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ORIGINAL ARTICLE

Asymmetric dimethylarginine represents a precious risk indicator for radial artery calcification in patients with advanced kidney disease

Short title: ADMA represents an indicator for artery calcification

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Abstract

Introduction Medial arterial calcification (MAC) is frequent in chronic kidney disease (CKD) patients and is also considered a risk factor for morbidity and mortality.

Objectives The aim of the study was to evaluate the correlation between asymmetric dimethyloarginin (ADMA) level, radial artery calcification (RAC) and common carotid intimal medial thickness (CCA-IMT).

Patients and methods The studied group included 51 CKD patients in whom arteriovenous fistula for hemodialysis (HD) access was created, allowing collection of radial artery samples for histological examination and 33 healthy volunteers who were recruited in order to assess the reference concentrations of ADMA. Concentrations of creatinine, albumin, calcium, phosphate, fibroblast growth factor 23 (FGF-23), osteoprotegerin (OPG), osteopontin (OPN), osteocalcin (OC), secreted protein, acidic and rich in cysteine (SPARC), interleukin-6 (IL-6), interleukin-18 (IL-18), pentraxin3 (PTX3), stromal cell-derived factor-1 alpha (SDF1α), thrombomodulin (TM), soluble necrosis factor receptor II (sTNF-R II), transforming growth factor-β (TGF-β) and matrix metalloproteinase-2 (MMP-2) were determined. Fragments of radial artery were stained for calcifications using alizarin red. Ultrasonography was performed to assess CCA-IMT.

Results CKD patients had significantly higher ADMA than healthy controls. Patients with ADMA levels above the median were older, had higher phosphate, FGF-23, OPG, OPN, PTX, sTNFR II, MMP-2, TM and they had more atherosclerotic plaques in CCA. In multiple regression, log(sTNFR II), log(MMP-2), and SDF1α were independent predictors of log(ADMA). Patients with calcifications in the radial artery had significantly higher ADMA. Similar correlation was observed between SDF1α and Alizarin red staining 0 to 3. In logistic regression, ADMA positively predicted the presence of radial artery calcifications independently of age, HD status, Framingham risk score and PTX3.

Conclusions Our study is the first to found that circulating ADMA is an indicator of MAC in CKD patients.

Key words

asymmetric dimethyloarginin, end-stage renal disease, medial artery calcification, mineral-bone disease, stromal cell-derived factor-1 alpha

Introduction Elevated plasma concentrations of asymmetric dimethylarginine (ADMA) are associated not only with endothelial dysfunction and atherosclerosis but predict mortality and cardiovascular (CV) complications.[1-4] In subjects with mild to advanced chronic kidney disease (CKD), plasma ADMA was inversely related to glomerular filtration rate (GFR) and was an independent risk marker for progression to end stage renal disease (ESRD) and mortality.[5] In hemodialysis (HD) patients, plasma ADMA is a strong and independent predictor of overall mortality and cardiovascular outcome.[6] ADMA is a natural inhibitor of nitric oxide (NO) synthase and serum ADMA levels are strongly correlated with impaired flow-mediated vasodilatation and with carotid intima-media thickness. NO plays a crucial role in vascular protection because it inhibits proliferation and migration of vascular smooth muscle cells,

expression of adhesion molecules and platelet aggregation.[7] Three- to 9-fold increase in plasma levels of ADMA would inhibit NO production by 30% to 70%.[6] Elevated ADMA levels lead to myocardial fibrosis and microvascular dropout through direct effects on endothelial cells and fibroblasts and by indirectly stimulating production of additional, potent angiogenesis inhibitors.[8]

The aim of the present study was to evaluate the correlation between circulating ADMA level and radial artery calcification in CKD patients. We also studied the associations between serum concentration of ADMA and selected markers of inflammation, mineral-bone disease, endothelial dysfunction as well as matrix metalloproteinase-2 (MMP-2).

Patients and methods The studied group included 51 CKD patients (20 women, 31 men, 21 pre-dialysis and 30 hemodialyzed patients). The mean (standard deviation, SD) age of patients was 62 (16) years in whom arteriovenous fistula (AVF) for HD access was created for the first time, allowing collection of radial artery samples for histological examination and 33 healthy volunteers of comparable age and sex who were recruited in order to assess the reference concentrations of ADMA. The autologous AVFs were done by one surgeon at a single medical center. Cross-sectional data were obtained immediately before that procedure, and included clinical assessment of patients, common carotid intimal medial thickness (CCA-IMT) measurements and assessment of laboratory parameters (markers of inflammation, oxidative stress, endothelial dysfunction and bone turnover). Patients with active infection, positive history of hepatitis B or C, HIV infection, renal transplantation, parathyroidectomy and neoplastic disease, were excluded. Detailed medical history including, diabetes mellitus, hypertension, dyslipidemia, current smoking, duration of dialysis, and medications was recorded.

At the start of the study, ten-year risk of CAD for the patients was calculated using Framingham risk score (FRS) in accordance with the published guidelines [FRS calculator

https://www.framinghamheartstudy.org/risk-functions/cardiovascular-disease/10-year-risk.php]. Additionally, CCA-IMT was assessed by ultrasonography (B presentation, Acuson 128 XP/10 apparatus equipped with linear head at 5/7 MHz). The measurements were performed bilaterally at 0.5 cm and 2 cm below the division of the common carotid artery during diastolic phase of the heart cycle. The results were expressed as the arithmetic means of the values obtained for the left and right arteries.

Ethics statement

The study was conducted according to the principles of the Declaration of Helsinki and in compliance with the International Conference on Harmonization/Good Clinical Practice regulations. The study was approved by the Bioethics Committee of the Jagiellonian University and all patients signed an informed consent for their participation.

Histology

Small fragments of radial artery wall were collected during the first creation of arteriovenous fistula for HD access. Briefly, tissue sections were stained with alizarin red to detect calcium deposits and then they were subjected to microscopic assessment. The stained sections were examined using an Olympus BX-50 microscope (Olympus, Tokyo, Japan) in brightfield mode and images were registered using Olympus DP-71 digital CCD camera controlled by Olympus AnalySIS FIVE software. An experienced histologist evaluated the sections in a blind manner. The advancement of vascular calcification was evaluated semiquantitatively and the degree of mineralization was classified according to the following scale: 0 - no mineral content, 1 - a few small dispersed concretions, 2 - numerous small dispersed concretions, 3 - larger granular concretions, 4 - large areas occupied by fused mineral deposits. The calcifications were found exclusively in the vascular media (Figure 1). The reproducibility of the morphological analysis

was confirmed by Bland-Altman method and by calculating intraclass correlation coefficient (ICC) which was 0.88.

Laboratory tests

In all patients, selected biochemical parameters were measured, including creatinine concentration, glucose, parathyroid hormone (iPTH), total calcium (Ca) and phosphate (Pi), ADMA, stromal cell-derived factor-1 alpha (SDF1α), and matrix metalloproteinase-2 (MMP-2), interleukin-6 (IL-6), interleukin-18 (IL-18), pentraxin3 (PTX3), soluble tumor necrosis factor receptor II (sTNF-R II), thrombomodulin (TM), osteoprotegerin (OPG), osteopontin (OPN), osteocalcin (OC), secreted protein, acidic and rich in cysteine (SPARC), fibroblast growth factor 23 (FGF-23). The eGFR was calculated by Modification of Diet in Renal Disease (MDRD) formula: eGFR = [186 x serum creatinine (μmol/l) x $0.0113]^{-1.154}$ x $age^{-0.203}$ x 114 × (0.742 for women).

A sample of peripheral venous blood was collected at fasting, in the morning prior to creation of AVF into ethylenediaminetetraacetic acid tubes and plasma was kept frozen at −70°C for subsequent biochemical analyses. Routine biochemical tests were carried out using automatic biochemical analyzers: Hitachi 917 (Hitachi, Japan) and Modular P (Roche Diagnostics, Mannheim, Germany).

ADMA levels in the platelet-poor EDTA plasma were measured by a commercially available ELISA (ADMA *Xpress* ELISA Kit - DLD Diagnostika GmbH., Hamburg, Germany). The detection limit for the assay was 0.04 μmol/l. The intra- and interassay precision was 10.8% and 7.9%, respectively. The cross reactivity of L-arginine and symmetric dimethylarginine was <0.02% and <0.6%, respectively. The reference range for ADMA is 0.26-0.64 μmol/l.

SDF1α levels in the platelet-poor EDTA plasma were measured by a commercially available ELISA (Human CXCL12/ SDF1α Immunoassay-R&D Systems, Minneapolis, MN, USA). The

minimum detectable dose for the assay was 0.018 ng/ml. The intra and interassay precision was 3.9% and 13.4%, respectively. The reference range for SDF1α is 1.3-2.9 ng/ml.

Inflammatory, calcification and endothelial dysfunction markers were assessed using ELISA micro-plate immunoassays and ELX808 automatic reader (BIO-TEK® Instruments Inc., Vermont, VT, USA). The following kits were applied: IL-6, IL-18, PTX3, TNF-R II, TM, OPG (BioVendor, Brno, Czech Republic); SPARC, OPN and MMP-2 (R&D Systems, Minneapolis, MN, USA); OC (Metra/Quidel, CA, USA), FGF-23 (Immunotopics Int., San Clemente, California, United States).

Statistical analysis

Number of patients (percentage of the respective group) was reported for categories. Contingency tables were analyzed with chi-squared test. Median (lower-upper quartile) was reported for non-normally distributed and mean (SD) for normally distributed quantitative variables, respectively. Distributions were tested for normality with Shapiro-Wilk test. Mann-Whitney test or unpaired t-test were used to assess differences between groups, according to distribution. Pearson correlation coefficients and linear regression models were calculated following log-transformation of right-skewed variables. In order to assess independent predictors of log (ADMA), backward stepwise linear regression was calculated. Standardized regression coefficients (beta) ± standard errors were reported for linear regression. Simple and multiple logistic regression (with pre-specified covariates) was used to study association between ADMA concentrations and vascular calcifications. Resulting odds ratios (OR) were reported with 95% confidence intervals (95%CI). The tests were two-tailed and $P \leq 0.05$ was considered significant. Statistica 12 (StatSoft, Tulsa, USA) software was used for calculations.

Results

Biochemical results

The relationships between ADMA concentrations and the markers of inflammation, turnover, endothelial dysfunction and MMP-2

The characteristics of the studied group is shown in Tables 1, 2 and 3.

The studied group included 21 predialysis patients with stage 5 CKD and 30 patients treated with maintenance hemodialysis (Table 1). Age, sex, and the prevalence of traditional cardiovascular risk factors did not differ significantly between the groups. Also, the groups were comparable with regard to the degree of radial artery calcifications, CCA-IMT values, and the presence of calcified atherosclerotic plaques in CCA (Table 1). Except for diuretics (less useful among HD patients) and erythropoietin (used more often in HD group), there were no significant differences in prescribed drugs (Table 1). Serum creatinine, calcium-phosphate product (but not PTH), OPG, and OPN (but not OC and SPARC) were significantly higher in HD group. Moreover, selected inflammatory factors (PTX-3, IL-6, and TNFR II) as well as TM and ADMA concentrations were higher in dialyzed stage 5 CKD patients (Table 1).

CKD patients had significantly higher ADMA concentrations as compared to healthy controls: 0.779 (0.672-0.947) vs 0.572 (0.481-0.602) μmol/l; $P<0.001$. ADMA concentrations were significantly higher in dialyzed than in pre-dialysis patients (Table 1), still, the pre-dialysis patients had significantly higher ADMA comparing to controls ($P<0.001$). Both dialysis status and serum creatinine independently predicted ADMA concentrations in multiple regression. Patients with ADMA concentrations above the median value were characterized by older age, higher concentrations of phosphate and higher calcium-phosphate product, higher concentrations of FGF-23, OPG, OPN, PTX, sTNFR II, MMP-2 and TM (Table 2 and Table 3). Among patients with ADMA above the median, calcifications of radial artery revealed by alizarin red staining

were more common, especially those with grades 1-3 (Figure 1). Furthermore, atherosclerotic plaques in common carotid artery were more prevalent in these patients.

ADMA above median was also associated with more prevalent female sex, and hemodialysis treatment (Table 2). There were no significant associations between drug administration in the studied CKD patients and ADMA concentrations.

Significant correlations were observed between log (ADMA) and:

- log (serum creatinine) (r = 0.38; P = 0.007),

- log (Pi) (r = 0.37; P = 0.008), log (Ca x Pi) (r = 0.43; P = 0.002), log (FGF-23) (r = 0.42; P = 0.003),

- log (OPG) (r = 0.47; P = 0.001), log (OPN) (r = 0.52; P <0.001), log (OC) (r = 0.31; P = 0.04),

- serum albumin (r = -0.33; P = 0.02), log (PTX3) (r = 0.31; P = 0.04), log (IL6) (r = 0.36; P = 0.01), log (IL18) (r = 0.29, P = 0.048), log (sTNFR II) (r = 0.59; P <0.001),

- log (MMP-2) (r = 0.42; P = 0.003), log (TM) (r = 0.30; P = 0.03), SDF1α (r = 0.39; P = 0.005).

The associations with Ca x Pi, FGF-23, OPG, OPN, serum albumin, sTNFR II, MMP-2 and SDF1α were independent of serum creatinine concentrations. In backward stepwise multiple linear regression, log (sTNFR II) (beta=0.36 (0.15); P = 0.02), log (MMP-2) (beta = 0.31 (0.10); P = 0.003), and SDF1α (beta = 0.24 (0.11); P = 0.04) were independent predictors of log (ADMA).

We have not observed significant associations between ADMA or SDF-1 and traditional CV risk factors. No differences were found in ADMA and SDF-1 concentrations between patients with diabetes, hypertension, dyslipidemia or active smoking. No correlations were observed between log(ADMA) or SDF-1 and age, BMI or Framingham score.

Histological findings

Patients with calcifications in the radial artery had significantly higher ADMA concentrations: 0.847 (0.684-0.978) vs 0.708 (0.650-0.852) μmol/l; $P = 0.029$. Among patients with calcification grade 0 to 3, there was significant correlation between ADMA concentration and the grade ($r = 0.54$; $P < 0.001$; Figure 2). In contrast, patients with most advanced calcifications (grade 4) had relatively low concentrations of ADMA: 0.724 (0.668-0.770) μmol/l (Figure 2). In logistic regression analysis, high ADMA predicted the presence of radial artery calcifications (especially grade 1-3) independently of HD status and other predictors of calcifications, i.e. age, Framingham risk score and PTX concentrations (Table 3).

Similar correlation (although weaker) was observed between SDF1α and Alizarin red staining 0 to 3 ($r = 0.33$; $P = 0.03$; Figure 3).

Ultrasonography of common carotid artery

Data from common carotid artery ultrasound were available in 44 (86%) patients, including 28 men and 16 women, aged 60 (17) years, of whom 26 (59%) were hemodialyzed at the start of the study. Of these patients, 23 had ADMA concentrations below 0.779 μmol/l (i.e. whole group median) and 21 had ADMA above the value. CCA-IMT values did not differ significantly between patients with ADMA below and above 0.779 μmol/l [0.87 (0.75-1.05) versus 0.95 (0.90-1.10); $P = 0.06$], nor were significantly correlated with ADMA concentrations ($r = 0.30$; $P = 0.06$). However, calcified atherosclerotic plaques in common carotid artery were more prevalent in patients with ADMA concentrations above 0.779 μmol/l [11 patients (52%) versus 3 patients (13%); $P = 0.005$].

Discussion

ADMA and arterial calcification Our studies have shown that elevated concentration of circulating ADMA was related to higher risk of medial arterial calcification (MAC) in patients with advanced renal disease. So far, the relationship between circulating ADMA and vascular

calcification assessed histologically has not been studied. Our data suggests that excessive accumulation of ADMA in serum is accompanied by MAC, especially of its lower grades. In contrast, high concentrations of ADMA did not correlate with high CCA-IMT recognized as an early marker of sub-clinical atherosclerosis, although the patients with high ADMA levels more frequently had calcified carotid plaques- intimal calcifications. We found an association between ADMA and bone turnover parameters which were increased in patients with ADMA concentrations above the median value. This finding highlights ADMA participation in the development of calcifications of both intima and media of the arteries. In recent years, there has been an increasing interest in ADMA. Clinical studies included in the recently published meta-analysis,[9] demonstrated that patients with myocardial infarction, as well as with stable and unstable angina pectoris had elevated plasma ADMA concentrations, thus supporting the suggestion that ADMA concentrations reflect coronary plaque vulnerability. Our research confirmed that ADMA concentrations were also higher in patients with atherosclerotic plaques in CCA. The importance of ADMA has also been emphasized in the study of Opalińska et al[10] in patients with advanced atherosclerosis. Authors identified the inflamed plaques by scintigraphy using IL-2 labelled 99mTc in the selected, high CV risk group of CKD patients. ADMA concentrations were found to be significantly higher in the group of patients with the highest values of IL-2 uptake in scintigraphy.[8] However, in contrast to other studies,[11,12] we have not found the relation between ADMA and IMT.

The ADMA concentrations in our patients were significantly higher than ADMA levels in healthy subjects of similar age from European populations and without evidence of CKD and atherosclerotic vascular disease. This corresponds with results of meta-analysis,[9] indicating that patients with artery disease showed higher ADMA levels than healthy controls. The ADMA levels increased with the progression of kidney disease: the pre-dialysis patients had significantly

higher ADMA comparing to healthy participants and ADMA concentrations were significantly higher in dialyzed than in pre-dialysis patients, as also confirmed by other reports.[13]

The available literature includes only a few articles on the relationship between ADMA and vascular calcification – this relationship is confirmed by imaging studies but not assessed by histology.[14-17] Coronary artery calcification score (CACS) is regarded as an indicator of the severity of atherosclerotic artery disease and may accurately identify high-risk asymptomatic dialysis patients at the start of dialysis.[14] In the *CARDIA* study[16] the median level of ADMA was significantly higher in cases with the presence of CAC (revealed by computed tomography, CT) than in controls. In logistic regression model adjusted for age, smoking status, alcohol consumption, BMI, waist circumference, hypertension, diabetes, LDL and HDL cholesterol, triglycerides, renal function and CRP, the highest tertile of ADMA (compared with the lowest tertile) was associated with increased odds (OR 1.8) of the presence of CAC. By linear regression, a significant independent relationship was also found between ADMA and the degree of CAC. Our study revealed a significant association of baseline ADMA with severity RAC in CKD patients. In agreement with our study, Kobayashi et al,[17] demonstrated that plasma ADMA levels were negatively correlated with GFR and positively correlated with CACS measured by multidetector-row CT according to Agatston score. The patients with severe calcifications had significantly higher values ADMA levels, insulin resistance measured by the homeostasis model assessment (HOMA-IR), and fibrinogen along with serum levels of phosphorus compared with patients with mild CACS.

In our study, positive correlation observed between RAC and ADMA was confirmed by multiple regression analysis, in which ADMA was shown to predict artery calcification independently of age, HD status, FRS as classical risk factors and PTX3 as an acute-phase reactant, which is synthesized locally, at the site of inflammation.

ADMA and selected markers of inflammation, endothelial dysfunction, mineral-bone disease and MMP-2

In hemodialysis patients, higher ADMA concentration is a strong and independent predictor of overall mortality and CV outcome.[6] Normal endothelial function depends on nitric oxide (NO) release by endothelial cells. Asymmetric dimethylarginine (ADMA), by competing with L-arginine, inhibits NO production and may cause endothelial dysfunction leading to atherosclerotic process.[7,18] Advanced renal failure is accompanied by accumulation of a naturally occurring inhibitors of NO synthase.[19] Endothelial dysfunction as assessed by ADMA levels and inflammation has been consistently linked to atherosclerosis, CV events and death in CKD patients.[20] In our study, there was a significant correlation between ADMA and the severity of radial artery calcifications in case of mild and moderate calcifications, expressed by alizarin red staining grades 1-3. Unexpectedly, patients with most advanced calcifications (alizarin red staining grade 4) had relatively low concentrations of ADMA. It suggests that ADMA-induced endothelial damage leading to calcium deposition in the arterial wall is most pronounced in earlier stages of the process and later the calcification progresses (probably influenced by other factors, e.g. those involved in bone turnover) even if ADMA levels decrease. We also found that markers of inflammation (IL6, IL18, sTNFR II) positively correlate with ADMA. These findings are in agreement with previous studies[20-22] and suggest that ADMA may be involved in the inflammatory reaction induced by uremia. Proinflammatory cytokines and metabolic abnormalities associated with systemic inflammation are considered one of principal mechanisms leading to endothelial dysfunction.[23] In the present study, SDF1α, sTNFR II and MMP-2 were independent predictors of increase ADMA level. According to the available

literature, there is no data on the relationship between ADMA and MMP-2 in patients with renal failure. Matrix metalloproteinases are proteolytic enzymes that degrade the extracellular matrix (ECM) and facilitate proliferation and migration of endothelial and vascular smooth muscle cells which are involved in the initiation of calcification.[24]

Results of the present study indicate that circulating ADMA seems to be a key biomarker of both, MAC and advanced atherosclerosis with calcified atherosclerotic plaques. Higher levels of calcium-phosphate product and bone-related proteins (FGF-23, OPG, OPN) were associated with high ADMA levels, independently of serum creatinine concentrations. Also, higher concentration of endothelial dysfunction marker SDF1α was correlated with higher serum ADMA. Moreover, SDF1α was predictor of increase ADMA level. Thus, ADMA seems to promote endothelial dysfunction. The endothelium, especially in early atherosclerosis, undergoes a constant process of injury and repair in order to maintain normal vascular function and structure.[25] SDF1α has been shown to induce neovascularization by recruiting endothelial progenitor cells (EPC) into ischemic tissues by increased SDF1α/CXC motif chemokine receptor 4 (CXCR4) coupling.[26] Gossl et al[27] identified a subgroup of bone marrow derived, circulating EPC with an osteogenic phenotype (expressing the osteoblast marker osteocalcin, OCN) and found that patients with coronary endothelial dysfunction have significantly higher numbers of circulating OCN(+) EPC compared with patients with normal endothelial function. In cell culture, circulating EPC with high expression of OCN were capable of forming mineralized deposits. Another study [28] showed that in contrast to controls, patients with coronary endothelial dysfunction retain osteogenic EPC within the coronary circulation and this retention is accompanied by the release of SDF1α and IL-8. The authors conclude that retention of osteogenic EPC for endothelial repair may lead to the induction and progression of coronary calcification rather than to normal repair.

In our study, similarly to the results obtained for ADMA, in patients with radial artery

calcification grades 1 to 3 there was a significant correlation between SDF1α concentration and the calcification grade, whereas patients with most advanced calcifications (grade 4) had comparatively low concentrations of SDF1α. Thus, in the uremic environment, elevated serum ADMA and SDF1α can serve as indicators of the vascular calcification process.

ADMA and angiotensin-converting enzyme inhibitors (ACEIs), or angiotensin receptor blockers (ARBs)

ADMA may not only be a marker but also an active player in CVD, which makes it a potential target for therapeutic interventions. The present study showed that the use of ACEIs, or ARBs were not significant for ADMA concentration while in the study Gamboa et al. [29] indicated that short-term ACE inhibition increases ADMA in HD patients whereas ARBs do not. Interestingly, in the general population without kidney injury, ACEIs and ARBs decrease ADMA levels.[30,31] In the current study, also, no correlations were found between the other drugs administration (β-blocker, calcium channel blocker, statin, vitamin D, calcium, erythropoietin) and ADMA concentrations.

Limitations of the study

Limitations of the present study include relatively low number of participants, small size of radial artery fragments and the fact that we relied on a single blood sample per patient, collected at the beginning of the study. We also acknowledge that it is not possible to demonstrate a cause-effect relationship on the basis of a cross-sectional study. Nevertheless, this is the first study on the effect of circulating ADMA levels on histologically assessed calcification in CKD patients. Our findings support the hypothesis that elevated plasma ADMA is an important risk factor for CVD in CKD patients. High costs limit the application of imaging diagnostic methods such as

CACS, which in many CKD patients might be replaced by the measurement of baseline ADMA concentration for the assessment of CV risk already at the beginning of dialysis.

Conclusions Our study is the first to found that circulating ADMA is an indicator of MAC in CKD patients and suggests that plasma ADMA could be a useful, simple biomarker of CVD risk in daily clinical practice.

Contribution statement MK and KK conceived the study, were the major participants in its design, coordination, interpretation of results and statistical analysis, they also prepared draft manuscript. MG carried out histological examinations. PD performed statistical analysis. KW and BG participated in the design of the study. GK, AD and JAL participated in design of the study and analyzed the data. WS participated in study design and coordination. All authors were involved in data collection, draft manuscript modifications and approved the final version of the manuscript.

Disclosure Statement

The manuscript has not been published elsewhere.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Table 1. Clinical characteristics of patients and the results of laboratory tests in predialysis stage 5 CKD patients and HD patients

	Predialysis stage 5 CKD patients (n = 21)	HD patients (n = 30)	*P* value
Age, years	62 (13)	62 (18)	0.8
Male sex, N (%)	13 (62)	18 (60)	0.9
Dialysis therapy duration, months	-	8 (3-38)	-
BMI, kg/m^2	27.3 (5.7)	25.6 (6.1)	0.2
Diabetes, N (%)	8 (38)	11 (37)	0.9
Hypertension, N (%)	20 (95)	25 (83)	0.2
Dyslipidemia, N (%)	12 (57)	20 (67)	0.5
Active smoking, N (%)	6 (29)	9 (30)	0.9
Framingham risk score, %	9 (8-20)	11 (6-22)	0.8
Radial artery calcifications: None, N (%) Stage 1-3, N (%) Stage 4, N (%)	 9 (43) 8 (38) 4 (19)	 11 (37) 15 (50) 4 (13)	0.7
CCA-IMT, mm[a]	0.90 (0.75-1.00)	1.00 (0.85-1.05)	0.1
Calcified atherosclerotic plaque in CCA, N (%)[b]	4 (22)	10 (38)	0.3
ACEI / ARB use, N (%)	4 (19)	6 (20)	0.9
β-blocker use, N (%)	13 (62)	21 (70)	0.5

Calcium channel blocker use, N (%)	12 (57)	12 (40)	0.2
Diuretic use, N (%)	19 (90)	14 (47)	0.001
Number of hypotensive drugs used	3 (2-4)	2 (1-4)	0.3
Statin use, N (%)	15 (71)	11 (37)	0.01
Vitamin D use, N (%)	6 (29)	12 (40)	0.4
Calcium use, N (%)	12 (57)	20 (67)	0.5
Erythropoietin use, N (%)	1 (5)	15 (50)	<0.001
Serum creatinine, μmol/l	389 (512-269)	506 (411-571)	<0.001
Ca, mmol/l	2.20 (0.28)	2.20 (0.19)	0.8
Pi, mmol/l	1.39 (1.27-1.54)	1.62 (1.35-1.86)	0.03
Ca x Pi, $mmol^2/l^2$	2.94 (2.69-3.15)	3.60 (2.96-4.16)	0.009
iPTH, pg/ml	303 (186-512)	237 (166-398)	0.6
FGF-23, RU/ml	476 (357-1021)	1184 (935-5066)	0.006
OPG, pmol/l	5.44 (3.03-7.77)	9.37 (7.15-14.15)	0.02
OPN, ng/ml	225 (185-315)	356 (270-621)	0.005
OC, ng/ml	41.9 (29.3-51.3)	41.6 (29.0-78.2)	0.4
SPARC, ng/ml	119 (76-159)	106 (79-167)	0.9
Serum albumin, g/l	42.1 (3.6)	40.0 (6.0)	0.3
PTX3, ng/ml	0.78 (0.52-2.11)	1.79 (1.08-2.93)	0.03
IL-6, pg/ml	2.97 (2.06-4.66)	5.82 (2.57-8.57)	0.05
IL-18, pg/ml	631 (461-775)	601 (496-905)	0.8
TNFR II, μg/ml	9.85 (8.92-11.92)	16.64 (11.91-22.34)	0.003
MMP-2, ng/ml	216 (187-295)	253 (187-358)	0.4

TM, ng/ml	15.4 (13.9-17.3)	19.4 (14.9-20.4)	0.007
SDF1α, ng/ml	2.90 (2.67-3.38)	3.06 (2.72-3.39)	0.6
ADMA, μmol/l	0.673 (0.655-0.779)	0.885 (0.745-0.978)	<0.001

[a] The results of CCA ultrasound were available for 44 patients, including 18 predialysis and 26 HD patients.

Abbreviations: ACE-I, angiotensin-converting-enzyme inhibitors; ARB, angiotensin II receptor blockers; BMI, body mass index; CCA-IMT, common carotid artery intima media thickness; Ca, calcium; FGF-23, fibroblast growth factor 23; IL-6, interleukin-6; IL-18, interleukin-18; iPTH, intact parathyroid hormone; MMP-2, matrix metalloproteinase-2; OPG, osteoprotegerin; OPN, osteopontin; OC, osteocalcin; Pi, phosphate; PTX3, pentraxin3; TNF-R II, tumor necrosis factor receptor II; TM, thrombomodulin; SDF1α, stromal cell-derived factor α; SPARC, secreted protein acidic and cysteine rich

Table 2. Clinical characteristics and the treatment used among stage 5 CKD patients with ADMA concentrations below and above the median value of 0.779 μmol/l

	ADMA<median (N=25)	ADMA≥median (N=26)	*P*-value
Age, years	57 (17)	67 (13)	0.03
Male sex, N (%)	19 (76)	12 (46)	0.03
Hemodialyzed, N (%)	10 (40)	20 (77)	0.007
Dialysis therapy duration, months[a]	18 (4-38)	6 (2-38)	0.6
BMI, kg/m^2	26.2 (5.6)	26.3 (6.4)	0.9
Diabetes, N (%)	8 (32)	11 (42)	0.4

Hypertension, N (%)	23 (92)	22 (85)	0.4
Dyslipidemia, N (%)	14 (56)	18 (69)	0.3
Active smoking, N (%)	7 (28)	8 (31)	0.8
Framingham risk score, %	11 (6-22)	11 (7-22)	0.7
Radial artery calcifications: none, N (%) Stage 1-3, N (%) Stage 4, N (%)	 13 (52) 6 (24) 6 (24)	 7 (27) 17 (65) 2 (8)	0.01
ACEI / ARB use, N (%)	6 (24)	4 (15)	0.4
β-blocker use, N (%)	18 (72)	16 (62)	0.4
Calcium channel blocker use, N (%)	15 (60)	9 (35)	0.07
Diuretic use, N (%)	17 (68)	16 (62)	0.6
Number of hypotensive drugs used	3 (2-4)	2 (1-3)	0.2
Statin use, N (%)	13 (52)	13 (50)	0.9
Vitamin D use, N (%)	7 (28)	11 (42)	0.3
Calcium use, N (%)	14 (56)	18 (69)	0.3
eEythropoietin use, N (%)	7 (28)	9 (35)	0.6

[a] values provided for hemodialyzed patients (n = 30)

Abbreviations: ACE-I, angiotensin-converting-enzyme inhibitors; ARB, angiotensin II receptor blockers; BMI, body mass index; CCA-IMT, common carotid artery intima media thickness

Table 3. The results of laboratory tests among stage 5 CKD patients with ADMA concentrations below and above the median value of 0.779 μmol/l

	ADMA<median (n = 25)	ADMA≥median (n = 26)	*P* value
Serum creatinine, μmol/l	408 (313-474)	468 (405-547)	0.08
Ca, mmol/l	2.20 (0.25)	2.20 (0.20)	0.8
Pi, mmol/l	1.38 (1.18-1.64)	1.57 (1.39-1.95)	0.02
Ca x Pi, $mmol^2/l^2$	2.91 (2.43-3.39)	3.58 (2.98-4.08)	0.003
iPTH, pg/ml	292 (206-524)	218 (154-343)	0.3
FGF-23, RU/ml	504 (292-1243)	1147 (935-3734)	0.02
OPG, pmol/l	5.44 (2.72-7.69)	10.00 (7.70-12.66)	0.002
OPN, ng/ml	258 (154-357)	354 (253-629)	0.02
OC, ng/ml	41.8 (28.0-59.5)	41.4 (29.4-69.5)	0.5
SPARC, ng/ml	104 (71-139)	126 (88-190)	0.2
Serum albumin, g/l	42.2 (3.9)	39.4 (6.0)	0.05
PTX3, ng/ml	0.95 (0.56-2.13)	1.55 (1.08-3.06)	0.03
IL-6, pg/ml	2.97 (1.69-6.51)	5.24 (2.89-8.01)	0.1
IL-18, pg/ml	575 (428-678)	695 (507-905)	0.09
TNFR II, μg/ml	9.85 (8.30-12.92)	17.65 (13.70-20.67)	<0.001
MMP-2, ng/ml	208 (177-256)	291 (229-391)	0.005
TM, ng/ml	15.4 (13.9-17.6)	18.9 (16.9-20.1)	0.05
SDF1α, ng/ml	2.98 (2.57-3.38)	3.11 (2.90-3.39)	0.1

Abbreviations: Ca, calcium; FGF-23, fibroblast growth factor 23; IL-6, interleukin-6; IL-18, interleukin-18; iPTH, intact parathyroid hormone; MMP-2, matrix metalloproteinase-2; OPG, osteoprotegerin; OPN, osteopontin; OC, osteocalcin; Pi, phosphate; PTX3, pentraxin3; TNF-R II, tumor necrosis factor receptor II; TM, thrombomodulin; SDF1α, stromal cell-derived factor α; SPARC, secreted protein acidic and cysteine rich

Table 4. Logistic regression analysis to predict radial artery calcifications as detected with alizarin red staining

Independent variable	OR (95%CI) for radial artery calcifications stage 1-4		OR (95%CI) for radial artery calcifications stage 1-3	
	Simple model	Multiple model	Simple model	Multiple model
ADMA, per 10 µmol/l	1.49 (1.02-2.16)	1.76 (1.03-3.03)	1.71 (1.12-2.61)	2.29 (1.15-5.86)
Age, per 1 year	-	1.03 (0.97-1.09)	-	1.07 (0.98-1.16)
Hemodialyzed	-	0.92 (0.17-4.99)	-	0.41 (0.04-4.50)
Framingham risk score, per 1 %	-	1.06 (0.96-1.18)	-	1.05 (0.94-1.16)
PTX3, per 1 ng/ml	-	0.97 (0.57-1.64)	-	0.87 (0.51-1.49)

Abbreviations: ADMA, asymmetric dimethyloarginin; PTX3, pentraxin3

Figure 1. Calcifications of various grades (RAC°1-a, RAC°2-b, RAC°3-c, RAC°4-d) in radial artery sections stained with alizarin red.

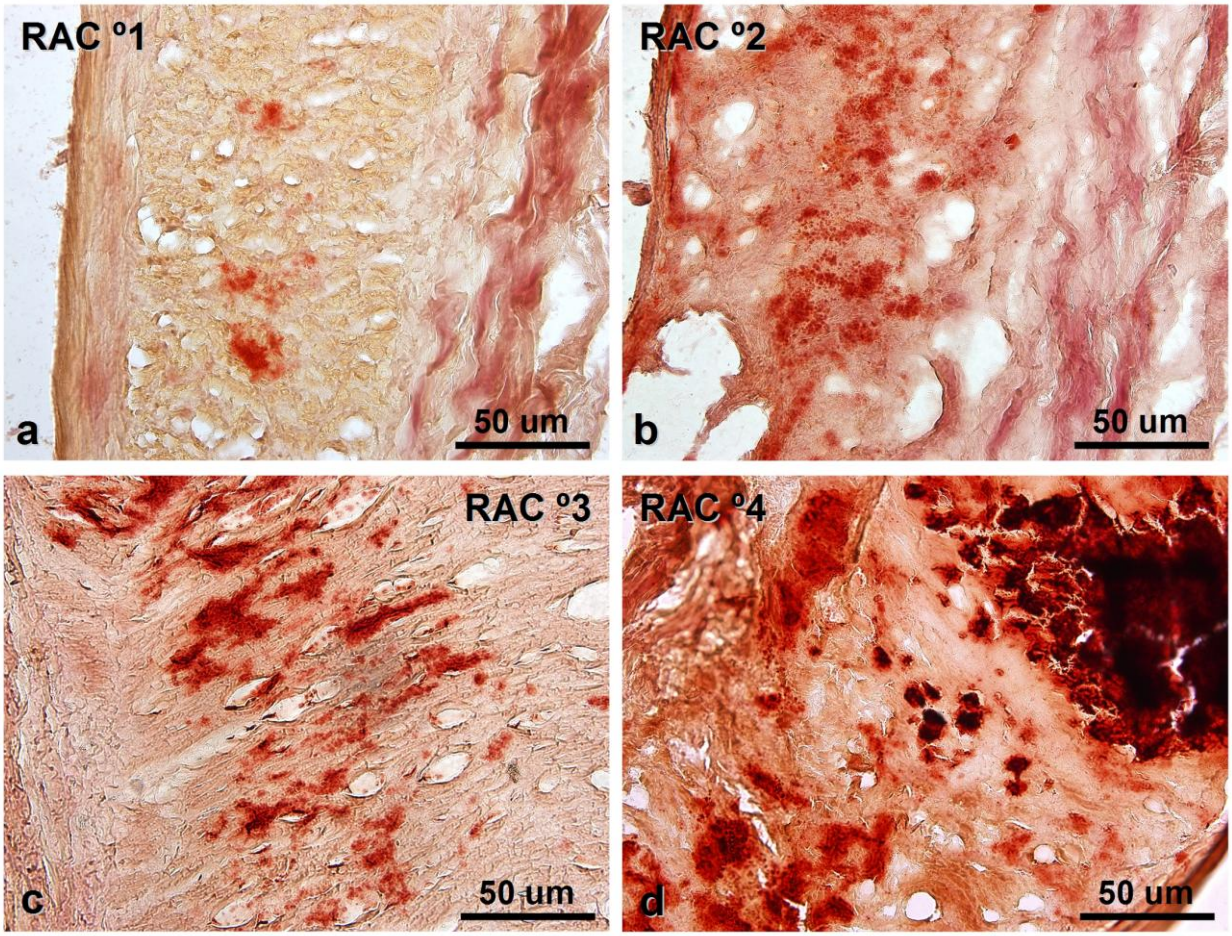

Figure 2. The association between serum ADMA concentrations and the advancement of radial artery calcifications

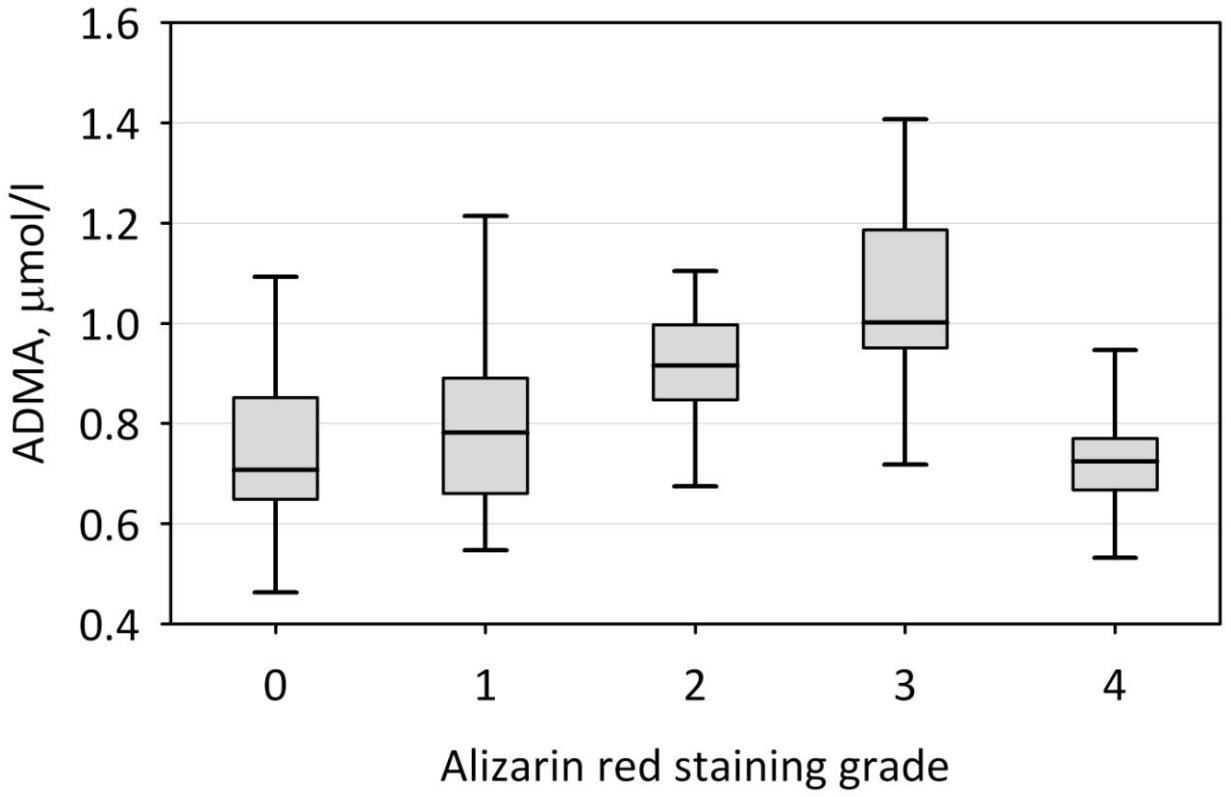

Figure 3. The association between serum SDF1α concentrations and grade of radial artery calcifications