ORIGINAL ARTICLE

Asymmetric dimethylarginine as a useful risk marker of radial artery calcification in patients with advanced kidney disease

Marcin Krzanowski[1], Katarzyna Krzanowska[1], Mariusz Gajda[2], Paulina Dumnicka[3], Grzegorz Kopeć[4], Bartłomiej Guzik[5], Karolina Woziwodzka[1] Artur Dziewierz[6], Jan A. Litwin[2], Władysław Sułowicz[1]

1 Department of Nephrology, Jagiellonian University Medical College, Kraków, Poland
2 Department of Histology, Jagiellonian University Medical College, Kraków, Poland
3 Department of Medical Diagnostics Jagiellonian University Medical College, Kraków, Poland
4 Department of Cardiac and Vascular Diseases, Institute of Cardiology Jagiellonian University, Medical College, Kraków, Poland
5 Department of Interventional Cardiology, Institute of Cardiology Jagiellonian University, Medical College, Kraków, Poland
6 2nd Department of Cardiology, Jagiellonian University Medical College, Kraków, Poland

KEY WORDS

asymmetric dimethylarginine, end-stage renal disease, medial artery calcification, mineral and bone disorder, stromal cell-derived factor 1α

ABSTRACT

INTRODUCTION Medial arterial calcification is common in patients with chronic kidney disease (CKD) and is considered a risk factor for morbidity and mortality.

OBJECTIVES We aimed to evaluate the correlation between asymmetric dimethylarginine (ADMA) levels, radial artery calcification, and common carotid artery intima–media thickness (CCA-IMT).

PATIENTS AND METHODS The study included 51 patients with CKD, in whom an arteriovenous fistula for hemodialysis access was created to collect radial artery samples for a histological examination, and 33 healthy volunteers, in whom the reference concentrations of ADMA were assessed. The concentrations of creatinine, albumin, calcium, phosphate, fibroblast growth factor 23, osteoprotegerin (OPG), osteopontin (OPN), osteocalcin, secreted protein acidic and rich in cysteine, interleukin 6, interleukin 18, pentraxin 3, stromal cell-derived factor 1α (SDF1α), thrombomodulin, soluble tumor necrosis factor receptor II (sTNFRII), and matrix metalloproteinase 2 (MMP-2) were determined. Radial artery fragments were stained for calcifications using alizarin red. The CCA-IMT was assessed by ultrasonography.

RESULTS Patients with CKD had higher ADMA levels than controls. Patients with ADMA levels above the median were older, had higher levels of phosphate, fibroblast growth factor 23, OPG, OPN, PTX3, sTNFRII, MMP-2, thrombomodulin, and they had more atherosclerotic plaques in the carotid artery. In multiple regression, log-transformed (log)sTNFRII, MMP-2, and SDF1α levels were independent predictors of log(ADMA). Patients with calcifications had higher ADMA levels. A similar correlation was observed between SDF1α and alizarin red staining grades 1 to 3. In logistic regression, ADMA levels positively predicted the presence of calcifications independently of age, hemodialysis status, Framingham risk score, and PTX3.

CONCLUSIONS Circulating ADMA levels indicate medial arterial calcification in patients with CKD.

Correspondence to:
Katarzyna Krzanowska, MD, PhD, Katedra i Klinika Nefrologii, Uniwersytet Jagielloński, Collegium Medicum, ul. Kopernika 15c, 31-501 Kraków, Poland,
phone: +48 12 424 78 00,
email: kasiajanda@op.pl

Received: November 17, 2017.
Revision accepted: February 05, 2018.
Published online: February 06, 2018.
Conflict of interest: none declared.
Pol Arch Intern Med. 2018; 128 (3): 157-165
doi:10.20452/pamw.4201

INTRODUCTION Elevated plasma concentrations of asymmetric dimethylarginine (ADMA) are associated not only with endothelial dysfunction and atherosclerosis but also predict mortality and cardiovascular complications.[1-4] In patients with mild to advanced chronic kidney disease (CKD), plasma ADMA levels were inversely correlated with glomerular filtration rate (GFR) and were an independent risk marker of progression to end-stage renal disease and mortality.[5] In patients on hemodialysis (HD), the plasma ADMA level 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 the common carotid artery intima–media thickness (CCA-IMT). 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] A 3- to 9-fold increase of plasma ADMA levels was shown to inhibit NO production by 30% to 70%.[6] Elevated ADMA levels lead to myocardial fibrosis and microvascular dropout by directly influencing endothelial cells and fibroblasts and by indirectly stimulating the production of additional potent angiogenesis inhibitors.[8]

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

PATIENTS AND METHODS

The study group included 51 patients with CKD (20 women, 31 men; 21 predialysis patients, 30 HD patients) and 33 healthy volunteers matched for age and sex who were recruited to assess the reference ADMA concentrations. The mean (SD) age of patients was 62 (16) years. Patients had an arteriovenous fistula for HD access created for the first time, allowing a collection of radial artery samples for a histological examination. The autologous arteriovenous fistulas were made by one surgeon at a single medical center. Cross-sectional data were obtained immediately before the procedure and included the clinical assessment of patients, CCA-IMT measurements, and laboratory tests (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, or neoplastic disease were excluded. Detailed medical history including diabetes mellitus, hypertension, dyslipidemia, current smoking, duration of dialysis, and medications was recorded.

At baseline, 10-year risk of CAD was calculated for all patients, using the Framingham risk score calculator in accordance with the published guidelines.[9] Additionally, the CCA-IMT was assessed by ultrasonography in B presentation, using Acuson 128 XP/10 (Siemens, United States) equipped with a 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 (CCA) during the diastolic phase of the heart cycle. The results were presented as the arithmetic means of the values obtained for the left and right arteries.

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 participation in the study.

Histology Small fragments of the radial artery wall were collected during the first creation of an arteriovenous fistula for HD access. Briefly, tissue sections were stained with alizarin red to detect calcium (Ca) deposits and then subjected to microscopic assessment. The stained sections were examined using an Olympus BX-50 microscope (Olympus, Tokyo, Japan) in the bright-field mode, and the images were registered using Olympus DP-71 digital CCD camera controlled by Olympus AnalySIS FIVE software. An experienced histologist evaluated the sections in a blinded 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; and 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 the Bland–Altman method and by calculating an intraclass correlation coefficient, which was 0.88.

Laboratory tests The levels of the following biochemical parameters were measured in all patients: creatinine, glucose, intact parathyroid hormone (iPTH), total Ca and phosphate (Pi), ADMA, stromal cell-derived factor 1α (SDF1α), MMP-2, interleukin 6 (IL-6), interleukin 18 (IL-18), pentraxin 3 (PTX3), soluble tumor necrosis factor receptor II (sTNFRII), thrombomodulin (TM), osteoprotegerin (OPG), osteopontin (OPN), osteocalcin (OC), secreted protein acidic and rich in cysteine (SPARC), and fibroblast growth factor 23 (FGF-23). The estimated GFR was calculated by the Modification of Diet in Renal Disease formula: estimated GFR = (186 × serum creatinine [μmol/l] × 0.0113)$^{-1.154}$ × age$^{-0.203}$ × 114 × (0.742 for women).

A sample of peripheral venous blood was collected at fasting into EDTA tubes in the morning prior to creation of the arteriovenous fistula, 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 platelet-poor EDTA plasma were measured by a commercially available ADMA ELISA kit (DLD Diagnostika GmbH, Hamburg, Germany). The detection limit for the assay was 0.04 μmol/l. The intra-assay and interassay precision was 10.8% and 7.9%, respectively. The cross reactivity of L-arginine and symmetric

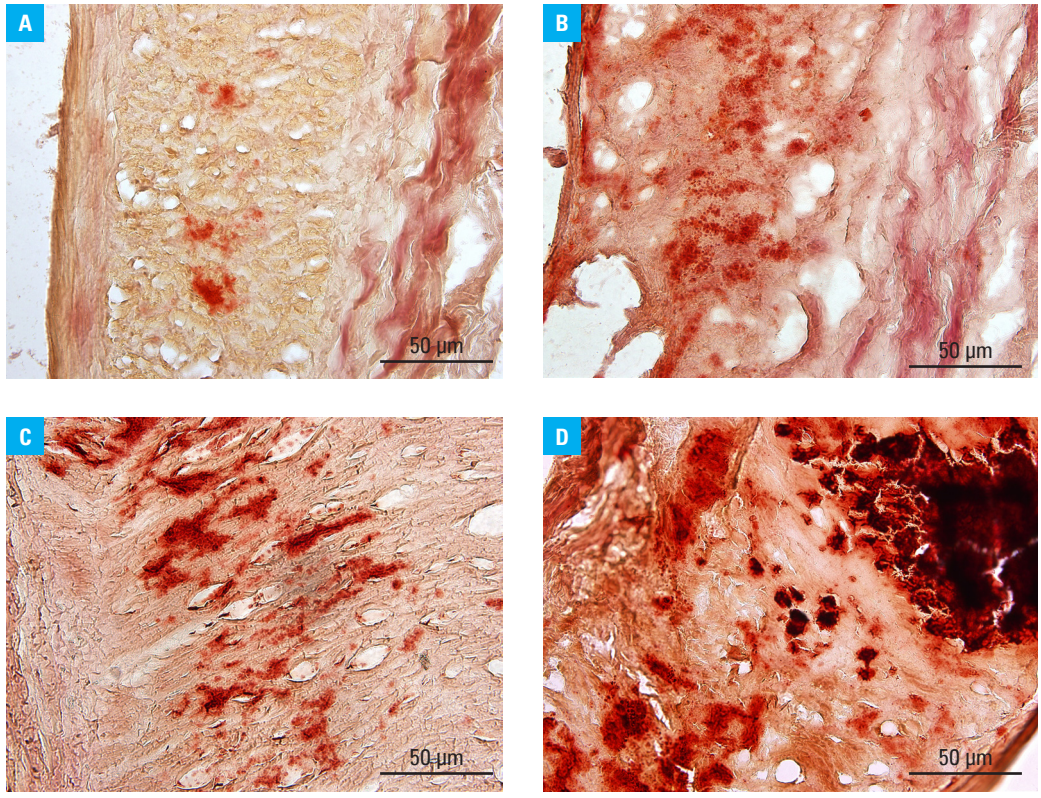

FIGURE 1 Radial artery sections stained with alizarin red showing calcification of various grades; **A** – grade 1; **B** – grade 2; **C** – grade 3; **D** – grade 4

dimethylarginine was less than 0.02% and less than 0.6%, respectively. The reference range for ADMA is 0.26 to 0.64 µmol/l.

SDF1α levels in platelet-poor EDTA plasma were measured by a commercially available Human CXCL12/SDF1α Immunoassay ELISA (R&D Systems, Minneapolis, Minnesota, United States). The minimum detectable dose for the assay was 0.018 ng/ml. The intra-assay and interassay precision was 3.9% and 13.4%, respectively. The reference range for SDF1α is 1.3 to 2.9 ng/ml.

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

Statistical analysis The number of patients (percentage of the respective group) was reported for categories. Contingency tables were analyzed with the χ^2 test. Medians and interquartile ranges (IQRs) were reported for nonnormally distributed and means (SD)—for normally distributed quantitative variables. Distributions were tested for normality with the Shapiro–Wilk test. The Mann–Whitney test or the unpaired *t* test was used to assess differences between the groups, according to the distribution. The Pearson correlation coefficients and linear regression models were calculated following logarithmic transformation (log) of right-skewed variables. To assess independent predictors of logarithm-transformed (log) (ADMA), backward stepwise linear regression was calculated. Standardized regression coefficients β (SE) were reported for linear regression. Simple and multiple logistic regression (with prespecified covariates) was used to analyze the association between ADMA concentrations and vascular calcifications. The resulting odds ratios were reported with 95% confidence intervals (CIs). The tests were 2-tailed and a *P* value of 0.05 or lower was considered significant. The Statistica 12 software (StatSoft, Tulsa, Oklahoma, United States) was used for calculations.

RESULTS

Biochemical test results. The characteristics of the study group are shown in TABLES 1, 2, and 3. The study group included 21 predialysis patients with stage 5 CKD and 30 patients treated with maintenance HD (TABLE 1). Age, sex, and the prevalence of traditional cardiovascular risk factors did not differ between the groups. Also, the groups were comparable in the degree of calcifications, CCA-IMT values, and the presence of calcified atherosclerotic plaques in the CCA (TABLE 1). Except for diuretics (less commonly used among patients on HD) and erythropoietin (used more often in the HD group), there were no significant differences in the prescribed drugs (TABLE 1). Serum creatinine, calcium–phosphate product (Ca × Pi) (but not iPTH), OPG, and OPN (but not OC and SPARC) levels were higher in the HD group. Moreover, selected inflammatory factors (PTX3, IL-6, and sTNFRII) as well as TM and ADMA concentrations were higher in dialyzed patients with stage 5 CKD (TABLE 1).

Patients with CKD had higher ADMA concentrations as compared with healthy controls

TABLE 1 Clinical characteristics of patients and the results of laboratory tests in predialysis patients with stage 5 chronic kidney disease and patients on hemodialysis

Parameter		Predialysis patients (n = 21)	Hemodialysis patients (n = 30)	*P* value
Age, y, mean (SD)		62 (13)	62 (18)	0.8
Male sex, n (%)		13 (62)	18 (60)	0.9
Dialysis therapy duration, mo		–	8 (3–38)	–
BMI, kg/m^2, mean (SD)		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, n (%)	None	9 (43)	11 (37)	0.7
	Grade 1–3	8 (38)	15 (50)	
	Grade 4	4 (19)	4 (13)	
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
ACEIs/ARBs, n (%)		4 (19)	6 (20)	0.9
β-blockers, n (%)		13 (62)	21 (70)	0.5
Calcium channel blockers, n (%)		12 (57)	12 (40)	0.2
Diuretics, n (%)		19 (90)	14 (47)	0.001
Number of antihypertensive drugs		3 (2–4)	2 (1–4)	0.3
Statins, n (%)		15 (71)	11 (37)	0.01
Vitamin D, n (%)		6 (29)	12 (40)	0.4
Ca, n (%)		12 (57)	20 (67)	0.5
Erythropoietin, n (%)		1 (5)	15 (50)	<0.001
Serum creatinine, µmol/l		389 (512–269)	506 (411–571)	<0.001
Ca, mmol/l; mean (SD)		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 × 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; mean (SD)		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
sTNFRII, 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

Data are presented as median (interquartile range) unless otherwise stated.

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

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ADMA, asymmetric dimethylarginine; ARB, angiotensin II receptor blocker; BMI, body mass index; CCA, common carotid artery; CCA-IMT, common carotid artery intima–media thickness; Ca, calcium; Ca × Pi, calcium–phosphate product; 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, pentraxin 3; SDF1-α, stromal cell-derived factor α; SPARC, secreted protein acidic and cysteine rich; sTNFRII, soluble tumor necrosis factor receptor II; TM, thrombomodulin

TABLE 2 Clinical characteristics and treatment in patients with stage 5 chronic kidney disease depending on the median asymmetric dimethylarginine level

Parameter		ADMA		*P* value
		<0.779 µmol/l (n = 25)	≥0.779 µmol/l (n = 26)	
Age, y, mean (SD)		57 (17)	67 (13)	0.03
Male sex, n (%)		19 (76)	12 (46)	0.03
Hemodialysis, n (%)		10 (40)	20 (77)	0.007
Dialysis therapy duration, mo, median (IQR)[a]		18 (4–38)	6 (2–38)	0.6
BMI, kg/m², mean (SD)		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, %, median (IQR)		11 (6–22)	11 (7–22)	0.7
Radial artery calcifications, n (%)	None	13 (52)	7 (27)	0.01
	Grade 1–3	6 (24)	17 (65)	
	Grade 4	6 (24)	2 (8)	
ACEIs/ARBs, n (%)		6 (24)	4 (15)	0.4
β-blockers, n (%)		18 (72)	16 (62)	0.4
Calcium channel blockers, n (%)		15 (60)	9 (35)	0.07
Diuretics, n (%)		17 (68)	16 (62)	0.6
Number of antihypertensive drugs, median (IQR)		3 (2–4)	2 (1–3)	0.2
Statins, n (%)		13 (52)	13 (50)	0.9
Vitamin D, n (%)		7 (28)	11 (42)	0.3
Ca, n (%)		14 (56)	18 (69)	0.3
Erythropoietin, n (%)		7 (28)	9 (35)	0.6

a Values provided for hemodialyzed patients (n = 30)

Abbreviations: see TABLE 1

(median [IQR], 0.779 [0.672–0.947] µmol/l vs 0.572 [0.481–0.602] µmol/l; P <0.001). ADMA concentrations were higher in HD patients than in predialysis patients (TABLE 1), and predialysis patients had higher ADMA levels than controls (P <0.001). The dialysis status and serum creatinine levels independently predicted ADMA concentrations in multiple regression.

Patients with ADMA concentrations above the median were characterized by older age and higher levels of Pi, Ca × Pi, FGF-23, OPG, OPN, PTX, sTNFRII, MMP-2, and TM (TABLES 2 and 3). Patients with ADMA levels above the median more often had calcifications, especially grades 1 to 3 (FIGURE 1). Furthermore, atherosclerotic plaques in the CCA were more prevalent in these patients. ADMA levels above the median were also associated with more prevalent female sex and HD treatment (TABLE 2). There were no associations between drug administration in patients with CKD and ADMA concentrations.

Correlations were observed between log(ADMA) and the following parameters: log(serum creatinine) (r = 0.38; P = 0.007); log(Pi) (r = 0.37; P = 0.008), log(Ca × 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(IL-6) (r = 0.36; P = 0.01), log(IL-18) (r = 0.29, P = 0.048), log(sTNFRII) (r = 0.59; P <0.001); log(MMP-2) (r = 0.42; P = 0.003), log(TM) (r = 0.30; P = 0.03), and SDF1α (r = 0.39; P = 0.005).

Serum creatinine concentrations were not correlated with Ca × Pi, FGF-23, OPG, OPN, serum albumin, sTNFRII, MMP-2, or SDF1α levels. In backward stepwise multiple linear regression, log(sTNFRII) (β [SE], 0.36 [0.15]; P = 0.02), log(MMP-2) (β [SE], 0.31 [0.10]; P = 0.003), and SDF1α (β [SE], 0.24 [0.11]; P = 0.04) were independent predictors of log(ADMA).

We did not observe any associations between ADMA or SDF-1 α levels and traditional cardiovascular risk factors. No differences were found in ADMA and SDF-1 α concentrations between patients with diabetes, hypertension, dyslipidemia, or active smokers. Moreover, no correlations were observed between log(ADMA) or SDF-1 α levels and age, body mass index (BMI), or the Framingham risk score.

Histological findings Patients with calcifications in the radial artery had higher ADMA concentrations (median [IQR], 0.847 µmol/l [0.684–0.978 µmol/l] vs 0.708 µmol/l [0.650–0.852 µmol/l]; P = 0.029). ADMA levels correlated with the calcification grade in patients with grades 1 to 3 (r = 0.54; P <0.001; FIGURE 2). In contrast, patients with most advanced calcifications (grade 4) had relatively low concentrations of ADMA (median [IQR], 0.724 µmol/l [0.668–0.770 µmol/l]; FIGURE 2). In the logistic regression analysis, high ADMA levels predicted the presence of radial artery calcifications (especially grades 1–3) independently of the HD status and other predictors of calcifications such as age, the Framingham risk score, and PTX3 concentrations (TABLE 4).

A similar correlation (although weaker) was observed between SDF1α and Alizarin red staining grades 1 to 3 (r = 0.33; P = 0.03; FIGURE 3).

Common carotid artery ultrasound Data from CCA ultrasound were available in 44 patients (86%), including 28 men and 16 women (mean [SD] age, 60 [17] years), of whom 26 (59%) were on HD at the start of the study. Of these patients, 23 had ADMA levels below a median value of 0.779 µmol/l (ie, the median from the whole group) and 21 had ADMA levels above the median. CCA-IMT values did not differ between patients with ADMA below and above the median of 0.779 µmol/l (median [IQR], 0.87 µmol/l [0.75–1.05] vs 0.95 µmol/l [0.90–1.10]; P = 0.06), and they were not correlated with ADMA concentrations (r = 0.30; P = 0.06). However, calcified atherosclerotic plaques in the CCA were more prevalent in patients with ADMA concentrations above 0.779 µmol/l (11 patients [52%] vs 3 patients [13%]; P = 0.005).

TABLE 3 Laboratory parameters in patients with stage 5 chronic kidney disease depending on the median asymmetric dimethylarginine level

Parameter	ADMA		*P* value
	<0.779 µmol/l (n = 25)	<0.779 µmol/l (n = 25)	
Creatinine, µmol/l	408 (313–474)	468 (405–547)	0.08
Ca, mmol/l, mean (SD)	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 × Pi, mmol²/l²	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
Albumin, g/l, mean (SD)	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
sTNFRII, 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

Data are presented as median (interquartile range) unless otherwise stated.

Abbreviations: see TABLE 1

DISCUSSION Asymmetric dimethylarginine and arterial calcification Our study showed that elevated concentrations of circulating ADMA levels were related to a higher risk of medial arterial calcification (MAC) in patients with advanced renal disease. So far, the relationship between circulating ADMA levels and vascular calcification assessed histologically has not been studied. Our data suggest that excessive accumulation of ADMA in serum is accompanied by MAC, especially its lower grades. In contrast, high concentrations of ADMA did not correlate with high CCA-IMT values recognized as an early marker of subclinical atherosclerosis, although patients with high ADMA levels more frequently had carotid plaques. We found an association between ADMA levels and bone turnover parameters, which were increased in patients with ADMA concentrations above the median value. This finding suggests that ADMA is involved in the development of calcifications in the intima and media of the arteries.

In recent years, there has been an increasing interest in ADMA. Clinical studies included in a recent meta-analysis demonstrated that patients with myocardial infarction as well as with stable and unstable angina pectoris had elevated plasma ADMA concentrations, thus supporting the hypothesis that ADMA concentrations reflect coronary plaque vulnerability.[10] Our research confirmed that ADMA concentrations were also higher in patients with atherosclerotic plaques in the CCA. The importance of ADMA has also been emphasized by Opalińska et al[11] in a study on patients with advanced atherosclerosis. The authors identified the inflamed plaques on scintigraphy using ^{99m}Tc-labelled interleukin 2 (IL-2) in the selected group of patients with CKD with high cardiovascular risk. ADMA concentrations were found to be higher in patients with the highest values of IL-2 uptake on scintigraphy.[8] However, in contrast to other studies, we did not observe a relation between ADMA levels and CCA-IMT.[12,13]

ADMA concentrations in our patients were higher than those in our healthy subjects of similar age from European populations and without evidence of CKD and atherosclerotic vascular disease. This corresponds with the results of the meta-analysis, indicating that patients with artery disease have higher ADMA levels than healthy controls.[10] ADMA levels increased with the progression of kidney disease: predialysis patients had higher ADMA levels compared with healthy participants, and ADMA concentrations were higher in patients on HD than in predialysis patients, which is in line with other reports.[14]

The available literature includes only a few articles on the relationship between ADMA and vascular calcification, which was confirmed by imaging studies but not by histological examination.[15-18] The coronary artery calcification (CAC) score is regarded as an indicator of the severity of atherosclerotic artery disease and may accurately identify high-risk asymptomatic patients at the start of dialysis.[15] In the CARDIA study,[17] the median value of the ADMA level was higher in patients with the presence of CAC on computed tomography than in controls. In the logistic regression model adjusted for age, smoking status, alcohol consumption, BMI, waist circumference, hypertension, diabetes, low- and high-density lipoprotein and cholesterol, triglycerides, renal function, and C-reactive protein, the highest tertile of ADMA (compared with the lowest tertile) was associated with an increased odds ratio (1.8) of the presence of CAC. By linear regression, an independent relationship was also found between ADMA and the degree of CAC.

Our study revealed an association of baseline ADMA levels with the severity of radial artery calcifications in patients with CKD. In agreement with our study, Kobayashi et al[18] demonstrated that plasma ADMA levels were negatively correlated with GFR and positively correlated with the CAC score measured by multidetector-row computed tomography according to the Agatston score. Patients with severe calcifications had higher ADMA levels, insulin resistance measured by the homeostasis model assessment of insulin resistance, and fibrinogen levels, along with the serum levels of phosphorus, compared with patients with mild CAC. In our study, a positive correlation observed between calcifications and ADMA was confirmed by the multiple regression analysis, which showed that ADMA levels predicted artery calcification independently of age, HD status, and the Framingham risk score as classic

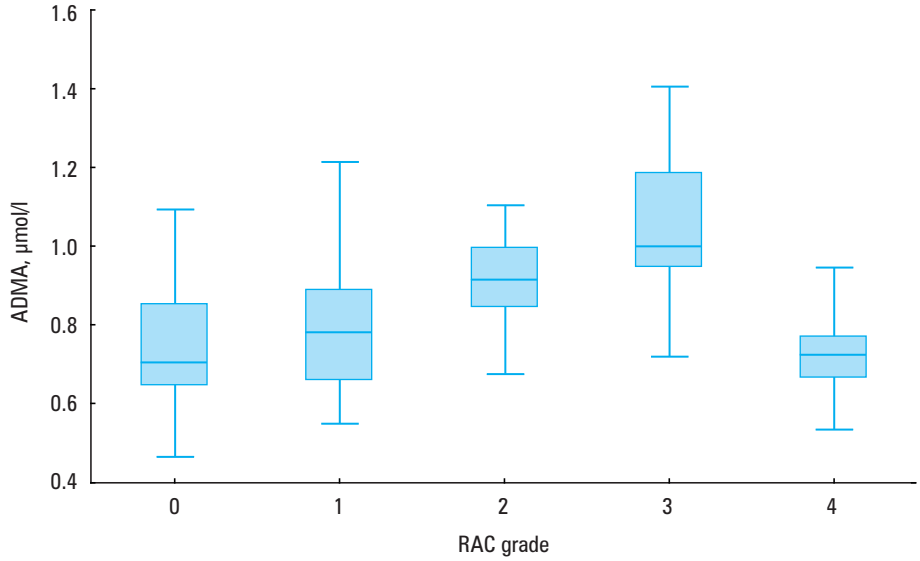

FIGURE 2 Association between serum asymmetric dimethylarginine concentrations and grade of radial artery calcification (RAC)

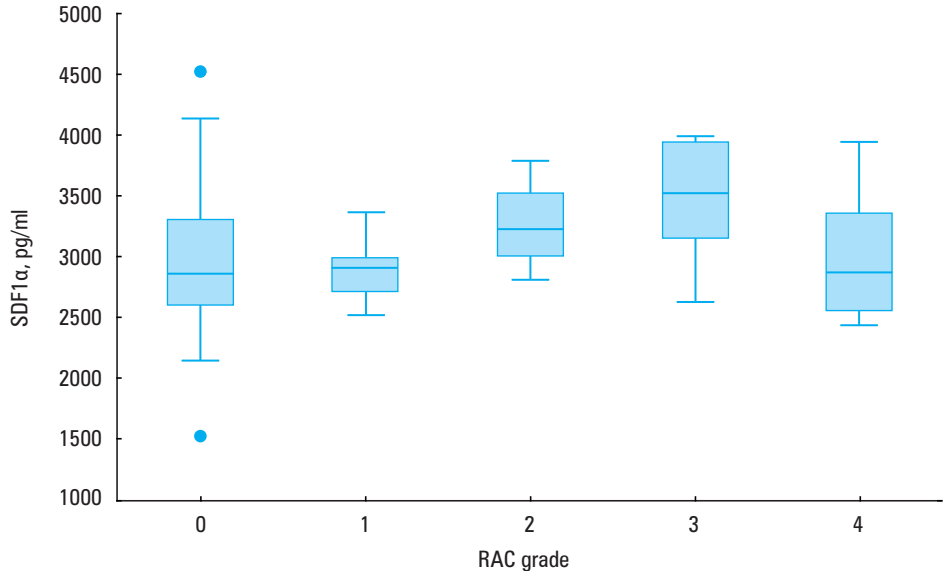

FIGURE 3 Association between serum concentrations of stromal cell-derived factor 1α and grade of radial artery calcification (RAC)

risk factors, and PTX3 as an acute-phase reactant synthesized locally at the site of inflammation.

Asymmetric dimethylarginine and selected markers of inflammation, endothelial dysfunction, mineral and bone disorder, and matrix metalloproteinase 2 In patients on HD, the higher ADMA concentration is a strong and independent predictor of overall mortality and cardiovascular outcome.[6] Normal endothelial function depends on NO release by endothelial cells. ADMA, by competing with L-arginine, inhibits NO production and may cause endothelial dysfunction leading to atherosclerotic process.[7,19] Advanced renal failure is accompanied by accumulation of a naturally occurring inhibitors of NO synthase.[20] Endothelial dysfunction, as assessed by ADMA levels and inflammation, has been consistently linked to atherosclerosis, cardiovascular events, and death in patients with CKD.[21] In our study, there was a correlation between ADMA and the severity of calcifications in the case of mild and moderate calcifications, expressed by alizarin red staining grades 1 to 3. Unexpectedly, patients with most advanced calcifications (grade 4) had relatively low concentrations of ADMA. It suggests that ADMA-induced endothelial damage leading to Ca deposition in the arterial wall is most pronounced in earlier stages of the process and in the further progression of calcification (probably influenced by other factors, eg, those involved in bone turnover) even if ADMA levels decrease.

We also found a positive correlation between inflammatory markers (IL-6, IL-18, sTNFRII) and ADMA. These findings are in agreement with previous studies, and suggest that ADMA may be involved in the inflammatory reaction induced by uremia.[21-23] Proinflammatory cytokines and metabolic abnormalities associated with systemic inflammation are considered one of the principal mechanisms leading to endothelial dysfunction.[24] In the present study, SDF1α, sTNFRII, and MMP-2 were independent predictors of an increased ADMA level. There are

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

Independent variable	Radial artery calcifications			
	Grade 1–4, OR (95% CI)		Grade 1–3, OR (95% CI)	
	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)
Hemodialysis	–	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: CI, confidence interval; OR, odds ratio; others, see TABLE 1

no data in the available literature on the relationship between ADMA and MMP-2 levels in patients with renal failure. Matrix metalloproteinases are proteolytic enzymes that degrade the extracellular matrix and facilitate proliferation and migration of endothelial and vascular smooth muscle cells which are involved in the initiation of calcification.[25]

The 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 Ca × Pi and bone-related proteins (FGF-23, OPG, and OPN) were associated with high ADMA levels, independently of serum creatinine concentrations. Also, higher concentrations of the endothelial dysfunction marker SDF1α were correlated with higher serum ADMA levels. Moreover, SDF1α was a predictor of an increased 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.[26]

SDF1α has been shown to induce neovascularization by recruiting endothelial progenitor cells (EPCs) into ischemic tissues by an increased coupling of SDF1α/C-X-C motif chemokine type-4 receptor.[27] Gossl et al[28] identified a subgroup of bone marrow–derived circulating EPCs with an osteogenic phenotype (expressing the osteoblast marker OC) and found that patients with coronary endothelial dysfunction have higher numbers of circulating OC(+) EPCs compared with patients with normal endothelial function. In cell culture, circulating EPCs with high expression of OC were capable of forming mineralized deposits. Another study showed that in contrast to controls, patients with coronary endothelial dysfunction retain osteogenic EPCs within the coronary circulation, and this retention is accompanied by the release of SDF1α and interleukin 8.[29] The authors concluded that retention of osteogenic EPCs 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, patients with calcification grades 1 to 3 showed a correlation between SDF1α concentrations 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 levels can serve as indicators of the vascular calcification process.

Asymmetric dimethylarginine and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers ADMA may not only be a marker but also an active player in cardiovascular disease, which makes it a potential target for therapeutic interventions. The present study showed that the use of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) was not significant for ADMA concentrations, while a study by Gamboa et al[30] indicated that short-term use of ACEIs increases ADMA levels in patients on HD whereas ARBs do not. Interestingly, in the general population without kidney injury, ACEIs and ARBs decrease ADMA levels.[31,32] Also in the current study, no correlations were found between the administration of other drugs (β-blocker, calcium channel blocker, statin, vitamin D, Ca, erythropoietin) and ADMA concentrations.

Limitations Limitations of the present study include a 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 baseline. We also acknowledge that it is not possible to demonstrate a cause and 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 patients with CKD. Our findings support the hypothesis that elevated plasma ADMA levels are an important risk factor for cardiovascular disease in patients with CKD. High costs limit the application of imaging diagnostic methods, such as the assessment of the CAC score, which in many patients with CKD might be replaced by the measurement of baseline ADMA concentrations for the evaluation of cardiovascular risk already at the beginning of dialysis.

Conclusions In conclusion, our study is the first to report that circulating ADMA levels are

an indicator of MAC in patients with CKD and suggests that plasma ADMA levels could be a useful, simple biomarker of cardiovascular risk in daily clinical practice.

ACKNOWLEDGMENTS We are grateful to all patients who participated in this study. Financial support was provided by the Jagiellonian University Medical College (a statutory grant No. K/ZDS/000 597; to KK).

CONTRIBUTION STATEMENT MK and KK conceived the concept for the study, were the major contributors to study design, coordination, interpretation of the results, statistical analysis, and manuscript drafting. MG conducted histological examinations. PD performed statistical analysis. KW and BG participated in the design of the study. GK, AD, and JAL contributed to study design and data analysis. WS contributed to study design and coordination. All authors were involved in data collection, drafted manuscript modifications, and approved the final version of the manuscript.

REFERENCES

1 Zhou S, Zhu Q, Li X, et al. Asymmetric dimethylarginine and all-cause mortality: a systematic review and meta-analysis. Sci Rep. 2017; 7: 44692.

2 Zelzer S, Enko D, Pilz S, et al. Myeloperoxidase, asymmetric dimethyl--arginine and the renin-angiotensin-aldosterone-system in cardiovascular risk patients: Cross-sectional findings from the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Clin Biochem. 2017; 50: 739-745.

3 Surdacki A, Kruszelnicka O, Rakowski T, et al. Asymmetric dimethylarginine predicts decline of glucose tolerance in men with stable coronary artery disease: a 4.5-year follow-up study. Cardiovasc Diabetol. 2013; 12: 64. doi: 10.1186/1475-2840-12-64.

4 Langsford D, Tang M, Cheikh Hassan HI, et al. The Association between biomarker profiles, etiology of chronic kidney disease and mortality. Am J Nephrol. 2017; 45: 226-234.

5 Aldamiz-Echevarria L, Andrade F. Asymetric dimethylarginine, endothelial dysfunction and renal disease. Int J Mol Sci. 2012; 13: 11 288-11 311.

6 Zoccali C, Bode-Böger S, Mallamaci F, et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet. 2001; 358: 2113-2117.

7 Brunet P, Gondouin B, Duval-Sabatier A, et al. Does uremia cause vascular dysfunction? Kidney Blood Press Res. 2011; 34: 284-290.

8 Charytan DM, Padera R, Helfand AM, et al. Increased concentration of circulating angiogenesis and nitric oxide inhibitors induces endothelial to mesenchymal transition and myocardial fibrosis in patients with chronic kidney disease. Int J Cardiol. 2014; 176: 99-109.

9 D'Agostino RB Sr, Vasan RS, Pencina MJ, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation. 2008; 117: 743-753.

10 Xuan C, Tian QW, Li H, et al. Levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, and risk of coronary artery disease: A meta-analysis based on 4713 participants. Eur J Prev Cardiol. 2016; 23: 502-510.

11 Opalińska M, Stompór T, Pach D, et al. Imaging of inflamed carotid artery atherosclerotic palques with use of 99mTc-HYNIC-IL-2 scintigraphy in end-stage renal disease patients. Eur J Nucl Med Mol Imaging. 2012; 39: 673-682.

12 Bai Y, Sun L, Du L, et al. Association of circulating levels of asymmetric dimethylarginine (ADMA) with carotid intima-media thickness: evidence from 6168 participants. Ageing Res Rev. 2013; 12: 699-707.

13 Morisawa T, Nakagomi A, Kohashi K, et al. Osteoprotegerin is associated with endothelial function and predicts early carotid atherosclerosis in patients with coronary artery disease. Int Heart J. 2015; 56: 605-612.

14 Napora M, Graczykowska A, Próchniewska K, et al. Relationship between serum asymmetric dimethyloarginine and left ventricular structure and function in patients with end-stage renal disease treated with hemodialysis. Pol Arch Med Wewn. 2012; 122: 226-233.

15 Janda K, Krzanowski M, Dumnicka P, et al. Risk stratification in dialysis patients: coronary artery calcification score combined with high sensitive C-reactive protein and Framingham score for cardiovascular risk prediction in asymptomatic subjects. J Clin Exp Cardiol. 2014; 5: 296.

16 Parma Z, Parma R, Brzoska J, Sosonowski M. Prognostic value of coronary artery calcium score in patients with symptoms suggestive of coronary artery disease. Results from the Silesian Calcium Score (SILICAS) study. Pol Arch Med Wewn. 2016; 126: 395-401.

17 Iribarren C, Husson G, Sydow K, et al. Asymmetric dimethyl-arginine and coronary artery calcification in young adults entering middle age: the CARDIA study. Eur J Cardiovasc Prev Rehabil. 2007; 14: 222-229.

18 Kobayashi S, Oka M, Maesata K, et al. Coronary artery calcification, ADMA and insulin resistance in CKD patients. Clin J Am Soc Nephrol. 2008; 3: 1289-1295.

19 Fuchs D, Kurz K, Gostner JM. Asymmetric dimethylarginine: a risk indicator or pathogenic factor? Pol Arch Med Wewn. 2016; 126: 621-622.

20 Vallance P, Leone A, Calver A, et al. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet. 1992; 7: 572-575.

21 Tripepi G, Mattace Raso F, Sijbrands E, et al. Inflammation and asymmetric dimethylarginine for predicting death and cardiovascular events in ESRD patients. Clin J Am Soc Nephrol. 2011; 6: 1714-1721.

22 Yano A, Nakao K, Sarai A, et al. Elevated serum interleukin-18 levels might reflect the high risk of hospitalization in patients on peritoneal dialysis. Nephrology (Carlton) 2005; 10: 576-582.

23 Chen MF, Xie XM, Yang TL, et al. Role of asymmetric dimethylarginine in inflammatory reactions by angiotensin II. J Vasc Res. 2007; 44: 391-402.

24 Krzanowski M, Krzanowska K, Gajda M, et al. Pentraxin 3 as a new indicator of cardiovascular-related death in patients with advanced chronic kidney disease. Pol Arch Intern Med. 2017; 127: 170-177.

25 Shanahan CM, Crouthamel MH, Kapustin A, Giachelli C. Arterial calcification in chronic kidney disease: key roles for calcium and phosphate. Circ Res. 2011; 109: 697-711.

26 Ross R. Atherosclerosis an inflammatory disease. N Engl J Med. 1999; 340: 115-126.

27 Hiasa K, Ishibashi M, Ohtani K, et al. Gene transfer of stromal cell--derived factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase--related pathway: next-generation chemokine therapy for therapeutic neovascularization. Circulation. 2004; 109: 2454-2461.

28 Gossl M, Modder UI, Atkinson EJ, et al. Osteocalcin expression by circulating endothelial progenitor cells in patients with coronary atherosclerosis. J Am Coll Cardiol. 2008; 52: 1314-1325.

29 Gossl M, Modder UI, Gulati R, et al. Coronary endothelial dysfunction in humans is associated with coronary retention of osteogenic endothelial progenitor cells. Eur Heart J. 2010; 31: 2909-2914.

30 Gamboa JL, Pretorius M, Sprinkel KC, et al. Angiotensin converting enzyme inhibition increases ADMA concentration in patients on maintenance hemodialysis-a randomized cross-over study. BMC Nephrol. 2015; 16: 167.

31 Ito A, Egashira K, Narishige T, et al. Asymmetric dimethylarginine and all-cause mortality: a systematic review and meta-analysis. Sci Rep. 2017; 7: 44692.

32 Celik T, Iyisoy A, Yuksel C, Jata B. The beneficial effects of angiotensin--converting enzyme inhibitors on serum asymmetric dimethylarginine levels in the patients with cardiovascular disease. Int J Cardiol. 2010; 142: 107-109.