Progression of coronary artery calcification and thoracic aorta calcification in kidney transplant recipients

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Abstract: 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.

DOI: https://doi.org/10.1053/j.ajkd.2011.07.019

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

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Running Title: Vascular calcification progression in RTR

Subject of manuscript: Kidney transplantation

Abstract word count: 299

Body word count: 3545

ABSTRACT

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

Study Design: Longitudinal.

Setting and participants: The Agatston score of coronary arteries and thoracic aorta was measured by 16-slice spiral computerized tomography (CT) in 281 RTR.

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

Outcomes and measurements: The Agatston score was again measured ≥ 3.5 years later.

Results: A repeat analyzable CT was available in 197 RTR (70 %) after 4.40 (±0.28) years; it was not in the remaining patients due to 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 AoC scores increased significantly (by a median of 11% and 4% per year, respectively) over follow-up. By multivariable linear regression, a higher baseline CAC score, a history of cardiovascular event, the use of a statin and a lower 25-(OH) vitamin D3 level were independent determinants of CAC progression. Independent determinants of AoC progression were higher baseline AoC score, higher

pulse pressure, use of a statin, older age, higher serum level of phosphate, use of aspirin and male gender. Significant regression of CAC/AoC was not observed in this cohort.

Limitations: cohort of prevalent RTR with potential survival bias; few diabetics and non Caucasians, limiting the generalizability of the results

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

Keywords : renal transplantation, vascular calcification, clinical determinants, cardiovascular disease

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of premature death in RTR, with a 3.5 to 5% annual risk of fatal or non-fatal CV events (CVE), much higher than in the general population despite adjustment for traditional risk factors (1, 2). A high prevalence of vascular calcification has been demonstrated in patients with chronic kidney disease (CKD), with higher calcification scores than in age- and gender-matched non-renal patients with coronary heart 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 advanced CKD and cannot be distinguished by imaging techniques, including computed tomography (CT) (6). Still, vascular calcification strongly predicts CVD and all-cause mortality not only in hemodialysis and peritoneal dialysis patients (7-9) but also in RTR (10-12). Moreover, coronary artery calcification (CAC) progression also predicts CVE and mortality in RTR (12). Only few small-sized studies relying on CT have assessed CAC progression in RTR (13-16).

In the present study a large prevalent RTR cohort underwent CT at inclusion and ~4 years later to assess the evolution of coronary artery and thoracic aorta calcification (AoC). The relationship between clinical, demographic, biological markers and calcification score at baseline and progression of vascular calcification was investigated. We hypothesized that vascular calcification progresses substantially in stable renal transplant recipients and that independent determinants of both CAC and AoC progression include both classical and non-classical cardiovascular (CV) risk factors.

PATIENTS AND METHODS

Patients

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

Spiral CT scan

At inclusion 281 patients underwent chest multi-slice spiral CT on a 16-slice scanner (Brillance 16, Philips Healthcare, www.healthcare.philips.com). The thoracic aorta and the four branches of the main coronary arteries were individually scored as previously described (17). Agatston scores of coronary arteries (CAC) and thoracic aorta (AoC) were measured using a manufacturer algorithm (Heart Beat CS, Philips Healthcare, www.healthcare.philips.com) and expressed in mg. Intra-reader variability was 3 % and 8 % respectively for CAC and AoC (17). The Agatston score was again measured ≥ 3.5 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 cardiovascular event (CVE) (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 in order to measure in blood or serum creatinine, cholesterol, triglyceride, glycemia (Synchron CX®, Beckman Coulter, www.beckman.com), fibrinogen (Sysmex® CA 7000, Siemens, www.medical.siemens.com), homocysteine (ARCHITECT®, Abbott, www.abbott.com), 25 (OH) and 1,25-$(OH)_2$ vitamin D3 (LIAISON®, DiaSorin, www.diasorin.com), iPTH (Nichols, www.nicholsinstitute.com). Serum analysis for high-sensitivity CRP (hsCRP) was performed by immunonephelometry using a standard (Dade Behring Holding GmbH, www.dadebehring.com). Serum fetuin-A level was measured by nephelometry as previously described (19). Proteinuria was measured on a 24h urine collection. Blood pressure was measured with an automatic validated device (Omron® M5-I, www.omronhealthcare.com) after 10 min rest according to the JNC VII recommendations (20). Glomerular filtration rate was estimated by the abbreviated MDRD formula at time of inclusion. Induction and maintenance immunosuppressive drugs and all drugs prescribed at inclusion were recorded.

Statistical analysis

Results are presented as means ± SD, median [P25-P75] or n (and %) as appropriate. Variables presenting a right skewed distribution were log-transformed.

Univariate analysis was performed using the t-test, Wilcoxon sign-rank test or Chi-square 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 AoC progression. We computed the annualized absolute rate of change of vascular calcification (CAC or AoC) as the difference of Agatston score between the first and second scan divided by the time between scans. The annualized absolute rates of change were log-transformed.

All variables reaching in univariate analysis the $p < 0.2$ level entered the 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 AoC either similar or greater than the cohort. Similar CAC or AoC progression was defined as 14% or 10% (median) progression respectively, over 4.4 years. Greater CAC or AoC progression was defined as 57% or 48% (third tertile) progression respectively, over 4.4 years.

We compared non progressors, slow and high progressors. For this analysis we computed the percentage annualized rate as the absolute rate divided by the initial score (CAC or AoC). The analysis comparing non progressors, slow and high progressors included only patients with a score of 0 or higher than 30 because with low (but different from 0) baseline scores, there is a substantial risk of overestimation of the percentage change of score over time (15,21). For patients with CAC or AoC at baseline scored 0 or higher than 30, progressors were divided in three groups (tertiles). Subjects with a CAC or AoC score of 0 and a follow-up score > 4 were considered as high progressors (14).

All statistical analyses were performed using SPSS 15.0 software. All tests were two-tailed and a p-value < 0.05 was considered as significant.

RESULTS

Out of the initial 281 patients' cohort, forty patients died before the appointment for the second CT and one had atrial fibrillation precluding a successful scan. Thirty-five declined to undergo a second CT. Two hundred and five patients thus had a repeat CT after 4.40 (± 0.28) years. Calcium scoring could not be performed in 8 patients of them, due to technical problems (n=4) or arrhythmias (n=4). Thus the study cohort included 197 patients (Figure 1). Patients resuming dialysis (n=14) were not censored.

1. Characteristics of the patients

The 197 patients were 98 % Caucasian, 57 % male, aged 52 ± 12 years, transplanted for 93 ± 78 months (Table 1). A history of CVE was recorded in 48 patients (25 %). Blood pressure was 134 ± 20 / 82 ± 12 mmHg. Drugs at inclusion were azathioprine in 27 % (n = 53), mycophenolate mofetil (MMF) in 45 % (n = 88), cyclosporin in 47 % (n = 93), tacrolimus in 42 % (n = 82) and sirolimus in 8 % (n = 15). The cause of end-stage renal disease was chronic 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 repeat analyzable CT with the 40 patients who died and the 40 without repeat analyzable CT for another reason (5 with arrhythmias and 35 who declined). They differed for age, history of CVE, systolic blood pressure,

pulse pressure, use of azathioprine and aspirin, time on dialysis, glucose, hsCRP, homocysteine, 25-(OH) vitamin D_3, 1,25-(OH) vitamin and PTH levels (Table 1).

2. Vascular calcification at baseline and follow-up

At baseline CAC score was: mean 616 ± 1164 mg, median 110 [1 – 582] (Figure 2). CAC score was equal to 0 in 24.4 % (n = 48) of the subjects. At baseline AoC score was: mean 2384 ± 5635 mg, median 222 [6 – 1564] (Figure 2). AoC score was equal to 0 in 18 % (n = 36) of the subjects.

The follow-up CAC score was: mean 957 ± 1941, median 202 [8 – 936] (Figure 2). The absolute annualized progression of CAC was: mean 79 (± 327), median 11 [1-58] ($p < 0.001$) (Figure 3). Among subjects with an initial CAC score equal to 0, 83.3 % (n = 40) again had a score of 0. The follow-up AoC score was: mean 2582 ± 5769, median 294 [9 – 1750] (Figure 1). The absolute annualized AoC progression was: mean 54 (± 404), median 5 [0-62] ($p < 0.001$) (Figure 3). Among subjects with an initial AoC score equal to 0, 75 % (n = 27) again had a score of 0.

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

3. Determinants of CAC progression

Among patients with an initial CAC score of 0 or higher than 30 (n = 172), 55 met the definition of high CAC progressors (Table 2). Age, gender, diabetes and homocysteine, parathyroid hormone and 25-(OH) vitamin D3 levels (but not graft function) were significantly different between non progressors, slow and high CAC progressors.

By univariate regression analysis, older age, male gender, history of CVE, systolic blood pressure, pulse pressure, use of statin, time on dialysis, 25-(OH) vitamin D3 and PTH levels, and CAC baseline score were associated with annualized absolute CAC progression (Table 3). In a multivariable regression model, CAC baseline score, history of CVE, use of statin and level of 25-(OH) vitamin D3 were independently associated with the 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 a low level of 25(OH)vitD3 and CAC progression was even strengthened in the greater progression analysis (Supplementary tables 1 and 2).

4. Determinants of AoC progression

Among patients with an AoC score of 0 or higher than 30 (n = 166), 60 met the definition of high AoC progression (Table 4). Age, history of CVE, use of statin and cyclosporine, and levels of phosphate and 25-(OH) vitamin D3 (but not graft function) were significantly different between non progressors, slow and high AoC progressors.

By univariate regression analysis, older age, male gender, history of CVE, diabetes, systolic blood pressure, pulse pressure, diabetes or hypertension as cause of ESRD, living donor TP, use of statin and aspirin, fetuin-A and phosphate levels as well as AoC baseline score were associated with annualized absolute AoC progression (Table 5). In a multivariable regression model AoC baseline score, pulse pressure, use of statin, age, phosphate level, use of aspirin and gender were independently associated with the annualized absolute AoC progression ($R^2 = 0.42$, $p<0.001$) (Table 5). Sensitivity analyses performed in 237 patients showed that determinants of AoC progression were largely unchanged in both the similar and greater progression analyses, with only phosphate level no longer reaching significance ($p = 0.06$) (Supplementary tables 3 and 4).

DISCUSSION

To the best of our knowledge, the current study is the first one to assess the progression of both CAC and AoC in a large population of stable RTR with a relatively long follow-up time. Our major finding is that vascular calcification progresses substantially within 4 years in prevalent RTR: CAC increased by a median of 11 % per year and AoC increased by a median of 4 % per year in RTR with a baseline vascular score higher than 30 (21). In 25 percent of patients the yearly increase of CAC and AoC

reached or exceeded 23 % and 17 % respectively. The independent determinants of both CAC and Aoc progression include both classical and non-classical CV risk factors, some of them modifiable.

Only four series previously assessed the progression of CAC in RTR (13-16). All these cohorts were much smaller and intervals between CT scans were shorter. Mazzaferro et al. compared the 2-year CAC changes in 41 prevalent RTR transplanted (since at least 6 months) and in 30 dialyzed patients. Interestingly, the score increased in the dialysis group whereas it remained stable in RTR. The relatively small size of this study and limited duration of follow-up very likely accounts for the discrepancy with our results (13). The other 3 series were in incident RTR. Schankel et al. performed an EBCT in 82 patients at time of transplantation and at least one year later. They observed that CAC continues to progress after renal transplantation at a median yearly rate of 10.7 % in recipients with baseline calcification; one quarter of subjects had an increase of 25 % per year (14). In a small study performed in 31 patients, Oschatz et al. measured CAC immediately after renal transplantation and at 6 and 12 months. They observed a significant progression within the first 6 months but no significant change between the 6^{th} and 12^{th} months (15) whereas by design, our study did not assess CAC and AoC progression in the first year after transplantation. In contrast to Oschatz et al. (15), Moe et al. did not observe any CAC progression in a cohort of 23 patients who underwent a CT scan at the time of renal transplantation and 15 to 20 months later (16). Regarding AoC progression, data in RTR patients are limited to the 23 patients studied by Moe et al. Again, no significant progression of AoC was detected, in contrast to our study, probably as a result of a small cohort and short follow-up time.

Interestingly, we observe for the first time in RTR a positive correlation between the absolute annualized progression of CAC and AoC. A few patients showed an apparent decrease of either CAC or AoC. 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 have recently been reported in a large cohort of 197 diabetics whose CAC and Abdominal aortic score were measured twice some 4 years apart (22). Of note, the exclusion of these paired scans with apparent regression does not change the independent determinants (see below) of CAC progression (data not shown) and very little those of Aoc progression (Table 5 versus Supplementary table 5).

Our second aim was to investigate the determinants of vascular calcification progression in RTR. For CAC, these include a higher baseline CAC score, a history of CVE, the use of a statin and a lower 25-(OH) vitamin D3 level. Restricting the analysis to patients with a baseline score > 0 (n = 149) does not change the results (data not shown). That a higher baseline CAC score is associated with CAC progression confirms previous studies both in RTR (14) and in hemodialysis patients (13). Studies in dialysis patients as well as in renal transplant recipients have demonstrated the strong association of CVE history with CAC, the latter most likely related to atherosclerosis (23, 24, 25, 19), a hypothesis supported by our results of CVE history 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 by the fact that when the analysis is restricted to patients without CVE history (n = 148), the relationship of

CAC/AoC progression with statin use is no longer significant. Moreover, whereas early observational studies performed in the general population and in diabetics demonstrated an association between the use of statin and slower CAC progression (26), more recent randomized trials demonstrated that statins do not change the progression of CAC (27). The fourth independent determinant of CAC progression was a low 25-(OH) vitamin D3 level. In addition to its role in mineral and bone metabolism, vitamin D has many other pleiotropic effects. Circulating concentrations of 25-(OH) vitamin D3 are a sensitive measure of vitamin D status. Previous studies observed a negative correlation between vitamin D levels and the onset of CAC in HD patients (28), the prevalence of CAC in RTR (17), as well as the pulse wave velocity in HD patients (29). Moreover, in 52 stable hemodialysis patients, London et al. showed a positive correlation of 25-(OH) vitamin D3 levels with brachial artery distensibility and flow-mediated dilation (29). 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 plays an important role in calcium deposition (30). Observational studies already showed that vitamin D deficiency is associated with increased risks for cardiovascular disease and death in the general population (31, 32), with heart failure in patients referred for coronary angiography (33) and with mortality in hemodialysis and CKD patients (34, 35). Moreover low 25-(OH) vitamin D3 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 are thus required to test the potential benefits of vitamin D treatment on those outcomes. In incident RTR, diastolic blood pressure, GFR, BMI (14)

as well as smoking (15) were determinants of CAC progression. In our cohort of prevalent RTR, these parameters were not independent determinants of CAC progression.

The independent determinants of AoC progression are a higher baseline AoC score, a higher pulse pressure, the use of a statin, older age, a higher level of phosphate, the use of aspirin and male gender. Restricting the analysis to patients with a baseline score > 0 (n = 161) did not change the results (data not shown). Older age and male gender are classical CV risk factors (23, 24, 18, 40). Like for CAC progression, use of statin and baseline score were associated with AoC progression. We also show an association of AoC progression with a higher pulse pressure, independently of baseline AoC. This association is of interest, as 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. A high pulse pressure (a surrogate for arterial stiffness) is indeed a well established cardiovascular risk factor (41) and both a cause and a consequence of atherosclerosis. The endothelium regulates the vascular tone and cardiovascular homeostasis. Endothelial dysfunction reduces 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 further supported by an *in vivo* study showing that exercise prevented the decrease in eNOS expression and NO production and reduced arterial calcification in ovariectomized rats (47). Furthermore, other studies have reported an association between high pulse pressure and endothelial dysfunction (48-50). It was also observed that NO may regulate arterial distensibility, and thus possibly pulse pressure (51). On the other hand, the elevation of pulse pressure may also exert a negative feedback on the endothelium (52). Overall, these results are in line with our findings and

suggest that a high pulse pressure may be a risk factor for AoC progression. The use of aspirin at inclusion predicts AoC progression in our study. Like for the statins, this observation is probably related to confounding by indication. We also identified a higher phosphate level as predictor of AoC progression, independently of eGFR. Studies in healthy adults (53) and CKD patients (54) already observed an association of higher phosphatemia with atherosclerosis and vascular calcification. Russo et al. established in a cohort of predialysis patients that higher phosphorus levels were associated with CAC progression (55). Another study performed in CKD patients observed an association between higher phosphorus levels and mortality (56). Again, hypotheses generated by observational studies require testing in randomized trials, in order in this case to assess the impact of phosphate binders on survival or AoC progression (57).

The strengths of our study, the first to examine the progression of both CAC and AoC in a large population of stable RTR, include the sample size, the 4-year follow-up, the use of an identical multi-slice spiral CT at baseline and follow-up. Some limitations should be acknowledged. First, the study population is typical of RTR followed in European centers, mostly Caucasian and with a lower prevalence of diabetes than in the United States. Second, all parameters (except the calcium scoring) were obtained once at inclusion, so that we could not perform a time-dependent covariate analysis. Third, there is certainly a selection bias. Indeed a repeat CT scan was available only in 205/281 patients and analyzable in 197/281 (70%). Since the patients without repeat scoring overall had a worse CV risk profile at baseline (Table 1), our results probably underestimate somewhat the progression of vascular calcification in RTR. Sensitivity

analyses however showed that our results are robust. Finally, the inclusion of prevalent rather than incident RTR patients may have introduced a survival bias.

In conclusion, the present study shows that vascular calcification progresses substantially in stable renal transplant recipients. The independent determinants of both CAC and Aoc progression include both classical and non-classical CV risk factors, some of them modifiable.

ACKNOWLEDGMENTS

The authors acknowledge Y.Pirson, and the members of the UCL Nephrology Collaborative group for the careful joint follow-up of the patients, Alain Vlassenbroek from Philips Healthcare for the thoracic CT-scan , Prof. W Jahnen-Dechent for the gift of the anti-fetuin-A antibody, and the financial support from the Fonds National de la Recherche Scientifique Médicale (FRSM), the Fondation Saint-Luc, the EU project grant GENECURE (FP6 LSHM CT 2006 037697), the 'Fondation Alphonse & Jean Forton', an Inter-university Attraction Pole (IUAP P6/05), and the DIANE project (Communauté Française de Belgique).

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Table 1: Characteristics of the renal transplant recipients cohort, compared with patients without a second analyzable CT scan (dead and excluded)

Variables	Study cohort : with a second analyzable CT N = 197 Mean ± SD Median [P25-P75] %	Patients who died during follow-up (without a second analyzable CT scan) N = 40 Mean ± SD Median [P25-P75] %	Patients who declined a second CT or had arrhythmias (without a second analyzable CT scan) N = 40 Mean ± SD Median [P25-P75] %	P-value
Demographics and comorbidity				
Age (y)	52 ± 12	63 ± 10	50 ± 14	<0.001
Male gender	57	72	68	0.1
BMI (kg/m²)	26 ± 5	27 ± 4	26 ± 5	0.6
Diabetes	13	23	12	0.3
History of CVE	25	69	27	<0.001
History of smoking	50	54	61	0.4
Current smoking	12	10	27	0.05
History of PTX	13	18	15	0.7
Physical examination				
Systolic BP (mmHg)	134 ± 20	146 ± 25	134 ± 16	0.01
Diastolic BP (mmHg)	82 ± 12	85 ± 17	82 ± 10	0.2
Pulse pressure (mmHg)	53 ± 17	60 ± 16	53 ± 11	0.04
Drugs				
Use of statin	37	41	44	0.7
Use of tacrolimus	42	44	39	0.9

Use of cyclosporin	47	46	54	0.7
Use of azathioprine	17	46	46	0.008
Use of sirolimus	8	15	7	0.3
Use of MMF	45	44	46	0.9
Use of aspirin	9	15	24	0.02
Use of calcium with/without vit D	35	56	42	0.05
Kidney function and RRT characteristics				
MDRD (ml/min/1.73m²)	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
Diab/HT as cause of ESRD	8	21	7	0.07
Living donor TP	14	13	15	0.9
Biological markers				
Glucose (mg/dL)	92 [85-103]	103 [90-121]	89 [83-102]	0.03
hsCRP (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/24h)	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)vitD3 (ng/mL)	15 [11-23]	10 [7-16]	15 [11-19]	0.01
1-25(OH)vitD3 (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

OPG (pmol/L)	14 ± 9	17 ± 14	14 ± 8	0.2

Note Table 1:

Conversion factors for units: serum creatinine in mg/dL to µmol/L, x88.4; glucose in mg/dL to mmol/L, x0.05551; hemoglobin in g/dL to g/L, x10; total cholesterol in mg/dL to mmol/L, x0.02586; HDL cholesterol in mg/dL to mmol/L, x0.02586; 25(OH) vitD3 in ng/mL to nmol/L, x2.496; 1-25(OH) vitD3 in pg/mL to pmol/L, x2.6; calcium in mg/dL to mmol/L, x0.2495; phosphate in mg/dL to mmol/L, x0.3229; magnesium in mEq/L to mmol/L, x0.5; PTH in pg/mL to µmol/L, x11.1.

Table 2: Comparison of non, slow and high CAC progressors (tertile 1: <1%; tertile 2: 1-15%; tertile 3: >15%).

Variables	**Non Progressors**	**Slow Progressors**	**High Progressors**	**p-value**
N=172 patients	**Tertile 1**	**Tertile 2**	**Tertile 3**	
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	
	Median [P25-P75]	**Median [P25-P75]**	**Median [P25-P75]**	
	%	**%**	**%**	
	N = 56	**N = 56**	**N = 60**	
Annualized Relative rate of change of CAC (%)	0 [-1 to 0.7]	7 [4-12]	29 [21-42]	
Demographics and comorbidity				
Age (y)	46 (14)	54 (9)	58 (10)	<0.001
Male gender	45	65	66	0.02
BMI (kg/m²)	27 (4)	27 (5)	26 (5)	0.9
History of CVE	18	28	31	0.3
Diabetes	3	23	18	0.01
History of smoking	45	49	58	0.4
Current smoking	12	11	9	0.9
History of PTX	17	14	11	0.7
Physical examination				
Systolic BP (mmHg)	131 (19)	136 (16)	137 (24)	0.3
Diastolic BP (mmHg)	81 (11)	83 (11)	79 (13)	0.2
Pulse pressure (mmHg)	50 (18)	53 (11)	58 (21)	0.06
Drugs				
Use of statin	27	49	40	0.06
Use of tacrolimus	45	25	55	0.01
Use of cyclosporin	48	58	33	0.05

Use of azathioprine	23	44	18	0.01
Use of sirolimus	5	4	16	0.04
Use of MMF	43	37	47	0.5
Use of aspirin	5	14	9	0.02
Use of calcium with/without vit D	33	32	40	0.6
Kidney function and RRT characteristics				
MDRD (ml/min/1.73m²)	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
Diab/HT as cause of ESRD	5	12	7	0.3
Living donor TP	20	11	11	0.3
Time of transplantation (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
hsCRP (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/24h)	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)vitD3 (ng/mL)	21 (10)	16 (8)	16 (7)	0.01
1-25(OH)vitD3 (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
OPG (pmol/L)	14 (9)	14 (8)	15 (8)	0.7

Note table 2

Conversion factors for units: serum creatinine in mg/dL to µmol/L, x88.4; glucose in mg/dL to mmol/L, x0.05551; hemoglobin in g/dL to g/L, x10; total cholesterol in mg/dL to mmol/L, x0.02586; HDL cholesterol in mg/dL to mmol/L, x0.02586; 25(OH) vitD3 in ng/mL to nmol/L, x2.496; 1-25(OH) vitD3 in pg/mL to pmol/L, x2.6; calcium in mg/dL to mmol/L, x0.2495; phosphate in mg/dL to mmol/L, x0.3229; magnesium in mEq/L to mmol/L, x0.5; PTH in pg/mL to µmol/L, x11.1.

Table 3: Univariate and multivariable linear regression analysis of risk factors of annualized change in CAC score

	Univariate Analysis			Multivariable analysis*		
Variables **N=197 patients**	**Univariate coefficient (SE)**	**95% CI**	**p-value**	**Multivariable coefficient (SE)**	**95% CI**	**p-value**
Demographics and cormobidity						
Age (y)	0.005 (0.001)	0.002 to 0.008	0.001			
Female gender	-0.11 (0.04)	-0.18 to -0.04	0.003			
History of CVE	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 (mmHg)	0.002 (0.001)	0.000 to 0.004	0.04			
Pulse pressure (mmHg)	0.003 (0.001)	0.001 to 0.0025	0.004			
Vascular calcification						
CAC baseline	0.09 (0.01)	0.07 to 0.12	<0.001	0.07 (0.01)	0.04 to 0.10	<0.001
Drugs						
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 characteristics						
Time on dialysis (y)	0.02 (0.01)	0.004 to 0.04	0.02			
Living donor TP	-0.08 (0.05)	-0.18 to 0.03	0.2			
Biological markers						
Homocysteine (µmol/L)	0.26 (0.15)	-0.033 to 0.55	0.08			
25(OH)vitD3 (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			

Notes Tables 3:

*R^2 = 0.29, $p < 0.001$; Factors with a p-value < 0.2 in the univariate analysis entered the multivariable stepwise linear regression

Conversion factors for units: 25(OH) vitD3 in ng/mL to nmol/L, x2.496; PTH in pg/mL to µmol/L, x11.1.

Table 4: Comparison of non, slow and high AoC progressors (tertile 1: <0%; tertile 2: 0-8%; tertile 3: > 8%)

Variables	**Non Progressors**	**Slow Progressors**	**High Progressors**	**p-value**
N=166 patients	**Tertile 1**	**Tertile 2**	**Tertile 3**	
	Mean (SD) or Median [P25-P75]	**Mean (SD) or Median [P25-P75]**	**Mean (SD) or Median [P25-P75]**	
	%	**%**	**%**	
	N = 53	**N = 53**	**N = 60**	
Annualized Relative rate of change of AoC (%)	-1 [-4 to 0]	2 [0-4]	21 [14-50]	
Demographics and comorbidity				
Age (y)	46 (14)	58 (9)	54 (10)	<0.001
Male gender	55	62	57	0.7
BMI (kg/m²)	26 (4)	27 (5)	27 (5)	0.8
History of CVE	22	42	17	0.01
History of smoking	44	68	42	0.05
Current smoking	9	14	10	0.7
Diabetes	7	18	13	0.3
History of PTX	16	12	13	0.8
Physical examination				
Systolic BP (mmHg)	133 (17)	135 (19)	136 (21)	0.7
Diastolic BP (mmHg)	81 (11)	79 (11)	83 (12)	0.2
Pulse pressure (mmHg)	52 (19)	57 (18)	53 (15)	0.4
Drugs				
Use of statin	24	46	50	0.01
Use of tacrolimus	53	46	30	0.05
Use of cyclosporin	33	44	62	0.01
Use of azathioprine	26	26	28	0.9

Use of sirolimus	11	6	3	0.3
Use of MMF	42	42	45	0.9
Use of aspirin	6	16	8	0.2
Use of calium with/without vit D	27	38	43	0.2
Kidney function and RRT characteristics				
MDRD (ml/min/1.73m²)	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
Diab/HT as cause of ESRD	9	8	7	0.9
Living donor TP	18	4	13	0.08
Time of transplantation (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
hsCRP (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)vitD3 (ng/mL)	19 (10)	16 (8)	15 (6)	0.01
1-25(OH)vitD3 (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

OPG (pmol/L)	14 (11)	16 (9)	13 (7)	0.2

Note table 4

Conversion factors for units: serum creatinine in mg/dL to µmol/L, x88.4; glucose in mg/dL to mmol/L, x0.05551; hemoglobin in g/dL to g/L, x10; total cholesterol in mg/dL to mmol/L, x0.02586; HDL cholesterol in mg/dL to mmol/L, x0.02586; 25(OH) vitD3 in ng/mL to nmol/L, x2.496; 1-25(OH) vitD3 in pg/mL to pmol/L, x2.6; calcium in mg/dL to mmol/L, x0.2495; phosphate in mg/dL to mmol/L, x0.3229; magnesium in mEq/L to mmol/L, x0.5; PTH in pg/mL to µmol/L, x11.1.

Table 5: Univariate and multivariable linear regression analysis of risk factors of annualized change in AoC score

	Univariate analysis			Multivariable analysis*		
Variables **N = 197 patients**	**Univariate coefficient (SE)**	**CI 95%**	**p-value**	**Multivariable coefficient (SE)**	**CI 95%**	**p-value**
Demographics and cormobidity						
Age (y)	0.013 (0.002)	0.01 to 0.02	<0.001	0.006 (0.002)	0.001 to 0.01	0.01
Female gender	-0.12 (0.05)	-0.22 to -0.03	0.01	-0.09 (0.04)	-0.17 to -0.01	0.03
History of CVE	0.21 (0.06)	0.11 to 0.34	<0.001			
Diabetes	0.15 (0.07)	0.01 to 0.30	0.04			
Vascular calcification						
AoC 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 (mmHg)	0.004 (0.001)	0.002 to 0.01	<0.001			
Pulse pressure (mmHg)	0.007 (0.001)	0.004 to 0.01	<0.001	0.003 (0.001)	0.001 to 0.01	0.01
Drugs						
Use of statin	0.22 (0.05)	0.13 to 0.32	0.01	0.11 (0.04)	0.02 to 0.20	0.01
Use of tacrolimus	-0.08 (0.05)	-0.18 to 0.02	0.0			
Use of cyclosporin	0.07 (0.05)	-0.02 to 0.17	0.1			
Use of 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 characteristics						
Time on dialysis (y)	0.02 (0.01)	-0.002 to 0.04	0.07			
Diab/HT as cause of ESRD	0.24 (0.09)	0.08 to 0.52	0.01			
Living donor TP	-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)vitD3 (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			
OPG (pmol/L)	0.005 (0.003)	0.000 to 0.01	0.06			

Notes Tables 5:

*$R^2 = 0.42$, $p < 0.001$; Factors with a p-value < 0.2 in the univariate analysis entered the multivariable stepwise linear regression

Conversion factors for units: glucose in mg/dL to mmol/L, x0.05551; 25(OH) vitD3 in ng/mL to nmol/L, x2.496; phosphate in mg/dL to mmol/L, x0.3229

Figure legends:

Figure 1: Flow chart describing movement of the patients through the study

Figure 2: Progression of CAC (A) and AoC (B) Agatson score (mg) in prevalent renal transplant recipients. Results are presented as median [P25-P75].

Figure 3: Distribution of the annualized rate of change of CAC (A) and AoC (B).

Figure 4: Correlation between the absolute CAC and AoC progression per year.

Supplementary materials:

Supplementary Figure 1: A and B) Axial CT image at time of inclusion (40 mg) (A) and at follow-up (270 mg) (B) at the level of the left main coronary artery. There is evidence of CAC progression. C and D) Sagittal CT image using a maximal intensity projection (MIP) at time of inclusion (317 mg) (C) and at follow-up (1284 mg) (D) at the level of the aorta. There is evidence of AoC progression.

Supplementary table 1: Sensitivity analysis assuming patients who died had 14% CAC progression

Supplementary table 2: Sensitivity analysis assuming patients who died had 57% CAC progression

Supplementary table 3: Sensitivity analysis assuming patients who died had 10% AoC progression

Supplementary table 4: Sensitivity analysis assuming patients who died had 48% AoC progression

Supplementary table 5: Independent determinants of AoC progression in renal transplant recipients by multivariable linear regression (after exclusion of 13 patients with apparent AoC regression)