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A high abdominal aortic calcification score by dual X-ray absorptiometry is associated with cardiovascular events after kidney transplantation

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ABSTRACT

Background. Aortic calcification is associated with an increased risk for cardiovascular events in renal transplant recipients. This study focused on the association of abdominal aortic calcification (AAC) and cardiovascular events assessed using a dual-energy X-ray absorptiometry (DXA) scoring methodology for AAC.

Methods. From 2008 to 2014, renal transplant recipients referred for a DXA procedure within 6 months after transplantation were included in a retrospective, single-centre study. The primary endpoint was the occurrence of cardiovascular events, defined as myocardial infarction, cerebrovascular accident or transient ischaemic attack, after transplantation. AAC was quantified using an 8-point scoring system and patients were divided into three groups; a control group (AAC = 0), a low AAC group (AAC = 1–3) and a high AAC group (AAC = 4–8).

Results. We evaluated 701 patients, 267 (38.1%) had detectable calcifications (low AAC 190 patients, high AAC 77 patients) and 434 (61.9%) had no calcifications. Cardiovascular events were seen in 37 (8.5%) patients in the control group, in 18 (9.5%) in the low AAC group and in 20 (26.0%) in the high AAC group. Univariate Cox proportional hazards analysis of the high AAC score showed a hazard ratio (HR) of 4.23 [95% confidence interval (CI) 2.44–7.33; P < 0.01] for cardiovascular events, while results were not significant for the low AAC score. Multivariate analysis showed an independent significant association between a high AAC score and cardiovascular events [HR 2.78 (95% CI 1.05–7.64); P = 0.04]. Assessment of the continuous net reclassification index (NRI), comparing the combined clinical variables with a model of both AAC scoring and clinical variables, showed an NRI of 0.76 (95% CI 0.65–0.86; P < 0.01).

Conclusions. An independent association between a high AAC score, assessed by DXA, and cardiovascular events was identified and provides an opportunity for early cardiovascular risk stratification in renal transplant recipients.

Keywords: cardiovascular diseases, dual-energy X-ray absorptiometry, kidney transplantation, survival analysis, vascular calcification

INTRODUCTION

The risk of cardiovascular events increases substantially with end-stage renal disease, and cardiovascular death is the leading cause of mortality after kidney transplantation [1]. The cumulative incidence of a myocardial infarction (MI) is 11.1% at 36 months after kidney transplantation and 6.8% for *de novo* cerebrovascular disease, including cerebrovascular accident (CVA) and transient ischaemic attack (TIA) [2, 3]. Cardiovascular risk factors such as hypercholesterolaemia, hypertension and diabetes mellitus are common in patients with end-stage renal disease, which contributes to a higher prevalence of cardiovascular disease when compared with the general population [4]. In addition, kidney transplant–specific risk factors, such as the duration of pre-transplant dialysis, are important factor to consider in this patient population [5].

The Kidney Disease: Improving Global Outcomes (KDIGO) 2017 clinical practice guideline recommends bone mineral density testing in the first 3 months following transplantation in patients with an estimated glomerular filtration rate (eGFR) >30 mL/min/1.73 m^2 if they receive corticosteroids or have risk factors for osteoporosis [6]. An interesting but often neglected feature of dual-energy X-ray absorptiometry (DXA) is that the

degree of calcification of the abdominal aorta can be assessed in the image that is obtained for vertebral fracture assessment.

Earlier studies showed that abdominal aortic calcification (AAC) data derived from DXA procedures are associated with a risk of future development of cardiovascular events in the general population [7]. A retrospective, single-centre study with patients from the general population referred for bone mineral density screening showed a significantly higher risk for cardiovascular events in patients with an increased AAC score compared with the control group with no visual AAC [8].

Studies in which vascular calcification was assessed using computed tomography (CT), lumbar X-ray or cardiac CT found significant associations of vascular calcification in both the aorta and coronary arteries with cardiovascular events in renal transplant recipients [9–13].

We performed a retrospective, single-centre study to evaluate the association between AAC assessed by DXA and cardiovascular events after kidney transplantation.

MATERIALS AND METHODS

Patients

Renal transplant recipients referred for a DXA procedure within 6 months after transplantation at the University Medical Center Groningen (UMCG), The Netherlands between 2008 and 2014 were included in the study. Patients' charts were screened for baseline characteristics, prior cardiovascular events (MI, CVA, TIA) and cardiovascular events after transplantation. Pre-transplant hypertension was defined as blood pressure >140/90 mmHg or current anti-hypertensive medication, pre-transplant hypercholesterolaemia was defined as total cholesterol levels >200 mg/dL or current use of lipid-lowering agents, pre-transplant diabetes mellitus (DM) was defined as the use of anti-diabetic medication and patients were considered smokers if they smoked at the time of transplant wait-listing admission [14]. The serum creatinine–based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate the eGFR. The primary endpoint of this study was the occurrence of fatal and non-fatal cardiovascular events, defined as MI, CVA or TIA, after transplantation. Causes of death during follow-up were subdivided as cardiovascular event (MI, CVA or TIA), cardiac failure, pulmonary disease, infection, cancer or unknown.

Patient data were processed and electronically stored according to the Declaration of Helsinki ethical principles for medical research involving human subjects and the institutional ethics review board gave approval for this study (Medical Ethical Committee UMCG 2017/457). The clinical and research activities were consistent with the principles of the Declaration of Istanbul as outlined in the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

DXA procedure and AAC scoring

Lateral single-energy images of the lumbar spine were obtained on a Discovery DXA System (Hologic, Bedford, MA, USA). Two independent imaging specialists, blinded to the patients' medical history, analysed the DXA images to quantify
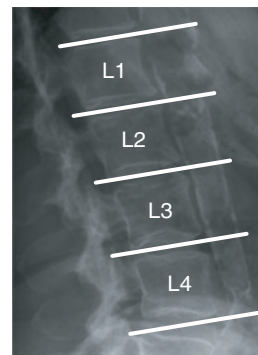

FIGURE 1: Example of a vertebral fracture assessment image from DXA with the lumbar vertebral bones L1–L4. This image shows double-sided aortic calcifications with an aggregate length up to the height of all four vertebrae, resulting in an AAC score of 8.

AAC using a visual 8-point scale, as previously described by Schousboe *et al.* [15]. This scale reflects the total length of calcification on the anterior and posterior aortic walls at the level of the L1–L4 vertebral bones. A point was given for a single-sided calcification with an aggregate length up to the height of one vertebra; subsequent points were given when calcifications reached the level of the three other vertebrae. The total score was the summation of anterior and posterior calcification scores and ranged from 0 to 8 (Figure 1). Based on AAC scoring outcomes, patients were stratified into three groups: the control group, with an AAC score of 0; the low AAC group, with a score between 1 and 3; and the high AAC group, with a score between 4 and 8.

Statistical analysis

Baseline characteristics and clinical follow-up results were presented as mean and standard deviation (SD) when normal distribution was assumed by means of a Q-Q plot or histogram, as median and interquartile range (IQR) for skewed data and as frequency and percentage when data were categorical. We compared patient characteristics between the control group, low AAC group and high AAC group, using a chi-square test and one-way analysis of variance (significant at a two-sided P-value <0.05). Cardiovascular events were shown for the three defined groups as numbers and percentages. We used univariate and multivariable Cox regression analysis to evaluate the association between the clinical covariates and the AAC scoring system. Multivariable analyses were performed with recipient age, recipient gender, pre-transplant DM, pre-transplant hypertension, pre-transplant hypercholesterolaemia, duration of pre-transplant dialysis, history of cardiovascular events and eGFR at 3 months post-transplantation as potential confounders. The hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) are reported. The event-free and overall survival were visualized using Kaplan–Meier survival curves. Differences between Kaplan–Meier curves were calculated using log-rank tests. The added value of the AAC scoring system for estimating the risk of cardiovascular events was assessed by examining the increase in Harrell's C-index and the change in −2 log likelihood. In addition, we assessed the continuous net reclassification index (NRI) for category-independent and time-dependent

Table 1. Patient characteristics grouped according to AAC group

Variables	Control group (n = 434)	Low AAC (n = 190)	High AAC (n = 77)	P-value
Male[a], n (%)	263 (58.4)	114 (60.0)	40 (52.0)	0.56[a]
Age (years), mean (SD)	47 ± 13	57 ± 11	60 ± 13	<0.01[b]
Hypertension, n (%)	344 (79.3)	160 (84.2)	64 (83.1)	0.31[a]
Hypercholesterolaemia, n (%)	166 (38.2)	99 (52.1)	37 (48.1)	<0.01[a]
Diabetes mellitus, n (%)	49 (11.3)	28 (14.7)	17 (22.1)	0.03[a]
Body mass index, mean (SD)	25 ± 4	26 ± 4	25 ± 4	0.52[b]
Smoking, n (%)	92 (21.2)	35 (18.4)	19 (24.7)	0.50[a]
Pre-emptive transplantation, n (%)	157 (36.2)	76 (40.0)	29 (37.7)	0.66[a]
Duration pre-transplant dialysis (months), median (IQR)	34 (20–51)	42 (26–62)	50 (26–66)	0.02[b]
eGFR 3 months post-transplant, median (IQR)	50 (43–60)	51 (40–59)	50 (38–64)	0.53[b]
Immunosuppressive therapy, n (%)				0.38[a]
Cyclosporin + MMF + corticosteroids	198 (45.6)	78 (41.1)	38 (49.4)	
Tacrolimus + MMF/MPS + corticosteroids	216 (49.8)	100 (52.6)	38 (49.4)	
Others	20 (4.6)	12 (6.3)	1 (1.3)	
History of MI, n (%)	33 (7.6)	22 (11.6)	19 (24.7)	<0.01[a]
History of CVA/TIA, n (%)	25 (5.8)	15 (7.9)	5 (6.5)	0.74[a]

[a]P-value by chi-square test.
[b]P-value by one-way analysis of variance.
MMF, mycophenolate mofetil; MPS, mycophenolate sodium.

model-based risk estimates of risk of cardiovascular events [16, 17]. Statistical analyses were performed with the Statistical Package for the Social Sciences (version 23; IBM, Armonk, NY, USA), STATA-SE (version 15; StataCorp, College Station, TX, USA) and R (version 3.4.3 for Mac OS X; R Foundation for Statistical Computing, Vienna, Austria). Univariate and multivariate analyses and the NRI analysis were performed using the survival, survC1 and survIDINRI packages from the R statistics program.

RESULTS

Characteristics

We evaluated 921 renal transplant recipients, of which 701 (76%) were referred for a DXA procedure within 6 months after transplantation and formed the basis for this analysis. At baseline, there were no significant differences between the patients with (n = 219) and without (n = 701) DXA with regard to sex (P = 0.78), age (P = 0.08), body mass index (P = 0.08), smoking status (P = 0.74), pre-transplant DM (P = 0.63), duration of pre-transplantation dialysis (P = 0.64) and history of cardiovascular events (P = 0.49). Of the included recipients, a total of 267 (38.1%) patients had detectable calcifications and 434 (61.9%) patients had no calcifications. Patients with detectable calcifications were stratified according to the AAC score into a low AAC group, consisting of 190 patients, and a high AAC group of 77 patients. Patient characteristics according to AAC status are shown in Table 1. The mean age was significantly different between the groups (P < 0.01), with 47 (SD 13) years in the control group, 57 (SD 11) years in the low AAC group and 60 (SD 13) years in the high AAC group. A significant difference between the groups was also seen for prevalence of hypercholesterolaemia (P < 0.01) and prevalence of DM (P = 0.03), with the highest prevalence in the high AAC group. No significant differences were seen between the control group, low AAC

Table 2. Number and percentage of cardiovascular events grouped according to AAC group

Events	Control group	Low AAC	High AAC
MI	28 (6.4)	15 (7.9)	14 (18.1)
CVA/TIA	9 (2.1)	3 (1.6)	6 (7.8)
Combined cardiovascular endpoint	37 (8.5)	18 (9.5)	20 (26.0)

Values presented as n (%).

group and high AAC group for gender (P = 0.56), hypertension (P = 0.31), body mass index (P = 0.52), eGFR at 3 months post-transplantation (P = 0.53), type of immunosuppressive therapy (P = 0.38) and smoking (P = 0.50). The percentage of patients undergoing a pre-emptive transplantation did not differ between groups (P = 0.66), while differences in the median duration of pre-transplantation dialysis were significant (P = 0.02) with durations of 34 (IQR 20–51), 42 (26–62) and 50 (26–66) months, for the control group, low AAC group and high AAC group, respectively. There was a significant difference regarding a history of MI, with 7.6%, 11.6% and 24.7% (P < 0.01), but not for CVA or TIA, with 5.8%, 7.9% and 6.5% (P = 0.74) for the control group, low AAC group and high AAC group, respectively.

Follow-up

The median follow-up for cardiovascular events was 5.4 (IQR 3.9–7.2) years. During follow-up, 75 cardiovascular events occurred, with an MI in 57 patients and a CVA or TIA in 18 patients. The cumulative incidence of cardiovascular events after transplantation was 8.5% in the control group, 9.5% in the low AAC group and 26.0% in the high AAC group (P < 0.01) (Table 2). A total of 137 (19.5%) patients died after transplantation: 14 (10.2%) due to cardiovascular events (10 due to an MI and 4 due to a CVA), 19 (13.9%) due to cardiac failure, 9 (6.6%)

due to pulmonary disease, 13 (9.5%) due to infections, 21 (15.3%) deaths were cancer related, 5 (3.6%) due to other causes and in 56 patients (40.9%) the cause of death was unknown. Eight (1.8%) patients in the control group, 3 (1.6%) in the low AAC group and 3 (3.9%) in the high AAC group died of cardiovascular events (P = 0.44).

Association between AAC scoring and cardiovascular events

Using univariate Cox proportional hazards analysis, a high AAC score was associated with a higher cardiovascular risk [HR 4.23 (95% CI 2.44–7.33); P < 0.01] (Table 3). There was a significant difference in cumulative event-free survival between the control group, low-AAC group and high AAC group (P < 0.01) (Figure 2). A low AAC score was not associated with a higher cardiovascular risk [HR 1.26 (95% CI 0.72–2.21);

P = 0.43]. Additional covariates with a significantly higher risk were age [HR 1.05 (95% CI 1.03–1.07); P < 0.01], hypercholesterolaemia [HR 1.99 (95% CI 1.26–3.15); P < 0.01], hypertension [HR 2.06 (95% CI 1.02–4.15); P = 0.04], duration of pre-transplant dialysis [HR 1.01 (95% CI 1.00–1.01); P = 0.03], a history of cardiovascular events [HR 2.73 (95% CI 1.63–4.58); P < 0.01] and eGFR at 3 months post-transplantation [HR 0.96 (95% CI 0.94–0.98); P < 0.01]. The covariates gender and DM did not contribute to a significantly higher risk. In a multivariable analysis with the *a priori* selected clinical variables (Table 4), a low AAC score was not associated with an increased risk [HR 1.02 (95% CI 0.42–2.59); P = 0.95], while the high AAC score was associated with an increased risk for a cardiovascular event [HR 2.78 (95% CI 1.05–7.64); P = 0.04].

Prognostic performance of the AAC scoring system for cardiovascular events

The combined clinical variables, as used for the multivariable analysis, had a C-index of 0.700 (95% CI 0.631–0.770) for

Table 3. Univariate Cox regression analysis of clinical covariates

Covariates	HR (95% CI)	P-value
AAC		
Low	1.26 (0.72–2.21)	0.43
High	4.23 (2.44–7.33)	<0.01
Gender, male	1.27 (0.79–2.03)	0.32
Age, years	1.05 (1.03–1.07)	<0.01
Hypercholesterolaemia	2.06 (1.02–4.15)	<0.01
Diabetes mellitus	1.65 (0.90–3.00)	0.10
Hypertension	2.06 (1.02–4.15)	0.04
Duration pre-transplant dialysis	1.01 (1.00–1.01)	0.03
History of cardiovascular event	2.73 (1.63–4.58)	<0.01
eGFR 3 months post-transplant	0.96 (0.94–0.98)	<0.01

Table 4. Multivariate Cox regression analysis comparing the control group, low AAC and high AAC for cardiovascular events

Event	Control group HR	Low-AAC		High-AAC	
		HR (95% CI)	P-value	HR (95% CI)	P-value
Multivariate	1.00	1.02 (0.42–2.59)	0.95	2.78 (1.05–7.64)	0.04

Multivariate analysis consists of age, gender, history of DM, history of hypertension, history of hypercholesterolaemia, duration of pre-transplant dialysis, history of cardiovascular events and eGFR 3 months post-transplant.
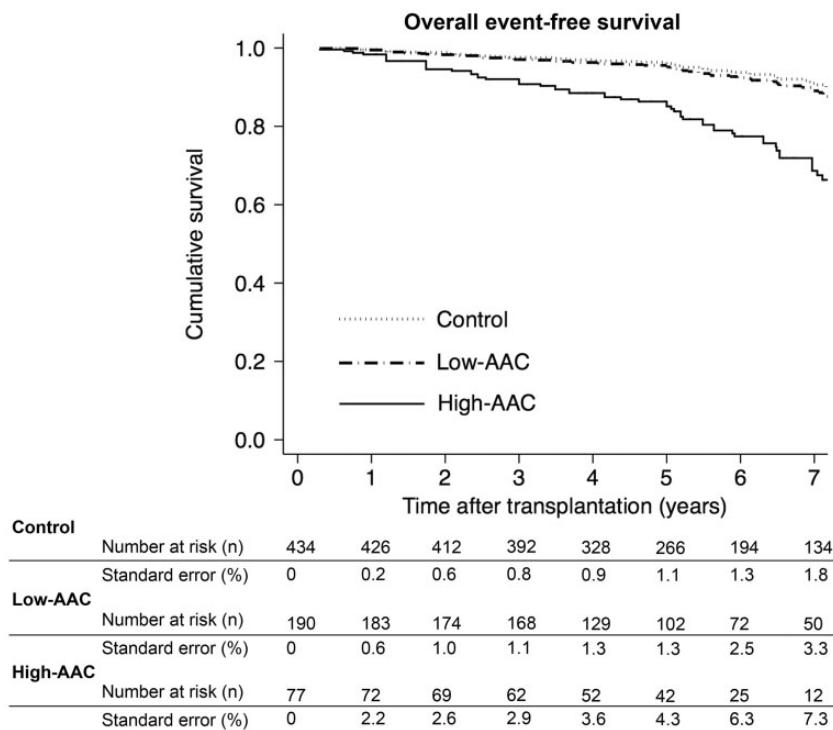

FIGURE 2: Kaplan–Meier curve for cumulative event-free survival for the control group, low AAC group and high AAC group, with a significant difference between the high AAC group and both the low AAC and control group (P < 0.01).
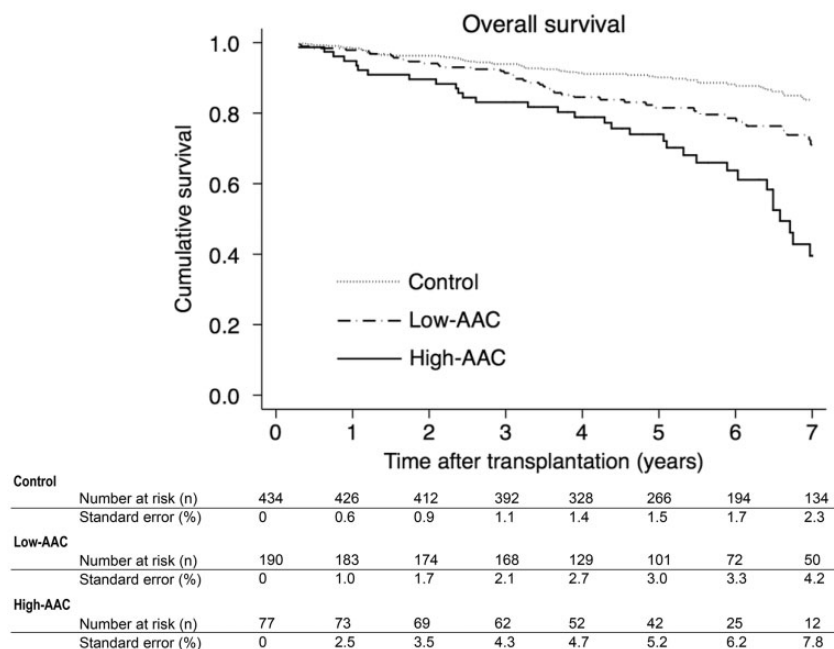
FIGURE 3: Kaplan–Meier curve for cumulative overall survival for the control group, low AAC group and high AAC group was significantly different between the three groups (P < 0.01).

estimating the risk of cardiovascular events. After implementation of the AAC scoring results, the C-index of this model increased to 0.731 (95% CI 0.663–0.800), resulting in a C-index gain of 0.031 (95% CI −0.003–0.065; P = 0.07). When investigating the change in −2 log likelihood of the clinical variable model with and without the AAC-scoring results, the −2 Log Likelihood improved significantly with AAC-scoring included in the model (from a −2 log likelihood of 505.8–494.9; P < 0.01). Assessment of the continuous NRI comparing the combined clinical variables with the model of both AAC scoring and clinical variables showed an NRI of 0.76 (95% CI 0.65–0.86; P < 0.01).

Association between AAC scoring and overall survival

Using univariate Cox proportional hazards analysis, both a low AAC score and a high AAC score were associated with a lower overall survival [HR 1.92 (95% CI 1.03–2.83); P < 0.01 and HR 4.01 (95% CI 2.62–6.12); P < 0.01, respectively]. The cumulative overall survival for the control group, low AAC group and high AAC group was significantly different between the three groups (P < 0.01) (Figure 3). Additional covariates with a significantly higher risk were age [HR 1.07 (95% CI 1.05–1.09); P < 0.01], hypercholesterolaemia [HR 1.82 (95% CI 1.30–2.55); P < 0.01], DM [HR 1.68 (95% CI 1.09–2.57); P = 0.02], duration of pre-transplant dialysis [HR 1.01 (95% CI 1.00–1.01); P < 0.01], history of cardiovascular events [HR 2.84 (95% CI 1.97–4.09); P < 0.01] and eGFR at 3 months posttransplantation [HR 0.97 (95% CI 0.96–0.98); P < 0.01]. The covariates gender and history of hypertension did not contribute to a significantly higher HR. In a multivariable analysis including the *a priori* selected clinical variables, the association between a low AAC score and all-cause mortality was not significant [HR 1.46 (95% CI 0.84–2.57); P = 0.19], while the

association with a high AAC score remained significant [HR 2.64 (95% CI 1.28–4.98); P < 0.01].

DISCUSSION

This study shows an association between a high AAC score, acquired on DXA for routine clinical evaluation of bone density, and both cardiovascular events and overall survival after kidney transplantation. The independent association between a high AAC score and cardiovascular events provides an opportunity for early cardiovascular risk stratification in renal transplant recipients. In patients with a high risk for cardiovascular events, interventions such as lipid control, treatment of hypertension and anti-platelet therapy could possibly lead to a risk reduction [14]. In addition, a cumulative incidence of cardiovascular events after transplantation of 26.0% urges for a more thorough cardiovascular follow-up in patients with a high AAC score.

The wide accessibility, extended experience and very low radiation burden (<0.01 mSv) contribute to the routine use of DXA procedures after kidney transplantation. Due to the KDIGO 2017 clinical practice guideline recommendation to evaluate bone density in the first 3 months following transplantation, a wide range of renal transplant recipients are eligible for DXA procedures [6]. Therefore, implementation of this 8-point AAC scale system will not lead to a greater patient burden while it provides the opportunity for early cardiovascular risk stratification and prevention.

Several previous studies have described the use of DXA images for the assessment of AAC in the general population. These studies reported a low interobserver variation, with an intra-class correlation coefficient of 0.9 (95% CI 0.8–0.9), and a good intra-observer reliability, with an intraclass correlation coefficient of 0.9 (95% CI 0.8–0.9) [15, 18]. With a significant association between cardiovascular events and both low AAC

scores [HR 4.2 (95% CI 1.2–15.4); P = 0.03] and high AAC scores [HR 6.1 (95% CI 1.7–21.6); P < 0.01] [8], the association between a high AAC score and cardiovascular events in our study is comparable with these earlier outcomes. However, we did not find an association between cardiovascular events and a low AAC score. In addition, it has been shown that in dialysis patients there is a significant correlation between aortic calcification as assessed using DXA images and aortic calcification as assessed using CT images [19].

We found a significant association between a high AAC score assessed by DXA scoring and future development of cardiovascular events in renal transplant recipients, which is in line with results of earlier studies in which AAC was assessed by other methods (i.e. CT and lumbar X-ray) [9, 12]. Similar results were seen for the association between coronary artery calcification and cardiovascular events in renal transplant recipients with increased coronary calcification [10, 11]. Our results with regard to overall survival were comparable to those of a study in which iliac artery calcification was assessed by means of CT, wherein 3-year survival in the non-calcification group was 94% versus 87% in the calcification group [13].

A study of pre-transplantation evaluation modalities and the occurrence of cardiovascular events within 1 year after transplantation reported a significant association with a history of cardiovascular events [HR 2.06 (95% CI 1.1–4.0); P = 0.03], echocardiographic left ventricular hypertrophy [HR 2.04 (95% CI 1.0–4.0); P = 0.04] and abnormal myocardial perfusion testing [HR 2.25 (95% CI 1.1–6.0); P = 0.03]. The pre-transplantation risk stratification methods showed that HRs were comparable to the AAC score. However, these were associations with cardiovascular events within 1 year after transplantation, whereas AAC scores were associated with events within a median follow-up of 5.4 (IQR 3.9–7.2) years.

When assessing the prognostic performance of AAC scoring for cardiovascular events, the Harrell's C-index was higher for the clinical variable model with AAC scoring included compared with the model without these scores. This difference did not reach statistical significance (P = 0.07). It should be realized, however, that Harrell's C-statistics are relatively insensitive for detection of differences between models. To avoid injudicious discarding of otherwise promising markers, it is currently recommended to perform additional analyses in which −2 log likelihood ratios are compared [20]. Using the −2 log likelihood analyses, we observed a significant (P < 0.01) improvement of the model with the AAC scoring included. When comparing the clinical variable model with the model of both AAC scoring and clinical variables, the NRI was 0.76 (95% CI 0.65–0.86), indicating an improved prognostic performance when implementing AAC scoring.

Some limitations of our study need to be addressed. First, the retrospective design of this study creates the risk of an underestimation of the total number of cardiovascular events due to incomplete patient records. The incidence of cardiovascular events is low compared with the literature in which a cumulative incidence of 11.1% for MI and 6.8% for CVA or TIA is reported at 36 months after kidney transplantation [2, 3]. The low rate of pre-transplantation DM (13.4%), compared with 44.7% in the study mentioned before, could be seen as an explanation for this discrepancy [2]. Thus we do not expect that this has altered our results since most patients were regularly seen in our clinic and the analysis has been based on a between-group comparison. Second, the study only included patients from a single centre. Therefore the presented results are not necessarily generalizable to a broader population of renal transplant recipients. Third, the patients in the high AAC group had a significantly greater baseline risk for future cardiovascular events compared with the control group, with a longer duration of pre-transplant dialysis, a higher age and a higher percentage of patients with pre-transplant DM and hypercholesterolaemia. We performed a multivariable analysis, adjusting for these clinical variables, and we present the predictive value of both the clinical variables alone and the combination of AAC scoring and clinical variables. Both these statistical analyses showed the independent value of AAC scoring after transplantation. Fourth, the number of patients in the high AAC group was small, hindering the inclusion of more clinical covariates in the performed multivariable analyses. Furthermore, the number of fatal cardiovascular events was low. These two factors for a comprehensive statistical analysis and the reason of death could not be defined for 22.8% of patients who died within 36 months after transplantation and for 40.9% of patients who died during the entire follow-up. Nevertheless, we have shown a significant association in a large cohort of renal transplant recipients, with no signs of selection bias.

In conclusion, an independent association between a high AAC score and cardiovascular events was identified. The combination of this DXA scoring methodology for AAC and the described clinical variables can be useful for early post-transplantation cardiovascular risk stratification and prevention. Due to the routine use and wide availability of DXA for evaluation of bone density after transplantation, AAC scoring can be added as a low-cost and easy-to-implement method for cardiovascular screening.

AUTHORS' CONTRIBUTIONS

S.B. acquired the data and was involved in data analysis and interpretation and writing the manuscript. R.A.P. was involved in data interpretation and contributed to the final adjustments to the manuscript after revising it critically for intellectual content. A.W.J.M.G. was involved in data interpretation and contributed to the final adjustments to the manuscript after revising it critically for intellectual content. I.W. was involved in acquiring the data and contributed to the final adjustments to the manuscript after revising it critically for intellectual content. S.P.B. was involved in data interpretation and contributed to the final adjustments to the manuscript after revising it critically for intellectual content. S.J.L.B. and R.H.J.A.S. initiated the study and contributed to the final adjustments to the manuscript after revising it critically for intellectual content.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest. The authors declare that the results presented in this paper have not been published previously in whole or part, except in abstract format.

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