

Vascular Calcification Slows But Does Not Regress After Kidney Transplantation



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Introduction: Medial arterial calcification is a common and progressive lesion in end-stage renal disease that is associated with poor cardiovascular outcomes. Whether this lesion can be arrested or reversed is unknown, and was examined retrospectively by measuring progression of breast arterial calcification before and after kidney transplantation.

Methods: Arterial calcification was measured on serial mammograms from patients with previous kidney transplantation and compared to measurements performed before transplantation or in patients on the active waitlist. Serum creatinine >2.0 mg/dl after transplantation or warfarin use were exclusions.

Results: Median (interquartile range) progression of arterial calcification was 12.9 mm/breast per year (5.9 to 32.6) in 34 patients before or awaiting transplantation compared to just 1.2 mm/breast per year (−0.54 to 5.1) in 34 patients after transplantation ($P < 0.001$). Slowing of progression was also seen in longitudinal analyses of patients with mammograms performed both before and after transplantation. Duration of end-stage renal disease before transplantation but not age, diabetes, baseline calcification, or serum chemistries correlated with progression after transplantation. Significant regression was not observed in any patient.

Conclusion: In this first quantitative study of the effect of kidney transplantation, medial arterial calcification appeared to slow to rates seen in patients with normal renal function, indicating that the effect of renal failure may be completely abrogated. Overall, however, there was no significant regression, suggesting that calcification is irreversible and emphasizing the importance of prevention. Duration of pre-transplant end-stage renal disease but not baseline calcification was a determinant of progression, consistent with cumulative, permanent changes to arteries that promote calcification.

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KEYWORDS: end-stage renal disease; transplantation; vascular calcification

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Chronic kidney disease and end-stage renal disease (ESRD) predispose to medial arterial calcification,^{1,2} a lesion histologically and pathophysiologically distinct from the intimal calcification in atherosclerosis. Medial calcification is presumably related to the altered mineral metabolism in these patients and correlates strongly with cardiovascular outcomes.^{3,4} Whether this lesion can be arrested or reversed is unclear, as existing calcifications or permanent changes to matrix proteins produced by uremia could enable progression despite normalization of renal function and mineral metabolism. Our previous studies in which calcified aortas from uremic mice were transplanted into normal

mice showed no evidence of regression,⁵ but the possibility of regression over longer periods of time remains unanswered and has important clinical implications.

Kidney transplantation provides an opportunity to directly assess changes in medial arterial calcification in human beings after reversal of renal failure. Extensive arterial calcification is present in many patients at the time of transplantation^{6,7} and may contribute to the high burden of cardiovascular disease after transplantation.^{8,9} However, little is known about the effect of kidney transplantation, and whether calcification stabilizes or even regresses is unclear. The paucity of data is due in large part to imaging that is restricted to coronary arteries and the aorta,⁹ sites dominated by atherosclerosis, even in ESRD.¹⁰ Because the 2 forms of calcification cannot be reliably distinguished radiologically, ongoing atherosclerosis, for which these patients are at risk, can obscure any effect on medial calcification. Thus, assessing changes in medial calcification requires imaging of a vascular bed devoid of atherosclerosis.

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Arterial calcification is easily discerned on routine mammograms and is exclusively medial,¹¹ as atherosclerosis does not occur in these vessels.^{11,12} Furthermore, this calcification correlates with medial calcification in other arteries¹³ and with cardiovascular disease, both in the general population^{14–16} and in ESRD patients,¹⁷ indicating that it is a marker of generalized medial arterial calcification. We have previously used mammography to identify and to quantify risk factors for medial arterial calcification,^{18–20} to demonstrate its clinical significance,¹⁷ and to measure its progression.¹⁹ In this study, we retrospectively measured progression of breast arterial calcification in patients before and after kidney transplantation at a single transplantation center.

MATERIALS AND METHODS

Subjects

The medical records of all women who received a transplanted kidney between 2010 and 2016 at Emory University Hospital were screened for the presence of at least 2 digital mammograms performed within Emory Healthcare before transplantation or at least 2 digital mammograms performed after transplantation (or up to 1 month before transplantation), separated by at least 1 year. Inclusion criteria were the presence of arterial calcification on the first mammogram, onset of ESRD within 6 months of the first pretransplantation mammogram, and a serum creatinine level <2 mg/dl at the time of the second posttransplantation mammogram. The exclusion of patients with no calcification on the first mammogram despite calcification on the second mammogram was necessary because the rate will be underestimated and arbitrarily determined by the mammogram interval. Current or past warfarin use was an exclusion. Some of the posttransplantation patients were included in a previous report.¹⁹ Additional patients with pretransplantation mammograms were obtained through a random search of the active transplant waiting list as of January 2018. Diabetes was defined as the use of a hypoglycemic medication during the mammogram interval as assessed by medical record review. This protocol was approved by the Institutional Review Board of Emory University.

Measurements

Arterial calcification was identified and measured as linear densities along vessel walls on standard cranial–caudal views as previously described.¹⁹ The lengths of calcified segments were summed and expressed as millimeters per breast. To measure progression, calcification was quantified on mammograms separated by the longest interval possible, but at least 12 months. Differences in breast coverage between

Table 1. Patient characteristics

Characteristic	Pretransplantation or waitlist	Posttransplantation
n	34	34
Age, yr, at most recent mammogram, mean ± SE	58.6 ± 1.6	60.4 ± 1.7
Diabetes, %	50	35
Serum creatinine, mg/dl, mean ± SE)		1.09 ± 0.05
Duration of RRT, yr, mean ± SE	4.8 ± 0.9	5.7 ± 0.7
Baseline BAC, mm/breast, median (IQR)	64 (14–137)	47 (9.7–83)
Mammogram interval, yr, mean ± SE	2.6 ± 0.2	2.8 ± 0.3

BAC, breast arterial calcification; IQR, interquartile range; RRT, renal replacement therapy; SE, standard error.

There were no significant differences between the groups.

mammograms were accounted for by measuring breast height and truncating images as needed. Serum creatinine, calcium, and phosphorus values obtained closest to the second mammogram were used for analysis (median of 14 days within the mammogram date).

Statistical Analysis

Data are presented as means and standard errors except for breast arterial calcification and progression, which are not normally distributed and are presented as medians and interquartile ranges. Significance was determined by *t* testing or the Mann–Whitney *U* and Wilcoxon tests for nonparametric data.

RESULTS

Assessment of changes in breast arterial calcification after restoration of normal renal function is necessarily limited to women with baseline calcification, at least 2 mammograms performed after transplantation at Emory Healthcare, and near normal renal function. Satisfying all of these criteria therefore required the screening of a large number of individuals. Of 614 female transplant recipients, 209 had at least 2 digital mammograms performed after transplantation. Of these, 156 had a serum creatinine <2.0 mg/dl at the time of a second mammogram, and 35 of these had arterial calcification on the earliest mammogram. One patient was excluded because of warfarin use. The second cohort with pretransplantation mammograms consisted of transplant patients with at least 2 digital mammograms before transplantation or patients on the active waiting list for transplantation with at least 2 mammograms. Pretransplantation mammograms were available in 68 of the transplant recipients, of whom 10 had arterial calcification on the earliest mammogram. An additional 24 subjects were obtained from the transplant waiting list. There were no significant differences between the cohorts in terms of age, prevalence of diabetes, duration of renal replacement therapy, baseline arterial calcification, or

Table 2. Parameters of mineral metabolism in the transplant patients

Parameter	Initial	Final
Mammograms, yrs after transplantation	0.95 (0.45–2.9)	4.28 (2.2–6.6)
Chemistries, yrs after transplantation	1.00 (0.54–2.7)	4.91 (2.2–6.7)
Serum calcium, mg/dl	9.56 ± 0.11	9.43 ± 0.10
Serum phosphorus, mg/dl	3.35 ± 0.14	3.33 ± 0.14
Serum parathyroid hormone, pg/ml	155 ± 24	148 ± 27

Data are presented as median (interquartile range) or as mean ± standard error.

the interval between mammograms (Table 1). Immunosuppression therapy for the transplant patients comprised tacrolimus (62%), belatacept (35%), rapamycin (3%), prednisone (94%), mycophenolate (91%), and azathioprine (3%). Parameters of mineral metabolism are shown in Table 2.

The time courses of arterial calcification in the 2 groups are shown in Figure 1, and the progression rates are shown in Figure 2. The rate of progression of arterial calcification was markedly reduced in the transplant patients (median = 1.2, IQR = −0.54 to 5.1 mm/breast per year) compared to the pretransplantation patients (median = 12.9, IQR = 5.9 to 32.6 mm/breast per year), with a P value of <0.0001. Longitudinal analysis was possible in 10 patients with at least 2 mammograms performed both before and after transplantation (Figure 3). Almost all patients showed a decrease in progression, but there was 1 patient with a very high rate of progression after transplantation that was more than 10-fold greater than the third quartile for the entire posttransplantation cohort. There was no apparent explanation for this, and when this outlier was omitted, the decrease in progression after transplantation was significant by nonparametric paired analysis ($P = 0.02$).

Progression rate after transplantation was similar in patients with diabetes (median = 0.83 mm/breast per year, IQR = −0.71 to 7.3) and without diabetes (median = 1.5 mm/breast per year, IQR = −0.54 to 4.7) and correlated weakly with age ($r = 0.19$) and baseline calcification ($r = 0.32$) but more strongly with time on dialysis before transplantation ($r = 0.54$). Only duration of pretransplantation dialysis was significantly correlated with progression of calcification ($P = 0.01$) in a multivariable model that also included age and baseline calcification. There was no correlation between progression and serum creatinine, calcium, phosphorus, or parathyroid hormone measured closest to the second mammogram.

Although the progression rate after transplantation approached 0, it was significantly ≥ 0 for the entire cohort ($P = 0.017$ by 1-sample Wilcoxon test). Negative rates were seen in 32% and these patients did not differ from those with positive rates in regard to age, diabetes, serum creatinine, calcium, phosphorus, duration of renal replacement therapy, or baseline calcification. The negative rates were usually small (median = 1.9 mm/breast per year; IQR = 0.9 to 6.1) and within the range of error for 2 measurements.¹⁹ To address this more accurately, additional measurements were made in the 9 patients with at least 4 mammograms after transplantation, with the change in calcification over time determined by linear regression in each patient. The median rate was 1.01 mm/breast per year (IQR = −0.52 to 2.62), almost identical to the rate in the entire cohort. The rate was negative in 3 patients, with the most negative being only −1.35 mm/breast per year.

Because the first mammogram was performed more than 2 years after transplantation in one-third of the

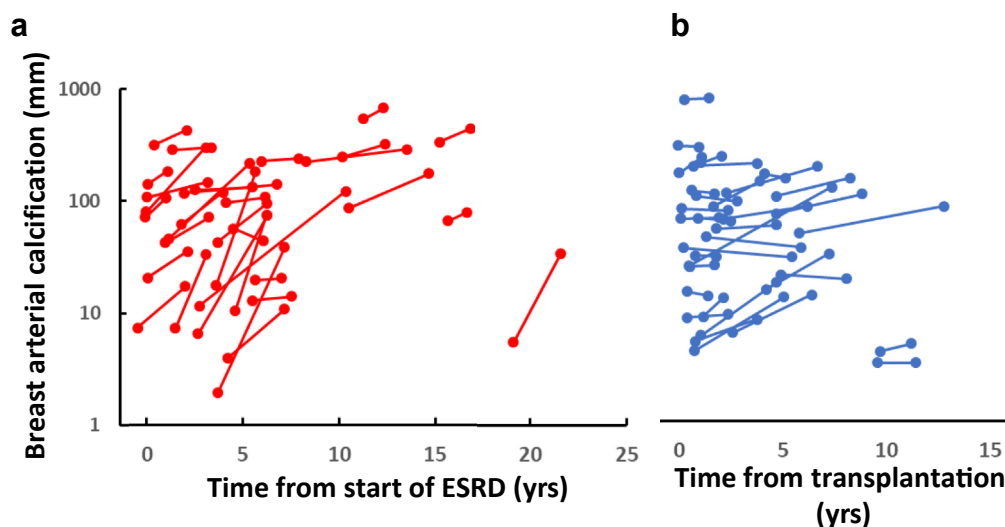


Figure 1. Progression of breast arterial calcification. (a) End-stage renal disease (ESRD) patients before transplantation or on waitlist for transplantation. (b) Patients after transplantation. Each pair of symbols represents a separate patient.

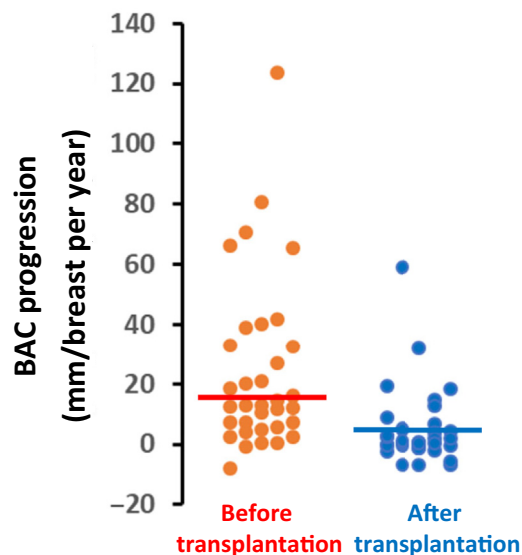


Figure 2. Rates of progression of breast arterial calcification (BAC). Horizontal lines indicate medians.

patients, an initial reversal of calcification could have been missed. This was addressed through a separate analysis of those patients in whom a mammogram was performed within 6 months of transplantation (range = -4.3 to 6 months; mean absolute difference = 2.2 months). This was restricted to patients without diabetes to eliminate other risk factors for calcification. As shown in Figure 4, there was no substantial reduction in calcification in any of the 10 patients.

DISCUSSION

This is the first quantitative assessment of medial arterial calcification before and after renal transplantation. We previously showed that this form of calcification, as assessed on mammograms, is markedly accelerated in advanced chronic kidney disease (CKD) and ESRD,¹⁹ and it is clear from the current cross-sectional and longitudinal data that this substantially slows after reversal of renal failure. The progression rate after transplantation was not significantly different from the rate in our previously reported cohort of control subjects without CKD,¹⁸ suggesting that successful kidney transplantation completely abrogates the effect of ESRD on medial arterial calcification. Previous studies examining coronary artery or aortic calcification have shown variable rates of progression after kidney transplantation,^{9,21} with the largest and truly quantitative studies showing significant progression.^{6,7,22,23} However, much of this calcification is atherosclerotic rather than medial, and because these patients are predisposed to atherosclerosis, the results likely reflect progression of atherosclerosis rather than medial calcification.

Progression of calcification after transplantation did not correlate with age or baseline calcification, similar to what is observed in subjects with normal renal function, CKD, or ESRD.¹⁹ Diabetes is a recognized risk factor for medial arterial calcification but did not affect progression after transplantation. This is consistent with our previous data showing that diabetes is a risk factor only in the presence of CKD.¹⁹ Progression did correlate with ESRD duration before transplantation, and as there was no correlation with baseline calcification, this may reflect other, permanent effects of renal failure on arterial structure.

Although progression of medial arterial calcification slowed after reversal of renal failure, there was no convincing evidence that the calcification regressed. The progression rate after transplantation was significantly greater than or equal to 0 for the entire cohort, and regression was not apparent in patients with multiple sequential mammograms or in patients with calcification measured close to the time of transplantation. This is unlikely to be explained by residual renal insufficiency, as the mean serum creatinine was 1.09 mg/dl and as we have previously shown that only advanced renal failure (estimated glomerular filtration rate <40 ml/min per 1.73 m²) accelerates breast arterial calcification.¹⁹ Hypercalcemia after transplantation was also not a factor, as only 2 patients exceeded the normal range and only by 0.1 mg/dl each. Furthermore, no relationship was seen between progression of

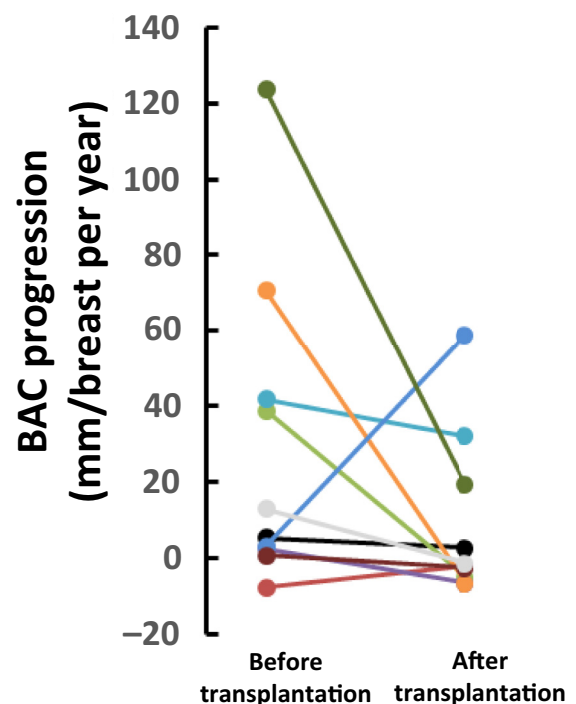


Figure 3. Change in progression of breast arterial calcification (BAC) in patients with mammograms before and after transplantation. Each set of symbols represents a separate patient.

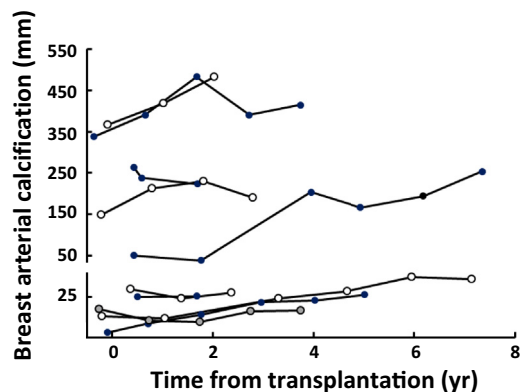


Figure 4. Time course of breast arterial calcification in patients with measurements within 6 months of transplantation.

calcification and serum calcium levels. Regression was also not observed in the previous studies of coronary artery and aortic calcification, and the results are consistent with animal studies in which there was no regression of medial calcification in aortas from uremic mice transplanted into normal mice.⁵ Given the association of medial arterial calcification with poor outcomes, the lack of regression emphasizes the importance of preventive strategies before transplantation.

This study has several limitations, including the exclusion of men, the small sample size, and its retrospective design. Unfortunately a comparable, accessible measurement site is not present in men. However, we have previously shown that risk factors for breast arterial calcification in women apply to peripheral arterial calcification in men,²⁴ and there is no reason to believe that calcification could reverse in men but not in women. The sample size is limited by the requirement for women with near normal renal function and with calcification at baseline, without which the onset of calcification is unknown and progression rates cannot be calculated. It is possible that calcification can regress in a small proportion of women that is not apparent in this sample, but it is clearly not common and would have to be explained by factors other than renal function or mineral metabolism. Although the retrospective, case-control design may have limited the ability to control for all potential variables, this was minimized by comparing transplant patients to ESRD patients either before transplantation or on the active transplant waitlist, thus avoiding any bias favoring healthier patients selected for transplantation. It is unlikely that the large and highly significant effect of transplantation can be explained by other variables. Furthermore, measurements of both pretransplantation and posttransplantation calcification rates in a subset of patients also showed slowing and a lack of significant regression.

Despite the limitations, the results suggest that medial arterial calcification decelerates substantially after reversal of renal failure. The data are not indicative of significant regression of calcification, but the possibility that this might occur slowly in some patients cannot be ruled out. The results further exemplify the utility of routine mammography as a means of quantifying and following the course of medial arterial calcification and form the basis for larger, prospective studies to definitively address the issue of regression and to identify risk factors in this population.

DISCLOSURE

All the authors declared no competing interests.

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