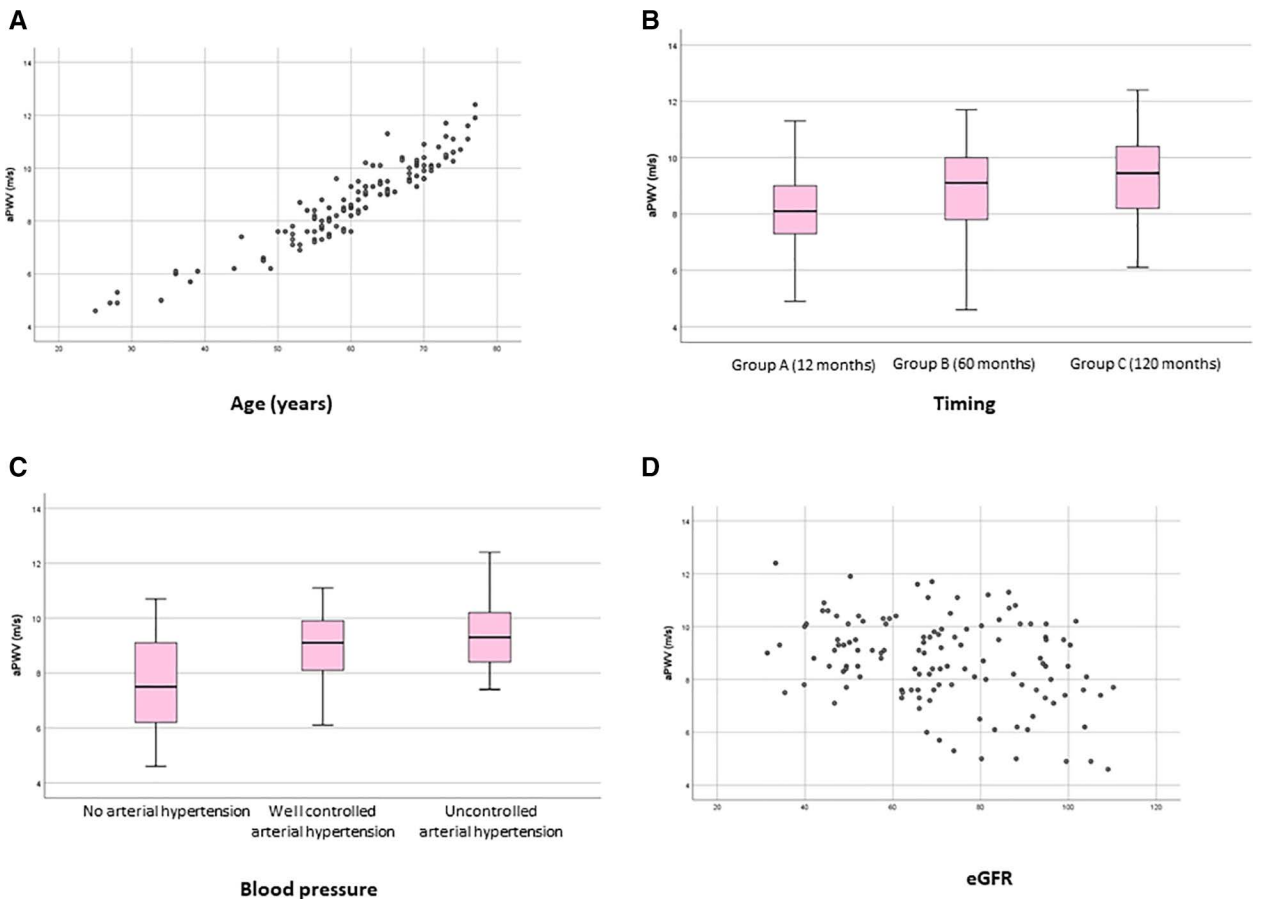


FIGURE 2. Dot plots or graph bars showing the association between aPWV and age (A), timing group (B), grade of control of blood pressure (C), and eGFR (D). aPWV, aortic pulse wave velocity; eGFR, estimated glomerular filtration rate.

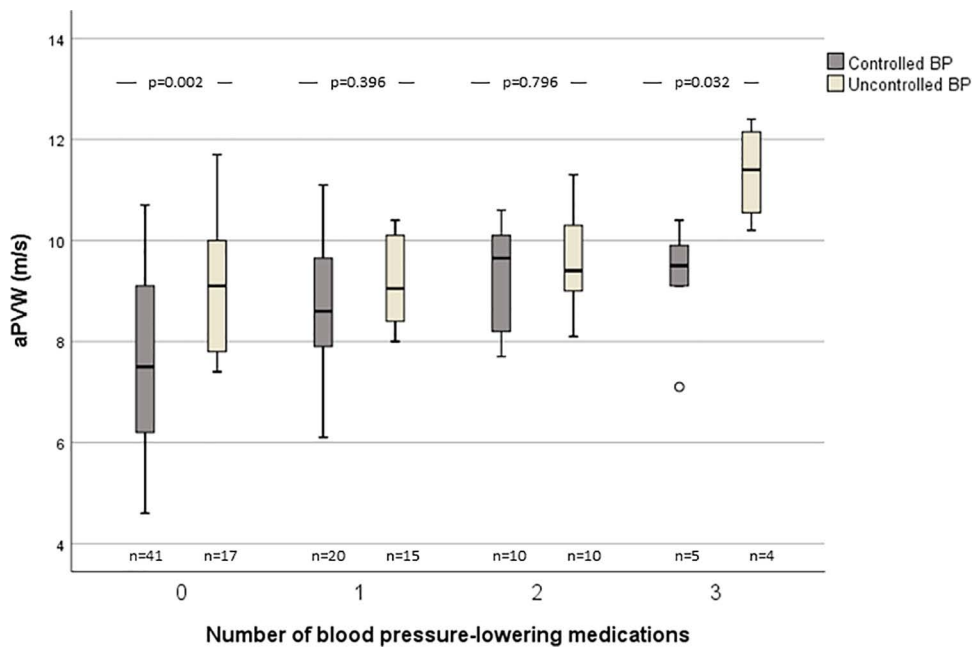


FIGURE 3. Box-plots showing aPWV in patients under 0, 1, 2, or 3 antihypertensive drugs according to the grade of control of blood pressure. aPWV, aortic pulse wave velocity; BP, blood pressure.

advantage of being a quantifiable, continuous parameter. Considering the potential association between aPWV and CV risk after LT suggested by our data, investigating its independent role in the prediction of CV events, as compared with

other classic estimators of CV risk, should be the focus of larger, probably multicenter, studies.

The relevance of long-term CV events in LT recipients is expected to increase in the near future. LT candidates are

TABLE 3.**Description of the patients who presented a CV event during follow-up**

	Sex	Age at aPWV (y)	Previous liver disease	Type of CV event	Months between aPWV and event		Smoking habit	Group	SCORE (%)	PCE (%)	eGFR	AHT	Controlled		HbA1C (%)	Dyslipemia	Previous CV events
					aPWV	aPWV (m/s)							BP	DM			
1	Male	62	HCV	Peripheral artery disease	32	8.5	Past	A	≥15	23.8	94.8	Yes	Yes	Yes	5.5	Yes	No
2	Male	72	Alcohol	Heart failure	22	10.1	Past	C	N/A	21.3	49.8	Yes	Yes	No	6	No	No
3	Male	60	HBV	Ischemic stroke	26	9.3	Past	B	≥15	≥20	34.2	Yes	No	Yes	8.7	Yes	Yes
4	Male	58	HCV	Peripheral artery disease	19	9.6	Past	A	≥15	27.5	67	Yes	No	Yes	7.6	Yes	No
5	Female	65	Alcohol	Heart failure	26	11.3	Never	A	≥15	21.3	86.4	Yes	No	Yes	5.6	Yes	No
6	Male	64	NASH	Heart failure	13	9	Past	A	≥15	≥20	31.4	Yes	Yes	Yes	9.8	Yes	Yes
7	Male	69	HCV	Heart failure	17	10.1	Past	B	7	19.2	58.4	Yes	No	No	5.5	Yes	No
8	Male	56	Alcohol	Acute coronary syndrome	3	8.8	Past	A	≥15	7.5	42	Yes	No	Yes	4.7	Yes	No
9	Male	70	Alcohol	Peripheral artery disease	7	10.9	Active	C	N/A	54.7	44.3	Yes	No	Yes	5.1	No	No
10	Female	61	Alcohol	Arrhythmia	11	9.5	Past	A	≥15	10.8	98.9	Yes	No	Yes	5.9	No	No
11	Male	66	Alcohol	Ischemic stroke	20	9.1	Past	B	≥15	20.7	52	No	Yes	Yes	6.3	Yes	No
12	Male	69	Alcohol	Acute coronary syndrome	33	10.2	Active	C	≥15	≥20	53.2	Yes	No	Yes	5.9	No	Yes
13	Female	57	NASH	Arrhythmia	29	7.5	Active	A	≥15	≥20	35.4	No	Yes	Yes	5.1	No	Yes
14	Male	77	HCV	Acute coronary syndrome	27	11.9	Never	C	N/A	≥20	50.3	Yes	No	Yes	7.2	Yes	Yes
15	Female	68	HCV	Arrhythmia	11	9.8	Past	B	≥15	≥20	69	Yes	Yes	No	5.1	No	Yes

AHT, arterial hypertension; aPWV, aortic pulse wave velocity; BP, blood pressure; CV, cardiovascular; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HBV, hepatitis B virus; HCV, hepatitis C virus; N/A, not applicable; NASH, nonalcoholic steatohepatitis; PCE, pooled cohort equation; SCORE, systematic coronary risk evaluation.

increasingly older and present more metabolic comorbidities and renal impairment,²⁴⁻²⁶ suggesting that the clinical impact of long-term CV events will be even more meaningful in the next few years. Importantly, a number of studies have shown that the grade of control of CV risk factors such as DM, AHT, or dyslipemia²⁷⁻²⁹ is suboptimal in this particular population. In contrast, a recent study demonstrated that, if achieved, an optimal BP control is associated with a decreased incidence of CV events and better survival of LT recipients, suggesting that there is a clear role for intensive monitoring and treatment of these risk factors.³⁰ However, the target levels (and thus the intensity of interventions) of HbA1c, LDL-cholesterol, or BP differ according to baseline CV risk.¹⁹ CV risk is usually estimated through clinical scores, whose usefulness in the LT setting has not been clearly demonstrated. In this regard, it seems clear that improving risk stratification in this population can be clinically relevant.

Pulse wave velocity is the most widely used noninvasive method to estimate arterial stiffness. Arterial walls are thickened and stiffened as a result of the loss of elastic fibers and increased fibrosis, consequence of age and repetitive stress.³¹ aPWV directly correlates with arterial stiffness or arteriosclerosis, and its measurement has been shown to improve the predictive ability of standard risk factors in the general population, potentially

contributing to a better identification of populations that might benefit from more aggressive management of CV risk factors.

In our cohort, aPWV correlated with several characteristics that have been shown to be associated with CV events in the general population and in LT recipients, and it was independently associated with age and BP level. These results are supported by the physiopathological mechanisms underlying arterial stiffness, which is intimately related with the degenerative changes that take place in the arteries as the consequence of aging and increased BP.^{11,31} Indeed, previous studies in the general (nontransplant) population, using either tonometry or the same oscillometric method that we used to evaluate arterial stiffness, have also shown a close association between aPWV and age and BP.^{32,33} It remains to be investigated whether the increase of aPWV with age is steeper in transplant recipients, hypothesizing a direct role of immunosuppression in a potentially higher increase of aPWV in these patients. This would be in line with the accelerated progression of atherosclerosis that possibly takes place in transplant recipients. In this regard, in our cross-sectional study, aPWV was associated with using CNI-free immunosuppression and with lower levels of cyclosporine A in patients using this drug in the univariate analysis. These results are probably related with the fact that those patients with a baseline higher CV (or

TABLE 4.
Univariate Cox-regression analysis of variables associated with the incidence of cardiovascular events during follow-up

Variable	HR	95% CI	P
Age (y)	1.060	0.998-1.126	0.060
Gender (male)	0.863	0.275-2.713	0.801
Etiology of liver disease (NASH)	1.567	0.354-6.944	0.554
Never smoker	0.206	0.046-0.917	0.038
aPWV (m/s)	1.595	1.119-2.273	0.010
SCORE, ≥15%^a	6.323	1.426-28.03	0.015
PCE, ≥20%^a	4.182	1.33-13.153	0.014
AHT	3.424	0.773-15.176	0.105
Controlled BP	0.371	0.132-1.102	0.059
Diabetes	4.703	1.327-16.670	0.016
HbA1C (mg/dL)	1.727	0.895-1.823	0.178
BMI (kg/m ²)	1.077	0.970-1.195	0.163
Waist circumference (cm)	1.049	0.999-1.101	0.053
Metabolic syndrome	2.171	0.742-6.352	0.157
Creatinine (mg/dL)	9.825	2.286-42.234	0.002
eGFR (mL/min/1.73 m²)	0.959	0.931-0.989	0.008
Total cholesterol (mg/dL)	1.012	1.001-1.024	0.032
LDL-cholesterol (mg/dL)	1.020	1.003-1.037	0.023
Triglycerides (mg/dL)	1.008	1.003-1.014	0.004
Previous CVE	3.651	1.297-10.278	0.014
Use of ASA	0.551	0.113-2.221	0.363

Bold indicates variables that showed statistical significance in univariate analysis.

^aPatients in whom SCORE and PCE are not calculated (those with diabetes/previous CV events for SCORE; previous CV events for PCE) are included in the very high-risk groups for each variable (≥15% for SCORE, ≥20% for PCE).

AHT, arterial hypertension; aPWV, aortic pulse wave velocity; ASA, acetyl salicylic acid; BMI, body mass index; BP, blood pressure; CI, confidence interval; CVE, cardiovascular events; eGFR, estimated glomerular filtration rate; HbA1C, glycosylated hemoglobin; HR, hazard ratio; LDL, low-density lipoprotein; NASH, nonalcoholic steatohepatitis; PCE, pooled cohort equation; SCORE, systematic coronary risk evaluation.

renal) risk are also those in which these regimens without CNI or with less exposure to these drugs are more frequently used to try to mitigate that increased risk. Longitudinal evaluation of arterial stiffness in transplant recipients will be useful to evaluate the actual role of immunosuppression in the progress of aPWV.

Notably, aPWV seemed to be able to identify patients with a higher risk of presenting new CV events after a median follow-up of 35 mo. Although the number of events must be taken into account when interpreting the results, we performed several multivariate models with clinical variables, all of which showed that higher aPWV and past or present smoking habit are independently associated with the incidence of CV events, with triglycerides serum levels also being independently associated with CV events if included in the analysis. As stated above, multivariate analysis must be interpreted with caution; however, our results are coherent and supported by the fact that smoking habit and serum triglycerides, well known CV risk factors,³⁴⁻³⁶ were consistently found as independent predictors of CV events. Additionally, when tested against a standard clinical algorithm like SCORE, aPWV maintained its independent predictive value, whereas neither PCE nor aPWV retained statistical significance when tested together. However, it must be stressed that such algorithms are intended to estimate long-term (10 y) risk, and our patients were followed for a median time of 35 mo. Thus, our results, although requiring confirmation in larger studies

with longer follow-up to prove whether aPWV independently adds to current clinical calculators, would favor including the evaluation of arterial stiffness in the investigation of CV risk of LT recipients. Additionally, it may be worthwhile to investigate the role of aPWV estimated before LT in the prediction of long-term CV events. Considering the increasingly worsening CV profile of LT candidates,³⁷ having a new tool to stratify risk before LT could be of great interest. Similarly, whether changes in aPWV according to the grade of control of BP improve the predictive ability of long-term CV events with respect to baseline measurements may also be clinically important, as well as to investigate the association of aPWV with post-LT kidney dysfunction.

We also examined the association between BP treatment and aPWV in this population. Although results are difficult to interpret, we did not find any obvious association between the type of antihypertensive used and aPWV. There is some evidence that drugs antagonizing the renin-angiotensin system may be more effective in decreasing arterial stiffness, probably because of a specific antifibrotic or anti-inflammatory effect on the matrix of the arterial wall^{38,39}; thus, it may be possible that the sample size and the difficulties in analyzing the different treatment regimens including combinations of different drugs have hampered our analysis.

There are limitations to our study. Particularly, it is a single-center study and the sample size is limited and includes patients with 3 different post-LT follow-up timings; however, this has also permitted us to study (and discard) the potential impact of time after LT in arterial stiffness. Additionally, there were relatively few CV events in the prospective follow-up. For this reason, confirmation of our results in larger studies is warranted before recommending to implement them in clinical practice. Finally, although we used a device that has been tested against intra-arterial measurements,¹⁴ the estimation of aPWV was done by oscillometry instead of using the gold standard noninvasive method (applanation tonometry). A recent large study has suggested that oscillometric methods may slightly overestimate aPWV in elderly and hypertensive patients with respect to tonometry, although these differences do not seem to be clinically relevant.⁴⁰ Additionally, there is increasing evidence of the usefulness of oscillometry in clinical practice,⁴¹ and, from a clinical point of view, the key issue would be the ability of any tool to provide an accurate estimation of future risk. In this regard, oscillometric-based 24-h aPWV estimation has been shown to be a strong predictor of cardiovascular events in hemodialysis patients.⁴² Not less importantly, oscillometric methods are less time-consuming and more user-friendly than tonometry, making their use easier in clinical practice. Indeed, the device used works as a standard sphygmomanometer and permits, in the same examination, to obtain BP measurement (including ambulatory 24-h measurements) and aPWV. Then, the results are downloaded and analyzed by a proprietary software. All these procedures can be easily performed in a post-LT medical or nursing clinic.

Although we recognize and have tried to overcome these limitations, it is clear that larger studies are needed to confirm our results, and indeed our work must be seen as a first step on the road. Indeed, even considering the pilot frame of the design, our results are consistent and encouraging, and may pave the path to evaluate these different forms of evaluation of CV risk in LT recipients.

In conclusion, we provide the first data showing that the estimation of arterial stiffness mirrors CV risk after LT. Further studies will be necessary as to investigate whether it can be implemented as a screening tool or used as a surrogate of CV risk in studies evaluating long-term outcomes.

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