

Matrix Gla-Protein: Overview

*Review Series
Immunodiagnostic Systems Limited*

Introduction

In recent years, the insights into the pathogenesis of Vascular Calcification (VC) have changed significantly. VC was considered a passive process, associated with an elevated calcium x phosphorus product in the extracellular fluid compartment. It is now widely acknowledged that VC is an actively regulated process where death and damage of Vascular Smooth Muscle Cells (VSMCs) occurs, with the transformation of VSMCs into osteoblast-like cells and deficiencies in calcification inhibitors.^{1,2} Amongst the proteins involved in vascular calcium metabolism, the vitamin K-dependent Matrix Gla-Protein (MGP) is the strongest local inhibitor of vascular calcification in the vessel wall.

MGP is the most potent inhibitor of tissue calcification presently known. MGP is γ -carboxylated 14 kDa protein comprising of 84 amino acids, which is mainly expressed and secreted by chondrocytes and vascular smooth muscle cells in the arterial media. Vitamin K serves as a co-factor for the enzyme γ -glutamate carboxylase that converts glutamate residues into γ -carboxyglutamate (Gla). These Gla-residues serve as calcium-binding groups, which are essential for the activity of all Gla-containing proteins including MGP. Besides carboxylation, MGP also undergoes post-translational serine phosphorylation during maturation. Whereas carboxylation is essential for its calcification inhibitory activity, its cellular secretion is enhanced by phosphorylation.^{3,4} At least four different MGP species are formed with varying states of phosphorylation and/or carboxylation (Figure 1): phosphorylated carboxylated MGP (p-cMGP), phosphorylated uncarboxylated MGP (p-ucMGP), desphospho-carboxylated MGP (dp-cMGP), and desphospho-uncarboxylated MGP (dp-ucMGP). Circulating forms of MGP have no known biological function, but reflect the extent of vascular calcification and availability of Vitamin K in the vessel wall.^{3,5-6}

Quick Facts

- Presently, there is no assay available to measure each individual circulating MGP species or even the total circulating MGP pool.
- The uncarboxylated MGP immunoassays are promising biomarkers for monitoring the cardiovascular calcification progression.
- The inactive MGP (dp-ucMGP) immunoassay detects the inactive dephosphorylated-uncarboxylated isoform of MGP, reflecting the status of vitamin K.
- The mid-active MGP (t-ucMGP) assay recognises the sum of p-ucMGP and dp-ucMGP. Through its calcium-binding capacity, t-ucMGP may become a useful monitoring marker for vascular calcification.
- Published data suggests that dp-ucMGP may become a risk marker for cardiovascular disease and mortality.

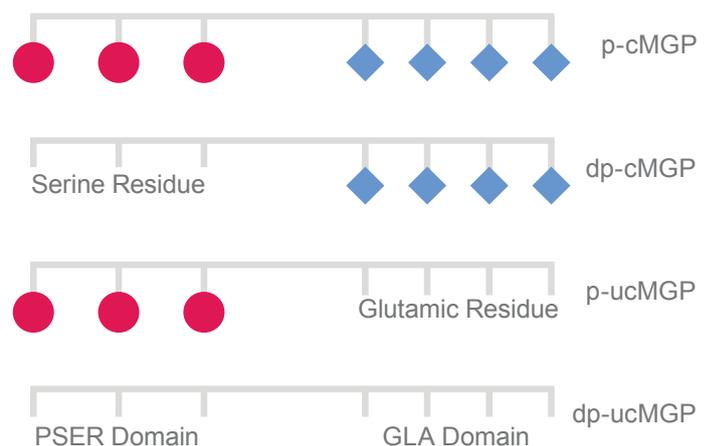


Figure 1. Four different conformations of MGP.

Phosphoserine (Pser) residues are depicted as circles; carboxylated glutamate (Gla) residues as triangles.

Selected References

The abnormal status of uncarboxylated matrix gla-protein species represents an additional mortality risk in heart failure patients with vascular disease.

Mayer O Jr, Seidlerová J, Vaněk J, Karnosová P, Bruthans J, Filipovský J, Wohlfahrt P, Cífková R, Windrichová J, Knapen MH, Drummen NE, Vermeer C. *Int J Cardiol.* 2016 Jan 15;203:916-22.

In a prospective 5 year follow-up study comprising 799 patients (mean age 65.1 years) with stable vascular disease, both desphospho-uncarboxylated and total uncarboxylated MGP (dp-ucMGP or t-ucMGP) were quantified by pre-commercial immunoassays (IDS Ltd., UK). Elevated (>100ng/L) circulating Brain Natriuretic Peptide (BNP) and abnormal status of plasma uncarboxylated MGP species (dp-ucMGP \geq 977pmol/L, t-ucMGP \leq 2825nmol/L) were all identified as robust predictors of all-cause 5-year mortality. The investigators observed the highest mortality risk in patients with elevated BNP and high dp-ucMGP, compared to those with normal BNP plus low dp-ucMGP. Similarly, the risk was increased when compared with patients with elevated BNP and low dp-ucMGP.

The concomitant abnormality of uncarboxylated MGP and mild elevation of BNP leads in chronic patients with vascular disease leads to two-fold increase of the relative mortality risk.

Desphospho-uncarboxylated matrix gla-protein is associated with increased aortic stiffness in a general population.

Mayer O Jr, Seidlerová J, Wohlfahrt P, Filipovský J, Vaněk J, Cífková R, Windrichová J, Topolčan O, Knapen MH, Drummen NE, Vermeer C. *J Hum Hypertens.* 2015 May 28.

[Epub ahead of print]

The authors investigated whether there is an association between dp-ucMGP and stiffness of elastic and muscular-type large arteries in a random sample from the general population of 1087 subjects from the Czech post-MONICA study. Aortic and femoro-popliteal Pulse Wave Velocities (PWVs) were measured using a Sphygmocor device. Dp-ucMGP concentrations were assessed using the pre-commercial IDS-iSYS InaKtif MGP (dp-ucMGP) (IDS Ltd., UK). Aortic PWV significantly ($P < 0.0001$) increased across the dp-ucMGP quartiles. After adjustment for all potential confounders, aortic PWV independently correlated with dp-ucMGP. In a categorised mode, subjects in the top quartile of dp-ucMGP (671 pmol/L) had a higher risk of elevated aortic PWV. No relation between dp-ucMGP and femoro-popliteal PWV was found.

Increased circulating levels of dp-ucMGP are independently associated with aortic stiffness.

Inactive matrix gla-protein is associated with arterial stiffness in an adult population-based study.

Pivin E, Ponte B, Pruijm M, Ackermann D, Guessous I, Ehret G, Liu YP, Drummen NE, Knapen MH, Pechere-Bertschi A, Paccaud F, Mohaupt M, Vermeer C, Staessen JA, Vogt B, Martin PY, Burnier M, Bochud M. *Hypertension.* 2015 Jul;66(1):85-92.

The researchers recruited 1001 participants (475 men and 526 women) from a multicenter family-based cross-sectional study in Switzerland. Dp-ucMGP was quantified via an immunoassay. Aortic Pulse Wave Velocity (PWV), a marker of aortic stiffness and an independent predictor of mortality, was determined by applanation tonometry. Multiple regression analysis was performed to estimate associations between PWV and dp-ucMGP, adjusting for age, renal function, and other cardiovascular risk factors. PWV was positively associated with dp-ucMGP both before and after adjustment for sex, age, body mass index, height, systolic and diastolic blood pressure (BP), heart rate, renal function, low and high-density lipoprotein, glucose, smoking status, diabetes mellitus, BP and cholesterol lowering drugs, and history of cardiovascular disease ($P \leq 0.01$).

High levels of dp-ucMGP are independently and positively associated with arterial stiffness.

Dephosphorylated-uncarboxylated matrix gla-protein concentration is predictive of vitamin K status and is correlated with vascular calcification in a cohort of hemodialysis patients.

Delanaye P, Krzesinski JM, Warling X, Moonen M, Smelten N, Médart L, Pottel H, Cavalier E. *BMC Nephrol.* 2014 Sep 4;15:145.

The authors reported the results from an observational study in a cohort of 165 haemodialysis (HD) patients, including 23 who were receiving Vitamin K Antagonist (VKA) treatment. The calcification score was determined (using the Kauppila method), and dp-ucMGP levels were measured using the automated IDS-iSYS InaKtif MGP (dp-ucMGP) method. The dp-ucMGP levels were much higher in patients being treated with VKA; small overlap was found with those not being treated. In the 137 untreated patients, dp-ucMGP levels were significantly ($p < 0.05$) associated both in the uni and multivariate analysis, with age, body mass index, plasma levels of albumin, C-reactive protein, FGF-23, and the vascular calcification score.

The concentration of dp-ucMGP was higher in HD patients being treated with VKA. The investigators observed a significant correlation between dp-ucMGP concentration and the calcification score. They confirmed the potential role of the inactive form of MGP in assessing the vitamin K status of the HD patients.

REFERENCES

1. Moe SM, Chen NX. Mechanism of vascular calcification in chronic disease. *J Am Soc Nephrol* 2008; 19: 213-216.
2. Schoppet M, Shroff RC, Hofbauer LC et al. Exploring the biology of vascular calcification in chronic kidney disease: what's circulating? *Kidney Int.* 2008; 384-390.
3. Cranenburg EC, Koos R, Schurgers LJ, Magdeleyns EJ, Schoonbrood TH, Landewé RB et al. Characterisation and potential diagnostic value of circulating matrix Gla protein (MGP) species. *Thromb Haemost.* 2010; 104: 811–822.
4. Schurgers LJ, Spronk HM, Skepper JN, Hackeng TM, Shanahan CM, Vermeer C et al. Post-translational modifications regulate matrix Gla protein function: importance for inhibition of vascular smooth muscle cell calcification. *J Thromb Haemost.* 2007; 5: 2503–2511.
5. Murshed M, Schinke T, McKee MD et al. Extracellular matrix mineralization is regulated locally; different roles of two gla-containing proteins. *J Cell Biol* 2004; 165:625-630.
6. Schurgers LJ, Cranenburg EC, Vermeer C. Matrix Gla-protein: the calcification inhibitor in need of vitamin K. *Thromb Haemost.* 2008; 100: 593–603.
7. Mayer O Jr, Seidlerová J, Vaněk J, Karnosová P, Bruthans J, Filipovský J, Wohlfahrt P, Cífková R, Windrichová J, Knapen MH, Drummen NE, Vermeer C. The abnormal status of uncarboxylated matrix Gla protein species represents an additional mortality risk in heart failure patients with vascular disease. *Int J Cardiol.* 2016 Jan 15;203:916-22.
8. Mayer O Jr, Seidlerová J, Wohlfahrt P, Filipovský J, Vaněk J, Cífková R, Windrichová J, Topolčan O, Knapen MH, Drummen NE, Vermeer C. Desphospho-uncarboxylated matrix Gla protein is associated with increased aortic stiffness in a general population. *J Hum Hypertens.* 2015 May 28. [Epub ahead of print]
9. Pivin E, Ponte B, Pruijm M, Ackermann D, Guessous I, Ehret G, Liu YP, Drummen NE, Knapen MH, Pechere-Bertschi A, Paccaud F, Mohaupt M, Vermeer C, Staessen JA, Vogt B, Martin PY, Burnier M, Bochud M. Inactive Matrix Gla-Protein Is Associated With Arterial Stiffness in an Adult Population-Based Study. *Hypertension.* 2015 Jul;66(1):85-92.
10. Delanaye P, Krzesinski JM, Warling X, Moonen M, Smelten N, Médart L, Pottel H, Cavalier E. Dephosphorylated-uncarboxylated Matrix Gla protein concentration is predictive of vitamin K status and is correlated with vascular calcification in a cohort of hemodialysis patients. *BMC Nephrol.* 2014 Sep 4;15:145.

