

The Circulating Inactive Form of Matrix Gla Protein Is a Surrogate Marker for Vascular Calcification in Chronic Kidney Disease: A Preliminary Report

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Background and objectives: Vitamin K-dependent matrix Gla protein (MGP) acts as a calcification inhibitor *in vitro* and *in vivo*. The present study was performed to (1) determine plasma levels of the inactive, dephosphorylated, uncarboxylated MGP (dp-ucMGP) in a cohort of patients at different stages of chronic kidney disease (CKD) and (2) evaluate the association between dp-ucMGP levels on one hand and aortic calcification and mortality on the other.

Design, setting, participants, & measurements: 107 patients (67 ± 13 years; 60% male; 32% at CKD stages 2 to 3, 31% at stages 4 to 5, 37% at stage 5D) were assayed for dp-ucMGP and underwent multislice spiral computed tomography scans to quantify aortic calcification at baseline. They were prospectively monitored for mortality.

Results: Plasma dp-ucMGP levels augmented progressively with CKD stage, with a significant difference from CKD stage 4. CKD stage, hemoglobin, age, and coumarin use were independently associated with plasma dp-ucMGP levels. Furthermore, plasma dp-ucMGP and age were positively and independently associated with the aortic calcification score. During follow-up (802 ± 311 days), 34 patients died (20 from cardiovascular events). In a crude analysis, [plasma dp-ucMGP] > 921 pM was associated with overall mortality; this association was lost after adjusting for both age and the calculated propensity score.

Conclusions: Plasma dp-ucMGP increased progressively in a CKD setting and was associated with the severity of aortic calcification. Plasma dp-ucMGP could thus be a surrogate marker for vascular calcification in CKD.

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Cardiovascular diseases account for 50% of all deaths in a chronic kidney disease (CKD) setting (1). Vascular calcification (VC) in the media-intimal arterial layers contributes significantly to the greater mortality in this population (2,3). In fact, it has been repeatedly demonstrated that patients suffering from advanced CKD present VC to a greater extent than individuals with normal renal function (4–7). Although many factors may influence the occurrence and progression of this condition, the fact that 20% to 40% of the patients in most CKD cohorts (8–10) do not develop detectable VC (despite exposure to well-known environmental triggers, such as uremia, diabetes, and hyperphosphatemia) suggests

that naturally occurring VC inhibitors have an important role in preventing this disease process.

Matrix Gla protein (MGP) is a 10-kD protein secreted by chondrocytes and vascular smooth muscle cells in the arterial media (11). It is the first protein known to act as a calcification inhibitor *in vivo* (12–14), probably by directly inhibiting calcium precipitation and crystallization in the vessel wall (15) and antagonizing bone morphogenetic protein-2 (which regulates osteoblast differentiation, and thus bone formation (16)). In particular, MGP only exerts its anticalcification activity after posttranslational γ -glutamyl carboxylation of five glutamate residues—a crucial activation step that depends on the availability of vitamin K (17,18). Moreover, there is evidence that to be adequately secreted and become fully active, MGP must undergo further phosphorylation (on three serine residues) (19).

Although recent studies have demonstrated an inverse association between total uncarboxylated MGP (ucMGP) and VC in hemodialysis patients (20–22), data for investigating an association with earlier stages of CKD are still lacking. Moreover, this measured total ucMGP level contains both phosphorylated and dephosphorylated fractions or fragmented MGP, which might

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exert distinct biologic functions. It is thus important to use an assay that enables the measurement of the circulating, inactive form; *i.e.*, dephosphorylated ucMGP (dp-ucMGP).

Hence, the objective of the present study was to specifically determine (for the first time) plasma levels of dp-ucMGP in a cohort of patients at different stages of CKD and to evaluate the association between plasma dp-ucMGP on the one hand and aortic calcification and mortality on the other.

Materials and Methods

Patient Selection

Over an 18-month period (from January 2006 to June 2007), a total of 150 Caucasian, prevalent CKD patients were recruited from the Nephrology Department's outpatient clinic at Amiens University Hospital. All patients gave their informed, written consent. The study was approved by the local independent ethics committee and was performed in accordance with the ethical principles of the Declaration of Helsinki.

Included patients had to be older than 40 years, with a confirmed diagnosis of CKD (defined as ongoing hemodialysis or having two previous estimated Cockcroft and Gault (23) creatinine clearance values <90 ml/min per 1.73 m^2 3 to 6 months apart). Stage 5D CKD patients had been receiving thrice-weekly hemodialysis for at least 3 months. The noninclusion criteria included the presence of chronic inflammatory disease, atrial fibrillation, complete heart block, abdominal aorta aneurysm, aortic and/or femoral artery prosthesis, primary hyperparathyroidism, kidney transplantation, or any other acute cardiovascular event in the 3 months before screening. The 107 patients who met all inclusion criteria and had available dp-ucMGP measurements were included in the present analysis. The 43 patients who were not included in the analyses did not differ from the analyzed patients in terms of age, sex, diabetes and cardiovascular disease status, body mass index, or CKD stage distribution.

Study Protocol

All patients were hospitalized for the day to perform the laboratory blood tests, BP measurements, a lateral lumbar x-ray, and multislice spiral computed tomography (CT) scan. Hence, for a given patient, all examinations were performed between 9 a.m. and 2 p.m. on the same day. Hemodialysis patients were preferentially seen on a dialysis-free day or, if this was not possible, the morning before the dialysis session. A patient interview focused on comorbidities and the personal disease history (especially any previous vascular events). The patients' medical records were reviewed to identify and describe any concomitant medications. Six patients were taking coumarins: three for thrombosis/stenosis of the internal carotid artery, two for deep venous thrombosis, and one after a cardiac valve replacement. For descriptive purposes, patients who reported the current or past use of insulin and/or oral hypoglycemic agents were considered to be diabetic. Previous cardiovascular disease was defined as a history of any of the following events: myocardial infarction, stroke, heart failure, angina pectoris, or surgical procedures for angina or coronary/peripheral artery disease (including percutaneous transluminal angioplasty).

Laboratory Procedures

Blood samples were collected on the same morning and before the other investigations were performed. Selected variables were measured after the samples had been frozen and stored at -80°C . Serum calcium, phosphate, cholesterol, hemoglobin, and creatinine (Scr) levels were assayed in an on-site biochemistry laboratory using standard autoana-

lyzer techniques (the Modular IIP® system from Roche Diagnostics, Basel, Switzerland). Serum-intact parathyroid hormone (iPTH 1 to 84) was determined in a chemiluminometric immunoassay (Liaison N-tact PTH CLIA®; Diasorin, Stillwater, MN). Plasma levels of dp-ucMGP were determined using a recently developed sandwich ELISA involving two anti-MGP monoclonal antibodies. An antibody directed against the nonphosphorylated sequence 3 to 15 in human MGP is used as capture antibody, whereas the antibody directed against the noncarboxylated sequence 35 to 49 is used as a detection antibody (VitaK BV, Maastricht, the Netherlands). The synthetic peptide dpMGP³⁻¹⁵-(AADO)-ucMGP³⁵⁻⁵⁴ was used as a standard. The intraassay and interassay variability values were 5.6% and 9.9%, respectively. Reference plasma dp-ucMGP values were determined in 77 healthy, age-matched controls (mean \pm SD: 466 ± 257 pM; median: 430 pM; interquartile range: 305 to 563 pM). C-reactive protein (normal value: 2.87 mg/L, ranging from nondetectable to 3.0 mg/L), albumin, and cystatin C (CysC) levels were determined by laser nephelometry (BNProSpec; Siemens Healthcare, Dade Behring, Marburg, Germany). To describe the true GFR as closely as possible, the estimated GFR combining Scr and CysC measurements was calculated for all nondialyzed patients according to the recently published (24) equation: $177.6 \times \text{Scr}^{-0.65} \times \text{CysC}^{-0.57} \times \text{age}^{-0.20}$ ($\times 0.82$ if female). Patients were then classified into CKD stages, according to the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines (25). Because there were low numbers of patients in CKD stages 2 and 5, the study population was stratified into CKD stages 2 to 3, 4 to 5, and 5D for descriptive and analytical purposes.

Abdominal Aorta Imaging with Plain Radiography

A technique similar to that described by Kauppila *et al.* (26) was used to obtain images of the lower abdominal aorta and generate an aortic calcification score. All x-rays were reviewed by two investigators, and a consensus on the interpretation was reached in all cases (17).

Multislice Spiral CT

To quantify the presence and extent of aortic calcifications, each patient underwent a multislice spiral CT scan. All examinations were performed with a 64-detector scanner (Lightspeed VCT®; GE Healthcare, Milwaukee, WI). Detailed technical information on the procedure is provided elsewhere (27).

Survival

Death records were compiled prospectively by considering all patients included at least 20 months before the study end date (March 1, 2009). Each set of medical records was reviewed, and the cause of death was assigned by a physician on the basis of the available clinical information. For out-of-hospital deaths, the patient's general practitioner was interviewed to gain pertinent information on the cause.

Statistical Analyses

Data were expressed as the mean \pm SD, median and range, or percentage, as appropriate. For analytical purposes, patients were divided into two groups according to the median [dp-ucMGP] (*i.e.*, above and equal to or below 921 pM). The CKD stages were entered as a continuous variable (recoded from 1 to 3, with 3 meaning stage 5D patients). Intergroup comparisons were performed using a χ^2 test for categorical variables and either *t* test or the Mann-Whitney test for continuous variables. For parameters presenting non-Gaussian distribution, logarithmic normalized values were considered in tests that assume normally distributed variables. Univariate and then multivariate linear regression analyses were used to select factors that were

independently associated with [dp-ucMGP]. Thereafter, to see whether higher dp-ucMGP levels could have contributed to a higher aortic calcification score in the study cohort, we performed a multiple linear regression analysis with the aortic calcification score as the dependent variable, and [dp-ucMGP] and selected clinical and biochemical characteristics (CKD stage, age, previous cardiovascular disease, and systolic arterial pressure) as covariates. Because there was a good correlation between the aortic calcification score on x-ray and the aortic calcification score on CT (Spearman correlation coefficient = 0.873, $P < 0.0001$), the latter was used as the reference measurement in all further analyses. The Kaplan-Meier actuarial method was used to estimate overall survival for the median dp-ucMGP level, and the log-rank test was used to compare survival curves. Univariate and multivariate analyses of mortality were performed by using a Cox proportional hazards model of death as a function of the plasma dp-ucMGP level (either categorized by the median or as a continuous variable). Variables that were significantly associated with death in the univariate analyses were fed into predefined, noncumulative models in the multivariate analysis. In view of the small size of the present cohort, additional Cox regression analyses included a propensity score adjustment; this takes account of each individual's probability of exposure to measured, confounding variables (*i.e.*, CKD stage, hemoglobin, and the aortic calcification score), as detailed elsewhere (28). A P value ≤ 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL), version 13.0 for Windows (Microsoft Corp., Redmond, WA).

Results

As shown in Figure 1, plasma dp-ucMGP levels increased with CKD stage. In comparison with age-matched controls, this increase became statistically significant from CKD stage 4 onward. When considering only predialysis patients at CKD stages 2 to 5 ($n = 67$), we still observed an inverse, exponential association between plasma dp-ucMGP levels and the estimated GFR (Figure 2).

Table 1 presents the main clinical and biochemical characteristics and vascular measurements for the entire study cohort

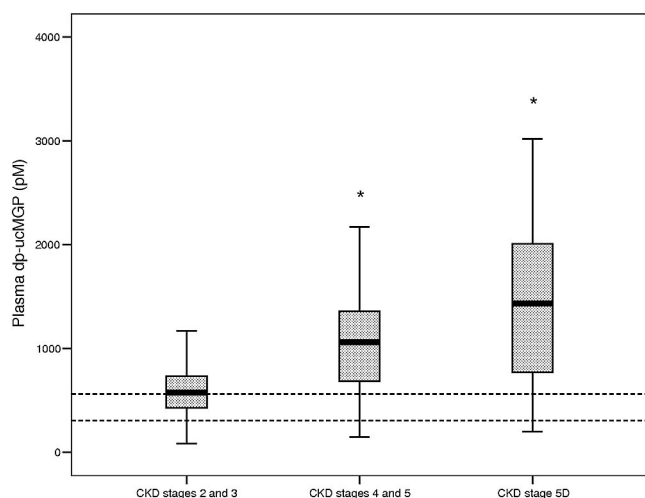


Figure 1. Plasma dp-ucMGP levels by CKD stage. * $P < 0.001$ versus CKD stages 2 and 3. The dotted lines indicate the upper and lower boundaries of the plasma dp-ucMGP range in age-matched controls.

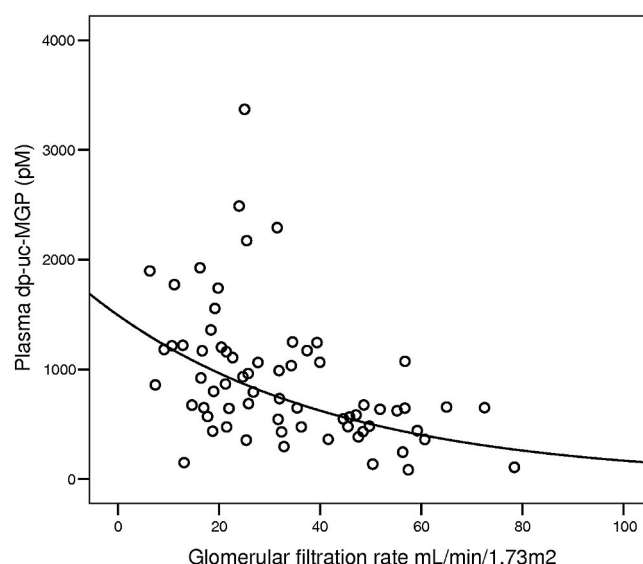


Figure 2. Exponential relationship between plasma dp-ucMGP levels and the estimated GFR, for patients at CKD stages 2 to 5 ($n = 67$), $r^2 = 0.268$, $P < 0.0001$.

and as a function of the median dp-ucMGP level (921 pM). Patients with higher plasma dp-ucMGP levels were significantly older, more likely to be taking coumarins, and more frequently in late-stage CKD (stages 4, 5, and 5D). They also had lower hemoglobin levels, higher C-reactive protein levels, and a higher aortic calcification score. Univariate linear regression analyses with plasma dp-ucMGP as a dependent variable yielded essentially the same results (Table 2). Furthermore, a multivariate linear regression analysis including all variables significantly associated with plasma dp-ucMGP levels in a univariate analysis (Table 2) identified the CKD stage ($P < 0.0001$), hemoglobin levels ($P < 0.0001$), age ($P = 0.002$), and current coumarin use ($P = 0.006$) as independent factors ($r^2 = 0.432$). This conclusion was not modified by excluding the six patients on coumarins from our analyses.

With respect to VC, there was a positive, statistically significant association between the aortic calcium score and plasma dp-ucMGP levels (Figure 3). Other variables significantly associated with the aortic calcium score are listed in Table 3. Furthermore, in a multivariate linear regression analysis (including all clinical and biochemical characteristics significantly associated with the aortic calcium score in the univariate analyses), only age and plasma dp-ucMGP levels were independently associated with the aortic calcium score (Table 4). In an analysis restricted to predialysis patients, the independent association between the aortic calcification score and plasma dp-ucMGP levels remained significant after adjustment for the estimated GFR ($n = 67$; $r^2 = 0.125$, $P = 0.018$).

During the follow-up period (mean duration: 802 ± 311 days; median: 846 days; range: 10 to 1129 days), 34 patients died (20 from cardiovascular events, 7 from infectious disease, and 7 from other causes). In a crude analysis, plasma [dp-ucMGP] > 921 pM predicted overall mortality ($P = 0.006$ in a log-rank test comparing the curves) (Figure 4). Further univariate and mul-

Table 1. Main clinical characteristics and baseline data as a function of plasma dp-ucMGP levels

	All	[dp-ucMGP] ≤ 921 pM	[dp-ucMGP] > 921 pM	P
<i>n</i>	107	53	54	
Age, yr	67 ± 13	65 ± 13	70 ± 11	0.049
Male gender, <i>n</i> (%)	64 (60)	35 (66)	29 (54)	0.193
Body mass index, kg/m ²	28 ± 6	27 ± 6	28 ± 6	0.421
SBP, mmHg	154 ± 27	149 ± 25	158 ± 28	0.075
DBP, mmHg	80 ± 12	80 ± 12	78 ± 12	0.447
Diabetes, <i>n</i> (%)	45 (42)	23 (43)	22 (41)	0.781
Smoking habit, <i>n</i> (%) ^a	40 (39)	20 (38)	20 (39)	0.937
Previous CVD, <i>n</i> (%)	35 (33)	18 (34)	17 (31)	0.784
Coumarin use, <i>n</i> (%)	6 (5.6)	0	6 (11.1)	0.027
Ca phosphate binder use, <i>n</i> (%)	43 (40)	19 (36)	24 (44)	0.365
Vitamin D use, <i>n</i> (%)	41 (38)	16 (30)	25 (46)	0.09
CKD stage, <i>n</i> (%)				0.004
2	4 (3.7)	4 (7.5)	0	
3	30 (28)	22 (41.5)	8 (14.8)	
4	25 (23.4)	10 (18.9)	15 (27.8)	
5	8 (7.5)	3 (5.7)	5 (9.3)	
5D	40 (37.4)	14 (26.4)	26 (48.1)	
Calcium, mmol/L	2.29 ± 0.18	2.31 ± 0.19	2.28 ± 0.17	0.443
Phosphate, mmol/L	1.30 ± 0.47	1.31 ± 0.43	1.30 ± 0.50	0.896
Intact PTH, pg/ml	123 ± 119	129 ± 135	117 ± 102	0.609
	(79; 43 to 164)	(80; 46 to 151)	(78; 40 to 200)	
Hemoglobin, g/dL	12 ± 1.7	12 ± 1.5	11 ± 1.6	<0.0001
Albumin, g/L	36 ± 6	37 ± 6	35 ± 5	0.102
C-reactive protein, mg/L	11 ± 24	5 ± 6	16 ± 33	0.042
	(3.5; 1.3 to 8.9)	(2.7; 1.1 to 6.7)	(4.0; 1.5 to 14.7)	
Triglycerides, mmol/L	2.1 ± 1.4 1.7	2.0 ± 1.6 1.5	2.2 ± 1.2 1.9	0.082
	(1.2 to 2.7)	(1.1 to 2.3)	(1.3 to 2.9)	
Total cholesterol, mmol/L	4.9 ± 1.21	4.9 ± 1.3	4.9 ± 1.1	0.907
Aortic calcification score on CT ^b	3.4 ± 3.2	2.4 ± 2.9	4.4 ± 3.1	<0.0001
	(2.28; 0.9 to 4.7)	(1.2; 0.5 to 3.8)	(3.7; 1.9 to 6.7)	
Aortic calcification score on x-ray ^c	7.3 ± 6.7	4.9 ± 5.4	9.4 ± 7.0	0.001
	(6.0; 2.0 to 11)	(3.0; 0 to 8.0)	(9.0; 4.0 to 14)	
[dp-ucMGP], pM	1100 ± 713	539 ± 205	1652 ± 591	—

Data are given as means ± SD (median; percentiles 25 to 75) for variables with a non-Gaussian distribution or as the number (percentage) for binary variables. SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; Ca, calcium-containing; PTH, parathyroid hormone.

^a*n* = 103.

^b*n* = 101.

^c*n* = 91.

tivariate, noncumulative Cox regression analyses (Table 5) including other variables that had been shown to significantly influence mortality risk in this population (age, per 1-year increment: risk ratio [RR] = 1.04; *P* = 0.013; hemoglobin, per 1-g/dL increment: RR = 0.73, *P* = 0.003; aortic calcification score, per 1-unit increment: RR = 1.23, *P* < 0.0001; CKD stage: RR = 1.84, *P* = 0.007) confirmed that the plasma dp-ucMGP level was an independent predictor of mortality, with attenuation after adjustment for the aortic calcification score (model 4). It is noteworthy that in cumulative models that considered either all these covariates together or included the calculated

propensity score (to better adjust for confounders, as detailed in Materials and Methods), the association between plasma dp-ucMGP level and mortality lost its significance (model 5).

Discussion

In the present study, we show for the first time that plasma dp-ucMGP levels augment progressively in a CKD setting. Moreover, renal dysfunction was the only independent determinant of higher circulating dp-ucMGP levels in our cohort. Importantly, these higher dp-ucMGP levels were indepen-

Table 2. Univariate linear regression analysis: variables associated with plasma dp-ucMGP levels

	β (95% CI)	r^2	P
CKD stage	381.7 (233.6 to 529.8)	0.199	<0.0001
Hemoglobin	−191.0 (−264.3 to −117.8)	0.203	<0.0001
Aortic calcification score on CT (logarithmic transformed)	209.7 (107.5 to 311.9)	0.143	<0.0001
C-reactive protein (logarithmic transformed)	132.2 (39.6 to 224.7)	0.071	0.006
Age	12.9 (2.3 to 23.6)	0.053	0.018
Coumarin use	631.6 (47.35 to 1215.9)	0.042	0.034

CI, confidence interval.

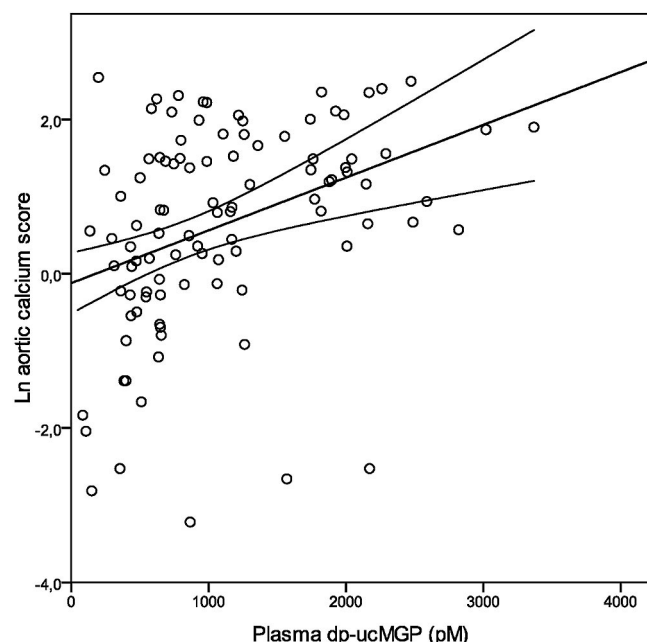


Figure 3. Linear relationship between aortic calcification score and plasma dp-ucMGP levels ($n = 101$), $r^2 = 0.143$, $P < 0.0001$.

dently associated with aortic calcification and showed a limited association with the overall mortality risk in the study cohort.

In contrast to our data, previous studies have reported significantly lower serum levels of ucMGP in dialyzed adult and pediatric patients than in healthy controls (20,29,30). Moreover, an inverse association between estimated GFR and ucMGP serum levels in patients with stable cardiovascular disease has been demonstrated (31). Recently, data from Cranenburg *et al.* (22) have evidenced that circulating levels of ucMGP were inversely correlated with the extent of coronary artery calcification in hemodialysis patients. It is important to note that these studies used an assay that did not differentiate between phosphorylated and dephosphorylated forms of ucMGP or fragments of MGP. In fact, it has been shown that ucMGP accumulates at arterial calcification sites (32), possibly by binding through its negatively charged phosphoserine residues (22). Consequently, up-regulation of MGP transcription in response to vascular stress (as well as absorptive forces in already calcified vessels) would influence ucMGP levels (31). Importantly, the present study featured a new assay that

distinguishes the phosphorylation status of ucMGP, so that only the dephosphorylated uncarboxylated form of MGP was specifically measured; this may explain the apparently conflicting results with respect to previous assays. Indeed, it was recently demonstrated that the formation of phosphoserine residues actively contributes to MGP's calcification inhibition activity (19). Furthermore, because of the absence of phosphoserine residues, dp-ucMGP is thought to be released more easily into the circulation than phosphorylated MGP forms; the latter usually present extremely low circulating levels in the highly calcified CKD population, which may limit their use as a biomarker.

Importantly, our present findings indicate that elevation of serum levels of the inactive form of MGP parallels the progression of CKD and thus seems to be an important contributor to (or even a biomarker of) the severity of the VC frequently observed in the CKD setting. The high dp-ucMGP levels could reflect a low dietary intake of vitamin K, resulting in lower metabolic availability and thus less MGP γ -glutamyl carboxylation in patients with more advanced CKD. Additionally, it has been suggested that vitamin K status in hemodialysis patients is also influenced by the apolipoprotein E genotype. Lipoprotein-bound apolipoprotein E regulates the clearance of circulating vitamin K in the liver and in peripheral tissues (33). It is interesting to note that the vitamin K requirement in extrahepatic tissues (for γ -glutamyl carboxylation of MGP, for example) appears to be greater than that for the liver itself (for the modification of coagulation factors). Therefore, a subclinical vitamin K deficiency could impair the function of vitamin K-dependent calcification modulators (such as MGP and osteocalcin) without greatly affecting coagulation. This hypothesis is reinforced by the observation of low phyloquinone (vitamin K1) concentrations and undercarboxylation of osteocalcin (another vitamin K-dependent protein) in dialysis patients, relative to the general population (34). Moreover, coumarin vitamin K antagonists (which are used as coagulation inhibitors) have been identified as a risk factor for VC in the general population (35,36). In contrast, high vitamin K intake has been shown to regress warfarin-induced medial calcinosis in rats (37), and a high dietary intake of menaquinone (vitamin K2) has been associated with (1) a lower risk of coronary heart disease in the general population (38) and (2) reduced coronary calcification in postmenopausal women (39). In our study, patients on coumarins presented significantly higher levels

Table 3. Univariate linear regression analysis: variables associated with the aortic calcification score on CT (logarithmic normalized)

	β (95% CI)	r^2	P
Age	0.057 (0.039 to 0.075)	0.290	<0.0001
[dp-ucMGP]	0.001 (0.000 to 0.001)	0.143	<0.0001
Previous CVD	0.570 (0.016 to 1.124)	0.040	0.044
CKD stage	0.173 (−0.019 to 0.365)	0.144	0.076

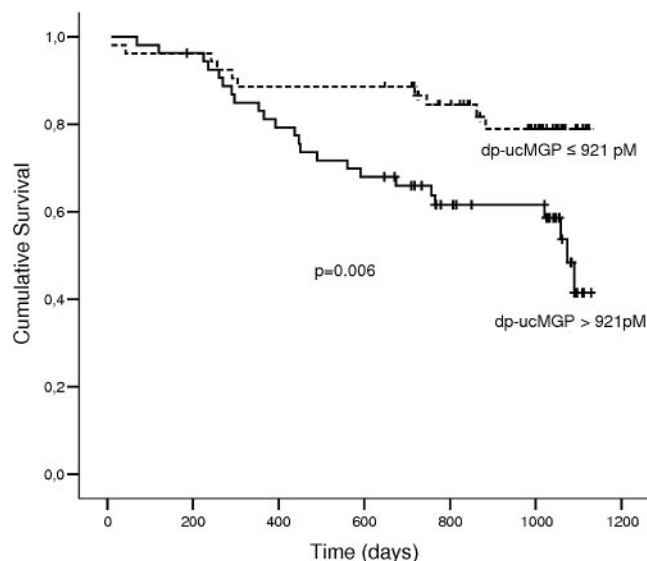
$n = 101$ patients. CI, confidence interval; CVD, cardiovascular disease.

Table 4. Multivariate linear regression analysis: variables independently associated with the aortic calcification score on CT (logarithmic normalized)

	β (95% CI)	P
Age	0.050 (0.032 to 0.068)	<0.0001
[dp-ucMGP]	0.000 (0.000 to 0.001)	0.003

$n = 101$ patients. CI, confidence interval.

Variables entered in the model: age, plasma dp-ucMGP, previous cardiovascular disease, and CKD stage; model's $r^2 = 0.381$.

**Figure 4.** Kaplan-Meier estimates of overall mortality as a function of the median plasma dp-ucMGP level.

of dp-ucMGP, which probably reflected an accumulation of this inactive form of MGP. In agreement with these observations, we recently reported (30) that plasma dp-ucMGP levels increase significantly with the use of vitamin K antagonists and, conversely, decrease after vitamin K supplementation. This finding indeed suggests that vitamin K has an important role in modulating dp-ucMGP levels. Additionally, a pilot study with vitamin K supplementation in CKD 5D patients clearly demonstrated a dose-dependent decrease in dp-ucMGP levels over time, with a return to baseline levels during the washout phase (Westenfeld *et al.*, ASN Renal week 2008, TH-FC044). Al-

ternatively, uremic toxins might impair the function of γ -glutamyl carboxylase. In such a case, a uremic state would affect MGP carboxylation even if vitamin K status were adequate. This latter hypothesis remains to be tested, however.

Our findings also demonstrate an association between plasma dp-ucMGP levels and overall mortality. However, this association was rather weak and was lost after cumulative adjustment for age and the calculated propensity score. This inconsistency may result (at least in part) from the small size of the study cohort and the complexity of the etiology of cardiovascular disease in a CKD setting. It seems also possible that plasma dp-ucMGP is simply a biomarker that reflects the VC burden. In theory, higher levels of the inactive form of MGP would initially favor VC. This high calcification burden would lead to a burst in MGP production, further exhausting vitamin K stores, limiting MGP activation, and resulting in additional VC. Additional research is needed to confirm and unravel the possible role of dp-ucMGP as a biomarker in CKD and to establish whether interventions that decrease plasma dp-ucMGP levels would result in better outcomes in a CKD setting.

Limitations of the present study include the small sample size and the fact that vitamin K intake and serum levels were not assayed. Moreover, plasma dp-ucMGP levels were measured at a single time point; at present, nothing is known about the time course of release of this biomarker and its persistence in the circulation. In contrast, one of the study's main strengths relates to the fact that this was the first specific analysis of circulating, inactive dp-ucMGP in a cohort of patients at different stages of CKD.

In conclusion, plasma dp-ucMGP levels augment progressively in a CKD setting and were directly correlated with the severity of aortic calcification in our study population. Plasma dp-ucMGP could thus be regarded as a surrogate marker for VC in CKD. Further experimental work on the potential relationship between vitamin K status and dp-ucMGP levels and interventional studies evaluating the influence of vitamin K repletion on dp-ucMGP levels, VC, and outcomes in the CKD setting are warranted.

Acknowledgments

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Table 5. Multivariate Cox regression analysis of risk factors at baseline for all-cause mortality

Models of Patient Survival (<i>n</i> = 34 events)	RR	95% CI	<i>P</i>
[dp-ucMGP], categorized by the median			
model 0 ^a	2.85 ^b	1.36 to 5.9	0.006
model 1 ^c	2.49 ^b	1.18 to 5.25	0.017
model 2 ^d	2.26 ^b	1.05 to 4.87	0.036
model 3 ^e	2.20 ^b	1.01 to 4.81	0.048
model 4 ^f	2.11 ^b	0.95 to 4.68	0.066
model 5 ^g	1.57 ^b	0.67 to 3.67	0.298
[dp-ucMGP] entered as a continuous variable			
model 0 ^a	1.07 ^h	1.03 to 1.11	0.001
model 1 ^c	1.06 ^h	1.02 to 1.10	0.005
model 2 ^d	1.05 ^h	1.01 to 1.10	0.024
model 3 ^e	1.05 ^h	1.00 to 1.10	0.041
model 4 ^f	1.05 ^h	1.00 to 1.09	0.032
model 5 ^g	1.02 ^h	0.97 to 1.08	0.319

RR, risk ratio; CI, confidence interval.

^aModel 0: unadjusted.

^bSummarizing the risk of having plasma [dp-ucMGP] > 921 pM.

^cModel 1: adjusted for age.

^dModel 2: adjusted for CKD stage.

^eModel 3: adjusted for hemoglobin levels.

^fModel 4: adjusted for the aortic calcification score.

^gModel 5: adjusted for age and the calculated propensity score.

^hSummarizing the risk of a 100-pM increment in log-normalized plasma dp-ucMGP levels for each model.

Disclosures

None.

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