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Arterial stiffness but not endothelial dysfunction is associated with multidrug antihypertensive therapy and nondipper blood pressure pattern in kidney transplant recipients

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Abstract

Vascular injury related to chronic kidney disease results in increased arterial stiffness and endothelial dysfunction which may affect arterial blood pressure (BP) and influence patient and graft survival in kidney transplant recipients (KTRs).

This cross-sectional study aims to elucidate the relationship between the above-mentioned measures of vascular damage and effectiveness of antihypertensive treatment in KTR.

One hundred forty-five KTRs 7.6±2.7 years after transplantation were enrolled in our study. Pulse wave velocity (PWV), flow-mediated dilation (FMD), and nitroglycerin-mediated dilation (NMD) were measured, and 24-hour ambulatory BP monitoring was performed.

Overall, there were 62 patients with well-controlled or borderline BP and 83 subjects who did not achieve target BP despite antihypertensive treatment. Patients with suboptimal BP control were characterized by greater PWV (median 9.6/interquartile range: 3.9 vs 8.0/3.3 m/s, $P = .002$), but borderline lower FMD (8.4%±5.0% vs 9.9%±5.7%; $P = .09$) as compared with the group with better BP control. When patients were allocated to subgroups based on the number of current antihypertensive medications, no differences in FMD and NMD were found. However, a significant trend was observed for higher PWV values and decreased proportion of dippers along with the increasing number of drugs. PWV, diabetes, and total cholesterol level, but not FMD or NMD, were explanatory variables for systolic BP in multivariate analysis.

Arterial stiffness but not endothelial dysfunction is associated with suboptimal BP control in stable KTRs. Less efficient antihypertensive treatment appears to be caused by inadequate control of nocturnal BP.

Abbreviations: ABPM = ambulatory blood pressure monitoring, BMI = body mass index, BP = blood pressure, BSA = body surface area, CABG = coronary artery bypass grafting, CKD = chronic kidney disease, DBP = diastolic blood pressure, ED = endothelial dysfunction, eGFR = estimated glomerular filtration rate, FMD = flow-mediated dilation, HDL = high-density lipoprotein, IMT = intima-media thickness, IQR = interquartile range, KTR = kidney transplant recipient, LDL = low-density lipoprotein, LVH = left ventricular hypertrophy, LVM = left ventricular mass, MACE = major adverse cardiovascular events, m-TOR = mammalian target of rapamycin, NMD = nitroglycerin-mediated dilation, PP = pulse pressure, PWV = pulse wave velocity, SBP = systolic blood pressure.

Keywords: blood pressure, dipper, flow-mediated dilation, 24-hour ambulatory monitoring, medications, pulse wave velocity

1. Introduction

Arterial hypertension is highly prevalent in kidney transplant recipients (KTRs) as a consequence of common pretransplant hypertension and as an additional effect of immunosuppressive medications.[1,2] In daily clinical practice, it was shown that blood pressure (BP) control is suboptimal [systolic BP (SBP) >140 mm Hg] in 50% of KTRs.[3–5] In addition, BP values were associated with reduced graft and patient survival.[5] BP values in 24-hour ambulatory BP monitoring (ABPM) seem to be a stronger predictor of renal graft damage than traditional immunologic factors.[6] Notably, the number of antihypertensive drugs at 1 year after kidney transplantation was significantly related to lower patient survival rates, which is independent of previous diagnosis of hypertension or diabetes, recipients age, and renal function at 1 year.[7]

Cardiovascular disease is the primary cause of mortality in kidney transplant population, mostly due to long-term consequences of chronic kidney disease (CKD).[8] CKD-related

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systemic inflammation, oxidative stress, and calcium-phosphate abnormalities promote endothelial dysfunction (ED), vascular calcification, and accelerated atherosclerosis.[9,10] The profound vascular injury caused by uremic *milieu* manifests as increased arterial stiffness (related to the structural changes of the vascular wall) and reduced flow-mediated dilation (FMD), that is, impaired endothelium-dependent relaxation of vascular tone.[11,12] Of note, kidney transplantation improves but not normalizes arterial function as measured by pulse wave velocity (PWV).[13] On the contrary, it was documented that BP is a major determinant of arterial stiffness and ED in CKD patients.[14] Moreover, long-term therapy with calcineurin inhibitors could further impair arterial distensibility and endothelial function in KTRs.[15]

In this cross-sectional study, we analyzed the structure of antihypertensive treatment and its efficacy in a cohort of stable KTRs. We also investigated the relationship between demographic, clinical, and metabolic factors and BP control as evaluated by ABPM. Finally, we assessed PWV and FMD, as well as nitroglycerin-mediated dilation (NMD) and examined the relationships between these measures of vascular injury and multidrug antihypertensive therapy in the kidney transplant population.

2. Methods

2.1. Study participants

This cross-sectional study enrolled 145 stable KTRs, transplanted in our center between 2005 and 2014, who still attended our outpatient clinic. Patients were randomly selected, and 145 out of 150 subjects agreed to participate in this study performed between January 2016 and April 2017. Patients with active infection, kidney graft artery stenosis, or unstable graft function in the preceding year were excluded. The study protocol was accepted by the Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/KB1/35/I/15), and all participants gave their written informed consent. The study was conducted in accordance with the Declaration of Helsinki. In addition to data retrieved from the center-operated transplant patient registry, we performed echocardiography, carotid ultrasound [including intima-media thickness (IMT) measurement and plaque evaluation], and PWV assessment in each patient. At the same time, both endothelial-dependent and endothelial-independent FMD were measured.

At the time of the study, patients have been receiving immunosuppressive therapy based on cyclosporine (n = 67) or tacrolimus (n = 73), antimetabolic drug (mainly mycophenolate mofetil or mycophenolate acid) or mammalian target of rapamycin inhibitors, and steroids (38.2%).

2.2. Clinical, anthropometric, and laboratory measurements

Body weight and height were measured following standard procedures, and body mass index (BMI) was then calculated (kg/m^2). Body surface area (BSA) was calculated according to DuBois formula [$0.20247 \times \text{weight (kg)}^{425} \times \text{height (m)}^{725}$] and was expressed in m^2.[16]

Serum concentrations of low-density lipoprotein (LDL) ≥130 mg/dL, serum high-density lipoprotein (HDL) <40 mg/dL for men and <50 mg/dL for women, serum triglycerides ≥150 mg/dL, or current use of statins or fibrates were scored as dyslipidemia.

Analysis of routine laboratory measurements (serum creatinine, uric acid, total cholesterol, and triglycerides concentrations) was performed in the hospital laboratory (Synchron Cx-9, Beckmann Coulter Inc, Fullerton, CA).

2.3. Echocardiography

Echocardiographic studies were performed by 1 experienced investigator, using the Toshiba Xario 100 Diagnostic Ultrasound System (Toshiba, Toshiba Medical System Corporation, Tochigi 324-8550, Japan). M-mode and 2-dimensional measurements were performed as per recommendations of the American Society of Echocardiography. These measurements included left ventricular end-diastolic and end-systolic diameters, intraventricular septum, and posterior wall end-diastolic thickness. Left ventricular mass (LVM) was calculated according to Devereux formula.[17] LVM was indexed for BSA.

2.4. Carotid artery intima-media thickness and carotid plaques assessment

Carotid ultrasound was performed by one investigator (AK), using a Siemens machine (Sonoline Antares, Mountain View, CA) equipped with a 4.0 to 9.0 MHz linear transducer. The evaluation included the common, internal, and external carotid arteries and the carotid bifurcation on each side. The common carotid artery IMT was measured proximal to the carotid bulb, about every 1 cm, omitting visible plaques. The results from 3 separate measurements on each side were then averaged. In addition, at each examined localization, the vessels were carefully evaluated in terms of the presence of plaques, which was classified based on the simplified scale: 0- no lesions, 1- noncalcified lesions, 2- at least 1 calcified lesion, 3- few calcified lesions, and 4- carotid bulb heavily covered by calcified lesions. The final plaque score was equal to the higher score obtained from both sides.

2.5. Carotid-femoral pulse wave velocity

Arterial stiffness measurements were performed in the morning, after at least 15 minutes of rest in the supine position, using a noninvasive tonometer (Sphygmo-Cor 2000, AtCor Medical, Sydney, Australia) placed over the carotid and femoral arteries. Pressure signals were calibrated using brachial BP, and PWV was calculated as the time of the pulse wave between the diagnosed points [distance (m)/time (s)].

2.6. Brachial artery flow-mediated dilation

Endothelial function was measured after 12 hours of fasting and after 10 minutes of lying in a quiet dimmed room with temperature of 20 to 25°C using Toshiba Xario 100 Diagnostic Ultrasound System (Toshiba). Before examination, the patients rested in a seated position, with their forearms and backs supported for at least 15 minutes; then, manual sphygmomanometer cuff was placed on the arm without arteriovenous fistula, and linear transducer was positioned 4 to 8 cm above the antecubital fossa to visualize the brachial artery and measure its diameter (lumen). After initial BP measurement, the cuff was inflated at approximately 50 mm Hg above the current systolic pressure for 5 minutes, in which a minimum of 200 mm Hg was applied. The measurements during diastole were recorded, and the widest dilation of the brachial artery was usually detected within 60 to 120 seconds of reactive hyperemia. FMD was

calculated as follows: FMD% = $(A - B)/B \times 100\%$, where A is diameter of artery after dilation and B is the diameter of artery before examination. In patients with arteriovenous fistula, the arm without hemodialysis shunt was chosen for brachial artery occlusion and subsequent FMD assessment.

NMD, which is a measure of nonendothelial dependent vasodilation, was measured thereafter. Similarly to FMD measurements, a vessel diameter was assessed before the use of nitroglycerine and 1, 2, and 5 minutes after sublingual nitroglycerin (400 μg) application [Nitromint (glyceroli trinitras), Proterapia, Poland].

2.7. 24-Hour ambulatory blood pressure monitoring

ABPM was performed using a device conforming to the British Hypertension Society recommendations (TM2430, Bosch+Sohn GmbH, Germany) with appropriate cuff sizes for each patient. In patients with arteriovenous fistula, BP was measured in the contralateral arm. All 24-hour recordings were carried out every 15 minutes during the day (6:00 AM-10:00 PM) and every 30 minutes during the night (10.00 PM-6.00 AM), with patients instructed to maintain their usual level of activity. On the basis of circadian BP, patients were classified into dippers ($\Delta SBP \geq 10\%$), nondippers ($\Delta SBP \geq 0\%$ and $<10\%$), or reverse dippers (with nighttime SBP rise).

2.8. Data and statistical analysis

The patients were divided into 2 study groups based on the therapeutic effect of antihypertensive treatment. BP was considered as well-controlled when the 24-hour ABPM values were lower than 130/80 mm Hg. For the purpose of this analysis, we defined borderline BP control as the ABPM values not exceeding the recommended target by >10 mm Hg. Finally, all patients were simply allocated into 2 groups: those with well-controlled or borderline BP and those who did not achieve therapeutic target. In addition, we analyzed the study cohort based on the number of antihypertensive medications currently used.

Pretransplant and post-transplant major adverse cardiovascular events (MACE) were defined as the episodes of myocardial infarct, stroke, or cardiac artery stenting/surgery.

Kidney graft function was measured by the estimated glomerular filtration rate (eGFR), which was calculated according to the abbreviated Modification of Diet in Renal Disease formula.

Statistical analyses were performed using the STATISTICA 12.0 PL for Windows software package (Stat Soft Polska, Kraków, Poland) and MedCalc 12.3.0.0 (Mariakerke, Belgium). The values were presented as mean values ± standard deviation (mean ± SD) or median values with interquartile range (IQR) for variables with nonparametric distribution. Differences in the distribution of qualitative variables between study subgroups were compared by χ^2 test and χ^2 test for trend, whereas that of quantitative variables was through analysis of variance or Mann-Whitney U test. Correlation coefficients were calculated using Pearson test. Backward stepwise multivariate linear and skew-n regression (in case of data skewness) analyses were performed for SBP, including age, BMI, eGFR, serum uric acid and total cholesterol levels, diabetes occurrence, PWV, FMD, and NMD as potential explanatory variables. Cook-Weisberg test was used to test heteroskedasticity and Remsey RESET test was used to test the linearity of regression. Variance inflation factor was calculated to check multicollinearity. The Shapiro-Wilk test with quantile-quantile plot was used to assess normality of the data distribution as well as regression residuals. In all statistical tests, "P" values $<.05$ were considered as statistically significant.

3. Results

3.1. Study patients

Overall, there were only 29 patients (20%) with well-controlled BP and 33 (23%) with borderline BP control; 83 (57%) subjects did not achieve the target BP despite antihypertensive treatment. Therefore, we performed our analysis in 2 groups of patients: those who did not reach the therapeutic BP goal and those with good or borderline BP control. The demographic and clinical characteristics of patients who did not achieve the BP target and those with borderline or well-controlled BP are shown in Table 1. There were more patients with diabetes in the group with suboptimal BP control. The percentage of ischemic heart disease diagnosed before kidney transplantation and the percentage of patients with previous MACE, coronary artery bypass grafting (CABG) or cardiac stenting was comparable. There were no significant differences in the age and BMI between groups; however, IMT and statin use were significantly greater in patients with suboptimal BP control, whereas post-transplant weight gain was significantly greater in patients with better BP control. The pretransplant dialysis vintage as well as the time after transplantation, proportion of retransplants, and percentage of calcified carotid plaques was similar. Interestingly, both groups did not differ in prevalence or duration of hypertension before transplantation: the concomitant number of antihypertensive drugs (mean 1.7 ± 1.3), Sokolow-Lyon index, and the grade of hypertensive retinopathy before transplantation were similar (data not shown) as well.

3.2. Potential risk factors for not achieving a BP goal

There were no differences between both groups (with suboptimal vs good or borderline BP control) in terms of kidney graft function, frequency of steroid use, type of calcineurin inhibitor used, and presence of functioning vascular access. Moreover, the prevalence of hyperlipidemia or hyperuricemia was similar. Of note, the frequency of left ventricular hypertrophy (LVH) was significantly higher in patients who have not achieved the BP treatment goal (45.8% vs 22.6%, $P = .007$). Patients taking steroids were characterized by significantly lower FMD (7.6% ± 4.6% vs 9.8% ± 5.6%, $P = .02$), whereas they did not differ in terms of age (53 ± 13 vs 56 ± 12 years, $P = .15$), NMD (median: 9.09/IQR: 7.36 vs 9.44/7.13, $P = .64$), and PWV (9.2/3.5 vs 8.9/4.1 m/s, $P = .87$) from patients without steroids. In contrast, there were no differences in age, FMD, NMD, and PWV values between patients treated with cyclosporine or tacrolimus (data not shown).

A significant trend was observed for increased LVH prevalence along the subgroups based on increased number of current antihypertensive medications (Table 2). In the latter analysis, the subgroups did not differ in terms of kidney graft function, frequency of steroid use, type of calcineurin inhibitor used, and the presence of functioning vascular access (Table 3). There was no difference in time spent on pretransplant dialysis therapy or time after transplantation; however, patients differed in regards to age at the time of the study. Moreover, there were increased numbers of overweight or obese patients with diabetes, ischemic heart disease, or previous MACE, CABG, or coronary stenting

Table 1

Demographic, clinical, and laboratory characteristics of study groups, defined by the effectiveness of antihypertensive treatment, based on the results of 24-hour ambulatory blood pressure measurement.

	All patients (n = 145)	Not achieving BP target (n = 83)	Borderline or well-controlled hypertension (n = 62)	P*
Age, yr	55 ± 12	56 ± 11	53 ± 13	.10
Sex, M/F	78/67	49/34	29/33	.19
BMI, kg/m^2	26.9 ± 4.8	27.1 ± 4.3	26.5 ± 5.5	.48
or obesity, %	62.8	65.1	59.7	.18
Diabetes, %	37.9	49.4	22.6	.012
IHD, %	12.4	15.7	8.1	.26
MACE/CABG/stent, %	15.2	19.3	9.7	.17
Dialysis vintage, mo	28 (15–43)	27 (16–42)	28 (13–45)	.79
Time after KTx, mo	91 ± 32	91 ± 33	92 ± 31	.96
Retransplant, %	9.0	6.0	12.9	.25
ECD, %	19.4	20.7	17.7	.66
Weight gain after KTx, %	9.5 ± 12.0	7.7 ± 10.9	11.8 ± 13.0	.25
Number of antihypertensive drugs	2.2 ± 1.4	2.5 ± 1.5	2.0 ± 1.4	.22
ACE/ARB, %	29.2	32.9	24.2	.34
CCB, %	42.4	43.9	40.3	.74
LBA, %	80.0	80.5	79.0	.99
Diuretics, %	32.6	37.8	25.8	.18
Steroids, %	38.2	43.9	30.6	.15
Hyperuricemia, %	55.6	59.8	50.0	.32
Uric acid, μM/L	390 ± 74	391 ± 80	389 ± 67	.88
Hyperlipidemia, %	50.0	53.7	45.2	.40
Total cholesterol, mM/L	5.4 ± 1.1	5.6 ± 1.2	5.3 ± 1.1	.13
Triglycerides, mM/L	1.6 (1.1–2.3)	1.8 (1.1–2.3)	1.7 (1.1–2.2)	.61
Statins, %	19.4	25.6	11.3	.17
Fibrates, %	9.0	9.8	8.1	.95
eGFR, mL/min/1.73 m^2	51.5 ± 23.4	49.0 ± 22.3	54.7 ± 24.6	.15
Functioning vascular access, %	28.3	33.7	21.0	.13
CNI, CyA/Tc	67/73	39/42	28/31	.73
LVH, %	35.9	45.8	22.6	.04
IMT, mm	0.64 ± 0.12	0.67 ± 0.12	0.60 ± 0.12	.01
Calcified plaques, %	56.6	61.4	50.0	.23
PWV, m/s	9.0 (7.4–11.1)	9.6 (8.0–11.9)	8.0 (6.9–10.2)	.01
FMD, %	9.0 ± 5.3	8.4 ± 5.0	9.9 ± 5.7	.09
NMD, %	9.4 (6.0–13.0)	8.1 (4.9–11.4)	10.4 (7.1–13.9)	.25

Data shown as means ± SD or medians with interquartile range or frequencies.

* Statistical significance was calculated using *t* student test, Mann-Whitney *U* test, or chi^2 test; as appropriate.

ACE/ARB = angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, BMI = body mass index, BP = blood pressure, CABG = cardiac artery by-pass graft, CCB = calcium channel blocker, CNI = calcineurin inhibitor, CyA = cyclosporine, ECD = extended criteria donor, eGFR = estimated glomerular filtration rate, FMD = flow-mediated dilation, IHD = ischemic heart disease, IMT = carotid intima-media thickness, KTx = kidney transplantation, LBA = beta-adrenergic blocker, LVH = left ventricular hypertrophy, MACE = major adverse cardiovascular event, NMD = nitroglycerin-dependent dilation, PWV = pulse wave velocity, Tc = tacrolimus.

along with the increasing number of antihypertensive medications currently used.

3.3. The antihypertensive treatment and BP control

At the time of the present study, the group of patients with suboptimally controlled BP was treated using a similar number of antihypertensive medications (2.0 ± 1.4 vs 2.5 ± 1.5, $P = .22$) in comparison with the group with good or borderline BP control. There was also no difference between the groups with regards to the structure of antihypertensive regimen, which included diuretics (Table 1). The number of antihypertensive drugs was associated with PWV ($r = 0.264$, $P = .001$) but not with FMD ($r = -0.131$, $P = .12$) and NMD ($r = -0.04$, $P = .65$). Of interest, PWV was significantly greater and FMD was significantly lower in patients using diuretics (PWV: 10.0/3.6 vs 8.8/3.8, $P = .003$; FMD: 7.0 ± 4.3 vs 10.0 ± 5.5, $P = .001$). As expected, patients from the group with better BP control had significantly lower SBP and diastolic BP (DBP), including 24-hour, day, and night measurements (24-hour: SBP 153 ± 11 vs 128 ± 8, DBP 88 ± 8 vs 77 ± 6; daytime: SBP 154 ± 11 vs 131 ± 8, DBP 89 ± 8 vs 79 ± 6; nighttime: SBP 148 ± 18 vs 120 ± 13, DBP 83 ± 11 vs 72 ± 8 mm Hg; all $P < .001$). Moreover, the drop in nocturnal mean SBP, but not DBP, was significantly greater in this group (8 ± 9 vs 4 ± 9, $P < .001$ and 9 ± 10 vs 7 ± 10 mm Hg, $P = .20$, respectively). Pulse pressure (PP) was significantly lower in the group with better BP control (52 ± 9 vs 65 ± 11 mm Hg, $P < .001$), with a subsequent difference in the nocturnal drop in PP (6 ± 11 vs 0 ± 13 mm Hg in the suboptimal BP control group, $P = .002$). Of note, 24-hour PP was significantly correlated with PWV ($r = 0.336$, $P < .001$) and with the number of antihypertensive drugs used ($r = 0.261$, $P = .002$).

Analysis of patients in subgroups based on the number of current antihypertensive medications, with the exception of patients untreated with antihypertensive drug, revealed that beta-blockers were the most frequently used type of antihypertensive drugs (89.1%) (Table 3). In addition, there was an increasing trend in the use of calcium channel blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and diuretics across study subgroups. It is noteworthy that

Table 2

The intima-media thickness measurements, kidney graft Doppler resistance index, left ventricular hypertrophy prevalence, flow-mediated dilation and arterial stiffness values, and 24-hour automated blood pressure monitoring measurements in study groups, defined by the number of antihypertensive drugs.

	Study groups according to the current antihypertensive treatment					
	Untreated (n = 16)	1 Drug (n = 32)	2 Drugs (n = 38)	3 Drugs (n = 33)	4 Drugs (n = 26)	ANOVA/chi^2
IMT, mm	0.61 ± 0.13	0.65 ± 0.1	0.64 ± 0.13	0.64 ± 0.14	0.66 ± 0.11	.83
Carotid plaques score	1.1 ± 1.5	1.8 ± 1.6	1.7 ± 1.8	2.4 ± 1.6	2.8 ± 1.4	.005
Calcified plaques, %	43.8	46.9	42.1	69.7	80.8	<.001*
LVH, %	12.5	34.4	23.7	51.5	53.8	.01*
FMD, %	9.8 ± 5.2	10.4 ± 6.3	8.6 ± 4.8	7.9 ± 4.9	8.6 ± 5.4	.38
FMD N, %	11.2 ± 4.9	10.1 ± 6.6	10.0 ± 6.7	10.6 ± 7.0	9.8 ± 5.2	.94
PWV, m/s	8.7 ± 2.9	8.9 ± 2.0	9.5 ± 3.0	9.4 ± 2.0	11.1 ± 3.5	.02
24-Hour SBP, mm Hg	139 ± 14	137 ± 17	141 ± 11	145 ± 15	148 ± 19	.09
24-Hour DBP, mm Hg	84 ± 7	81 ± 9	83 ± 7	84 ± 10	84 ± 9	.68
Daytime SBP, mm Hg	142 ± 15	139 ± 16	143 ± 15	146 ± 15	149 ± 18	.15
Daytime DBP, mm Hg	86 ± 7	83 ± 9	85 ± 7	86 ± 10	84 ± 9	.76
Nocturnal SBP, mm Hg	128 ± 16	128 ± 22	133 ± 14	142 ± 21	147 ± 24	.002
Nocturnal DBP, mm Hg	77 ± 8	75 ± 12	77 ± 8	80 ± 12	82 ± 13	.09
SBP night drop, mm Hg	10.1 ± 7.7	7.8 ± 9.5	7.4 ± 7.3	3.3 ± 8.2	1.3 ± 10.3	.004
DBP night drop, mm Hg	10.5 ± 6.3	10.5 ± 10.2	9.3 ± 7.2	6.2 ± 10.2	2.1 ± 13.0	.06
PP, mm Hg	55 ± 10	57 ± 13	58 ± 10	61 ± 13	65 ± 14	.06
Daytime PP, mm Hg	56 ± 10	48 ± 12	58 ± 10	60 ± 12	65 ± 14	.1
Nocturnal PP, mm Hg	51 ± 10	56 ± 15	56 ± 13	62 ± 16	64 ± 17	.01
PP night drop, %	9.0	3.7	5.3	−2.1	0.3	.02
Dippers, %	43.7	43.7	43.2	27.3	15.4	<.001*
Reverse dippers, %	12.5	18.8	16.2	27.3	38.5	.03*
Uncontrolled hypertension, %	8.5	17.1	28.1	26.8	19.5	.06*

Data shown as means ± SD or frequencies.

IMT = intima-media thickness, LVH = left ventricular hypertrophy, FMD = flow-mediated dilation, FMD N = FMD after nitrite administration, BP = blood pressure, PWV = pulse wave velocity, SBP = systolic blood pressure, DBP = diastolic blood pressure, PP = pulse pressure.

* For trend.

Table 3

Demographic, clinical, and laboratory characteristics of study groups, defined by the number of antihypertensive drugs.

	Study groups according to the current antihypertensive treatment					
	Untreated (n = 16)	1 Drug (n = 32)	2 Drugs (n = 38)	3 Drugs (n = 33)	>3 Drugs (n = 26)	*P*
Age, yr	47 ± 13	56 ± 12	53 ± 13	55 ± 10	59 ± 11	.02
Sex, M/F	6/10	13/19	21/17	21/12	17/7	.09
BMI, kg/m^2	24.2 ± 2.6	26.4 ± 6.0	26.6 ± 4.0	27.9 ± 5.4	28.1 ± 4.1	.04
Overweight or obesity, %	31.2	59.4	65.8	63.4	76.9	.02*
Diabetes, %	6.3	34.4	34.2	36.4	65.4	<.001
IHD, %	0	6.3	13.2	15.2	23.1	.01
MACE/CABG/stent, %	0	3.1	13.2	24.2	26.9	<.001
Dialysis vintage, mo	42 (10–72)	37 (17–49)	30 (17–41)	20 (14–33)	21 (10–36)	.28
ECD, %	0	19.4	13.2	21.2	42.3	.005
Time after KTx, mo	94 ± 40	96 ± 29	83 ± 33	86 ± 31	104 ± 26	.08
Weight gain after KTx, %	7.1 ± 9.8	10.3 ± 13.1	8.8 ± 12.0	10.7 ± 13.3	8.1 ± 11.9	.83
ACE/ARB, %	–	3.1	21.1	48.5	69.2	<.001*
CCB, %	–	6.2	52.6	63.6	73.1	<.001*
LBA, %	–	87.5	81.6	93.9	96.2	.13
Diuretics, %	–	0	21.1	51.5	84.6	<.001*
Steroids, %	37.5	34.4	39.5	45.5	30.8	.81
Hyperuricemia, %	31.2	53.1	47.4	60.6	76.9	.005*
Uric acid, μmol/L	355 (320–395)	384 (354–422)	390 (332–417)	377 (323–428)	398 (351–424)	.49
Hyperlipidemia, %	25.0	62.5	42.1	54.5	57.9	.10
Total cholesterol, mmol/L	5.0 ± 0.9	5.5 ± 1.1	5.5 ± 1.3	5.5 ± 1.1	5.4 ± 1.2	.64
Triglycerides, mmol/L	1.1 (0.8–1.4)	2.0 (1.1–2.3)	1.6 (1.1–2.0)	1.5 (1.1–2.5)	1.8 (1.2–2.9)	.003
Statins, %	6.2	12.5	23.7	24.2	26.9	.05
Fibrates, %	0	15.6	5.3	12.1	7.7	.33
eGFR, mL/min/1.73 m^2	62.0 ± 27.2	50.5 ± 19.7	49.5 ± 19.6	52.9 ± 24.5	46.9 ± 27.8	.32
Functioning vascular access, %	25.0	28.1	26.3	33.3	26.9	.96
CNI, CyA/Tc	31.3/62.5	40.6/50	42.1/44.7	48.5/42.4	61.5/34.6	.87

Data shown as means ± SD, or medians with interquartile range, or frequencies. Statistical significance was calculated using analysis of variance (ANOVA) or chi^2 tests, as appropriate.

ACE/ARB = angiotensin converting enzyme inhibitor/angiotensin receptor blocker, BMI = body mass index, CABG = cardiac artery by-pass graft, CCB = calcium channel blocker, CNI = calcineurin inhibitor, CyA = cyclosporine, ECD = extended criteria donor, eGFR = estimated glomerular filtration rate, IHD = ischemic heart disease, KTx = kidney transplantation, LBA = beta-adrenergic blocker, MACE = major adverse cardiovascular event, Tc = tacrolimus.

* For trend.

despite such treatment strategy, 24-hour SBP values differed significantly across these subgroups of patients and is mostly due to a significant increasing trend in nocturnal SBP, whereas the daytime SBP values remained comparable. In line with this, the percentage of dippers decreased, whereas that of reverse dippers increased, along with the increase in number of antihypertensive medications.

3.4. The morphologic and functional assessment of vascular system

Patients from the group with suboptimal BP control were characterized by greater PWV (9.6/3.9 vs 8.0/3.3 m/s, $P=.002$) but lower FMD (8.4% ± 5.0% vs 9.9% ± 5.7%; $P=.09$) as compared to the group with better BP control, whereas NMD values were similar in both groups (8.1/6.5 vs 10.4/6.8, $P=.2$; Fig. 1).

Interestingly, when we analyzed the patients subgroups based on the number of current antihypertensive medications, no differences were found between FMD and NMD. However, we observed a significant trend of higher PWV values along with the increase in number of medications used (Table 2). Contrary to the analysis of patients who did or did not achieve BP goal, in the subgroups concerning the number of antihypertensive medications, there was no difference found in IMT between groups; however, the prevalence of carotid plaques (both overall and calcified) significantly increased along the groups.

As expected, there was a positive correlation between age and PWV ($r=0.426$, $P<.001$), and reverse correlations between age and both FMD ($r=-0.192$, $P=.02$) and NMD ($r=-0.305$, $P<.001$).

When analyzing the association between SBP and the measures of vascular function, the strongest correlation was found with PWV ($r=0.289$, $P<.001$), whereas reverse correlations were found between FMD ($r=-0.195$, $P=.02$) and NMD ($r=-0.205$, $P=.013$) (Fig. 2). Backward stepwise multivariate skew-*t* regression analysis for SBP values, including age, BMI, eGFR, serum uric acid and total cholesterol levels, diabetes, PWV, FMD, and NMD, revealed that only PWV [$\beta=1.14$, SE(β)$=0.40$; $P=.002$], diabetes [$\beta=6.30$, SE(β)$=2.48$; $P=.01$], and total cholesterol level [$\beta=2.10$, SE(β)$=0.98$; $P=.014$], were explanatory variables for SBP values. In addition, the correlation between PWV and SBP night drop was of borderline significance ($r=-0.154$, $P=.06$).

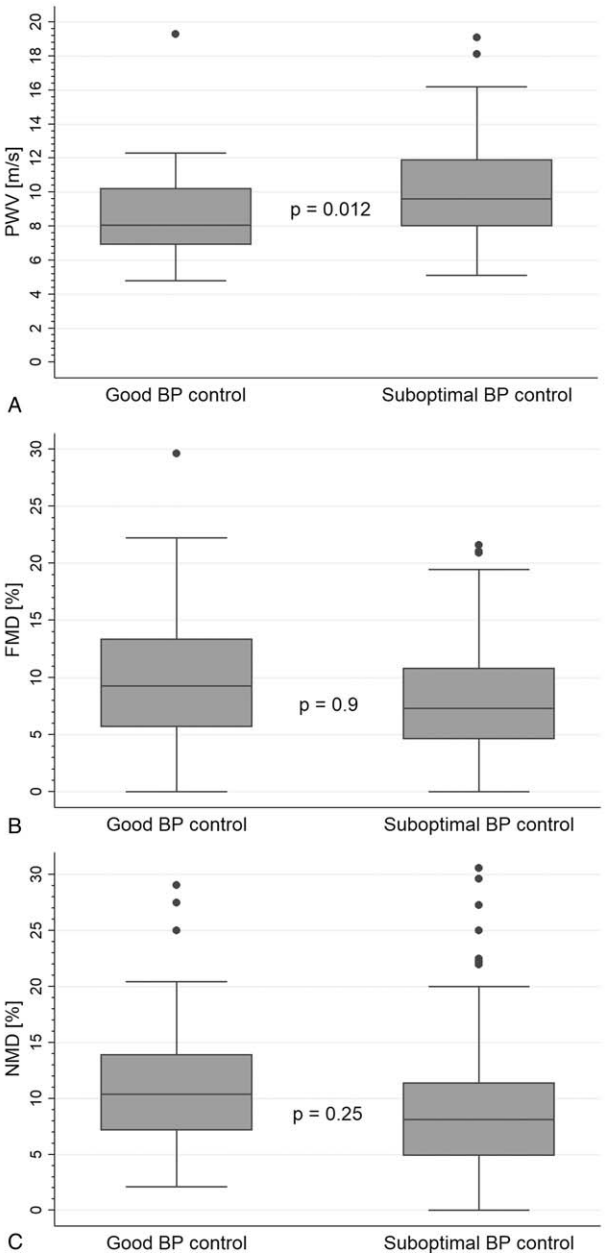

Figure 1. The values of arterial function measures in patients from the groups with good and suboptimal BP control. A, PWV. B, FMD. C, NMD. BP = blood pressure, FMD = flow-mediated dilation, NMD = nitroglycerin-mediated dilation, PWV = pulse wave velocity.

4. Discussion

In kidney transplant patients, hypertension is associated with decreased allograft survival, MACE, and worse patient survival.[5,18,19] Furthermore, lowering SBP, even after several years of post-transplantation hypertension, appears to improve long-term graft and patient survival.[19] On the contrary, BP values were higher in KTRs than in patients with CKD with similar kidney function, mainly as a result of increased nocturnal SBP.[4] Moreover, in a small prospective observation, both SBP and DBP values improved post-transplantation.[13] Nevertheless, successful kidney transplantation, despite the restoration of renal homeostasis, did not normalize the elastic and structural properties of arteries.[20] In fact, previous studies on PWV changes after transplantation yielded conflicting results.[13,21,22] Likewise, a cross-sectional study showed significantly lower FMD values in KTRs than in dialysis subjects,[12] although a recent prospective study demonstrated stable FMD values between first and 24th month post-transplantation.[23] Furthermore, Sharma et al[24] reported significant FMD improvement after transplantation; however, the exact time-point of post-transplant FMD measurement was unknown. Finally, increased sympathetic nerve activity in KTRs was proven to be related to decreased distensibility of the muscular type arteries, which contributes to arterial stiffness.[25]

In our present study on stable KTRs, we found that arterial stiffness increased with the increase in the number of antihypertensive medications used and that endothelial function was not associated with the number of medications used. Furthermore, patients who did not achieve satisfactory BP control during treatment were characterized by greater arterial stiffness, and

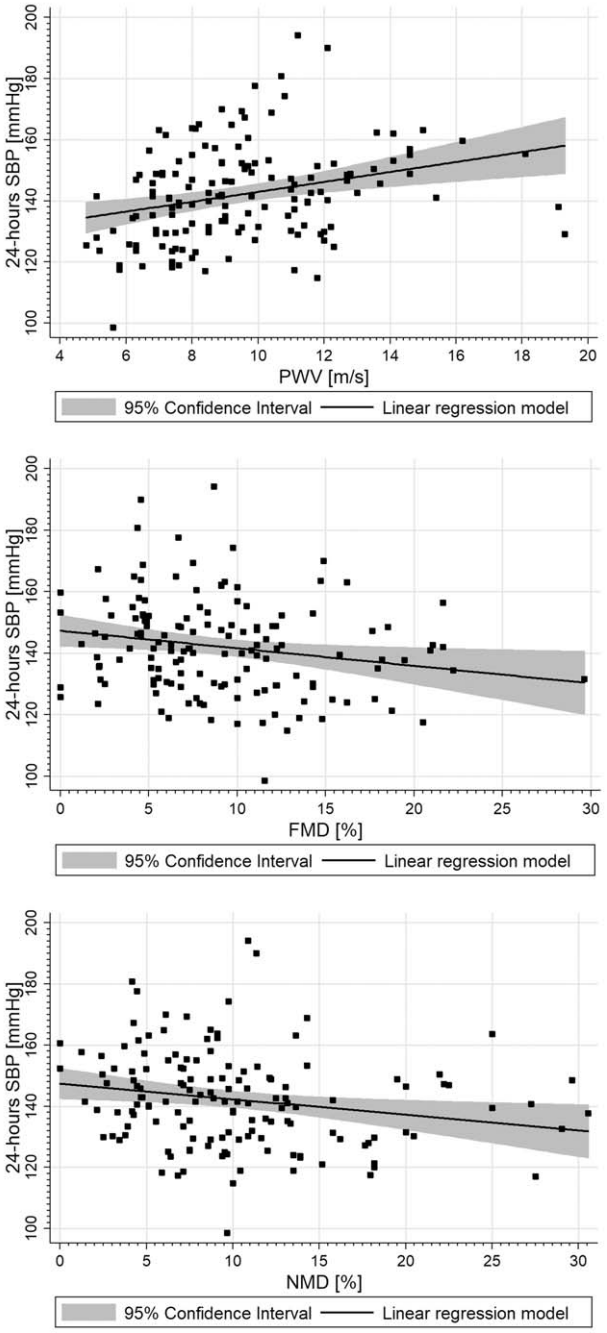

Figure 2. The correlations between 24-hour systolic BP and the values of arterial function measures in study patients. A, The association between SBP and PWV. B, The association between SBP and FMD. C, The association between SBP and NMD. BP = blood pressure, FMD = flow-mediated dilation, NMD = nitroglycerin-mediated dilation, PWV = pulse wave velocity, SBP = systolic blood pressure.

only slightly lower endothelium-dependent dilation values. Moreover, the correlation coefficient between BP and PWV was greater than that with both FMD and NMD. Finally, PWV, *diabetes, and total cholesterol level*, but not FMD or NMD, *were* among the significant explanatory factors for SBP in multivariate analysis. All above-mentioned evidences may suggest that arterial stiffness, rather than impaired endothelium function, is involved in the BP regulation of stable KTRs in long-term follow-up period. *It is worth to notice that total cholesterol level remained an independent explanatory variable despite the fact that approximately one fifth of our patients were on the statin treatment at the time of the study.*

Another important aspect of BP characteristics after transplantation is the presence of a circadian BP pattern. In general, CKD, age, and eGFR are the risk factors for nondipping hypertension.[26–29] On the contrary, nondipper status was shown to be an independent predictor of incident CKD.[30] In addition, reverse dipper BP pattern was closely related to severe renal and cardiovascular damage in patients with CKD and constitutes an independent predictor of kidney graft outcome, when identified 3 months after transplantation.[31,32] In our study, we found a decreased proportion of dippers, with a reciprocal trend among reverse dippers, along with increased number of antihypertensive drugs and PWV values. Notably, there was no concomitant difference in FMD between groups. Consequently, the number of antihypertensive drugs was associated with PWV but not with FMD; in contrast, diuretics use was more prevalent in patients with greater PWV but lower FMD. The recipients' age and prevalence of diabetes and LVH were increased among those groups. These finding are in line with our recent results involving a separate transplant cohort, wherein hypertension treated with ≥2 drugs, PP >50 mm Hg, diabetes, cardiovascular disease, and LVH were the only traditional risk factors for PWV values.[8] Likewise, other authors confirmed the association between age, LVH, and nondipper BP pattern.[33] The analysis of other potential covariates of BP dipping pattern revealed no differences in pretransplant dialysis vintage, current eGFR, type of calcineurin inhibitor, steroid use, or the presence of functioning arteriovenous fistula. In addition, there were no differences in PWV and FMD values in patients treated with cyclosporine or tacrolimus.

As our work was not based on a clinical trial, there was no universal BP goal for all participants, treated with antihypertensive medication upon physician discretion. However, our study analyzed the specific population of KTRs, excluded by definition from clinical trials and characterized by severe vascular damage. Therefore our findings are specific for this population and cannot be generalized.

The limitation of PWV analysis is the lack of data concerning the calcium-phosphate disturbances and their medication before transplantation that might cause irreversible damage to the vascular wall affecting BP control thereafter. *Another limitation is the lack of information concerning LDL and HDL concentrations.* In addition, the analysis of dyslipidemia and hyperuricemia is biased by the concomitant medication (the use of allopurinol and statins).

To summarize, the presented data support the hypothesis that arterial stiffness, rather than ED, is associated with multidrug antihypertensive therapy and nondipper BP pattern in stable KTRs.

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