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Original Investigation

Progression of Coronary Artery Calcification and Thoracic Aorta Calcification in Kidney Transplant Recipients

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Background: Vascular calcification independently predicts cardiovascular disease, the major cause of death in kidney transplant recipients (KTRs). Longitudinal studies of vascular calcification in KTRs are few and small and have short follow-up. We assessed the evolution of coronary artery (CAC) and thoracic aorta calcification and their determinants in a cohort of prevalent KTRs.

Study Design: Longitudinal.

Setting & Participants: The Agatston score of coronary arteries and thoracic aorta was measured by 16-slice spiral computed tomography in 281 KTRs.

Predictors: Demographic, clinical, and biochemical parameters were recorded simultaneously.

Outcomes & Measurements: The Agatston score was measured again 3.5 or more years later.

Results: Repeated analyzable computed tomographic scans were available for 197 (70%) KTRs after 4.40 ± 0.28 years; they were not available for the rest of patients because of death (n = 40), atrial fibrillation (n = 1), other arrhythmias (n = 4), refusal (n = 35), or technical problems precluding confident calcium scoring (n = 4). CAC and aorta calcification scores increased significantly (by a median of 11% and 4% per year, respectively) during follow-up. By multivariable linear regression, higher baseline CAC score, history of cardiovascular event, use of a statin, and lower 25-hydroxyvitamin D_3 level were independent determinants of CAC progression. Independent determinants of aorta calcification progression were higher baseline aorta calcification score, higher pulse pressure, use of a statin, older age, higher serum phosphate level, use of aspirin, and male sex. Significant regression of CAC or aorta calcification was not observed in this cohort.

Limitations: Cohort of prevalent KTRs with potential survival bias; few patients with diabetes and nonwhites, limiting the generalizability of results.

Conclusion: In contrast to previous small short-term studies, we show that vascular calcification progression is substantial within 4 years in prevalent KTRs and is associated with several traditional and nontraditional cardiovascular risk factors, some of which are modifiable.

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INDEX WORDS: Renal transplantation; vascular calcification; clinical determinants; cardiovascular disease.

Cardiovascular (CV) disease is the leading cause of premature death in kidney transplant recipients (KTRs), with a 3.5%-5% annual risk of fatal or nonfatal CV events, much higher than in the general population despite adjustment for traditional risk factors.[1,2] A high prevalence of vascular calcification has been shown in patients with chronic kidney disease (CKD), with higher calcification scores than in age- and sex-matched patients with coronary heart disease but not kidney disease.[3-5] Vascular calcification may involve either the intima, in association with inflammation and atherosclerosis, or the media, causing vascular stiffness. Both processes often coexist in patients with advanced CKD and cannot be distinguished by imaging techniques, including computed tomography (CT).[6] Still, vascular calcification strongly predicts CV disease and all-cause mortality not only in hemodialysis and peritoneal dialysis patients,[7-9] but also in KTRs.[10-12] Moreover, coronary artery calcification (CAC) progression also predicts CV events and mortality in KTRs.[12] Only a few small studies relying on CT have assessed CAC progression in KTRs.[13-16]

In the present study, a large prevalent KTR cohort underwent CT at inclusion and ~4 years later to assess the evolution of CAC and thoracic aorta calcification. The relationship between clinical, demographic, and biological markers and calcification score at baseline and progression of vascular calcification was investigated. We hypothesized that vascular calci-

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fication progresses substantially in stable KTRs and that independent determinants of CAC and aorta calcification progression include both classic and nonclassic CV risk factors.

METHODS

Patients

The prevalent Brussels Renal Transplant Cohort was initiated from February 3, 2004, to January 27, 2005. All KTRs with a functional transplant for 1 year or longer attending the outpatient clinic of the Cliniques universitaires Saint Luc (Brussels) for their annual or biannual in-depth control were asked to enter the study. The protocol was approved by the Ethics Committee of the Cliniques universitaires Saint Luc Medical School, and written informed consent was obtained from all patients. Exclusion criteria were age younger than 18 years, residing abroad, or being a recipient of a multiorgan transplant. Three hundred nineteen patients were contacted, 300 of whom entered the study.

Spiral CT

At inclusion, 281 patients underwent multislice spiral CT of the chest on a 16-slice scanner (Brillance 16; Philips Healthcare, www.healthcare.philips.com). The thoracic aorta and the 4 branches of the main coronary arteries were scored individually as previously described.[17] Agatston scores of CAC and thoracic aorta calcification were measured using a manufacturer algorithm (Heart Beat CS; Philips Healthcare) and expressed in milligrams. Intrareader variability was 3% and 8%, respectively, for CAC and aorta calcification.[17] The Agatston score was measured again 3.5 or more years later. All CT studies were performed at baseline and follow-up on the same machine using the same conditions of CT acquisition and the same scoring software.

Clinical and Biological Parameters

At baseline, demographic, clinical, and medical history parameters, including history of a CV event (defined as myocardial, cerebrovascular, or lower-limb necrosis or revascularization or documented transient ischemic attack[18]) were recorded by reviewing medical charts. Blood samples were obtained at inclusion to measure creatinine, cholesterol, triglycerides, glycemia (Synchron CX; Beckman Coulter, www.beckman.com), fibrinogen (Sysmex CA 7000; Siemens, www.medical.siemens.com), homocysteine (ARCHITECT; Abbott, www.abbott.com), 25-hydroxyvitamin D_3 ($25[OH]D_3$) and 1,25-dihydroxyvitamin D_3 ($1,25[OH]2D_3$]; LIAISON; DiaSorin, www.diasorin.com), and intact parathyroid hormone (Nichols, www.nicholsinstitute.com) in blood or serum. Serum analysis for high-sensitivity C-reactive protein was performed by immunonephelometry using a standard (Dade Behring Holding GmbH, www.dadebehring.com). Serum fetuin A was measured by nephelometry as previously described.[19] Proteinuria was measured on a 24-hour urine collection. Blood pressure was measured with an automatic validated device (Omron M5-I; www.omronhealthcare.com) after 10 minutes of rest according to Seventh Report of the Joint National Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC 7) recommendations.[20] Glomerular filtration rate was estimated by the 4-variable Modification of Diet in Renal Disease (MDRD) Study equation at the time of inclusion. Induction and maintenance immunosuppressive drugs and all drugs prescribed at inclusion were recorded.

Statistical Analysis

Results are presented as mean ± standard deviation, median (25th-75th percentile), or number (percentage), as appropriate. Variables presenting a right-skewed distribution were log-transformed. Univariate analysis was performed using *t* test, Wilcoxon sign-rank test, or χ^2 test, as applicable.

Multiple stepwise linear regression using annualized absolute rate of change as a continuous variable was performed to identify determinants of CAC or aorta calcification progression. We computed the annualized absolute rate of change in vascular calcification (CAC or aorta calcification) as the difference in Agatston score between the first and second scan divided by the time between scans. Annualized absolute rates of change were log-transformed.

All variables in univariate analysis reaching the $P < 0.2$ level entered multivariable models. To handle the major issue of competing risk for having a follow-up scan versus death, we performed sensitivity analyses with dead patients classified as having progression of CAC or aorta calcification either similar to or greater than that of the cohort. Similar CAC or aorta calcification progression was defined as 14% or 10% (median) progression, respectively, during 4.4 years. Greater CAC or aorta calcification progression was defined as 57% or 48% (third tertile) progression, respectively, during 4.4 years.

We compared nonprogressors and slow and fast progressors. For this analysis, we computed the annualized rate percentage as absolute rate divided by the initial score (CAC or aorta calcification). Analysis comparing nonprogressors and slow and fast progressors included only patients with a score of 0 or >30 mg because with low (but different from 0) baseline scores, there is substantial risk of overestimation of the percentage of change in score over time.[15,21] For patients with CAC or aorta calcification at baseline who scored 0 or >30 mg, progressors were divided into tertiles. Participants with a CAC or aorta calcification score of 0 mg and a follow-up score >4 mg were considered fast progressors.[14]

All statistical analyses were performed using SPSS, version 15.0, software (www-01.ibm.com/software/be/analytics/spss). All tests were 2-tailed and $P < 0.05$ was considered significant.

RESULTS

Study Cohort

Of the initial 281 patients in the cohort, 40 died before the appointment for the second CT; one had atrial fibrillation, thus precluding a successful scan; and 35 declined to undergo the second CT. Therefore, 205 patients underwent repeated CT after 4.40 ± 0.28 years. Calcium scoring could not be performed in 8 of these patients because of technical problems (n = 4) or arrhythmias (n = 4). Thus, the study cohort included 197 patients (Fig 1). Patients resuming dialysis therapy (n = 14) were not censored.

Characteristics of Patients

The 197 patients were 98% white, 57% men, and aged 52 ± 12 years and had a transplant for 93 ± 78 months (Table 1). A history of CV events was recorded in 48 patients (25%). Blood pressure was 134 ± 20/82 ± 12 mm Hg. Drugs at inclusion were azathioprine in 27% (n = 53), mycophenolate mofetil in 45% (n = 88), cyclosporin in 47% (n = 93), tacrolimus in 42% (n = 82), and sirolimus in 8% (n = 15). Causes of end-stage renal disease were chronic

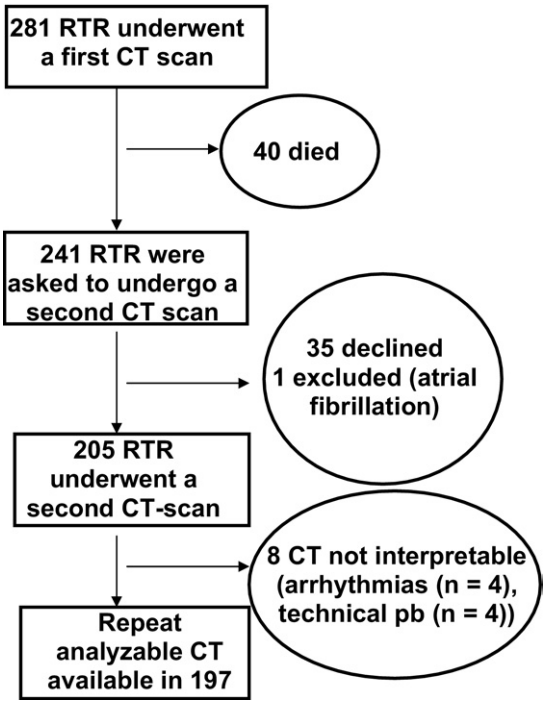

Figure 1. Flow chart of movement of patients through the study. Abbreviations: CT, computed tomographic; pb, problem; RTR, renal transplant recipient.

glomerulonephritis (n = 66; 34%), chronic interstitial nephropathy (n = 60; 30%), polycystic kidney disease (n = 38; 19%), nephrosclerosis (n = 9; 5%), diabetic nephropathy (n = 6; 3%), and others/unknown (n = 18; 9%).

We compared the 197 patients with a repeated analyzable CT scan with the 40 patients who died and the 40 without a repeated analyzable CT scan for another reason (5 with arrhythmias and 35 who declined). They differed for age, history of CV events, systolic blood pressure, pulse pressure, use of azathioprine and aspirin, time on dialysis therapy, and glucose, high-sensitivity C-reactive protein, homocysteine, $25(OH)D_3$, $1,25(OH)2D_3$, and parathyroid hormone levels (Table 1).

Vascular Calcification at Baseline and Follow-up

At baseline, mean CAC score was 616 ± 1,164 (standard deviation) mg and median score was 110 (25th-75th percentile, 1-582; Fig 2). CAC score was 0 mg in 24.4% (n = 48) of participants. At baseline, mean aorta calcification score was 2,384 ± 5,635 mg and median score was 222 (25th-75th percentile, 6-1,564) mg (Fig 2). Aorta calcification score was 0 mg in 18% (n = 36) of participants.

Mean follow-up CAC score was 957 ± 1,941 mg, and median score was 202 (25th-75th percentile, 8-936) mg (Fig 2). Mean absolute annualized progression of CAC was 79 ± 327 mg, and median was 11 (25th-75th percentile, 1-58) mg ($P < 0.001$; Fig 3). In participants with an initial CAC score of 0 mg, a total of 83.3% (n = 40) again had a score of 0 mg. Mean follow-up aorta calcification score was 2,582 ± 5,769 mg, and median score was 294 (25th-75th percentile, 9-1,750) mg (Fig 1). Mean absolute annualized aorta calcification progression was 54 ± 404 mg, and median score was 5 (25th-75th percentile, 0-62) mg ($P < 0.001$; Fig 3). In participants with an initial aorta calcification score of 0 mg, a total of 75% (n = 27) again had a score of 0 mg.

Absolute annualized CAC and aorta calcification progression were highly correlated ($P < 0.001$; Fig 4). A few individuals had decreases in CAC (n = 5) and/or aorta calcification (n = 13). In some, these changes were (very) small and consistent with the variability of the measure. In others, careful review of paired scans ascribed apparent regressions to artifacts. Overall, there was no solid evidence of regression of either CAC or aorta calcification in any patient.

Determinants of CAC Progression

In patients with an initial CAC score of 0 or >30 mg (n = 172), 55 met the definition of fast CAC progressors (Table 2). Age, sex, diabetes, and homocysteine, parathyroid hormone, and $25(OH)D_3$ levels (but not transplant function) were significantly different between nonprogressors and slow and fast CAC progressors.

By univariate regression analysis, older age, male sex, history of CV events, systolic blood pressure, pulse pressure, use of statin, time on dialysis therapy, $25(OH)D_3$ and parathyroid hormone levels, and CAC baseline score were associated with annualized absolute CAC progression (Table 3). In a multivariable regression model, CAC baseline score, history of CV events, use of statin, and $25(OH)D_3$ level were associated independently with annualized absolute CAC progression ($R^2 = 0.29$; $P < 0.001$; Table 3). Sensitivity analyses performed in 237 patients did not change the determinants of CAC progression. The relationship between low $25(OH)D_3$ level and CAC progression was even strengthened in the greater progression analysis (Tables S1 and S2, available as online supplementary material).

Determinants of Aorta Calcification Progression

In patients with an aorta calcification score of 0 or >30 mg (n = 166), 60 met the definition of fast aorta calcification progression (Table 4). Age, history of CV event, use of statin and cyclosporin, and phosphate and $25(OH)D_3$ levels (but not transplant function) were significantly different between nonprogressors and slow and fast aorta calcification progressors.

Table 1. Characteristics of the KTR Cohort Versus Patients Without a Second Analyzable CT

Variable	Patients With Second Analyzable CT (n = 197)	Patients Without Second Analyzable CT		P
		Died During Follow-up (n = 40)	Declined Scan or Had Arrhythmias (n = 40)	
Demographics and comorbid conditions				
Age (y)	52 ± 12	63 ± 10	50 ± 14	<0.001
Men	57	72	68	0.1
BMI (kg/m^2)	26 ± 5	27 ± 4	26 ± 5	0.6
DM	13	23	12	0.3
History of CV events	25	69	27	<0.001
History of smoking	50	54	61	0.4
Current smoking	12	10	27	0.05
History of parathyroidectomy	13	18	15	0.7
Physical examination				
Systolic BP (mm Hg)	134 ± 20	146 ± 25	134 ± 16	0.01
Diastolic BP (mm Hg)	82 ± 12	85 ± 17	82 ± 10	0.2
Pulse pressure (mm Hg)	53 ± 17	60 ± 16	53 ± 11	0.04
Drug therapy				
Statin	37	41	44	0.7
Tacrolimus	42	44	39	0.9
Cyclosporin	47	46	54	0.7
Azathioprine	17	46	46	0.008
Sirolimus	8	15	7	0.3
MMF	45	44	46	0.9
Aspirin	9	15	24	0.02
Calcium ± vitamin D	35	56	42	0.05
Kidney function and RRT				
eGFR (mL/min/1.73 m^2)	53 ± 20	47 ± 18	52 ± 21	0.3
Time on dialysis (y)	2 ± 2	3 ± 3	2 ± 3	0.01
Creatinine (mg/dL)	1.6 ± 0.8	1.7 ± 0.9	1.6 ± 0.7	0.6
DM/HTN as cause of ESRD	8	21	7	0.07
Living-donor Tx	14	13	15	0.9
Biological markers				
Glucose (mg/dL)	92 [85; 103]	103 [90; 121]	89 [83; 102]	0.03
hs-CRP (mg/L)	1.43 [0.57; 3.01]	3.35 [0.77; 7.19]	1.51 [0.46; 3.18]	0.02
Hemoglobin (g/dL)	13 ± 2	13 ± 2	13 ± 1	0.5
Homocysteine (μmol/L)	15 [13; 18]	17 [14; 23]	15 [13; 19]	0.02
Proteinuria (g/24 h)	0.12 [0.07; 0.25]	0.17 [0.08; 0.48]	0.15 [0.07; 0.27]	0.4
Total cholesterol (mg/dL)	200 [175; 226]	206 [185; 244]	192 [175; 216]	0.4
HDL cholesterol (mg/dL)	58 [47; 72]	59 [49; 73]	54 [47; 63]	0.3
25(OH)D_3 (ng/mL)	15 [11; 23]	10 [7; 16]	15 [11; 19]	0.01
1,25(OH)2D_3 (pg/mL)	33 [24; 46]	28 [16; 31]	28 [18; 38]	<0.001
Calcium (mg/dL)	10 ± 1	10 ± 1	10 ± 1	0.9
Phosphate (mg/dL)	3 ± 1	3 ± 1	3 ± 1	0.8
PTH (pg/mL)	40 [28; 58]	60 [43; 86]	42 [31; 66]	0.01
Fetuin A (g/L)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 1	0.8
Osteoprotegerin (pmol/L)	14 ± 9	17 ± 14	14 ± 8	0.2

Note: Continuous variables given as mean ± standard deviation or median [25th; 75th percentile]; categorical variables given as percentage. Conversion factors for units: serum creatinine in mg/dL to μmol/L, ×88.4; glucose in mg/dL to mmol/L, ×0.05551; hemoglobin in g/dL to g/L, ×10; total and HDL cholesterol in mg/dL to mmol/L, ×0.02586; 25(OH)D_3 in ng/mL to nmol/L, ×2.496; 1,25(OH)2D_3 in pg/mL to pmol/L, ×2.6; calcium in mg/dL to mmol/L, ×0.2495; phosphate in mg/dL to mmol/L, ×0.3229; magnesium in mEq/L to mmol/L, ×0.5; PTH in pg/mL to μmol/L, ×11.1; eGFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, ×0.01667.

Abbreviations: 25(OH)D_3, 25-hydroxyvitamin D_3; 1,25(OH)2D_3, 1,25-dihydroxyvitamin D_3; BMI, body mass index; BP, blood pressure; CT, computed tomography; CV, cardiovascular; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HTN, hypertension; KTR, kidney transplant recipient; MMF, mycophenolate mofetil; PTH, parathyroid hormone; RRT, renal replacement therapy; Tx, transplant.

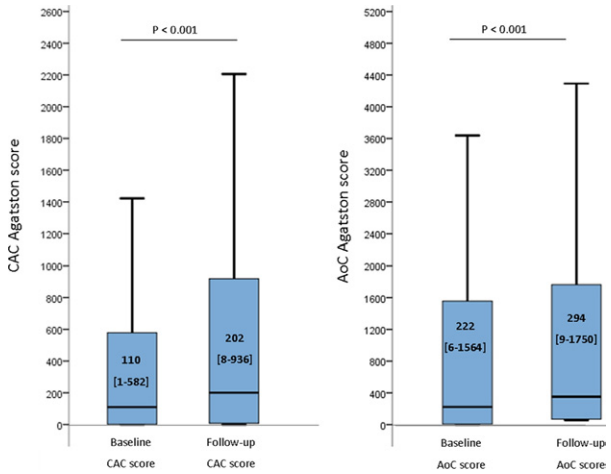

Figure 2. Progression of (A) coronary artery calcification (CAC) and (B) aorta calcification (AoC) Agatston score (milligrams) in prevalent renal transplant recipients. Results are presented as median [25th-75th percentile].

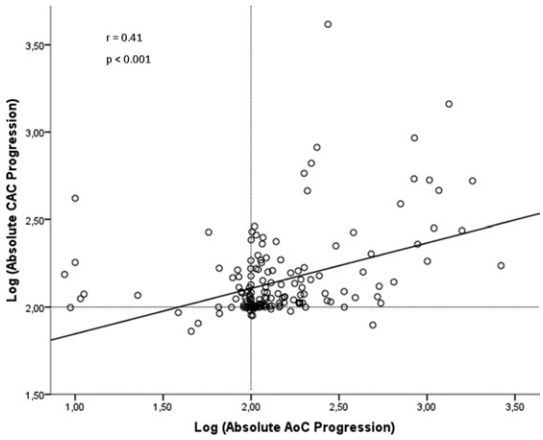

Figure 4. Correlation between the absolute coronary artery calcification (CAC) and aorta calcification (AoC) progression per year.

By univariate regression analysis, older age, male sex, history of CV event, diabetes, systolic blood pressure, pulse pressure, diabetes or hypertension as cause of end-stage renal disease, living donor transplant, use of statin and aspirin, fetuin A and phosphate levels, and baseline aorta calcification score were associated with annualized absolute aorta calcification progression (Table 5). In a multivariable regression model, aorta calcification baseline score, pulse pressure, use of a statin, age, phosphate level, use of aspirin, and sex were independently associated with annualized absolute aorta calcification progression ($R^2 = 0.42$; $P < 0.001$; Table 5). Sensitivity analyses performed in 237 patients showed that determinants of aorta calcification progression were largely unchanged in both the similar and greater progression analyses, with only phosphate level no longer reaching significance ($P = 0.06$; Tables S3 and S4).

DISCUSSION

To our knowledge, the present study is the first to assess the progression of both CAC and aorta calcification in a large population of stable KTRs with a relatively long follow-up. Our major finding is that vascular calcification progresses substantially within 4 years in prevalent KTRs: CAC increased by a median of 11% per year and aorta calcification increased by a median of 4% per year in KTRs with a baseline vascular score >30 mg.[21] In 25% of patients, the yearly increase in CAC and aorta calcification was ≥23% and ≥17%, respectively. Independent determinants of both CAC and aorta calcification progression include both classic and nonclassic CV risk factors, some of them modifiable.

Only 4 series previously assessed CAC progression in KTRs.[13-16] All these cohorts were much smaller and intervals between CT were shorter. Mazzaferro et al[13] compared 2-year CAC changes in 41 prevalent KTRs with a transplant for at least 6 months and in 30

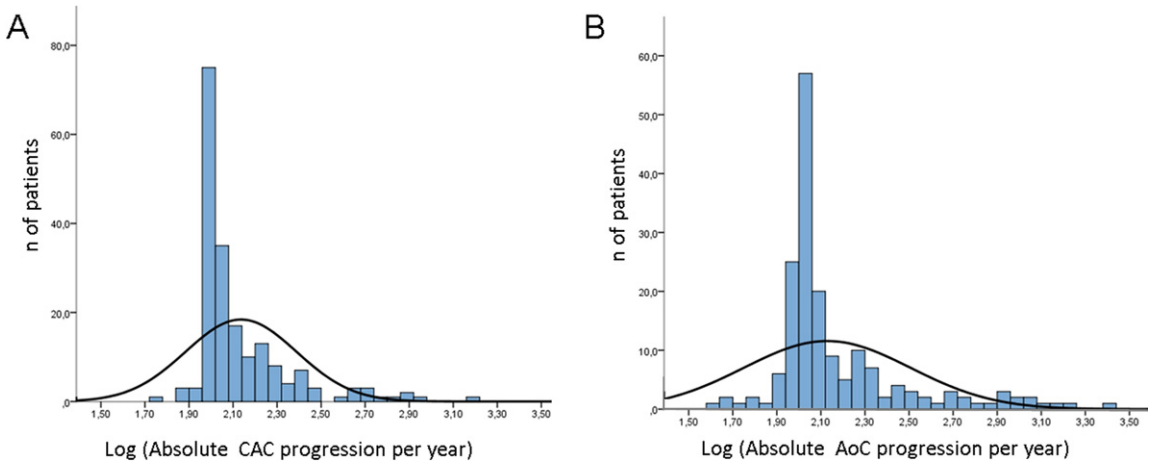

Figure 3. Distribution of annualized rate of change in (A) coronary artery calcification (CAC) and (B) aorta calcification (AoC).

Table 2. Comparison by CAC Tertile

Variable	Tertile 1 (n = 56)	Tertile 2 (n = 56)	Tertile 3 (n = 60)	*P*
Annualized relative rate of change in CAC (%)	0 [−1; 0.7]	7 [4; 12]	29 [21; 42]	
Demographics and comorbid conditions				
Age (y)	46 ± 14	54 ± 9	58 ± 10	<0.001
Men	45	65	66	0.02
BMI (kg/m^2)	27 ± 4	27 ± 5	26 ± 5	0.9
History of CV events	18	28	31	0.3
DM	3	23	18	0.01
History of smoking	45	49	58	0.4
Current smoking	12	11	9	0.9
History of parathyroidectomy	17	14	11	0.7
Physical examination				
Systolic BP (mm Hg)	131 ± 19	136 ± 16	137 ± 24	0.3
Diastolic BP (mm Hg)	81 ± 11	83 ± 11	79 ± 13	0.2
Pulse pressure (mm Hg)	50 ± 18	53 ± 11	58 ± 21	0.06
Drug therapy				
Statin	27	49	40	0.06
Tacrolimus	45	25	55	0.01
Cyclosporin	48	58	33	0.05
Azathioprine	23	44	18	0.01
Sirolimus	5	4	16	0.04
MMF	43	37	47	0.5
Aspirin	5	14	9	0.02
Calcium ± vitamin D	33	32	40	0.6
Kidney function and RRT characteristics				
eGFR (mL/min/1.73 m^2)	55 ± 21	55 ± 18	50 ± 20	0.3
Time on dialysis (y)	1.9 ± 1.6	2.5 ± 2.3	2.3 ± 2.7	0.3
Creatinine (mg/dL)	1.5 ± 0.6	1.5 ± 0.5	1.7 ± 0.9	0.07
DM/HTN as cause of ESRD	5	12	7	0.3
Living-donor Tx	20	11	11	0.3
Time since Tx (y)	6.8 ± 6.0	9.5 ± 7.2	5.3 ± 4.5	0.06
Biological markers				
Glucose (mg/dL)	93 ± 15	107 ± 44	99 ± 18	0.05
hs-CRP (mg/L)	3.0 ± 5.1	2.0 ± 2.0	3.5 ± 5.3	0.3
Hemoglobin (g/dL)	13 ± 2	13 ± 1	13 ± 2	0.9
Homocysteine (μmol/L)	15 ± 4	16 ± 3	17 ± 4	0.01
Proteinuria (g/24 h)	0.2 ± 0.4	0.2 ± 0.2	0.5 ± 1.7	0.2
Total cholesterol (mg/dL)	196 ± 44	203 ± 36	212 ± 53	0.2
HDL cholesterol (mg/dL)	60 ± 18	60 ± 19	62 ± 20	0.8
25(OH)D_3 (ng/mL)	21 ± 10	16 ± 8	16 ± 7	0.01
1,25(OH)2D_3 (pg/mL)	38 ± 19	35 ± 19	37 ± 17	0.6
Calcium (mg/dL)	9.5 ± 0.4	9.6 ± 0.6	9.4 ± 0.5	0.05
Phosphate (mg/dL)	3.0 ± 0.6	3.0 ± 0.6	3.3 ± 0.9	0.1
Magnesium (mEq/L)	1.5 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	0.06
PTH (pg/mL)	42 ± 31	49 ± 32	64 ± 52	0.01
Fetuin A (g/L)	0.59 ± 0.13	0.56 ± 0.12	0.58 ± 0.16	0.4
Osteoprotegerin (pmol/L)	14 ± 9	14 ± 8	15 ± 8	0.7

Note: N = 172 patients. Tertile 1, CAC progression <1% (nonprogressor); tertile 2, CAC progression of 1%-15% (slow progressor); and tertile 3, CAC progression >15% (fast progressor). Continuous variables given as mean ± standard deviation or median [25th; 75th percentile]; categorical variables given as percentage. Conversion factors for units: serum creatinine in mg/dL to μmol/L, ×88.4; glucose in mg/dL to mmol/L, ×0.05551; hemoglobin in g/dL to g/L, ×10; total and HDL cholesterol in mg/dL to mmol/L, ×0.02586; 25(OH)D_3 in ng/mL to nmol/L, ×2.496; 1,25(OH)2D_3 in pg/mL to pmol/L, ×2.6; calcium in mg/dL to mmol/L, ×0.2495; phosphate in mg/dL to mmol/L, ×0.3229; magnesium in mEq/L to mmol/L, ×0.5; PTH in pg/mL to μmol/L, ×11.1; eGFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, ×0.01667.

Abbreviations: 25(OH)D_3, 25-hydroxyvitamin D_3; 1,25(OH)2D_3, 1,25-dihydroxyvitamin D_3; BMI, body mass index; BP, blood pressure; CAC, coronary artery calcification; CV, cardiovascular; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HTN, hypertension; MMF, mycophenolate mofetil; PTH, parathyroid hormone; RRT, renal replacement therapy; Tx, transplant.

Table 3. Linear Regression Analysis of Risk Factors for Annualized Change in CAC Score

Variables	Univariate Analysis			Multivariable Analysis[a]		
	Univariate Coefficient ± SE	95% CI	*P*	Multivariable Coefficient ± SE	95% CI	*P*
Demographics and comorbid conditions						
Age (y)	0.005 ± 0.001	0.002 to 0.008	0.001			
Women	−0.11 ± 0.04	−0.18 to −0.04	0.003			
History of CV events	0.18 ± 0.04	0.10 to 0.26	<0.001	0.11 ± 0.04	0.03 to 0.18	0.005
History of smoking	0.05 ± 0.04	−0.02 to 0.13	0.1			
Physical examination						
Systolic BP (mm Hg)	0.002 ± 0.001	0.000 to 0.004	0.04			
Pulse pressure (mm Hg)	0.003 ± 0.001	0.001 to 0.0025	0.004			
CAC baseline	0.09 ± 0.01	0.07 to 0.12	<0.001	0.07 ± 0.01	0.04 to 0.10	<0.001
Use of statin	0.16 ± 0.04	0.09 to 0.23	<0.001	0.09 ± 0.03	0.02 to 0.16	0.01
Kidney function and RRT						
Time on dialysis (y)	0.02 ± 0.01	0.004 to 0.04	0.02			
Living-donor Tx	−0.08 ± 0.05	−0.18 to 0.03	0.2			
Biological markers						
Hcy (μmol/L)	0.26 ± 0.15	−0.033 to 0.55	0.08			
25(OH)D_3 (ng/mL)	−0.23 ± 0.08	−0.37 to -0.06	0.008	−0.15 ± 0.07	−0.28 to 0.00	0.05
PTH (pg/mL)	0.20 ± 0.07	0.06 to 0.33	0.004			
Fetuin A (g/L)	−0.21 ± 0.14	−0.48 to 0.06	0.1			

Note: N = 197 patients. Conversion factors for units: 25(OH)D_3 in ng/mL to nmol/L, ×2.496; PTH in pg/mL to μmol/L, ×11.1.

Abbreviations: 25(OH)D_3, 25-hydroxyvitamin D_3; BP, blood pressure; CAC, coronary artery calcification; CI, confidence interval; CV, cardiovascular; Hcy, homocysteine; PTH, parathyroid hormone; RRT, renal replacement therapy; SE, standard error; Tx, transplant.

[a] $R^2 = 0.29$, $P < 0.001$; factors with $P < 0.2$ in univariate analysis entered the multivariable stepwise linear regression.

dialyzed patients. Interestingly, scores increased in the dialysis group, whereas they were stable in KTRs. The relatively small size of this study and limited duration of follow-up very likely account for the discrepancy with our results.[13] The other 3 series were of incident KTRs. Schankel et al[14] performed electron beam CT in 82 patients at the time of transplant and at least one year later. They observed that CAC continues to progress after kidney transplant at a median yearly rate of 10.7% in recipients with baseline calcification; one-quarter of participants had an increase of 25% per year.[14] In a small study of 31 patients, Oschatz et al[15] measured CAC immediately after kidney transplant and at 6 and 12 months. They observed significant progression within the first 6 months, but no significant change between the months 6 and 12,[15] whereas by design, our study did not assess CAC and aorta calcification progression in the first year after transplant. In contrast to Oschatz et al,[15] Moe et al[16] did not observe CAC progression in a cohort of 23 patients who underwent CT at the time of kidney transplant and 15 to 20 months later. Regarding aorta calcification progression, data for KTRs are limited to the 23 patients studied by Moe et al.[16] Again, no significant progression of aorta calcification was detected, in contrast to our study, probably as a result of a small cohort and short follow-up.

Interestingly, we observe for what we believe is the first time in KTRs that there is a positive correlation between absolute annualized progression of CAC and aorta calcification. A few patients showed an apparent decrease in either CAC or aorta calcification. However, careful review of the few paired scans showing a decrease exceeding the variability of the measure did not confirm true regression, but rather artifacts or inaccurate or at least slightly different technical aspects accounting for this apparent regression(s). Similar findings recently have been reported in a large cohort of 197 patients with diabetes for whom CAC and abdominal aortic scores were measured twice about 4 years apart.[22] Of note, exclusion of these paired scans with apparent regression does not change the independent determinants (discussed next) of CAC progression (data not shown) and very few of those of aorta calcification progression (Table 5 vs Table S5).

Our second aim was to investigate determinants of vascular calcification progression in KTRs. For CAC, these include higher baseline CAC score, history of CV events, use of a statin, and a lower 25(OH)D_3 level. Restricting the analysis to patients with a base-

Table 4. Comparison by Aorta Calcification Progression Tertile

Variable	Tertile 1 (n = 53)	Tertile 2 (n = 53)	Tertile 3 (n = 60)	*P*
Annualized relative rate of change in aorta calcification (%)	−1 [−4; 0]	2 [0; 4]	21 [14; 50]	
Demographics and comorbid conditions				
Age (y)	46 ± 14	58 ± 9	54 ± 10	<0.001
Men	55	62	57	0.7
BMI (kg/m^2)	26 ± 4	27 ± 5	27 ± 5	0.8
History of CV events	22	42	17	0.01
History of smoking	44	68	42	0.05
Current smoking	9	14	10	0.7
DM	7	18	13	0.3
History of parathyroidectomy	16	12	13	0.8
Physical examination				
Systolic BP (mm Hg)	133 ± 17	135 ± 19	136 ± 21	0.7
Diastolic BP (mm Hg)	81 ± 11	79 ± 11	83 ± 12	0.2
Pulse pressure (mm Hg)	52 ± 19	57 ± 18	53 ± 15	0.4
Drug therapy				
Statin	24	46	50	0.01
Tacrolimus	53	46	30	0.05
Cyclosporin	33	44	62	0.01
Azathioprine	26	26	28	0.9
Sirolimus	11	6	3	0.3
MMF	42	42	45	0.9
Aspirin	6	16	8	0.2
Calcium ± vitamin D	27	38	43	0.2
Kidney function and RRT				
eGFR (mL/min/1.73 m^2)	55 ± 21	51 ± 20	51 ± 21	0.5
Time on dialysis (y)	2.0 ± 1.9	2.7 ± 2.8	2.0 ± 2.0	0.2
Creatinine (mg/dL)	1.5 ± 0.6	1.5 ± 0.5	1.7 ± 1.0	0.4
DM/HTN as cause of ESRD	9	8	7	0.9
Living-donor Tx	18	4	13	0.08
Time since Tx (y)	6.9 ± 6.8	7.6 ± 6.6	7.6 ± 5.8	0.8
Biological markers				
Glucose (mg/dL)	95 ± 32	99 ± 22	99 ± 17	0.2
hs-CRP (mg/L)	4.2 ± 7.8	2.9 ± 5.3	2.8 ± 2.5	0.6
Hemoglobin (g/dL)	13 ± 2	14 ± 2	13 ± 1	0.1
Homocysteine (μmol/L)	15 ± 4	16 ± 4	16 ± 4	0.1
Proteinuria (g/24h)	0.2 ± 0.3	0.2 ± 0.2	0.6 ± 1.6	0.2
Total cholesterol (mg/dL)	196 ± 34	206 ± 46	210 ± 47	0.3
HDL cholesterol (mg/dL)	59 ± 16	60 ± 19	64 ± 22	0.4
25(OH)D_3 (ng/mL)	19 ± 10	16 ± 8	15 ± 6	0.01
1,25(OH)2D_3 (pg/mL)	38 ± 18	34 ± 16	35 ± 21	0.6
Calcium (mg/dL)	9.5 ± 0.5	9.5 ± 0.5	9.5 ± 0.7	0.9
Phosphate (mg/dL)	3.0 ± 0.7	3.1 ± 0.6	3.3 ± 0.9	0.04
Magnesium (mEq/L)	1.5 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	0.2
PTH (pg/mL)	51 ± 50	57 ± 42	60 ± 49	0.6
Fetuin A (g/L)	0.59 ± 0.11	0.57 ± 0.15	0.58 ± 0.13	0.8
Osteoprotegerin (pmol/L)	14 ± 11	16 ± 9	13 ± 7	0.2

Note: N = 166 patients. Tertile 1, aorta calcification progression <0% (nonprogressor); tertile 2, aorta calcification progression of 0%-8% (slow progressor); and tertile 3, aorta calcification progression >8% (fast progressor). Continuous variables given as mean ± standard deviation or median [25th; 75th percentile]; categorical variables given as percentage. Conversion factors for units: serum creatinine in mg/dL to μmol/L, ×88.4; glucose in mg/dL to mmol/L, ×0.05551; hemoglobin in g/dL to g/L, ×10; total and HDL cholesterol in mg/dL to mmol/L, ×0.02586; 25(OH)D_3 in ng/mL to nmol/L, ×2.496; 1,25(OH)2D_3 in pg/mL to pmol/L, ×2.6; calcium in mg/dL to mmol/L, ×0.2495; phosphate in mg/dL to mmol/L, ×0.3229; magnesium in mEq/L to mmol/L, ×0.5; PTH in pg/mL to μmol/L, ×11.1; eGFR in mL/min/1.73 m^2 to mL/s/1.73 m^2, ×0.01667.

Abbreviations: 25(OH)D_3, 25-hydroxyvitamin D_3; 1,25(OH)2D_3, 1,25-dihydroxyvitamin D_3; BMI, body mass index; BP, blood pressure; CV, cardiovascular; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HTN, hypertension; MMF, mycophenolate mofetil; PTH, parathyroid hormone; RRT, renal replacement therapy; Tx, transplant.

Table 5. Linear Regression Analysis of Risk Factors for Annualized Change in Aorta Calcification Score

	Univariate Analysis			Multivariable Analysis[a]		
Variables	Univariate Coefficient ± SE	95% CI	*P*	Multivariable Coefficient ± SE	95% CI	*P*
Demographics and comorbid conditions						
Age (y)	0.013 ± 0.002	0.01 to 0.02	<0.001	0.006 ± 0.002	0.001 to 0.01	0.01
Women	−0.12 ± 0.05	−0.22 to −0.03	0.01	−0.09 ± 0.04	−0.17 to −0.01	0.03
History of CV events	0.21 ± 0.06	0.11 to 0.34	<0.001			
DM	0.15 ± 0.07	0.01 to 0.30	0.04			
Aorta calcification baseline	0.12 ± 0.02	0.09 to 0.16	<0.001	0.06 ± 0.02	0.02 to 0.10	0.008
Physical examination						
Systolic BP (mm Hg)	0.004 ± 0.001	0.002 to 0.01	<0.001			
Pulse pressure (mm Hg)	0.007 ± 0.001	0.004 to 0.01	<0.001	0.003 ± 0.001	0.001 to 0.01	0.01
Drug therapy						
Statin	0.22 ± 0.05	0.13 to 0.32	0.01	0.11 ± 0.04	0.02 to 0.20	0.01
Tacrolimus	−0.08 ± 0.05	−0.18 to 0.02	0.0			
Cyclosporin	0.07 ± 0.05	−0.02 to 0.17	0.1			
Aspirin	0.27 ± 0.09	0.10 to 0.44	0.002	0.16 ± 0.07	0.02 to 0.31	0.03
Kidney function and RRT						
Time on dialysis (y)	0.02 ± 0.01	−0.002 to 0.04	0.07			
DM/HTN as cause of ESRD	0.24 ± 0.09	0.08 to 0.52	0.01			
Living-donor Tx	−0.24 ± 0.07	−0.36 to −0.09	0.001			
Biological markers						
Glucose (mg/dL)	0.48 ± 0.28	−0.08 to 1.10	0.09			
25(OH)D_3 (ng/mL)	−0.006 ± 0.003	−0.09 to 0.001	0.09			
Phosphate (mg/dL)	0.07 ± 0.03	0.01 to 0.13	0.03	0.47 ± 0.20	0.08 to 0.86	0.02
Fetuin A (g/L)	−0.41 ± 0.18	−0.76 to −0.05	0.03			
Osteoprotegerin (pmol/L)	0.005 ± 0.003	0.000 to 0.01	0.06			

Note: N = 197 patients. Conversion factors for units: glucose in mg/dL to mmol/L, ×0.05551; 25(OH)D_3 in ng/mL to nmol/L, ×2.496; phosphate in mg/dL to mmol/L, ×0.3229.

Abbreviations: 25(OH)D_3, 25-hydroxyvitamin D_3; BP, blood pressure; CI, confidence interval; CV, cardiovascular; DM, diabetes mellitus; ESRD, end-stage renal disease; HTN, hypertension; RRT, renal replacement therapy; SE, standard error; Tx, transplant.

[a] $R^2 = 0.42$, $P < 0.001$; factors with $P < 0.2$ in univariate analysis entered the multivariable stepwise linear regression.

line score >0 mg (n = 149) does not change results (data not shown). That higher baseline CAC score is associated with CAC progression confirms previous studies of both KTRs[14] and hemodialysis patients.[13] Studies of dialysis patients and KTRs have shown the strong association of history of CV event with CAC, the latter most likely related to atherosclerosis,[19,23-25] a hypothesis supported by our results of a history of CV events predicting CAC progression. Statin treatment at inclusion also predicts CAC progression in our cohort. This very likely reflects confounding by indication. This possibility is supported because when the analysis is restricted to patients without a history of CV events (n = 148), the relationship of CAC/aorta calcification progression with statin use is no longer significant. Moreover, whereas early observational studies of the general population and patients with diabetes showed an association between statin use and slower CAC progression,[26] more recent randomized trials showed that statins do not change the progression of CAC.[27] The fourth independent determinant of CAC progression was low 25(OH)D_3 level. In addition to its role in mineral and bone metabolism, vitamin D has many other pleiotropic effects. Circulating concentrations of 25(OH)D_3 are a sensitive measure of vitamin D status. Previous studies observed a negative correlation between vitamin D level and CAC onset in hemodialysis patients,[28] the prevalence of CAC in KTRs,[17] and pulse wave velocity in hemodialysis patients.[29] Moreover, in 52 stable hemodialysis patients, London et al[29] showed a positive correlation of 25(OH)D_3 level with brachial artery distensibility and flow-mediated dilation. These results support an association between vitamin D deficiency and arteriosclerosis, as well as endothelial dysfunction. Interestingly, vitamin D treatment of osteoblastic cells inhibits calcification by decreasing type 1 collagen production, which has an important

role in calcium deposition.[30] Observational studies already showed that vitamin D deficiency is associated with increased risks of CV disease and death in the general population,[31,32] with heart failure in patients referred for coronary angiography,[33] and with mortality in hemodialysis patients and those with CKD.[34,35] Moreover, low 25(OH)D_3 levels are associated with traditional atherosclerosis risk factors.[36-39] These studies suggest a potential role for vitamin D therapy in these populations. Randomized controlled trials thus are required to test the potential benefits of vitamin D treatment on those outcomes. In incident KTRs, diastolic blood pressure, glomerular filtration rate, body mass index,[14] and smoking[15] are determinants of CAC progression. In our cohort of prevalent KTRs, these parameters were not independent determinants of CAC progression.

Independent determinants of aorta calcification progression are higher baseline aorta calcification score, higher pulse pressure, use of a statin, older age, higher phosphate level, use of aspirin, and male sex. Restricting the analysis to patients with a baseline score >0 mg (n = 161) did not change results (data not shown). Older age and male sex are classic CV risk factors.[18,23,24,40] As for CAC progression, use of a statin and baseline score were associated with aorta calcification progression. We also show an association of aorta calcification progression with higher pulse pressure independently of baseline aorta calcification. This association is of interest because it suggests that a stiffer aorta could be a risk factor for the progression of aortic calcification independently of the initial extent of the latter. High pulse pressure (a surrogate for arterial stiffness) is a well-established cardiovascular risk factor[41] and both a cause and consequence of atherosclerosis. The endothelium regulates vascular tone and cardiovascular homeostasis. Endothelial dysfunction decreases nitric oxide (NO) bioavailability and is associated with the onset and progression of atherosclerosis[42-43] and with arterial calcification, resulting in arterial stiffness.[44-46] This link is supported further by an in vivo study showing that exercise prevents the decrease in endothelial NO synthase expression and NO production and decreases arterial calcification in ovariectomized rats.[47] Furthermore, other studies have reported an association between high pulse pressure and endothelial dysfunction.[48-50] It also has been observed that NO may regulate arterial distensibility and thus possibly pulse pressure.[51] However, the elevation in pulse pressure also may exert negative feedback on the endothelium.[52] Overall, these results are in line with our findings and suggest that high pulse pressure may be a risk factor for aorta calcification progression. Use of aspirin at inclusion predicts aorta calcification progression in our study. As for the statins, this observation probably is related to confounding by indication. We also identified higher phosphate level as a predictor of aorta calcification progression independently of eGFR. Studies of healthy adults[53] and patients with CKD[54] already observed an association of higher phosphatemia with atherosclerosis and vascular calcification. Russo et al[55] established in a cohort of predialysis patients that higher phosphorus levels were associated with CAC progression. Another study of patients with CKD observed an association between higher phosphorus levels and mortality.[56] Again, hypotheses generated by observational studies require testing in randomized trials in this case to assess the impact of phosphate binders on survival or aorta calcification progression.[57]

Strengths of our study, the first to examine the progression of both CAC and aorta calcification in a large population of stable KTRs, include sample size, 4-year follow-up, and use of identical multislice spiral CT at baseline and follow-up. Some limitations should be acknowledged. First, the study population is typical of KTRs followed up in European centers, mostly white and with a lower prevalence of diabetes than in the United States. Second, all parameters (except calcium scoring) were obtained once at inclusion and therefore we could not perform time-dependent covariate analysis. Third, there certainly is selection bias. Repeated CT was available for only 205 of 281 patients and scans were analyzable in 197 of 281 (70%). Because patients without repeated scoring overall had a worse CV risk profile at baseline (Table 1), our results probably underestimate the progression of vascular calcification in KTRs. However, sensitivity analyses showed that our results are robust. Finally, the inclusion of prevalent rather than incident KTRs may have introduced survival bias.

In conclusion, the present study shows that vascular calcification progresses substantially in stable KTRs. Independent determinants of both CAC and aorta calcification progression include both classic and nonclassic CV risk factors, some of them modifiable.

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SUPPLEMENTARY MATERIAL

Table S1: Sensitivity analysis assuming patients who died had 14% CAC progression.

Table S2: Sensitivity analysis assuming patients who died had 57% CAC progression.

Table S3: Sensitivity analysis assuming patients who died had 10% aorta calcification progression.

Table S4: Sensitivity analysis assuming patients who died had 48% aorta calcification progression.

Table S5: Independent determinants of aorta calcification progression in KTRs by multivariable linear regression.

Figure S1: Axial CT image at time of inclusion and at follow-up at the level of the left main coronary artery; sagittal CT image using a maximal intensity projection at time of inclusion and at follow-up at the level of the aorta.

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