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## Association between aortic calcification, cardiovascular events, and mortality in kidney and pancreas-kidney transplant recipients

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1 **Association between aortic calcification, cardiovascular events and mortality in kidney**  
2 **and pancreas-kidney transplant recipients.**

3

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34 **Abstract**

35 **Background:** Cardiovascular (CV) disease is the leading cause of death in kidney and  
36 simultaneous pancreas-kidney (SPK) transplant recipients. Assessing abdominal aortic  
37 calcification (AAC), using lateral spine x-rays and the Kaupilla 24-point AAC (0-24) score,  
38 may identify transplant recipients at higher CV risk.

39 **Methods:** Between the years 2000-2015, 413 kidney and 213 SPK first transplant recipients  
40 were scored for AAC at time of transplant and then followed for CV events (coronary heart,  
41 cerebrovascular or peripheral vascular disease), graft-loss and all-cause mortality.

42 **Results:** The mean age was  $44 \pm 12$  years (SD) with 275 (44%) having AAC (26% moderate:  
43 1-7 and 18% high:  $\geq 8$ ). After a median of 65 months (IQR 29-107 months), 46 recipient's  
44 experienced CV events, 59 died and 80 suffered graft loss. For each point increase in AAC,  
45 the unadjusted hazard ratios (HR) for CV events and mortality were 1.11 (95% CI 1.07-1.15)  
46 and 1.11 (1.08-1.15). These were similar after adjusting for age, gender, smoking, transplant  
47 type, dialysis vintage and diabetes: aHR 1.07 (95% CI 1.02-1.12) and 1.09 (1.04-1.13). For  
48 recipients with high versus no AAC, the unadjusted and fully-adjusted HR for CV events  
49 were 5.90 (2.90-12.02) and 3.51 (1.54-8.00), for deaths 5.39 (3.00-9.68) and 3.38 (1.71-6.70),  
50 and for graft loss 1.30 (0.75-2.28) and 1.94 (1.04-3.27) in age and smoking history-adjusted  
51 analyses.

52 **Conclusion:** Kidney and SPK transplant recipients with high AAC have 3-fold higher CV  
53 and mortality risk and poorer graft outcomes than recipients without AAC. AAC scoring may  
54 be useful in assessing and targeted risk-lowering strategies.

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57 **KEY WORDS;** vascular calcification, cardiovascular disease, kidney transplant,  
58 simultaneous pancreas-kidney transplant, mortality.

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## 67 **Introduction**

68 Patients with chronic kidney disease (CKD) have increased atherosclerosis, large-vessel  
69 remodelling or arteriosclerosis[1], a propensity for arterial calcification[2] and substantially  
70 higher risks for cardiovascular (CV) events[3]. Although kidney transplant recipients have  
71 improved survival compared to patients on dialysis, they nevertheless have 3-5 times higher  
72 CV risk than people from the age-matched general population[4,5]. For patients undergoing  
73 simultaneous pancreas-kidney (SPK) transplantation, this risk may be even greater due to the  
74 cumulative vascular burden associated with type 1 diabetes mellitus and end-stage kidney  
75 disease (ESKD)[6]. Age and prior CV events are predictive of outcomes[7], but further  
76 means to stratify risk and to target treatment strategies are poorly understood.

77 In the general population abdominal aortic calcification (AAC) is commonly present as focal  
78 intimal or atherosclerotic calcifications[8,9] and is associated with increased cardiovascular  
79 (CV) risk[10]. The development and progression of AAC is a dynamic, actively regulated  
80 process, and is commonly described in patients with ESKD [11,12], in whom the process of  
81 vascular calcification is exacerbated by abnormal mineral metabolism and alterations to local  
82 and systemic inhibitors of calcification[13]. By the time patients require kidney or SPK  
83 transplantation, endothelial dysfunction and atherosclerosis is common, with 50-60% having  
84 detectable vascular calcification [6,7,14]. Even after kidney transplantation, calcification of  
85 the thoracic aorta and coronary arteries progresses at median rates of 4 and 11%, respectively,  
86 per year[15].

87 AAC can be assessed from lateral spine X-rays that include the abdominal aorta, or from  
88 dual-energy X-ray absorptiometry (DXA) lateral spine images, using the Kauppila AAC-24  
89 point semi-quantitative scale[16]. For this scoring method, the anterior and posterior walls of  
90 the aorta are divided into 4 regions corresponding to the L1-L4 vertebrae (8 segments). Each  
91 segment is scored zero if there is no calcification, 1 for  $\leq 1/3^{\text{rd}}$  of the segment calcified, 2 for  
92  $1/3$  to  $\leq 2/3^{\text{rds}}$  calcified, and 3 for  $> 2/3^{\text{rds}}$  calcified. Scores for the 8 segments are then added  
93 for a maximum score of 24/24. In a study of 531 patients admitted for kidney (69%) and  
94 SPK (31%) transplantation[7], AAC was significantly associated with age, dialysis vintage,  
95 smoking, pre-existing vascular disease, and with diabetes for patients undergoing kidney only  
96 transplants[7].

97 To date, a number of studies have reported associations of AAC to cardiovascular risk and  
98 mortality. These include a retrospective study of 253 patients with 3 years median follow up  
99 [6], a study using dual-energy X-ray absorptiometry for AAC assessment [17], a study of 134

100 patients commencing 7 to 9 years after transplantation [18] and a retrospective study of 119  
101 patients, which reported ACC was not predictive of patient survival in multivariable analyses  
102 [19]. To date, no study has reported on the association of AAC with cardiovascular or  
103 mortality outcomes in SPK recipients.

104 The primary aim of this study was to determine whether AAC scores at the time of kidney or  
105 SPK transplantation were associated with cardiovascular events and all-cause mortality. A  
106 secondary aim was to determine the association of AAC with death-censored kidney and/or  
107 pancreas graft loss.

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## 133 **Materials and Methods**

134 The manuscript complies with the STROBE reporting guidelines for observational studies  
135 [20].

### 136 **Statement of ethics**

137 The study was approved by the Western Sydney Local Health District Human Research  
138 Ethics Committee (LNR/13/WMEAD/64) and complies with the ‘Declaration of Istanbul on  
139 Organ Trafficking and Transplant Tourism’.

### 140 **Study population**

141 Recruitment of this cohort has been described previously [7]. Briefly, a lateral lumbar spine  
142 X-ray including the abdominal aorta was performed within four weeks of transplantation on  
143 695/900 (77%) consecutive patients admitted to Westmead Hospital, Sydney, for kidney or  
144 SPK transplantation between 2000 and 2015. Patients without a lateral spine X-ray included  
145 those with post-operative complications, those who returned soon after transplantation to  
146 their referring renal unit and those who did not attend their scheduled visit to the clinic. Only  
147 first transplant recipients were included and patients with suspected or proven CV disease  
148 (coronary artery disease, cerebrovascular disease or peripheral vascular disease) and heart  
149 failure were excluded. The final dataset consisted of 623 transplant recipients (**Figure 1A**).

150

### 151 **Study population demographics and comorbidities**

152 Pre-transplant demographic and clinical data was collected directly from transplant recipients  
153 during their clinic visit within four weeks of transplant, and included body mass index (BMI),  
154 intercurrent illnesses including diabetes (none, type I and type II), information on smoking  
155 status (never, former and current) and pre-transplant medications. Prevalent co-morbidities  
156 and racial background were obtained through data linkage to the Australia and New Zealand  
157 Dialysis and Transplant (ANZDATA) registry. Blood was collected within the 24 hours  
158 preceding transplantation for routine biochemistry, hormonal studies including intact-  
159 parathyroid hormone (PTH) and bone turnover markers as described previously[7].

160

### 161 **Abdominal aortic calcification 24 scores (AAC24)**

162 Lateral spine X-rays (example in **Figure 1B**) including the abdominal aorta were performed  
163 within 4 weeks of transplantation. AAC was quantified using an established 24 point scoring  
164 scale [16], assigned prospectively by an experienced clinician (GJE, MVD) at the immediate  
165 post-transplant consultation, with high blinded inter-observer consistency (Spearman’s

166 rho=0.91, P<0.001), as described previously [7]. Suspected and proven CV disease was an  
167 exclusion criterion for this study. AAC was examined in three ways; i) semi-continuous  
168 variable on the AAC 24 point scale, ii) present vs. absent and iii) using previously published  
169 cut points from a prior study of kidney transplant recipients of no AAC, moderate AAC  
170 (score 1-7) and high AAC (score 8-24), respectively [6].

171

### 172 **Cardiovascular events and all-cause mortality**

173 The primary endpoints were CV events (coronary artery disease, cerebrovascular disease or  
174 peripheral vascular disease) and/or deaths (events) and all-cause mortality obtained from  
175 ANZDATA. For CV events, patients are classified as ‘yes’, ‘no’ or ‘suspected’. Patients with  
176 ‘suspected’ or confirmed CV or a CV-related death after transplantation were deemed to have  
177 an incident CV event. Time to event data was calculated from transplant date to date of first  
178 CV event, date of death or the 31<sup>st</sup> of December 2015, whichever came first. Death-censored  
179 kidney graft loss, death-censored pancreas graft loss and any death-censored graft loss  
180 (kidney and/or pancreas) was considered a secondary outcome. Data on kidney and pancreas  
181 graft outcomes was obtained from the ANZDATA or the Australian and New Zealand Islet  
182 and Pancreas Transplant Registries and graft failure dates corresponded to dates of graft  
183 removal or return to dialysis (kidney) or the use of insulin more than 0.5 units per kg per day  
184 over a period of 90 consecutive days (pancreas).

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### 187 **Statistical analysis**

188 Baseline data are presented as mean  $\pm$  SD, median and IQR or number and (%) where  
189 appropriate. The association of the presence of AAC (yes or no) or AAC24 severity (none,  
190 moderate or high) with incident CV events (hospitalization or death) was assessed in  
191 unadjusted and multivariable-adjusted Cox proportional hazards regression analyses. For the  
192 primary analyses for CV events, we treated deaths as censored. For the primary analyses with  
193 all-cause mortality we did not censor for graft loss. As a further analysis we censored at the  
194 time of kidney graft failure (death with a functioning kidney graft outcome, n=44 or death  
195 with any functioning kidney or pancreas graft outcome, n=41). To avoid overfitting models  
196 with low event rates (n=41-60), we performed multivariate Cox proportional hazards  
197 regression analyses adjusted for either age, or age, gender, smoking, transplant type, dialysis  
198 vintage and diabetes. In addition, multivariable models for graft loss included donor factors;

199 organ source (deceased or living), age and ischemia time (hours). For secondary outcomes of  
200 kidney graft loss or any graft loss (kidney and/or pancreas) we undertook forward stepwise  
201 Cox regression with age, gender, smoking history, transplant type, dialysis vintage, diabetes,  
202 organ source, donor age, ischemia time and HLA mismatch (0-6, number). The final model  
203 for both kidney and any graft loss (kidney and/or pancreas) included age and smoking  
204 history. We tested for linear trends across AAC categories by using the median AAC24 value  
205 for each category (low = 0, moderate = 3, or high = 11). Additionally, dose-response  
206 relationships between AAC scores and cardiovascular disease events and all-cause mortality  
207 outcomes were examined with restricted cubic splines [21]. Given the high risk of non-CV  
208 death, we performed competing-risks analyses based on Fine and Gray's proportional sub-  
209 distribution hazards model [22]. Cox proportional hazards assumptions were tested with the  
210 Schoenfeld residuals. No violations of the Cox proportional hazards assumptions were  
211 detected. Analyses were undertaken using SPSS Version 22 (2012, Armonk, NY: IBM Corp),  
212 STATA Version 13 (StataCorp LP, College Station, TX) or R (R Foundation for Statistical  
213 Computing, Vienna, Austria).

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## 228 **Results**

### 229 **Cohort characteristics**

230 An overview of the study recruitment is provided in **Figure 1A**. The median follow up of  
231 transplant recipients was 65 months (IQR 29-107 months). SPK recipients were younger, had  
232 lower BMI, shorter dialysis vintage and were less ethnically diverse, all  $P < 0.05$ , (**Table 1**).  
233 Medication data was available in 440 recipients. The percentage of patients prescribed  
234 calcium-based phosphate binders, calcitriol and cinacalcet prior to transplant was 75.9%,  
235 44.6% and 5.2% respectively. There were no statistically significant differences in the risk of  
236 CV events (7.5% vs. 7.3%,  $P = 0.930$ ) or deaths (10.3% vs. 9.0%,  $P = 0.598$ ) between SPK and  
237 kidney transplant recipients. AAC24 scores were skewed in both the kidney transplant and  
238 the SPK transplant recipients.

239

### 240 **Presence of AAC and cardiovascular events and all-cause mortality**

241 Of 623 kidney and SPK transplant recipients, 275 (44%) had detectable and 348 (66%) had  
242 no detectable AAC at time of transplantation. For transplant recipients with no detectable  
243 AAC, 3.7% had a CV event while 5.5% died of any cause. For transplant recipients with  
244 detectable AAC, 12.0% had a CV event while 14.5% died of any cause. Kaplan Meier  
245 survival plots for time to CV events and to death are presented in **Figure 2 a & b**. When  
246 investigating kidney and SPK transplant recipients separately, similar findings were seen  
247 (**Figure 3 a-d**). No interaction was observed between AAC24 scores and transplant type for  
248 CV events ( $P = 0.578$ ) or all-cause mortality ( $P = 0.153$ ). Transplant recipient groups were  
249 therefore combined for the main analyses.

250

### 251 **AAC scores as continuous measures**

252 In unadjusted analyses for each 1 point increase in AAC24 score there was a 11% increase in  
253 risk of CV events (HR 1.11 95%CI [1.07-1.15],  $P < 0.001$  and all-cause mortality HR 1.11  
254 95%CI [1.08-1.15],  $P < 0.001$ ). For each 1 point increase in AAC24 score there was a 7-8%  
255 increase in age and multivariable-adjusted risk of CV events (age-adjusted HR 1.08 95%CI  
256 [1.04-1.13],  $P < 0.001$  and multivariable-adjusted HR 1.07 95%CI [1.02-1.12],  $P = 0.005$ ).  
257 Similarly, for each 1 point increase in AAC24 score there was an 8-9% increase in age and  
258 multivariable-adjusted risk of all-cause mortality (age-adjusted HR 1.09 95%CI [1.06-1.13],  
259  $P < 0.001$  and multivariable-adjusted HR 1.08 95%CI [1.04-1.13],  $P < 0.001$ ). In the  
260 multivariable-adjusted restricted cubic spline regression model there was an increasing

261 gradient of estimated CV event and all-cause mortality risk with increasing AAC24 scores  
262 (Figure 4 a & b).

263

#### 264 **Presence of AAC and CV events and all-cause mortality**

265 In recipients with no AAC, the rate of CV events was 6.2, 95%CI (3.6-10.7) per 1000 person  
266 years and deaths was 9.0, 95%CI (5.7-14.1) per 1000 person years. By comparison, in  
267 recipients with AAC, the rates of CV events and deaths were 21.3, 95%CI (15.1-29.9) and  
268 24.7, 95%CI (18.1-33.6) respectively. In univariate analysis recipients with any AAC had a  
269 HR of 3.50 95%CI (1.82-6.58) for CV events and 2.80 95%CI (1.62-4.83) for all-cause  
270 mortality (both P<0.001). In age and multivariable-adjusted analyses the presence of AAC  
271 was associated with at least twice the relative hazard of having a cardiovascular event or  
272 dying from any cause (Table 2).

273

#### 274 **Severity of AAC and cardiovascular events and all-cause mortality**

275 Of the 623 patients, 348 (55.9%) had no AAC, 162 (26.0%) had moderate AAC (score 1-7)  
276 and 113 (18.1%) had high AAC (score 8-24). Using these cut points, Kaplan Meier survival  
277 estimates for CV events and all-cause mortality by the severity of AAC are presented in  
278 **Supplementary Figure 1**. The rate of CV events per 1000 person years was 6.2, 95%CI (3.6-  
279 10.7) in recipients with no AAC, 14.3, 95%CI (8.5-24.1) in recipients with moderate AAC  
280 and 33.2, 95%CI (21.2-52.1) in recipients with high AAC. The rate of all-cause mortality per  
281 1000 person years was 9.0, 95%CI (5.7-14.1) in recipients with no AAC, 11.9, 95%CI (6.7-  
282 20.9) in recipients with moderate AAC and 45.8, 95%CI (31.7-66.4) in recipients with high  
283 AAC. In unadjusted Cox proportional hazards analysis, transplant recipients with high AAC  
284 had 5-6 times higher relative hazards of CV events and all-cause mortality (5.90 95%CI [2.9,  
285 12.02] and 5.39 95%CI [3.00-9.68], both P<0.001). Recipients with moderate AAC had  
286 higher relative hazards for CV events than those with no AAC HR 2.22 95%CI (1.04-4.73),  
287 P=0.039. In age-adjusted models, transplant recipients with high AAC had at least 3 times  
288 the relative hazards for CV events and all-cause mortality compared to recipients with no  
289 AAC. This remained significant in multivariable-adjusted models (Table 2).

290

#### 291 **Graft loss**

292 Overall 80 recipients lost any graft, comprising 42 [10.2%] of kidney recipients and 38  
293 [17.8%] of SPK recipients, (P=0.007). Kidney graft loss occurred in 60 transplant recipients  
294 (kidney only 42 [10.2%] and SPK 18 [8.5%], P=0.567), while 20 SPK recipients lost their

295 pancreas graft and 4 lost both the pancreas and kidney. There was no significant increase in  
296 age-adjusted risk for death-censored a) kidney, b) pancreas, or c) any graft loss graft loss per  
297 1 point increase in AAC24 score (age-adjusted HR 1.04 95%CI [0.99-1.09], P=0.136, HR  
298 1.03 95%CI [0.94-1.12], P=0.552 and HR 1.04 95%CI [1.00-1.09], P=0.069, respectively).  
299 This was similar in multivariable-adjusted analyses. For transplant recipients with detectable  
300 versus no detectable AAC, there was no difference in graft loss in unadjusted (data not  
301 shown) age-adjusted or multivariable-adjusted analyses (**Supplementary Table 1**). In  
302 unadjusted analyses recipients with the most severe AAC (AAC24  $\geq$ 8) did not have increased  
303 relative hazards for graft loss compared to those with no AAC (HR 1.30 95%CI [0.75-2.28],  
304 P=0.351). However in age and smoking history-adjusted analyses, recipients with severe  
305 AAC had 1.9 times higher relative hazard for graft loss compared to those with no AAC  
306 (**Supplementary Table 1**).

307

### 308 **Further analyses**

309 When death from any non-CV cause was considered in competing risk analyses for CVD  
310 outcomes, findings were similar to the primary analyses (**Supplementary Table 2**).

311 Similarly, when death from any cause was included in competing risks analyse for kidney  
312 graft loss or any graft loss, there was no alteration of the overall results (**Supplementary**  
313 **Table 2**). Finally, including death with a functioning kidney graft or death with any  
314 functioning graft in analyses, strengthened the observed association between AAC and  
315 mortality (severe AAC vs no AAC, aHR 4.42 [2.04-9.62], p<0.001).

316

317

## 318 **Discussion**

319 This prospective study highlights several important and novel findings. Firstly, for  
320 patients undergoing kidney and SPK transplantation, the presence and the severity of AAC  
321 was associated with CV events and mortality, with no significant differences between kidney  
322 and SPK transplant recipients over time. Secondly, we report a continuous, positive  
323 relationship of AAC scores to cardiovascular risk and mortality. Thirdly, we report for the  
324 first time that severe AAC is associated with kidney and/or pancreas graft loss. High AAC  
325 scores may be surrogates for reduced graft perfusion or a greater propensity for vascular  
326 damage.

327 Conventional risk factors that are markers of pathological processes leading to blood  
328 vessel damage consistently underestimate CV risk [23] or do not improve net reclassification  
329 compared to age, gender and eGFR in kidney transplant recipients[24]. Vascular calcification  
330 on the other hand is a structural measure of the amount of damage to the blood vessel and has  
331 been shown to be strongly associated with increased risk of CV events[6,17]. The increased  
332 risks in this study were particularly evident in those with AAC24 scores  $\geq 8$ , similar to some  
333 earlier reports of transplant recipients. However, unlike those studies, we excluded people  
334 with prior transplantation or a history of CV disease, both of which could influence the  
335 accuracy of AAC to predict CV outcomes.

336 In a retrospective study of 253 kidney transplant recipients [6], the authors reported a  
337 relative HR of 1.09 per 1 point increase in AAC24 score with a univariate HR of 3.1 in  
338 kidney transplant recipients with high AAC. In a retrospective study of 119 older kidney  
339 transplant recipients [19], AAC24 scores  $>2$  were associated with a relative risk of 3.8 for CV  
340 events, compared to those with AAC24 scores of 0-2. A study of 701 kidney transplant  
341 recipients using DXA images to assess AAC[17], found that after adjusting for CV risk  
342 factors, transplant recipients with higher AAC had an increased relative hazard of 2.78 for  
343 cardiovascular events, compared to recipients with no AAC. Taken together, these data and  
344 the current study support the concept that transplant recipients with AAC, and particularly  
345 those with high AAC scores, are at high risk for future CV events.

346 The Kidney Disease Improving Global Outcomes (KDIGO) CKD-MBD guidelines suggest  
347 that a lateral abdominal radiograph can be used to detect the presence or absence of vascular  
348 calcification in patients with CKD G3a–G5D, and that patients with known vascular  
349 calcification are at highest CV risk[25]. However, neither the KDIGO CKD-MBD nor  
350 transplantation guidelines[26] comment on the value of AAC screening to identify

351 individuals at high CV risk at the time of transplantation. While lateral spine radiographs are  
352 relatively low radiation, equivalent to approximately 20 days of natural background radiation  
353 [27], lateral DXA may also be used to evaluate AAC, with a much lower radiation  
354 exposure[24].

355         Because capturing these images is safe, simple and inexpensive, this study supports  
356 AAC screening in patients undergoing kidney or SPK transplantation. Once identified,  
357 patients at greatest risk could be targeted for intensive modification of ‘traditional’ CV risk  
358 factors, which may have greater impact on outcomes once the calcifying ESKD milieu  
359 improves. In addition, these patients could be targeted to assess modifications in the  
360 prescription of calcium and activated vitamin D, the addition of supplemental vitamin K or  
361 magnesium, and introduction of drugs with potential to delay progressive vascular  
362 calcification. Patients with high AAC scores might also be considered for more intensive  
363 monitoring of graft function

364         For patients with type 1 diabetes, successful SPK transplantation removes the need for  
365 insulin injections, restores normal glycaemic control and reverses systemic microvascular  
366 structural abnormalities [28]. SPK recipients have slower progression of coronary  
367 atherosclerosis (minimum obstruction diameter loss 0.037 mm/year vs 0.061 mm/year).  
368 When patients with type 1 diabetes undergoing SPK or kidney-only transplantation are  
369 compared, SPK recipient have greater regression of atherosclerosis (38% vs. 0%) [29].  
370 Compared to kidney-only transplantation, these SPK recipients have improved long-term  
371 survival, predominantly from 5 years after transplantation [30]. However, we observed  
372 similar associations between AAC scores and incident CV events and mortality in both  
373 kidney-only and SPK recipients, suggesting that pre-transplant vascular damage is a major  
374 driver of later CV and mortality risk. Therefore strategies to monitor and intervene early to  
375 reduce pre-transplant AAC are warranted. Furthermore, our findings support the concept that  
376 AAC is a stable marker of long-term CV and all-cause mortality risk in both groups of  
377 transplant recipients.

378         This study has some limitations and a number of strengths. Firstly, AAC prevalence in  
379 this study is likely to underestimate that of older dialysis patients, patients with type 2  
380 diabetes mellitus or prevalent CV disease, who are often excluded from transplantation.  
381 Therefore our findings may underestimate the association of AAC, CV events and mortality  
382 in the general dialysis population and for transplant recipients with pre-existing CV disease.  
383 As indicated in the methods, some transplanted patients were not included in these analyses,  
384 which may have introduced selection bias. Also, the relatively young age of these kidney and

385 SPK recipients resulted in a low rate of CV events and deaths. Furthermore, we did not  
386 collect or adjust for pre-transplant CV risk factors such as low density lipoprotein cholesterol  
387 or CV medication use such as statins, which may have attenuated the relationship between  
388 AAC and CV events. Due to patient numbers, we undertook minimally adjusted models for  
389 associations of AAC to graft survival that did not include induction therapy or  
390 immunosuppressive medications and pre-transplant laboratory data. Including these may have  
391 attenuated the observed associations. Finally, our findings may not be generalisable to studies  
392 using other imaging modalities or scoring systems.

393 Study strengths include it being the largest and first prospective study using standard  
394 X-ray to assess patient and graft outcomes, and the first to include a large cohort of SPK  
395 recipients. Secondly, we describe the continuous, positive relationship between AAC scores  
396 and cardiovascular event and all-cause mortality risk. Additional strengths include  
397 comprehensive, accurate pre and post-transplant data collection in the clinic and in real time  
398 by the ANZDATA Registry. Finally, this is the first study to perform competing risks  
399 analysis or death with a functioning graft analyses.

400 In conclusion, in a large cohort of kidney and SPK transplant recipients, AAC on  
401 lateral spine radiographs identified transplant recipients at high risk of cardiovascular events  
402 and all-cause mortality. For secondary outcomes, recipients with severe AAC at  
403 transplantation also had significantly poorer graft outcomes compared to those with no AAC.  
404 In patients with high AAC scores detected at the time of kidney or SPK transplantation,  
405 future studies should evaluate whether, compared to usual care, intensive interventions aimed  
406 to reduce CV risk and progressive vascular calcification might impact incident CV events and  
407 mortality.

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424 **Data sharing statement:** Due to ethical restrictions related to patient privacy, raw data are  
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426 Elder g.elder@garvan.org.au.

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432 GW, MVD and JL. Data analysis: JL. Data interpretation: GE, GW and JL. Draft of the  
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434 version of the submitted manuscript. JL takes responsibility for the integrity of the data.

**Table 1.** Baseline characteristics of the study population stratified by abdominal aortic calcification (AAC) scores.

	<b>Whole cohort* (n=623)</b>	<b>Kidney only (n=410)</b>	<b>Simultaneous pancreas- kidney (n=213)</b>
<b>Age, years, mean ± SD</b>	44.1 ± 11.9	47.0 ± 12.8	38.5 ± 7.0
<b>Gender, (%)</b>			
<b>Female (%)</b>	257 (41.3)	161 (39.3)	96 (45.1)
<b>Male (%)</b>	366 (58.7)	249 (60.7)	117 (54.9)
<b>Body mass index, kg/m<sup>2</sup>, mean ± SD†</b>	25.9 ± 4.4	26.6 ± 4.6	24.5 ± 3.5
<b>Dialysis type, number (%)**</b>			
<b>Haemodialysis (%)</b>	329 (53.8)	230 (57.1)	99 (47.6)
<b>Peritoneal dialysis (%)</b>	185 (30.3)	119 (29.5)	66 (31.7)
<b>Pre-emptive (%)</b>	97 (15.9)	54 (13.4)	43 (20.7)
<b>Dialysis vintage, months, median [IQR]</b>	20.0 [6.0-48.0]	29.0 [9.0-60.5]	12.0 [3.0-30.0]
<b>Ethnicity, number (%)</b>			
<b>Asian (%)</b>	80 (12.8)	77 (18.8)	3 (1.4)
<b>Caucasian (%)</b>	496 (79.6)	297 (72.4)	199 (93.4)
<b>Other (%)</b>	47 (7.5)	36 (8.8)	11 (5.2)
<b>Smoking history, number (%)‡</b>			
<b>Never (%)</b>	389 (63.1)	261 (64.1)	128 (61.2)
<b>Previous (%)</b>	180 (29.2)	114 (28.0)	66 (31.6)
<b>Current (%)</b>	47 (7.6)	32 (7.9)	15 (7.2)
<b>Diabetes, number (%)</b>			
<b>Never, (%)</b>	352 (56.5)	352 (85.9)	0 (0)
<b>Type I (%)</b>	225 (36.1)	12 (2.9)	213 (100)
<b>Type II (%)</b>	46 (7.4)	46 (11.2)	0 (0)
<b>Calcium, (mmol/L), median [IQR]</b>	2.30 [2.19-2.43]	2.30 [2.19-2.44]	2.31 [2.21-2.39]
<b>Phosphorus, (mmol/L), median [IQR]</b>	1.60 [1.28-1.95]	1.55 [1.24-1.89]	1.69 [1.37-2.02]
<b>Parathyroid hormone, (pmol/L), median [IQR]</b>	34.2 [15.5-63.8]	35.8 [14.5-70.7]	32.7 [18.0-51.7]
<b>Alkaline phosphatase, (U/L), median [IQR]</b>	102 [75-145]	92 [70-138]	120 [93-154]
<b>Donor factors</b>			
<b>Donor age, years, mean ± SD</b>	41.3 ± 16.0	48.5 ± 13.7	27.3 ± 9.4
<b>Donor ischemia time, hours, mean ± SD</b>	8.4 ± 4.7	7.5 ± 5.1	10.4 ± 2.8
<b>Donor type</b>			



<b>Deceased donor,</b>	421 (67.6)	208 (50.7)	213 (100)
<b>Living donor,</b>	202 (32.4)	202 (49.3)	0 (0)
<b>HLA-mismatches</b>	3.6 ± 1.8	3.2 ± 1.8	4.3 ± 1.2

Data expressed as mean ± SD, median [IQR=interquartile range] or number and (%). \* For patients undergoing simultaneous pancreas-kidney transplants, the cause of ESKD was type 1 diabetes mellitus. For patients undergoing kidney only transplants, the most common causes of ESKD were glomerulonephritis, polycystic kidney disease and vesicoureteric reflux.

†Data available for 488 recipients. \*\*Data available for 611 recipients. ‡Data available for 616 recipients.

**Table 2.** Cox regression for CV events or all-cause mortality stratified by the presence and severity of abdominal aortic calcification (AAC).

	Number (%)	Age-adjusted HR (95% CI)	P value	Multivariable-adjusted HR (95% CI)*	P value
<b>Presence of AAC (any)</b>					
<b>Cardiovascular event</b>	46				
No AAC (AAC24 score 0)	13/348 (3.7)	1 (reference)		1 (reference)	
Any AAC (AAC24 score 1-24)	33/275 (12.0)	<b>2.53 (1.29-4.96)</b>	<b>0.007</b>	<b>2.23 (1.11-4.50)</b>	<b>0.024</b>
<b>All deaths</b>	59				
No AAC (AAC24 score 0)	19/348 (5.5)	1 (reference)		1 (reference)	
Any AAC (AAC24 score 1-24)	40/275 (14.5)	<b>2.07 (1.16-3.68)</b>	0.014	1.81 (0.99-3.31)	0.053
<b>Severity of AAC</b>					
<b>Cardiovascular event</b>	46				
No AAC (AAC24 score 0)	13/348 (3.7)	1 (reference)		1 (reference)	
Moderate AAC (AAC24 score 1-7)	14/162 (8.6)	1.79 (0.83-3.87)	0.141	1.71 (0.78-3.75)	0.183
High AAC (AAC24 score ≥8)	19/113 (16.8)	<b>4.04 (1.89-8.67)</b>	<b>&lt;0.001</b>	<b>3.51 (1.54-8.00)</b>	<b>0.003</b>
P for trend†		<b>&lt;0.001</b>		<b>&lt;0.001</b>	
<b>Any death</b>	59				
No AAC (AAC24 score 0)	19/348 (5.5)	1 (reference)		1 (reference)	
Moderate AAC (AAC24 score 1-7)	12/162 (7.4)	1.09 (0.52-2.28)	0.815	1.05 (0.50-2.22)	0.902
High AAC (AAC24 score ≥8)	28/113 (24.8)	<b>3.88 (2.05-7.34)</b>	<b>&lt;0.001</b>	<b>3.38 (1.71-6.70)</b>	<b>&lt;0.001</b>
P for trend†		<b>&lt;0.001</b>		<b>&lt;0.001</b>	

Abbreviations: AAC; Abdominal aortic calcification, CV; Cardiovascular event, HR; hazard ratio. \*Cox proportional hazards regression analyses were adjusted for age or age, gender, smoking history, type of kidney transplant (kidney only or SPK), dialysis vintage and diabetes. Values in bold p<0.05 compared to recipient with no AAC. †P values are a trend test using the median values of each AAC category in the Cox proportional hazards models.

## Figure legend

**Figure 1.** Overview of study including; A) Flowchart of the study participants, B) Example of lateral spine radiograph showing predominantly linear AAC typical of chronic kidney disease.

**Figure 2.** Kaplan Meier Survival plots (n=623) by the presence or absence of AAC for a) cardiovascular (CV) events (n=46) and b) deaths (n=59). Vertical lines indicate censored individuals.

**Figure 3.** Kaplan Meier Survival plots by presence of AAC for cardiovascular (CV) events A) kidney only (n=410) and B) simultaneous pancreas-kidney transplant (n=213) and all cause mortality C) kidney only (n=410) and D) simultaneous pancreas-kidney transplant (n=213). Vertical lines indicate censored individuals.

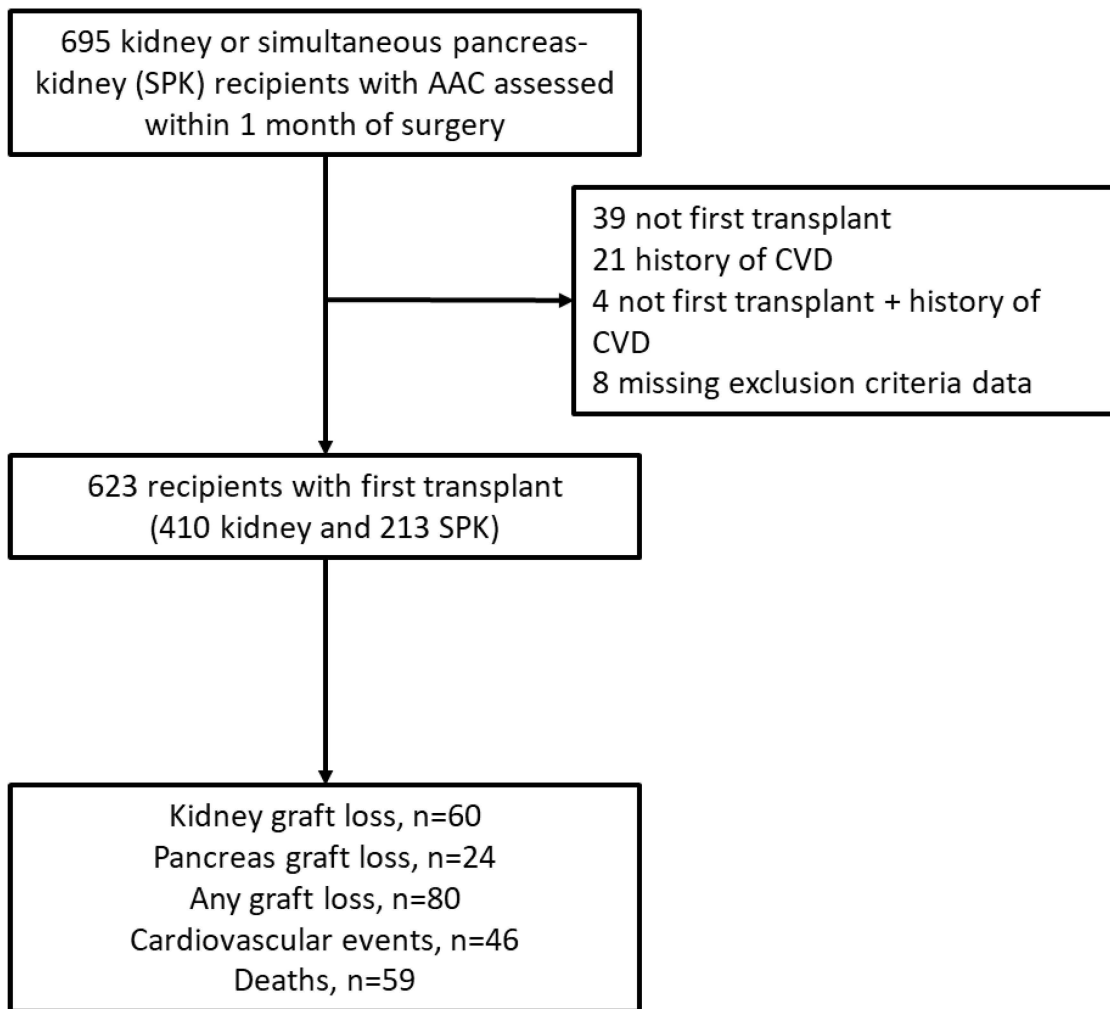
**Figure 4.** Multivariable-adjusted restricted cubic spline regression for the association between AAC24 scores and a) cardiovascular events and b) all-cause mortality. Adjusted for age, gender, smoking history, type of kidney transplant (kidney only or SPK), dialysis vintage and diabetes.

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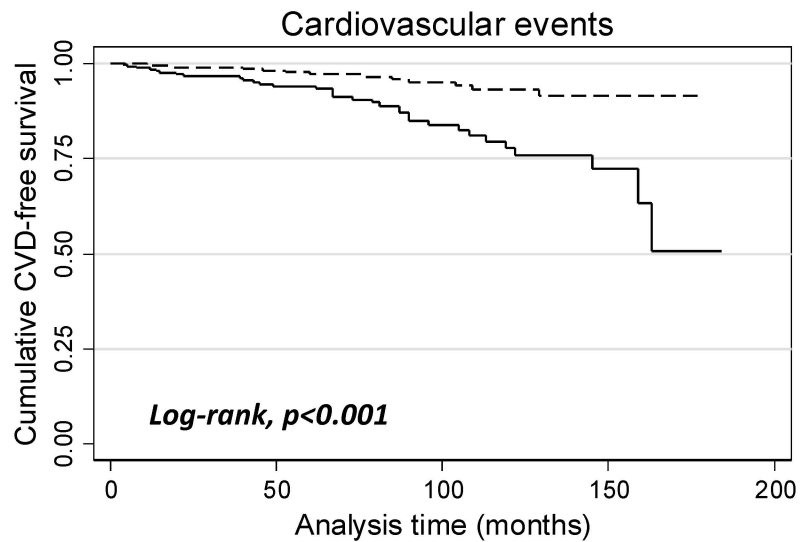
A)



B)



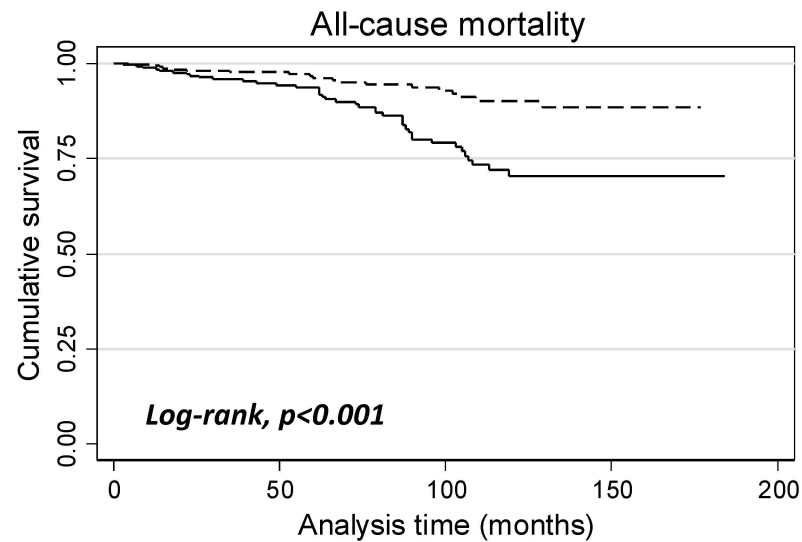
A)



Number at risk					
AAC = No AAC	348	212	107	23	0
AAC = Any AAC	275	162	69	15	0

----- AAC = No AAC      ——— AAC = Any AAC

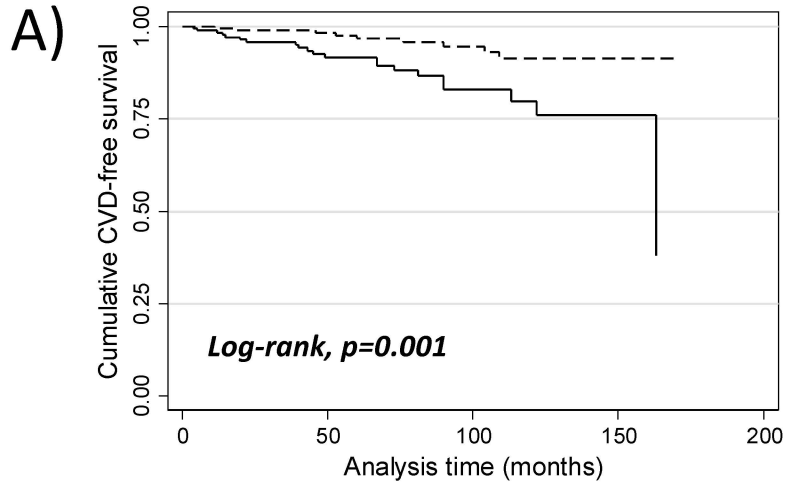
B)



Number at risk					
AAC = No AAC	348	215	108	24	0
AAC = Any AAC	275	170	73	19	0

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## Kidney only

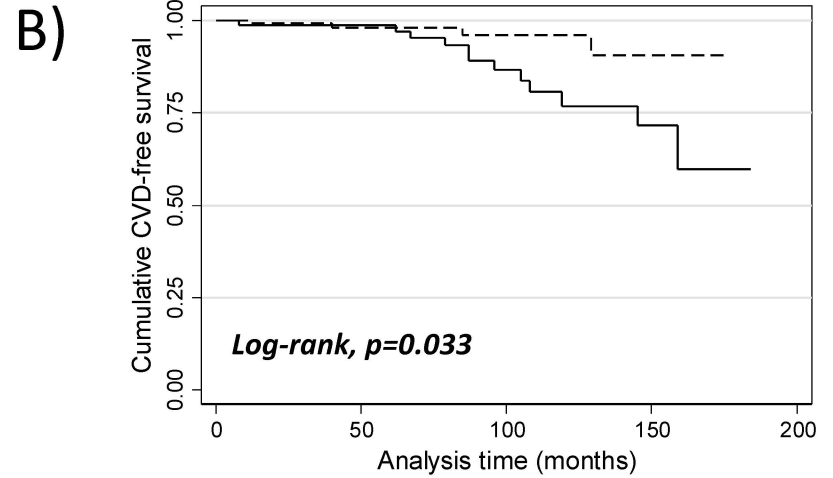


Number at risk

AAC = No AAC	220	142	67	16	0
AAC = Any AAC	190	103	38	6	0

----- AAC = No AAC      ——— AAC = Any AAC

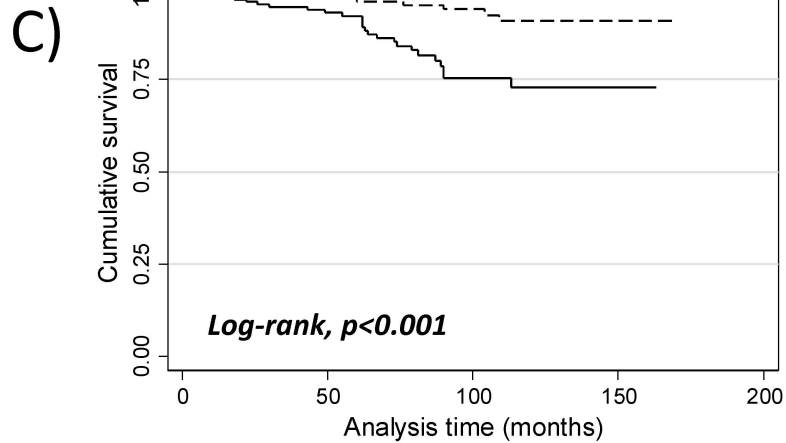
## Simultaneous pancreas-kidney



Number at risk

AAC = No AAC	128	70	40	7	0
AAC = Any AAC	85	59	31	9	0

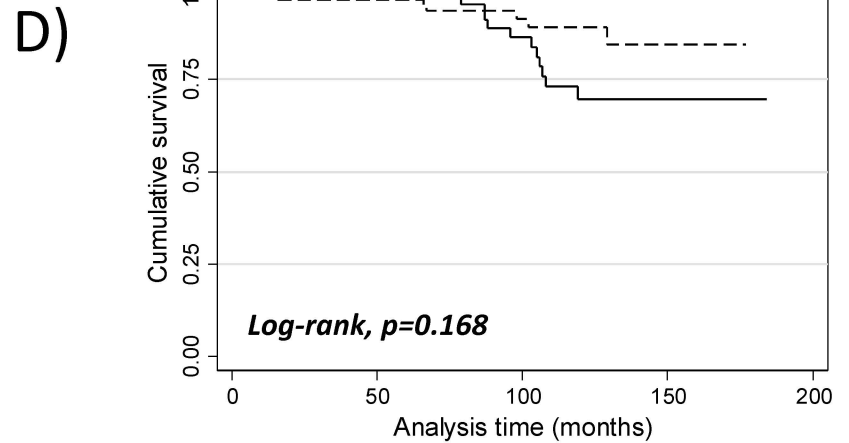
----- AAC = No AAC      ——— AAC = Any AAC



Number at risk

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----- AAC = No AAC      ——— AAC = Any AAC



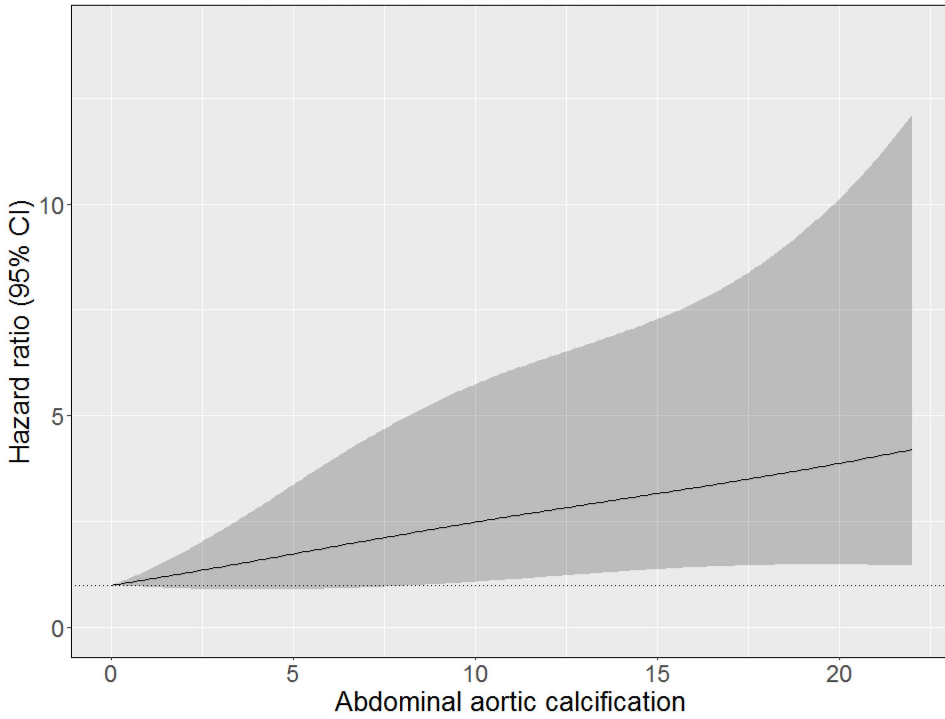
Number at risk

AAC = No AAC	128	71	41	8	0
AAC = Any AAC	85	60	32	10	0

----- AAC = No AAC      ——— AAC = Any AAC



Abdominal aortic calcification and CV events



Abdominal aortic calcification and mortality

