Nephrol Dial Transplant (2018) 1–9
doi: 10.1093/ndt/gfy352

ndt
Nephrology Dialysis Transplantation

ORIGINAL ARTICLE



# Inverse J-shaped relation between coronary arterial calcium density and mortality in advanced chronic kidney disease

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## ABSTRACT

**Background.** The coronary artery calcium (CAC) score from cardiac computed tomography (CT) is a composite of CAC volume and CAC density. In the general population, CAC volume is positively and CAC density inversely associated with cardiovascular disease (CVD) events, implying that decreased CAC density reflects atherosclerotic plaque instability. We analysed associations of CAC indices with mortality risk in patients with end-stage renal disease [chronic kidney disease Stage 5 (CKD5)].

**Methods.** In 296 CKD5 patients undergoing cardiac CT (median age 55 years, 67% male, 19% diabetes, 133 dialysed), the Framingham risk score (FRS), presence of CVD and protein-energy wasting (PEW; subjective global assessment) and high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) were determined at baseline. During follow-up for a median of 35 months, 51 patients died and 75 patients underwent renal transplantation. All-cause mortality risk was analysed with competing-risk regression models. Vascular calcification was analysed in biopsies of the arteria epigastrica inferior in 111 patients.

**Results.** Patients in the middle tertile of CAC density had the highest CAC score, CAC volume, age, CVD, PEW, FRS, hsCRP and IL-6. In competing risk analysis, the middle {subhazard ratio [sHR] 10.7 [95% confidence interval (CI) 2.0–57.3]} and high [sHR 8.9 (95% CI 1.5–51.8)] tertiles of CAC density associated with increased mortality, independent of CAC volume. The high tertile of CAC volume, independent of CAC density, associated with increased mortality [sHR 8.9 (95% CI 1.5–51.8)]. Arterial media calcification was prominent and associated with CAC volume and CAC density.

**Conclusions.** In CKD5, mortality increased linearly with higher CAC score and CAC volume whereas for CAC density an inverse J-shaped pattern was observed, with the crude mortality rate being highest for the middle tertile of CAC density. CAC volume and CAC density were associated with the extent of arterial media calcification.

**Keywords:** chronic kidney disease, coronary artery calcium density, coronary artery calcium score, coronary artery calcium volume, mortality risk

## ADDITIONAL CONTENT

An author video to accompany this article is available at: https://academic.oup.com/ndt/pages/author_videos.

## INTRODUCTION

The Agatston coronary artery calcium (CAC) score [1] is an independent risk predictor in the general population and in chronic kidney disease (CKD) patients [2–6]. It adds to the Framingham risk score (FRS) for cardiovascular disease (CVD) and improves risk stratification in the general population [7, 8] and in CKD [9]. However, the contribution of the two components of the CAC score, i.e. CAC volume and CAC density for risk prediction, is unclear in CKD.

Less calcified plaques may increase the risk of ruptures, leading to acute thrombosis and acute coronary syndromes [10–14], and outpatients with calcified plaques had a lower risk of coronary events compared with those without calcified plaques [15]. In the general population, CAC volume was positively and CAC density negatively associated with CVD events [8] across all levels of CAC volume and across multiple strata of other risk variables [16].

Patients with CKD are at high risk of both media and intima calcification. Media calcification (arteriosclerosis) is especially common in CKD and associates with arterial stiffness, hypertension, ventricular hypertrophy, poor cardiac perfusion and mortality [17, 18], while intima calcification (representing atherosclerosis with development of plaques) is the predominant

form of calcification in the general population [17–20]. Recently Bellasi *et al.* [21] reported that increased plaque density was an independent predictor of increased all-cause mortality in haemodialysis (HD) patients, suggesting that high-density CAC does not reflect atherosclerotic stabilization in the uraemic milieu.

However, the association between CAC density and clinical outcome has not been studied in other CKD cohorts. Moreover, the correlation between CAC and the extent of media calcification in arterial biopsies has not been studied. We investigated the association of CAC density with all-cause mortality in models that concomitantly included CAC density and CAC volume in carefully phenotyped CKD patients. Furthermore, in a subset of 111 patients we investigated the relation between the extent of media calcification in arterial biopsies and CAC score, volume and density.

## MATERIALS AND METHODS

We analysed CAC scores obtained by cardiac computed tomography (CT) in 296 clinically stable chronic kidney disease Stage 5 (CKD5) patients, including 163 non-dialysed (CKD5-ND) patients and 133 dialysed (CKD5-D) patients undergoing peritoneal dialysis (PD; $n = 92$) or HD ($n = 41$). The patients (ages 19–87 years) were enrolled in ongoing cohort studies described below. Exclusion criteria were age <18 years, signs of overt clinical infection and unwillingness to participate. All patients were followed until renal transplantation or death or until completing 60 months of follow-up. None of the patients were lost to follow-up. The Ethics Committee of the Karolinska Institutet at Campus Flemingsberg, Stockholm, Sweden, approved study protocols. Studies adhered to the Declaration of Helsinki. Informed written consent was obtained from each participant.

CKD5-ND patients ($n = 163$) were recruited from an ongoing prospective cohort study of patients investigated close to initiation of dialysis [22] and from an ongoing study on vascular changes in CKD5 patients investigated in conjunction with living donor renal transplantation [23]. The a etiologies of CKD were chronic glomerulonephritis ($n = 50$), hypertension and renovascular disease ($n = 21$), diabetic nephropathy ($n = 22$) and others or unknown causes ($n = 70$). Their median estimated glomerular filtration rate (according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation) was 6.0 (10th–90th percentile 4.2–10.0) mL/min/1.73 $m^2$.

CKD5-D patients ($n = 133$) were recruited from two cross-sectional studies with follow-up that aimed at evaluating variation in inflammatory markers in prevalent PD [24] and HD patients [23]. The median duration of preceding dialysis ('dialysis vintage') of PD patients was 11.5 months. PD patients received biocompatible glucose-based or amino acid–based solutions or, for long dwells, icodextrin-based solutions. The causes of CKD were chronic glomerulonephritis ($n = 17$), hypertension and renovascular disease ($n = 5$), diabetic nephropathy ($n = 11$) and others or unknown causes ($n = 59$). The median dialysis vintage of HD patients was 13.2 months. They were treated by conventional maintenance HD. The causes of CKD were chronic glomerulonephritis ($n = 15$), hypertension and renovascular disease ($n = 4$), diabetic nephropathy ($n = 1$) and others or unknown causes ($n = 21$). Supplementary data, Table S1 shows the clinical and laboratory characteristics of patients undergoing conservative treatment and dialysis therapies.

### Coronary artery calcification imaging and quantification

All patients underwent a non-contrast multidetector cardiac CT (LightSpeed VCT or Revolution CT; GE Healthcare, Milwaukee, WI, USA) with standard electrocardiogram-gated protocol to determine the Agatston score and CAC volume score. We used a semi-automatic software (syngo.via CT CaScoring, Siemens Healthcare, Forchheim, Germany). CAC was assessed as a lesion with an area >1 $mm^2$ and peak intensity >130 Hounsfield units (HU) based on the Agatston method and expressed in Agatston units (AU) [1]. The Agatston scoring method for CAC measures each discrete plaque area ($mm^2$). The discrete plaque area is multiplied by 1, 2, 3 or 4, depending on the highest density in the plaque. Plaques with a maximum density of 130–199 HU are multiplied by 1, those with 200–299 HU by 2, those with 300–399 HU by 3 and those with ≥400 HU by 4. These plaque-specific scores are summed for all slices of the heart to give the Agatston score. The Agatston score of each coronary artery was summed to determine the CAC score (total Agatston score). We calculated the area score ($mm^2$) by dividing the CAC volume score ($mm^3$) by slice thickness (2.5 mm). Then the CAC density score (score 1–4) was calculated as the CAC score (total Agatston score) divided by the area score, representing the average calcified lesion density for all CT slices [8]. Typical axial CT images are shown in Supplementary data, Figure S1A and S1B.

### Histological assessment of arterial media calcification

The extent of media calcification was assessed by a pathologist in vascular biopsies obtained from the inferior epigastric artery in 111 CKD5 patients [23] (see Supplementary text).

### Biochemical assessments

Blood biochemistry including high-sensitivity C-reactive protein (hsCRP) was analysed by routine methods. Interleukin-6 (IL-6) was analysed using commercial kits (see Supplementary text).

### Clinical assessments

CVD was defined based on clinical history or signs of ischaemic cardiac disease and/or the presence of peripheral vascular disease and/or cerebrovascular disease. According to the subjective global assessment (SGA) score, patients were classified as well-nourished (SGA = 1) or as having mild (SGA = 2), moderate (SGA = 3) or severe (SGA = 4) malnutrition [25]. For simplicity, patients were combined into two groups: malnourished (SGA > 1) and well-nourished (SGA = 1). Handgrip strength (HGS) was evaluated in the non-fistula arm using a Harpenden dynamometer (Yamar, Jackson, MI, USA) and repeated three times, and the greatest value was recorded and expressed in kilograms. HGS was expressed as the percentage of healthy individuals, considering the differences between the sexes, when included in the statistical analyses. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in metres.

### Framingham CVD risk score

The Framingham CVD risk score (FRS), an estimate of 10-year risk of developing CVD, was calculated from sex- and age-stratified tables with scores for systolic blood pressure (SBP), diabetes, anti-hypertensive medication, total cholesterol, high-density lipoprotein (HDL) cholesterol and smoking status [26].

### Statistical analyses

Data are expressed as median (10th–90th percentile) or percentage, as appropriate. Statistical significance was set at the level of $P < 0.05$. Comparisons between groups were assessed with the non-parametric Kruskal–Wallis test for continuous variables and chi-square test for nominal variables. Non-parametric Spearman rank correlation analysis was used to determine associations between variables. Tukey's multiple comparison analysis method was used to test each tertile of CAC volume and CAC density against patients with a CAC score of 0. We performed multinomial logistic regression analysis to examine factors associated with CAC density. The patients were followed from the inclusion date until renal transplantation or death or completing 60 months of follow-up. Causes of death were established by the death certificate issued by the attending physician. We used competing-risk regression models with transplantation as a competing risk to establish cumulative incidence curves [27]. Risk estimates for patients with a CAC score >0 were expressed as subhazard ratios (sHRs) for each tertile of the CAC score, CAC volume and CAC density, with patients with a CAC score of 0 serving as the reference. We used Fine and Gray models and these were adjusted for confounders [28]. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) and Stata 15.1 (StataCorp, College Station, TX, USA).

## RESULTS

Of the 296 CKD5 patients [median age 55 years, males 67%, CVD 22%, diabetes mellitus (DM) 19% and PEW (SGA >1) 34%] in the present study, 89 patients (30%) had a CAC score of 0 and 207 patients (70%) had a CAC score >0. The patients with a CAC score >0 had a median CAC score of 508 AU, median CAC volume of 416 $mm^3$ and median CAC density score of 3.17. Clinical and biochemical characteristics of patients with a CAC score of 0 and according to tertiles of CAC density and CAC volume among the patients with a CAC score >0 are shown in Tables 1 and 2.

### CAC density

Patients in the middle CAC density tertile had a higher CAC score and CAC volume; were older; had a higher prevalence of CVD, DM and PEW (SGA >1); had lower HDL and had higher BMI, FRS, hsCRP and IL-6 levels than the patients in other tertiles (Table 1).

### CAC volume

Patients in the high CAC volume tertile had a higher CAC score; were older; had a higher prevalence of DM, CVD and PEW; had higher FRS, hsCRP, IL-6 and had lower HGS as compared with the other tertiles (Table 2). Statin usage was more frequent in the high CAC volume tertile than in the other tertiles.

### Univariate associations between CAC measurements and risk markers

The CAC score and CAC volume were significantly associated with almost all investigated CVD risk markers, including FRS and markers of inflammation and PEW, whereas CAC density was significantly associated with age, male sex and PEW (Supplementary data, Text S1).

### Multivariate analysis of factors associated with CAC density

In multivariate logistic regression analysis of factors associated with CAC density (Table 3) (low CAC density tertile = reference), only the middle tertile was associated with CVD ($P = 0.002$) while both the high and middles tertiles were associated with PEW ($P = 0.03$).

### Crude mortality rate/1000 person-years for all-cause death

For patients with a CAC score of 0, the crude mortality rate/1000 person-years for all-cause death during the observation period was 7.6 [95% confidence interval (CI) 1.9–30.4] (Figure 1).

**Total CAC score.** Crude mortality rate in the low tertile was 16.2 (95% CI 5.2–50.4), middle tertile 78.8 (95% CI 47.5–130.7) and high tertile 145.1 (95% CI 102.1–206.4) (Figure 1A).

**CAC volume.** Crude mortality rate in the low tertile was 16.3 (95% CI 5.2–50.4), middle tertile 77.8 (95% CI 46.9–129.1) and high tertile 146.6 (95% CI 103.1–208.4) (Figure 1B).

**CAC density.** Crude mortality rate in the low tertile was 44.1 (95% CI 22.1–88.2), middle tertile 122.6 (95% CI 83.4–180.0) and high tertile 76.9 (95% CI 46.3–127.5) (Figure 1C).

### Multivariate competing risk analysis for all-cause mortality

During follow-up for a median of 35 months, 51 (17%) of 296 patients died and 75 (25%) patients underwent renal transplantation. Multivariate competing risk analysis for all-cause mortality taking renal transplantation into account and with patients with a CAC score of 0 serving as the reference group showed a different risk profile for CAC density as compared with total CAC score and CAC volume (Figure 2A–D).

**Total CAC score.** After adjustments for confounders, the high CAC score tertile associated with sHR = 8.4; 95% CI (1.6–44.0), middle tertile with sHR = 3.8; 95% CI (0.7–19.8) and low tertile with sHR = 2.0; 95% CI (0.3–12.3) (Figure 2A).

**CAC volume.** After adjustments for confounders and CAC density, the high CAC volume tertile associated with sHR = 8.9 (95% CI 1.5–51.8), the middle tertile with sHR = 3.9 (95% CI 0.7–20.9) and the low tertile with sHR = 2.5 (95% CI 0.4–16.0) (Figure 2B).

**CAC density.** After adjustments for confounders and CAC volume, the high CAC density tertile had sHR = 8.9 (95% CI 1.5–51.8), the middle tertile had sHR = 10.7 (95% CI 2.0–57.3)

**Table 1. Baseline clinical and biochemical characteristics in 296 CKD5 patients in relation to CAC density**

| Demographic and clinical characteristics | CAC score = 0 (*n* = 89) | CAC density | | | P-value |
|---|---|---|---|---|---|
| | | Low tertile (*n* = 68) | Middle tertile (*n* = 69) | High tertile (*n* = 70) | |
| Age (years) | 33 (22–62) | 55 (40–73)* | 63 (48–77)* | 60 (43–75)* | <0.001 |
| Males, *n* (%) | 54 (61) | 52 (76) | 52 (75) | 41 (29) | 0.03 |
| Diabetes mellitus, *n* (%) | 5 (6) | 16 (24)* | 21 (30)* | 15 (21)* | <0.001 |
| Cardiovascular disease[a], *n* (%) | 8 (9) | 10 (15) | 30 (43)* | 17 (24)* | <0.001 |
| Current smoker, *n* (%) | 2 (2) | 7 (10) | 7 (10) | 6 (9) | 0.10 |
| Systolic blood pressure (mmHg) | 142 (117–166) | 144 (119–177) | 140 (112–179) | 147 (118–180) | 0.26 |
| Framingham CVD risk score (%) | 3.7 (0.8–15.8) | 15.7 (4.9–47.1)* | 24.6 (7.1–52.2)* | 17.4 (4.6–57.3)* | <0.001 |
| Nutritional status | | | | | |
| PEW (SGA>1), *n* (%) | 33 (38) | 13 (20) | 28 (41) | 26 (38) | 0.03 |
| Body mass index (kg/m$^2$) | 23.2 (19.7–28.8) | 25.4 (21.0–30.2)* | 25.1 (21.2–31.4)* | 24.9 (20.3–30.9) | <0.001 |
| HGS[b] (%) | 98 (61–120) | 81 (56–114) | 81 (52–103)* | 81 (58–112) | <0.001 |
| Laboratory values | | | | | |
| Haemoglobin (g/L) | 109 (94–130) | 112 (87–133) | 114 (99–131) | 113 (99–129) | 0.09 |
| Albumin (g/L) | 35 (28–40) | 33 (26–40) | 33 (26–38) | 33 (28–38) | 0.19 |
| Triglyceride (mmol/L) | 1.3 (0.8–2.8) | 1.6 (0.8–2.8) | 1.5 (0.7–2.9) | 1.6 (1.0–2.7) | 0.41 |
| Total cholesterol (mmol/L) | 4.5 (3.4–5.9) | 4.4 (3.0–6.2) | 4.4 (3.0–6.6) | 4.7 (3.2–6.3) | 0.59 |
| HDL cholesterol (mmol/L) | 1.3 (0.9–1.9) | 1.3 (0.7–2.0) | 1.2 (0.9–2.0) | 1.2 (0.8–2.2) | 0.45 |
| LDL cholesterol (mmol/L) | 2.4 (1.3–3.9) | 2.4 (1.1–3.7) | 2.2 (1.3–4.1) | 2.5 (1.4–4.0) | 0.69 |
| Calcium (mmol/L) | 2.3 (2.0–2.5) | 2.3 (2.0–2.6) | 2.3 (2.0–2.5) | 2.3 (2.1–2.5) | 0.92 |
| Phosphate (mmol/L) | 1.7 (1.0–2.4) | 1.8 (1.2–2.4) | 1.7 (1.3–2.4) | 1.8 (1.2–2.5) | 0.52 |
| Intact PTH (ng/L) | 226 (103–546) | 273 (49–730) | 320 (94–618) | 255 (69–555) | 0.26 |
| hsCRP (mg/L) | 0.8 (0.2–9.8) | 1.3 (0.3–12.0) | 2.4 (0.6–17.4) | 2.4 (0.6–10.4) | <0.001 |
| IL-6 (pg/mL; *n* = 200) | 1.7 (0.05–8.3) | 3.3 (0.2–13.0) | 5.1 (1.6–13.9)* | 4.5 (0.6–14.8)* | <0.001 |
| Medications, *n* (%) | | | | | |
| β-blockers | 42 (47) | 51 (75)* | 50 (72)* | 46 (66) | <0.001 |
| Calcium channel blocker | 47 (53) | 32 (47) | 36 (53) | 36 (51) | 0.89 |
| ACEi/ARB | 58 (65) | 42 (62) | 39 (57) | 54 (77) | 0.06 |
| Statins | 24 (27) | 29 (43) | 35 (51)* | 25 (36) | 0.02 |
| CAC | | | | | |
| CAC score (AU) | 0 | 123 (3–1, 882)* | 1189 (113–3, 889)* | 392 (13–2, 869)* | <0.001 |
| CAC volume (mm$^3$) | 0 | 104 (3–1, 660)* | 936 (90–3, 016)* | 282 (9–2, 196)* | <0.001 |
| CAC density score (range) | – | 1.21–3.09 | 3.09–3.24 | 3.24–4.00 | – |

Values are presented as median (10th–90th percentile) unless stated otherwise. Range is given as minimum and maximum values.
[a]Defined as clinical history or signs of ischaemic cardiac disease and/or the presence of peripheral vascular disease and/or cerebrovascular disease.
[b]HGS is defined as the percentage of values for healthy individuals.
*P < 0.01 compared with the patients with a CAC score of 0.
LDL, low-density lipoprotein [LDL is calculated based on the Friedewald formula: (total cholesterol) – (HDL cholesterol) – (triglycerides/2.2)]; HDL, high-density lipoprotein; PTH, parathyroid hormone; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

and the low tertile had sHR = 5.0 (95% CI 0.8–29.9) (Figure 2C).

As a sensitivity test, we repeated the analysis for CAC density for subjects with inflammation, defined as hsCRP >1.5 mg/L (*n* = 142). The cumulative incidence curve in this group, after adjustments for CAC volume and other confounders, showed the highest mortality risk for the high tertile of CAC density [sHR = 5.6 (95% CI 0.8–36.7), P = 0.08] compared with the reference group (CAC score = 0) (Figure 2D).

Furthermore, we repeated the analyses after excluding patients with CAC score = 0; now with patients in the low tertile serving as the reference (Supplementary data, Figure S3). While cumulative incidence curves showed the highest mortality risk for the highest tertiles of CAC score (after adjusting for confounders; Supplementary data, Figure S3A) and CAC volume (after adjusting for CAC density + confounders; Supplementary data, Figure S3B), for CAC density the highest mortality was instead seen (Supplementary data, Figure S3C) for the middle tertile [sHR = 2.25 (95% CI 0.95–5.36); P = 0.06].

### Association between medial vascular calcification and CAC

In a subgroup of 111 CKD5 patients undergoing living donor renal transplantation, vascular biopsies were obtained from the inferior epigastric artery and scored by a pathologist (MS). Both CAC volume ($\chi^2 = 172$, $P < 0.001$) and CAC density ($\chi^2 = 177$, $P < 0.001$) were linearly associated to the degree of medial arterial calcification (histological score 0–3) (Figure 3). Some calcification was occasionally observed in the intima, but as the extent was minimal in comparison with the media, it was not scored separately.

## DISCUSSION

In CKD5 patients undergoing CAC scoring by cardiac CT, the mortality rate increased linearly with increasing total CAC score and CAC volume. In contrast, the relationship between CAC density and mortality had an inverse J-shaped form with the middle CAC density tertile being associated with the highest mortality. Indeed, patients within the middle CAC density

**Table 2. Baseline clinical and biochemical characteristics in 296 CKD5 in relation to CAC volume**

| Demographic and clinical characteristics | CAC score = 0 (*n* = 89) | CAC volume | | | P-value |
|---|---|---|---|---|---|
| | | Low tertile (*n* = 68) | Middle tertile (*n* = 69) | High tertile (*n* = 70) | |
| Age (years) | 33 (22-62) | 51 (38-69)* | 59 (47-75)* | 67 (51-79)* | <0.001 |
| Males, *n* (%) | 54 (61) | 44 (65) | 42 (61) | 59 (84)* | 0.004 |
| Diabetes mellitus, *n* (%) | 5 (6) | 6 (9) | 20 (29)* | 26 (37)* | <0.001 |
| Cardiovascular disease[a], *n* (%) | 8 (9) | 6 (9) | 17 (25) | 34 (49)* | <0.001 |
| Current smoker, *n* (%) | 2 (2) | 3 (4) | 10 (14)* | 7 (10) | 0.02 |
| Systolic blood pressure (mmHg) | 142 (117–166) | 144 (122–171) | 149 (125–181) | 140 (112–176) | 0.06 |
| Framingham CVD risk score (%) | 3.7 (0.8–15.8) | 10.3 (3.5–25.3) | 23.8 (6.4–58.8)* | 28.2 (10.7–56.1)* | <0.001 |
| Nutritional status | | | | | |
| PEW (SGA>1), *n* (%) | 33 (38) | 12 (18)* | 25 (37) | 30 (43) | 0.009 |
| Body mass index (kg/m$^2$) | 23.2 (19.7–28.8) | 25.1 (21.0–30.7)* | 25.3 (20.8–30.3) | 25.2 (20.6–31.4)* | <0.001 |
| HGS[b] (%) | 98 (61–120) | 89 (66–123) | 81 (53–107)* | 71 (53–98)* | <0.001 |
| Laboratory values | | | | | |
| Haemoglobin (g/L) | 109 (94–130) | 114 (94–133) | 113 (92–129) | 113 (98–132) | 0.17 |
| Albumin (g/L) | 35 (28–40) | 35 (29–40) | 32 (27–38) | 33 (26–38) | <0.001 |
| Triglyceride (mmol/L) | 1.3 (0.8–2.8) | 1.5 (0.7–2.5) | 1.8 (0.7–3.4) | 1.5 (0.9–2.6) | 0.17 |
| Total cholesterol (mmol/L) | 4.5 (3.4–5.9) | 4.5 (3.2–6.3) | 4.6 (3.2–7.1) | 4.3 (2.9–5.9) | 0.28 |
| HDL cholesterol (mmol/L) | 1.3 (0.9–1.9) | 1.4 (0.9–2.2) | 1.3 (0.9–2.0) | 1.1 (0.8–1.8) | 0.005 |
| LDL cholesterol (mmol/L) | 2.4 (1.3–3.9) | 2.4 (1.2–3.7) | 2.5 (1.2–4.1) | 2.2 (1.4–3.7) | 0.86 |
| Calcium (mmol/L) | 2.3 (2.0–2.5) | 2.3 (2.0–2.5) | 2.3 (2.0–2.5) | 2.3 (2.0–2.6) | 0.49 |
| Phosphate (mmol/L) | 1.7 (1.0–2.4) | 1.8 (1.1–2.3) | 1.8 (1.2–2.6) | 1.8 (1.2–2.4) | 0.30 |
| Intact PTH (ng/L) | 226 (103–546) | 255 (79–631) | 296 (94–610) | 279 (62–553) | 0.72 |
| hsCRP (mg/L) | 0.8 (0.2–9.8) | 1.2 (0.2–4.0) | 3.4 (0.6–18.3) | 3.3 (0.7–19.8) | <0.001 |
| IL-6 (pg/mL; *n*=200) | 1.7 (0.1–8.3) | 2.0 (0.2–7.2) | 4.9 (1.6–14.9)* | 5.9 (1.7–16.1)* | <0.001 |
| Medications, *n* (%) | | | | | |
| β-blockers | 42 (47) | 44 (65) | 51 (74)* | 52 (74)* | <0.001 |
| Calcium channel blocker | 47 (53) | 29 (43) | 41 (60) | 34 (49) | 0.21 |
| ACEi/ARB | 58 (65) | 45 (66) | 47 (68) | 43 (61) | 0.87 |
| Statins | 24 (27) | 19 (28) | 29 (42) | 41 (59)* | <0.001 |
| CAC | | | | | |
| CAC score (AU) | 0 | 33 (2–101) | 491 (186–919)* | 2120 (1219–4413)* | <0.001 |
| CAC density score | – | 3.09 (2.22–3.98) | 3.20 (3.00–3.46) | 3.17 (3.02–3.30) | 0.07[c] |
| CAC volume range (mm$^3$) | 0 | 1–111 | 113–818 | 828–6468 | – |

Values are presented as median (10th–90th percentile) unless stated otherwise. Range is given as minimum and maximum values.
[a]Defined as a clinical history or signs of ischaemic cardiac disease and/or the presence of peripheral vascular disease and/or cerebrovascular disease.
[b]HGS is defined as the percentage of values for healthy individuals.
*P < 0.01 compared with patients with a CAC score of 0.
LDL, low-density lipoprotein [LDL is calculated based on the Friedewald formula: (total cholesterol) – (HDL cholesterol) – (triglycerides/2.2)]; HDL, high-density lipoprotein; PTH, parathyroid hormone; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

**Table 3. Multinominal logistic regression analysis of factors associated with the middle tertile and high tertile of CAC density (*n* = 207, pseudo $R^2$=0.06)**

| | Middle tertile of CAC density | | High tertile of CAC density | |
|---|---|---|---|---|
| | Coefficient (± SE) | P-value | Coefficient (± SE) | P-value |
| 1 SD increase of FRS | 0.29 ± 0.19 | 0.13 | 0.16 ± 0.19 | 0.39 |
| Statin use (no = 0, yes = 1) | 0.13 ± 0.37 | 0.74 | −0.36 ± 0.37 | 0.32 |
| PEW (SGA > 1, no = 0, yes = 1) | **0.88 ± 0.42** | **0.03** | **0.88 ± 0.41** | **0.03** |
| Presence of CVD (no = 0, yes = 1) | **1.36 ± 0.45** | **0.002** | 0.65 ± 0.47 | 0.17 |
| 1 SD increase of hsCRP, mg/L | 0.02 ± 0.17 | 0.91 | −0.17 ± 0.20 | 0.39 |

The low tertile served as a reference. CVD is defined as a clinical history or signs of ischaemic cardiac disease and/or presence of peripheral vascular disease and/or cerebrovascular disease. Significant associations are in bold.
SE, standard error; SD, standard deviation.

tertile had the highest CAC score, highest CAC volume and presented with the highest burden of CVD risk factors, including higher FRS and higher prevalence of inflammation and PEW, compared with the low and high CAC density groups. Histological examination of vascular biopsies revealed that both higher CAC volume and CAC density significantly associated with the extent of arterial media calcification.

Whereas high CAC volume associates with worse outcomes in the general population [2–5, 21], it has been postulated that increased density in the arterial wall may reflect stabilization of atherosclerotic plaques [11–14], leading to a reduced risk of coronary events [15] in the general population [29]. However, in type 2 DM patients, the density of calcified plaques was not associated with mortality [30], while in HD patients, increased

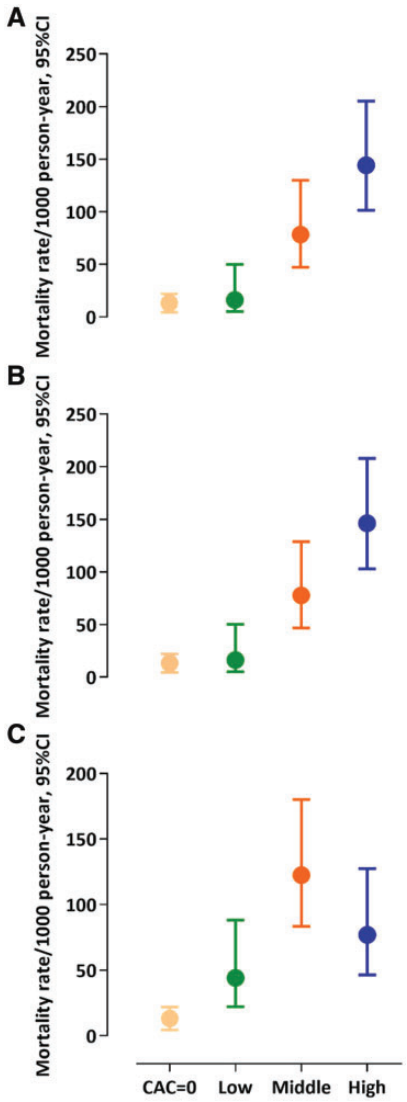


**FIGURE 1:** Crude mortality rate/1000 patient-years (95% CI) according to tertiles of (**A**) CAC score, (**B**) CAC volume and (**C**) CAC density ($n = 207$) and in patients with a CAC score of 0 ($n = 89$).

density of calcified plaques independently predicted increased all-cause mortality [21]. Here we report that in CKD5-ND and CKD5-D patients, the middle tertile of CAC density associated with the worst survival (and worst CVD risk profile) while high CAC density formed an intermediate risk group. It has been suggested [29] that patients within the middle tertile of CAC density may have clinical characteristics that are more similar to those for mixed plaques as compared with the low and upper tertiles of CAC density.

On the other hand, there may be multiple uraemia-related specific reasons for a different predictive role of high CAC density in CKD [21] compared with non-CKD patients [8, 16] or type 2 DM patients [30]. The vascular calcification process is complex in the uraemic milieu [31] and involves alterations in calcium, phosphate, magnesium, parathyroid hormone, fibroblast growth factor 23 and Klotho [32–34] and deficiency of calcification inhibitor proteins such as fetuin-A [35], matrix Gla protein (MGP) [36], osteoprotegerin [37] and vitamin K required for carboxylation of MGP [38, 39]. Other factors contributing to uraemic vascular calcification are oxidative stress and accumulation of advanced glycated end-products [31]. Recent evidence suggests a key role for serum- and glucocorticoid-inducible kinase 1 and nuclear factor κB signalling in the uraemic vascular smooth muscle cell (VSMC) calcification process [40]. We observed that increased CAC volume associated with inflammation, poor nutritional status and low HGS. The combination of sarcopenia and increased vascular calcification may reflect a process of premature biological ageing [41, 42] in the inflamed uraemic milieu [43]. In the present study we observed that the presence of inflammation modifies the relationship between CAC density and outcome (Figure 2D), which supports the concept of a catalytic effect of inflammation on cardiovascular risk factors in the uraemic milieu [44]. Statin therapy may contribute to plaque stabilization by promoting calcification [45]. In our study, similar to the general population [46, 47], statin usage associated with higher CAC volume. Although this finding may be attributed to 'confounding by indication', a study using intravascular ultrasound demonstrated that statin therapy leads to delipidation and VSMC calcification, which may promote plaque stability [45]. The inhibitory effects of statins on vitamin K (menaquinine-4) synthesis may be an alternative mechanism by which statins promote vascular calcification [48].

Intimal and medial calcification are common findings of the uraemic phenotype [49], and while the latter is thought to be a major contributor to poor clinical outcomes [17–20], intimal rather than medial vascular calcification was reported to be involved in the atherosclerotic disease process in CKD [50]. Based on histological scoring of vascular biopsies, we report that the media was the major arterial site affected by calcification, and that the extent of arterial media calcification significantly associated with both CAC volume and CAC density. It is noteworthy that 14% of patients with extensive histological arterial media calcification had no signs of coronary calcification (Figure 3). Thus the magnitude and propensity for calcification seems to differ in different arterial sites [51]. The finding by Bellasi *et al.* [21] that increased plaque density predicted all-cause mortality in HD patients suggests that high CAC density does not reflect plaque stabilization in the uraemic milieu. We calculated CAC density using the formula applied by Bellasi *et al.* [21] and excluding patients with a CAC score of 0. The cumulative incidence curves of 5-year mortality in 207 patients, using the low tertile as reference, showed that the adjusted sHR for the middle tertile of CAC density was increased, albeit not statistically significant ($P = 0.07$), while sHR for the high tertile was clearly not significantly increased (Supplementary data, Figure S2).

Some limitations of our study should be noted. The observational design precludes conclusions about causality. Inclusion of both incident and prevalent clinically stable dialysis patients may limit the interpretation. Measurements of CAC at a single time point may not reflect the ideal time for risk prediction. The relatively low number of patients is another limitation and

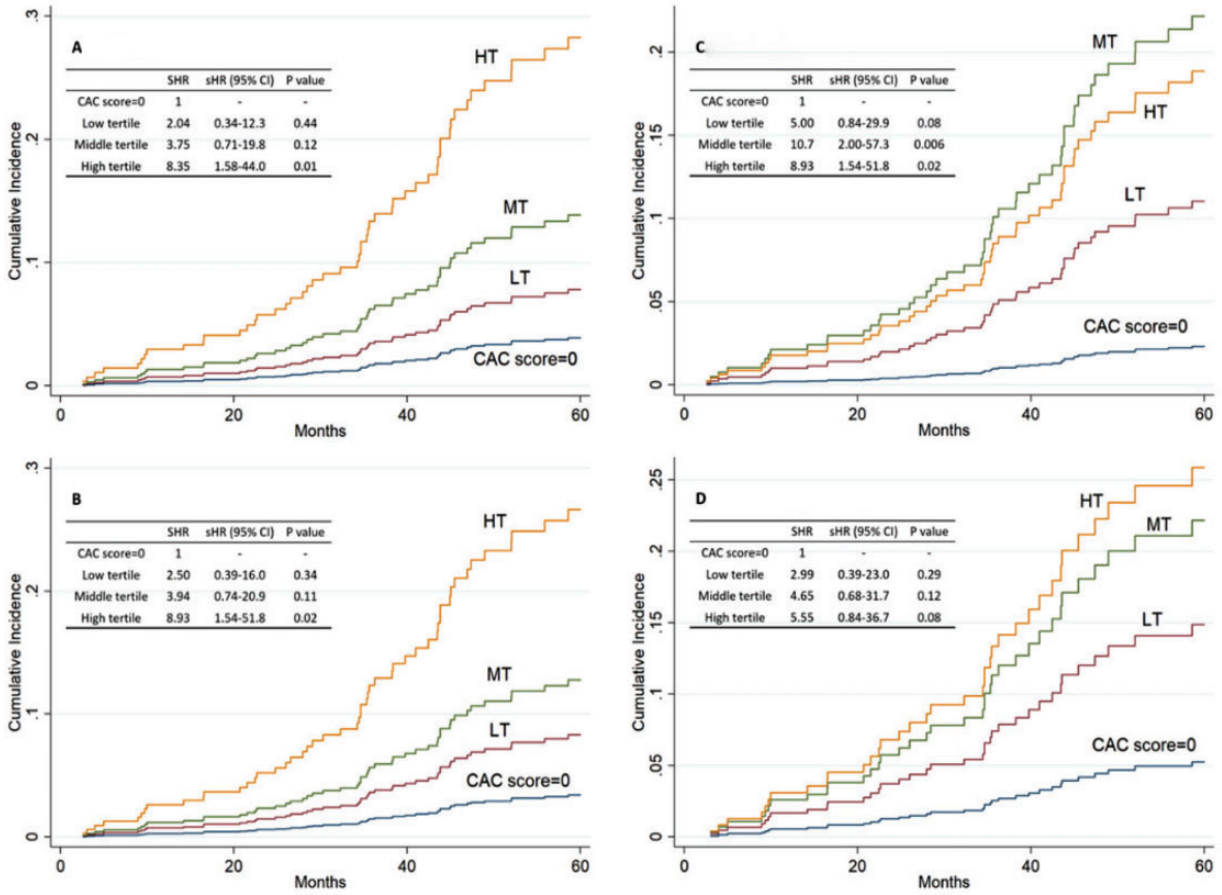


**FIGURE 2:** Cumulative incidence curves of 5-year mortality in relation to indices of CAC. (**A**) Tertiles of CAC score after adjusting for confounders. (**B**) Tertiles of CAC volume after adjusting for CAC density + confounders. (**C**) Tertiles of CAC density after adjusting for CAC volume + confounders. (**D**) Tertiles of CAC density in inflamed (hsCRP > 1.5 mg/L; $n = 142$) patients after adjusting for CAC volume + confounders. Adjustments for confounders included FRS, presence of CVD and PEW, levels of plasma albumin, hsCRP and HGS and statin use. The group of patients with a CAC score of 0 served as a reference. LT, low tertile; MT, middle tertile; HT, high tertile.

we did not analyse the incidence of CVD events or CVD-related mortality; however, this is, to the best of our knowledge, the largest study to date of the association of CAC volume and CAC density with outcome in CKD. Increased mortality among CKD patients with high CAC density before they reached CKD stage 5 could have contributed to survival bias. A lack of data on duration of kidney disease and residual renal function in prevalent dialysis patients also limits the study. Cardiac CT does not provide data about the composition of plaques, such as differentiation between medial and intimal calcification. However, based on histological examination, we observed that medial calcification was the predominant type of calcification in the arteria epigastrica. Assessment of CAC density provides only average density of calcified lesions, not the density of each lesion; however, low- and high-density calcifications may co-exist in the same patient, and the extent of non-calcified plaques is not known. In the Agatston method, each CAC density assessment is based on the highest density pixel within each calcified lesion. HD and haemodiafiltration (HDF) may affect circulating biomarkers of inflammation differently [52], therefore implications for CAC of different dialysis therapies—not investigated here—should be explored in future studies. Since vascular calcification processes may be heterogeneous within the arterial tree [51], comparisons between coronary and epigastric calcification may be spurious. Finally, the independent nature of the observed inverse J-shaped relation of CAC density with crude mortality rate needs to be confirmed by additional studies.

The present study also has some strengths worth mentioning. First, the phenotyping was detailed and included both traditional and several non-traditional risk factors. Moreover, because many observational studies in CKD have reported that associations of risk factors with mortality are J- or U-shaped, and analysis of only two groups may disguise such a relationship [21], we divided the patients into four groups. Furthermore, in a subgroup of patients we examined the extent of concomitant vascular calcification by both histological scoring of the arteria epigastrica and cardiac CT. Although the uraemic calcification process may differ between different arterial sites [51], our observations suggest that the extent of media calcification is linearly related to CAC volume and CAC density (Figure 3). Further studies in larger cohorts on the relationship between CAC density, CAC volume and clinical outcome in CKD patients should consider additional biochemical and clinical confounders, such as renal failure duration and residual renal function, as well as attempt to elucidate the localization of calcification in the arterial wall.

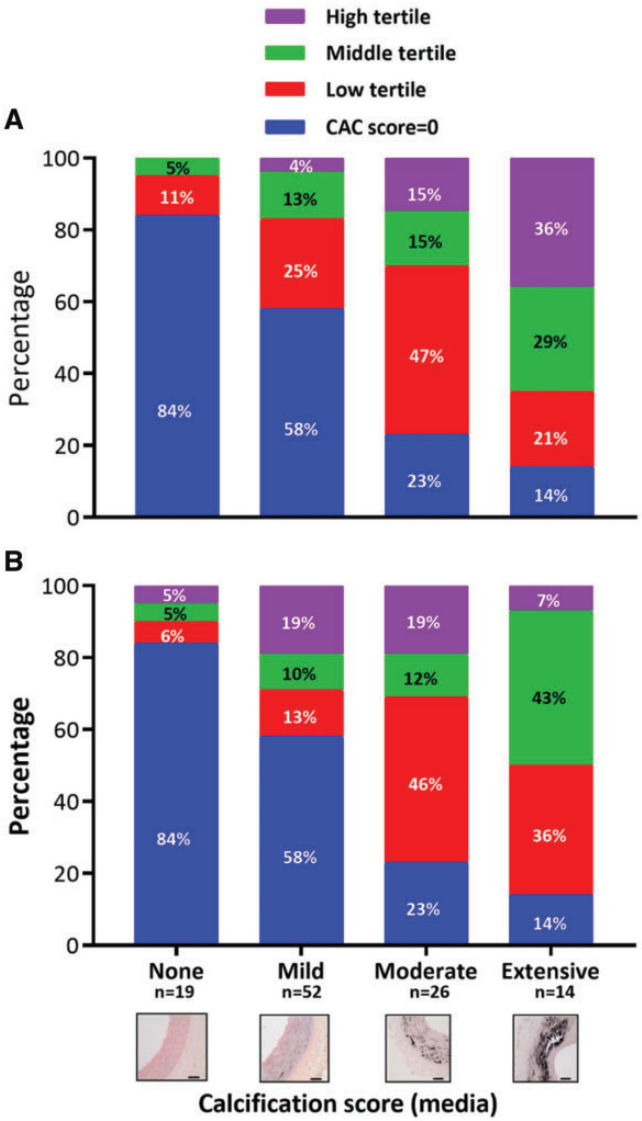


**FIGURE 3:** Relationship of CAC (**A**) volume and (**B**) density with the degree of medial vascular calcification in the epigastric artery scored as 0 (none), 1 (mild), 2 (moderate) or 3 (extensive) medial vascular calcification among 111 CKD5 patients. The four categories of degree of medial vascular calcification are illustrated by representative histological pictures (von Kossa staining).

In summary, in CKD5 patients, the crude mortality rate increased linearly with higher CAC score and CAC volume, being highest for the highest tertiles. For CAC density, the pattern was more complex, with an inverse J-shaped relation of CAC density versus crude mortality rate, with mortality as well as CVD risk factor burden (FRS, inflammation, PEW) being the highest for the middle tertile of CAC density. The reason for this is not clear, although survival bias in patients with low or high CAC density could have contributed. Furthermore, as inflammation appeared to aggravate the impact of higher CAC density on mortality, it should be taken into account when evaluating the relation between CAC density and outcome. Both CAC volume and CAC density correlated with the extent of histological signs of arterial media calcification in vascular biopsy specimens. Evaluation of CAC density may add important information for the prediction of outcome in CKD.

## SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

## ACKNOWLEDGEMENTS

We thank all patients who participated in the study and those who carried out the extensive clinical and laboratory work in the clinical investigational unit and the renal laboratory in the Department of Renal Medicine, Karolinska University Hospital.

## FUNDING

This study was supported by a grant from Baxter Healthcare to Baxter Novum, Department of Clinical Science, Intervention and Technology, Karolinska Institutet. The study also benefited from generous support from Amgen (P.S.), the Karolinska Institutet Diabetes Theme Center (P.S.), the Swedish Research Council (P.S.), the Heart and Lung Foundation (P.S.), Njurfonden (P.S.) and Westmans Foundation (P.S.). This study also received funding from the European Union's Horizon2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement 722609 https://www.intricare.eu/.

## CONFLICT OF INTEREST STATEMENT

B.L. is employed by Baxter Healthcare. M.S. is affiliated with AstraZeneca. None of the other authors declare any conflict of interest.

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*Received: 21.6.2018; Editorial decision: 28.9.2018*