Calciprotein particles are a sensitive marker predicting vascular calcification in patients with chronic kidney disease

Hidekazu Moriya (h_moriya@shonankamakura.or.jp)
Shonan Kamakura General Hospital
Yasuhiro Mochida
Shonan Kamakura General Hospital
Kunihiro Ishioka
Shonan Kamakura General Hospital
Machiko Oka
Shonan Kamakura General Hospital
Kyoko Maesato
Shonan Kamakura General Hospital
Sumi Hidaka
Shonan Kamakura General Hospital
Takayasu Ohtake
Shonan Kamakura General Hospital
Yutaka Miura
Jichi Medical University
Makoto Kuro-o
Jichi Medical University
Shuzo Kobayashi
Shonan Kamakura General Hospital

Research Article

Keywords: Calciprotein particle, Chronic kidney disease, Vascular calcification

Posted Date: December 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-123757/v1

Calciprotein particles are a sensitive marker predicting vascular calcification in patients with chronic kidney disease

Hidekazu Moriya[1)], Yasuhiro Mochida[1)], Kunihiro Ishioka[1)], Machiko Oka[1)], Kyoko Maesato[1)], Sumi Hidaka[1)], Takayasu Ohtake[1)], Yutaka Miura[2)], Makoto Kuro-o[2)], Shuzo Kobayashi[1)]

1) Department of Kidney Disease and Transplant Center, Shonan Kamakura General Hospital, Kamakura, Kanagawa, Japan
2) Division of Anti-Ageing Medicine, Center for molecular Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan

Corresponding author
Hidekazu Moriya
Department of Kidney Disease and Transplant Center, Shonan Kamakura General Hospital, Kamakura, Kanagawa, Japan
Address: 1370-1, Okamoto, Kamakura, Kanagawa, Japan 247-8533
Phone: +81-467-46-1717
Fax: +81-467-45-9792
E-mail: h_moriya@shonankamakura.or.jp

Abstract

Background: In chronic kidney disease (CKD) patients, vascular calcification occurs in the early stage of CKD, before the titer of existing CKD mineral bone disorder (CKD-MBD)-related markers exceed the threshold. Calciprotein particles (CPPs) are crystals in which excessive phosphorus and calcium in the blood form colloidal particles and are associated with vascular calcification. However, it is unknown whether CPPs are a more sensitive marker for detecting calcification than others.

Methods: In a prospective cohort study of 58 patients with CKD we examined CKD-MBD markers, including CPPs. Vascular arterial calcification score (ACS) of the lower extremities was measured with the Agatston score. One year later, the markers and ACS were reevaluated, and the relationship between the degree of progression of vascular calcification and CKD-MBD markers was evaluated.

Results: The CPP titer was significantly correlated with that of serum phosphate and corrected calcium. However, the CPP titer showed no correlation with eGFR or ACS in the lower extremities. After one year, the basic titers of serum creatinine, eGFR, FGF-23, intact-PHT, 1.25-dihydroxyvitamin D3 and CPP were not significantly correlated with ACS changes in the lower extremities. There was a significant correlation between the rate of change in ACS and that in CPP ($r = 0.292$, $p = 0.0258$). CPP was an independent risk factor for the progression of calcification in the lower extremities in a multivariate analysis ($p = 0.0144$).

Conclusions: CPP is a more sensitive marker of arterial calcification than other CKD-MBD markers in patients with CKD.

Key words: Calciprotein particle, Chronic kidney disease, Vascular calcification

Background

Chronic kidney disease (CKD) is associated with mineral bone disorder (MBD) from an early stage of CKD. CKD-MBD is not only related to bone changes and a biochemical increase in phosphate, but also causes vascular calcification and arteriosclerosis, leading to the onset of cardiovascular disease and poor prognosis. Generally, in stages 3-5 of CKD, the optimal range of serum phosphorus concentration is set to be the normal reference value (approximately 4.5 mg/dl or less) at each institution.

However, the phosphate level increases only when GFR falls below approximately 30 mL/min/1.73m^2, at that which point vascular calcification, arteriosclerotic disease, and lacunar infarction have already occurred [1,2]. It is too late to start intervention to control CKD-MBD at that point.

Several hormones, including PTH, vitamin D, and FGF-23, are involved in the metabolism of phosphate. FGF-23 acts as a phosphate diuretic hormone secreted by the bone in response to the phosphate overload. The titer of FGF-23 is already increased before the phosphate concentration exceeds the normal range. In addition, excess calcium and phosphate in the blood are captured by mineral-binding proteins such as fetuin-A, which forms calciprotein particles (CPPs). CPPs are microcrystals of calcium phosphate, and a defense mechanism works to prevent these crystals from growing large. However, CPPs are also considered to cause chronic inflammation and damage to the blood vessels, including in various organs such as the heart and kidney [3].

FGF-23 and CPPs increase, and fetuin-A decreases, according to the progression of CKD stages. In the CKD rat model, CPPs increased prior to aortic calcification [4], and it has been reported that CPP is more strongly associated with ectopic calcification than fetuin-A [5]. However, it is unknown which markers of CKD-MBD are most sensitive to the formation of arteriosclerosis in humans, and there are uncertainties regarding the correlation between the titer of FGF-23 and that of CPPs in CKD.

FGF-23 is considered to increase from the point at which eGFR is < 75 mL/min/1.73m^2, but if CPPs increase earlier, before FGF-23 changes, it could be possible to predict vascular calcification lesions earlier and start therapeutic intervention by using CPPs as a marker of vascular calcification.

In this study, we examined how CPPs associate with other markers of CKD-MBD and vascular calcification in patients with CKD.

Methods

Study design and population

To determine the relationship between CKD-MBD markers and vascular calcification, we conducted a prospective cohort study in Shonan Kamakura General Hospital. The inclusion criteria were as follows: 1) consecutive recruitment between July 2014 and December 2016 from our clinic; 2) change in serum creatinine level within 0.3 mg/dL for the previous three months. The exclusion criteria were as follows: 1) refusal to provide informed consent; 2) contraindication of multidetector-row computed tomography (MDCT); 3) presence of hemodialysis, malignancy, congestive heart failure, and infections.

Laboratory analyses

We carefully obtained patients' history and laboratory data, including those concerning serum creatinine, calcium, phosphate, C-reactive protein, intact-parathyroid hormone (i-PTH), 1.25-dihydroxyvitamin D3, urinary protein, fractional excretion of phosphate, and FGF-23 levels. FGF-23 was measured using an intact FGF-23 ELISA kit (Kinos, Tokyo, Japan) according to the manufacturer's protocols. All other samples were analyzed at the local Department of Clinical Biochemistry in our hospital. Blood tests were performed again after one year.

Examination by multidetector computed tomography

We calculated the Agatston score using MDCT (LightSpeed Ultrafast 16, General Electric Medical System, Tokyo, Japan), in which the score is well correlated with that measured by electron-beam CT [1]. Volumetric data of the entire extremities were obtained in the helical mode with scanning parameters of 1.25 mm collimation width 16 detectors, a gantry rotation speed of 0.5 s per rotation, 120 kV, and 100 mA. Images of 2.5 mm thickness with the center of the temporal window corresponding to 80% of the R-R interval were reconstructed with 2.5 mm spacing. Calcium score, volume, and mass were determined on a commercially available external workstation (Adventure Windows, version 4.4.1, General Electric Medical System, Tokyo, Japan) using CAC-scoring software (version 3.5, Smartscore, Tokyo, Japan), with MDCT. According to the Agatston method, we defined the regions of interest by vessel and slice with the threshold option for pixels greater than 130 Hounsfield units to measure the area and peak density of plaques. MDCT was conducted at the start of the study and after one year.

CPP assay

Quantification of CPPs in the plasma was performed as previously reported [6]. Briefly, a fluorescent probe that binds to CaPi crystals (OsteoSense 680EX; PerkinElmer Inc.,

Waltham, MA) was added to heparin plasma samples. After incubation at 25℃ for 60 minutes, the sample was applied to a gel-filtration spin column to remove unbound CaPi crystals. The fluorescent intensity of the flow-through was quantified using an infrared fluorescence scanner (Odyssey CLx; LI- COR Biosciences, Lincoln, NE) and expressed as an arbitrary unit (AU).

Statistical analysis

Continuous variables are presented as the mean ± SD or median (interquartile range; IQR), as appropriate. Spearman's correlation coefficients and multiple logistic regression analyses were used as appropriate to test correlation between variables.

All analyses were conducted in SPSS Statistics 19. Results were considered significant if P was < 0.05.

Results

A total of 58 CKD patients were included in the present study. Of these 58 patients, 24 (41.3%) had type 2 diabetes mellitus and 45 (77.5%) had hypertension. The mean age of the participants was 70.0 ± 12.0 years (45 men and 13 women). The basic characteristics of the participants are shown in Table 1. Participants with CKD had a median creatinine level of 1.42 mg/dL and a median eGFR of 39.7 mL/min/1.73m^2. The median FGF-23 level was 63.7 pg/mL and the median CPP level was 16616 AU. The median ACS of the lower extremities was 655 (IQR: 0-706).

CPP level correlated well with serum phosphate and corrected calcium (r=0.390 p <0.0001, r=0.329 p<0.0001, respectively). However, CPP level showed no significant relationship with eGFR (Figure 1A, 1B, 1C). CPP level also showed no significant relationship with age or FGF-23 level.

In univariate analyses, urinary protein and fractional excretion of phosphate correlated significantly with ACS in the lower extremities. However, these factors showed no correlation after adjusting for age and kidney function (Table 2). Smoking, diabetes mellitus, and systolic blood pressure were significantly correlated with calcification score in a multivariate analysis. No CKD-MBD markers, including CPPs, correlated with ACS in the lower extremities (Figure 2).

After one year, we reevaluated the calcification score and examined the relationship between basic factors and the change in ACS. However, the change in calcification score was not significantly correlated with any factors related to CKD-MBD (Table 3). In the next step, when evaluating the relationship between the rate of change in calcification score and

the rate of change in FGF-23, i-PTH, and 1.25-dihydroxyvitamin D3, there was no significant correlation. However, the rate of change in CPP did show a significant relationship with ACS in the univariate analysis (r=0.292, p=0.0258), and in the multivariate analysis when adjusting for age and kidney function (r=0.298, p=0.0144) (Table 4).

Discussion

This study revealed that CPPs could be the only marker of CKD-MBD for assessing changes in vascular calcification. Excessive calcium and phosphate in the blood are captured by mineral-binding proteins such as fetuin-A to form CPPs, which are microcrystals of calcium and phosphate. When calcium and phosphate exceed the solubility limit, the crystals appear in the blood. Defense mechanisms work to prevent amorphous and primary CPPs from growing into large and secondary CPPs in the extracellular space. However, when CPPs become large, they cause vascular calcification in the heart, kidneys, brain, and lower extremities. A small CPP containing only amorphous calcium phosphate is a physiological CPP that induces the production of FGF-23 in osteoblasts, whereas a large CPP containing calcium phosphate crystals causes cytotoxicity to vascular endothelial cells and renal tubular cells, resulting in calcification in vascular smooth muscle cells [7]. It therefore becomes a pathological CPP with the ability to induce hyperplasia and calcification in vascular smooth muscle cells and innate immune responses in macrophages [8].

When a CPP is added to vascular smooth muscle cells, BMP-2 and osteopontin are inducted and osteoblast-like changes occur in these cells. However, a high concentration of phosphate alone does not induce similar changes in vascular smooth muscle cells. It is therefore suggested that it is not phosphate but CPPs, formed by an overload of phosphate, that cause vascular calcification [9]. In fact, in the presence of agents that inhibit the formation of CPPs, an increase in phosphate concentration does not cause cell damage. In the present study, the concentration of phosphate does not show a significant association with the vascular calcification score of the lower extremities.

PTH, 1.25-dihydroxyvitamin D3, and FGF-23 are involved in mineral metabolism. FGF-23 is secreted from osteocytes/osteoblasts and acts as a hormone that increases urinary phosphate excretion in the kidney in response to phosphate load from the diet and is a counterregulatory hormone for 1.25-dihydroxyvitamin D3. The level of FGF-23 is increased to adjust the phosphate level before the blood phosphate level exceeds the normal limit.

In this study, CPPs, rather than FGF-23, 1,25-dihydroxyvitamin D3, or intact PTH, sharply predicted changes in vascular calcification score in the lower extremities, suggesting that CPPs change earlier in response to mineral metabolism.

The serum calcification propensity test (T_{50}) is an *in vitro* assay that quantifies the transformation time from primary to secondary CPP in the serum when challenged with exogenous calcium and phosphate. It has been used recently as an index of calcification risk. Low T_{50} values have been reported to be associated with all-cause mortality in patients with CKD and in kidney transplant patients and are also predictors of cardiovascular mortality [10-13]. However, it is reported that when adjusting for eGFR, there is no independent relationship between T_{50} and mortality in patients with CKD [14]. In addition, another report revealed that there is no direct relationship between vascular calcification and the values of T_{50} [15].

Conversely, it has been reported that the accuracy of prognosis prediction might be improved by evaluating the change in T_{50} via continuous measurement, rather than one point measurement [16]. Regarding CPPs in this study, we were able to predict the progress of vascular calcification by evaluating the change in CPP level, rather than using one point measurement.

Conclusion

CPP level was not correlated with eGFR or vascular calcification score based on one point measurement. However, change in the CPP level was significantly correlated with change in vascular calcification. CPPs have a protective effect on vascular calcification by trapping calcium and phosphate. Therefore, CPP is a more sensitive marker of arterial calcification than other CKD-MBD markers such as FGF-23.

Abbreviations

CKD: Chronic Kidney Disease; CKD-MBD: CKD Mineral Bone Disorder; CPP: Calciprotein Particle; ACS: Arterial Calcification Score; eGFR: estimated Glomerular Filtration Ratio; FGF-23: Fibroblast Growth Factor-23; PTH: Parathyroid Hormone; MDCT: Multidetector-row Computed Tomography; BMP-2: Bone Morphogenetic Protein-2

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Tokushukai Group Ethics Committee (TGE00434-024). The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all subjects or, if subjects are under 18, from a parent and/or legal guardian.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

None.

Authors' contributions

HM coordinated the study, analyzed the data and wrote the manuscript. YM, KI, MO, KM, SH, TO, YM and MK participated in the study design and coordination, analyzed the data. SK is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final Manuscript.

Acknowledgments

Not applicable

Author details

1) Kidney Disease and Transplant Center, Shonan Kamakura General Hospital, 1370-1, Okamoto, Kamakura 247-8533, Japan. 2) Division of Anti-Ageing Medicine, Center for molecular Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan

References

1) Kobayashi S, Oka M, Maesato K, Ikee R, Mano T, Moriya H, et al. Coronary artery calcification, ADMA, and insulin resistance in CKD patients. Clin J Am Soc Nephrol. 2008; 3: 1289-1295.

2) Kobayashi S, Ikeda T, Moriya H, Ohtake T, Kumagai H. Asymptomatic cerebral lacunae in patients with chronic disease. Am J Kidney Dis. 2004; 44: 35-41.

3) Kuro-o M. A phosphate-centric paradigm for pathophysiology and therapy of chronic kideny disease. Kidney Int Suppl. 2013; 3: 420-426.
4) Smith ER, Ford ML, Tomlinson LA, Rajkumar C, McMahon LP, Holt SG. Phosphorylated fetuin-A-containing calciprotein particles are associated with aortic stiffness and a procalcific milieu in patients with pre-dialysis CKD. Nephrol Dial Transplant. 2012; 27: 1957-1966.
5) Hamano T, Matsui I, Mikami S, Tomida K, Fujii N, Imai E, et al. Fetuin- mineral complex reflects extraosseous calcification stress in CKD. J Am Soc Nephrol. 2010; 21: 1998-2007.
6) Miura Y, Iwazu Y, Shiizaki K, Akimoto T, Kotani K, Kurabayashi M, et al. Identification and quantification of plasma calciprotein particles with distinct physical properties in patients with chronic kidney disease. Sci Rep. 2018; 8: 1256.
7) Akiyama K, Miura Y, Hayashi H, Sakata A, Matsumura Y, Kojima M, et al. Calciprotein particles regulate fibroblast growth factor-23 expression in osteoblasts. Kidney Int. 2020; 97: 702-712.
8) Kuro-o M. Klotho, phosphate and FGF-23 in ageing and distributed mineral metabolism. Nat Rev Nephrol. 2013; 9: 650-660.
9) Sage AP, Lu J, Tintut Y, Demer L. Hyperphosphatemia-induced nanocrystals upregulate the expression of bone morphogenetic protein-2 and osteopontin genes in mouse smooth muscle cells in vitro. Kiney Int. 2011; 79: 414-422.
10) Smith ER, Ford ML, Tomlinson LA, Bodenham E, McMahon L, Farese s, et al. Serum calcification propensity predicts all-cause mortality in predialysis CKD. J Am Soc Nephrol. 2014; 25: 339-348.
11) Dahle DO, Asberg A, Hartmann A, Holdaas H, Bachtler M, Jenssen TG, et al. Serum calcification propensity is a strong and independent determinant of cardiac and all-cause mortality in kidney transplant recipients. Am J Transplant. 2016; 16: 204–212.
12) Pasch A, Block GA, Bachtler M, Smith ER, Jahnen-Dechent W, Arampatzis S, et al. Blood calcification propensity, cardiovascular events, and survival in patients receiving hemodialysis in the EVOLVE Trial. Clin J Am Soc Nephrol. 2017; 12: 315–322.
13) Bostom A, Pasch A, Madsen T, Roberts MB, Franceschini N, Steubl D, Garimella PS, et al. Serum calcification propensity and fetuin-A: Biomarkers of cardiovascular disease in kidney transplant recipients. Am J Nephrol. 2018: 48: 21–31.

14) Bundy JD, Cai X, Mehta RC, Scialla JJ, de Boer IH, Hsu Chi-Yuan, et al. Serum Calcification Propensity and Clinical Events in CKD. Clin J Am Soc Nephrol. 2019; 14: 1562–1571.

15) Chen W, Anokhina V, Dieudonne G, Abramowitz M, Kashyap R, Yan C, et al. Patients with advanced chronic kidney disease and vascular calcification have a large hydrodynamic radius of secondary calciprotein particles. Nephrol Dial Transplant. 2019; 34: 992-1000.

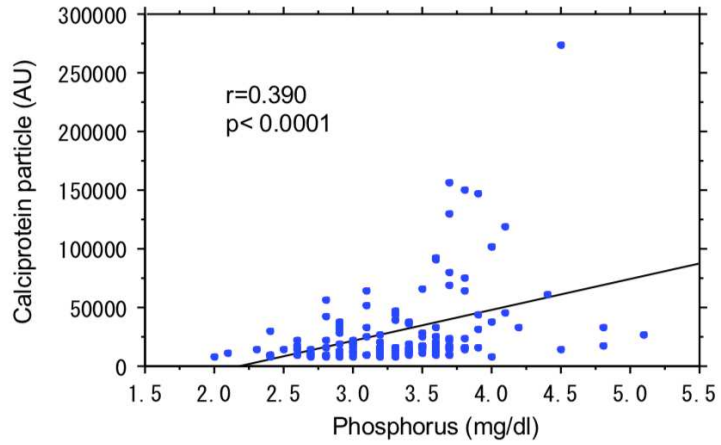
16) Lorenz G, Steubl D, Kemmner S, Pasch A, Koch-Sembdner W, Pham D, et al. Worsening calcification propensity precedes all-cause and cardiovascular mortality in haemodialyzed patients. Sci Rep. 2017; 7: 13368.

Figure legends

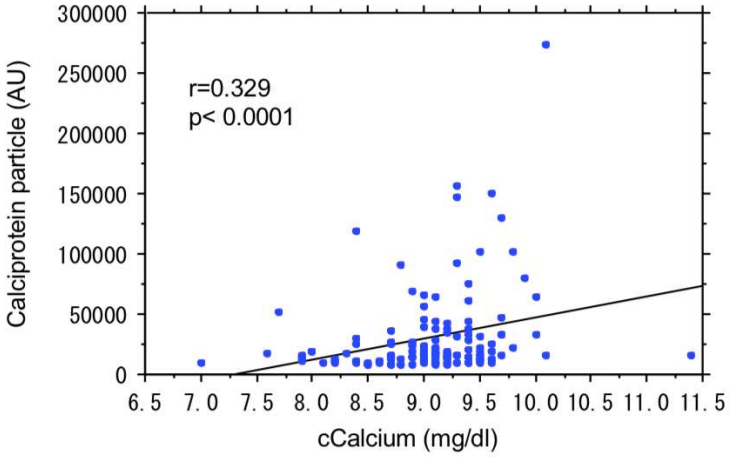
Fig 1. Relationship between CPP and phosphate (A), corrected calcium (B), eGFR (C). Phosphate and corrected calcium showed significant relationship with CCP, but eGFR did not show.

Fig 2. Relationship between CPP and Log ACS is shown. There is no significant correlation.

A


r=0.390
p< 0.0001
Calciprotein particle (AU)
Phosphorus (mg/dl)

B


r=0.329
p< 0.0001
Calciprotein particle (AU)
cCalcium (mg/dl)

C

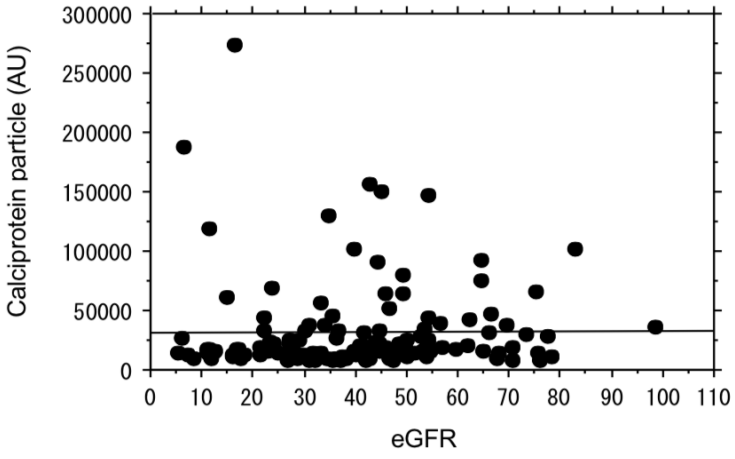

Calciprotein particle (AU)
eGFR

Figure 1

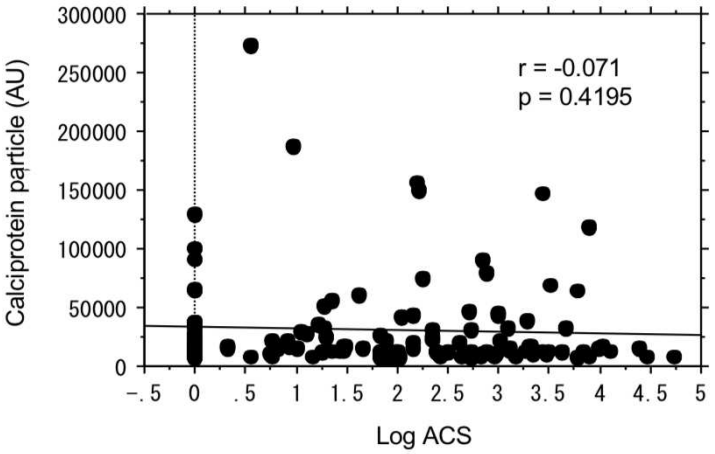

Calciprotein particle (AU)
300000
250000
200000
150000
100000
50000
0
r = -0.071
p = 0.4195
−.5
0
.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Log ACS

Figure 2

Table 1. Basic characteristics of patients (n=58)

Age	70.0±12.3
Sex	45 (77.5%)
Current smoking	9（15.5%）
BMI	23.8±4.0
History of diabetes	24 (41.3%)
History of CVD	17 (29.3%)
History of hyperlipidemia	35 (60.3%)
History of hypertension	45 (77.5%)
Systolic BP (mmHg)	131.7±17.8
Diastolic BP (mmHg)	77.7±9.7
Total protein (g/dl)	7.1±0.6
Albumin (g/dl)	4.1±0.4
BUN (mg/dl)	25.2±11.1
Creatinine(mg/dl)	1.42 (1.10-1.86)
eGFR (mL/min/1.73m^2)	39.7 (24.7-50.1)
corrected calcium (mg/dl)	9.3±0.4
phosphate (mg/dl)	3.4±0.6
HCO^3- (mmol/L)	24.5±2.5
Hemoglobin (g/dl)	13.3±1.7
CRP (mg/dl)	0.06 (0.03-0.18)
i-PTH (pg/ml)	67 (50-105)
1.25(OH_2) VitD3	50.1±22.4
FGF-23 (pg/ml)	63.7 (41.7-115.5)
CPP (AU)	16616 (11894-29152)
Urinary protein (g/gcr)	0.36 (0.09-1.19)
FE P (%)	23.5±14.7
ACS	655 (0-706)

BMI: body mass index, CVD: cardiovascular disease, BP: blood pressure, CRP: C-reactive protein, i-PTH: intact parathyroid hormone, VitD3: vitamin D3, FEP: fractional excretion of phosphate, ACS: arterial calcification score

Table 2. Univariate and multivariate analysis of relationship between ACS in lower extremities and CKD-MBD markers

	univariate		multivariate	
	r	*p*	*r*	*p*
Age	0.503	< 0.0001		
Smoking	0.291	0.0007	0.552	0.0004
Diabetes mellitus	0.388	< 0.0001	0.790	0.0009
Systolic BP	0.312	0.0003	0.017	0.0064
Albumin	-0.060	0.4943		
Creatinine	0.196	0.0238		
eGFR	-0.295	0.0005		
HCO^3-	-0.162	0.0634		
cCalcium	0.019	0.8307		
Phosphate	0.021	0.8074		
Log CRP	0.072	0.4102		
i-PTH	0.034	0.6983		
Hemoglobin	-0.178	0.0406	-0.018	0.7315
Urinary protein	0.172	0.0497	0.096	0.0766
1.25 (OH_2) VitD3	-0.141	0.1072		
FE P	0.175	0.0467	0.004	0.9971
FGF-23	0.096	0.2825		
Log CPP	-0.071	0.4195		

ACS: arterial calcification score, BP: blood pressure, CRP: C-reactive protein, i-PTH: intact parathyroid hormone, VitD3: vitamin D3, FE P: fractional excretion of phosphate

Table 3. Relationship between basic factors and change of ACS for one year

	r	*p*
Age	0.089	0.5102
Smoking	-0.134	0.3161
DM	-0.041	0.7608
Systolic BP	0.041	0.7607
Albumin	0.150	0.2620
Creatinine	-0.011	0.9334
eGFR	-0.007	0.9557
HCO^3-	-0.042	0.7549
cCalcium	-0.030	0.8257
Phosphate	0.195	0.1434
Log CRP	0.067	0.6162
i-PTH	-0.038	0.7805
Hemoglobin	-0.044	0.7450
Urinary protein	-0.159	0.2342
1.25 (OH_2) VitD3	0.043	0.7505
FE P	-0.090	0.5031
FGF-23	0.027	0.8440
Log CPP	0.073	0.5860

ACS: arterial calcification score, BP: blood pressure, CRP: C-reactive protein, i-PTH: intact parathyroid hormone, VitD3: vitamin D3, FE P: fractional excretion P,

Table 4. Univariate and multivariate analysis of relationship between rate of change of ACS and other factors

	univariate		multivariate	
	r	*p*	r	P
Change rate of Creatinine	-0.005	0.9712		
Change rate of eGFR	-0.042	0,7544		
Change rate of FGF-23	0.046	0.7411		
Change rate of CPP	0.292	0.0258	0.298	0.0144
Change rate of i-PTH	-0.124	0.3592		
Change rate of 1.25 (OH_2) VitD3	0.042	0.7606		

ACS: arterial calcification score, i-PHT: intact parathyroid hormone, VitD3: vitamin D3

Figures

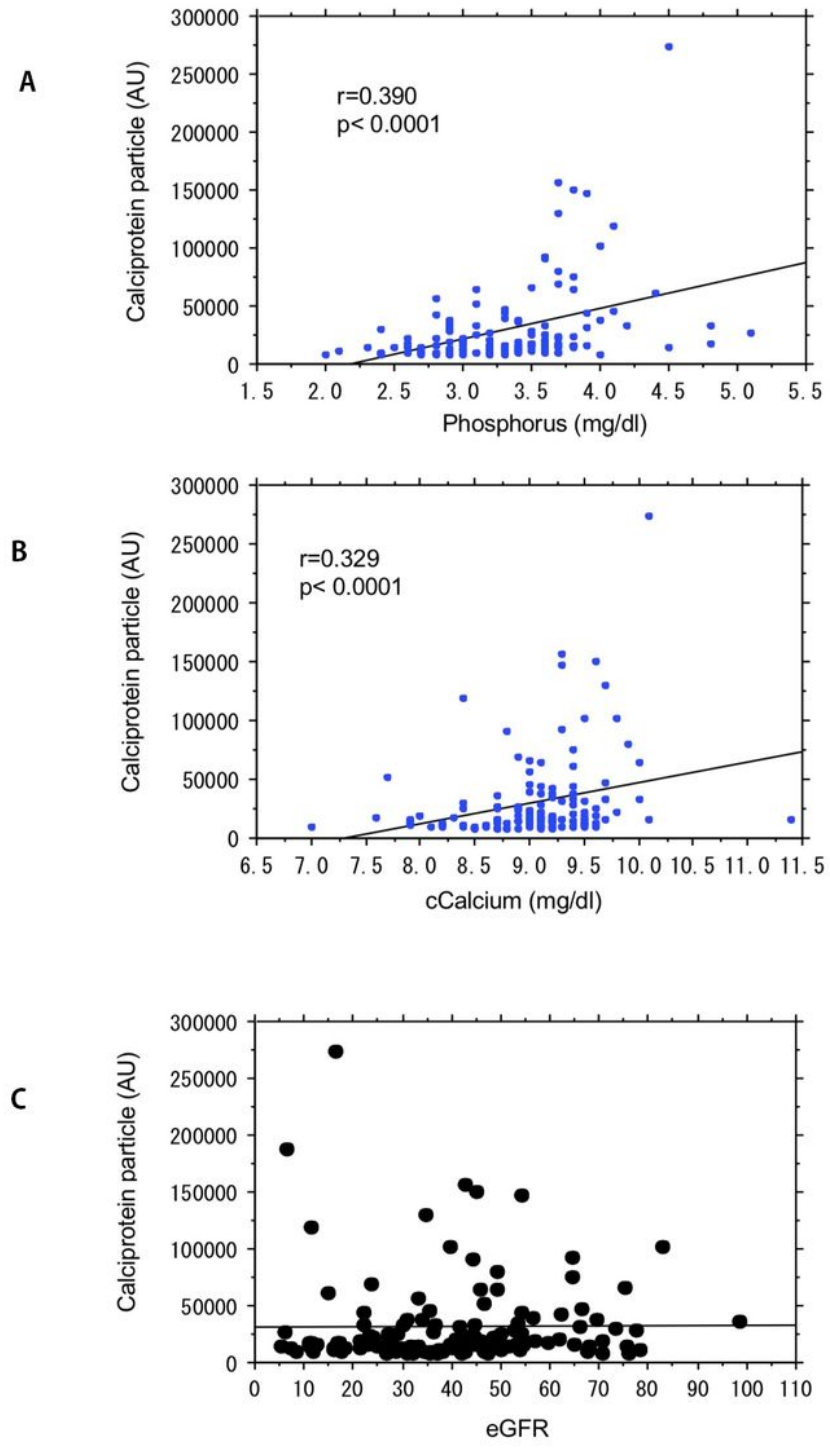


Figure 1

Relationship between CPP and phosphate (A), corrected calcium (B), eGFR (C).Phosphate and corrected calcium showed significant relationship with CCP, but eGFR did not show.

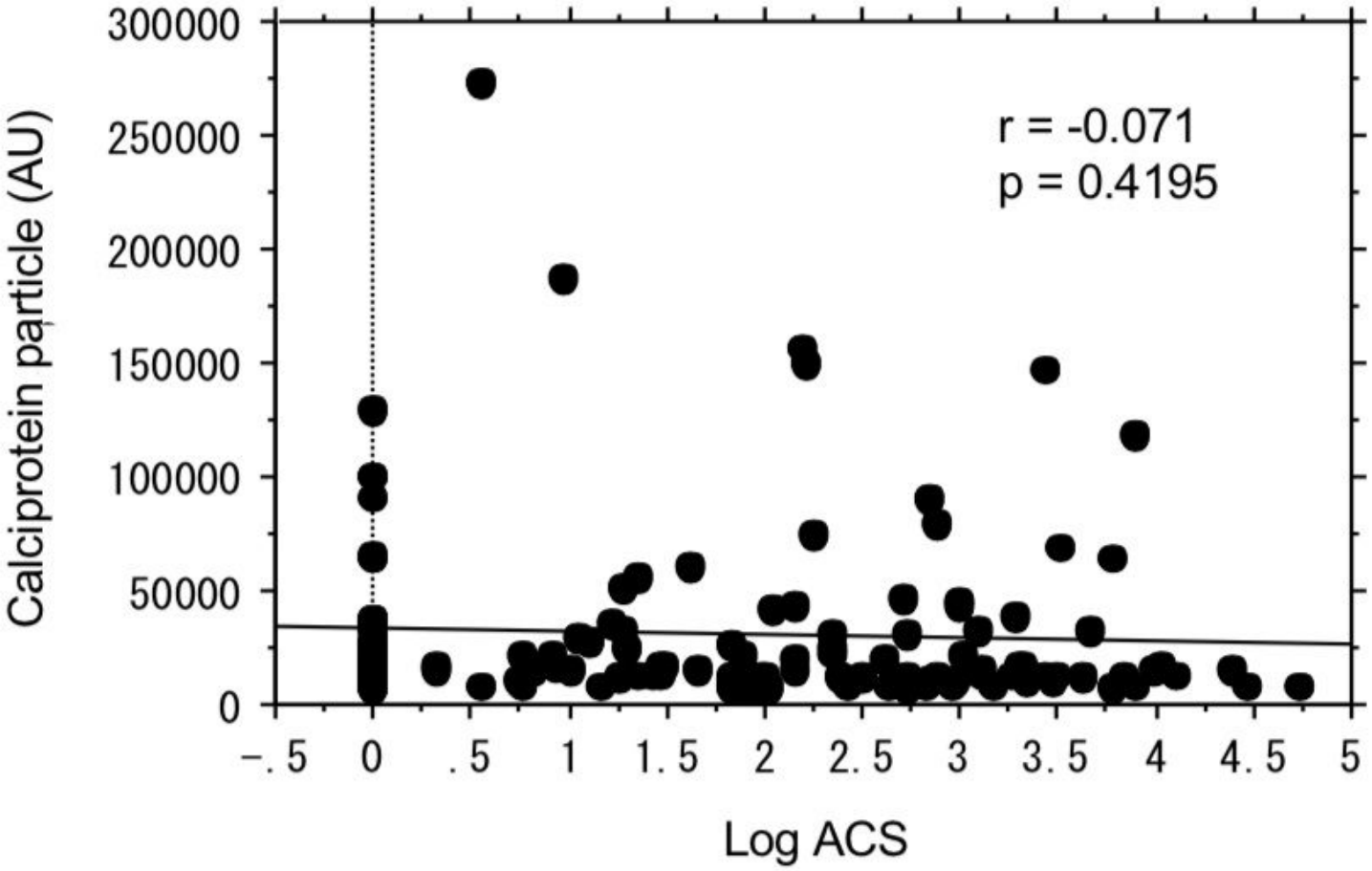


Figure 2

Relationship between CPP and Log ACS is shown. There is no significant correlation.