Bone Markers and Vascular Calcification in CKD-MBD

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Conflict of interest

• Honorarium (speaker or travel grant) : Fresenius, Menarini, Sanofi, Amgen, Roche

• Consultancy:
  Immunodiagnostic Systems limited
Two facts in dialysis patients...

Jadoul M, Kidney Int, 2006, p1358

Coronary calcifications in dialysis patients:

- Very frequent (over 50%) and severe
- Early and more rapidly progressive

*Goodman WG, N Engl J Med, 2000, p1478*
Relationship between

• Cardiovascular mortality and mineral metabolism markers (P, Ca, and PTH)
Relationship between
- Several mineral metabolism markers and VC

Relationship between
- VC and cardiovascular mortality

Is it causal?
Bone health in CKD patient
(turnover versus volume versus mineralization)

Moorthi N, Kidney Int, 2013, p886
Bone turnover is associated with VC

Osteoporosis is associated with VC

High Parathyroid Hormone Level and Osteoporosis Predict Progression of Coronary Artery Calcification in Patients on Dialysis

Hartmut H. Malluche,* Gustav Blomquist,† Marie-Claude Monier-Faugere,‡ Thomas I. Cantor,§ and Daniel L. Davenport∥

*Division of Nephrology, Bone and Mineral Metabolism and Departments of †Radiology and ‡Surgery, University of Kentucky, Lexington, Kentucky; and ∥Scalectibody Laboratory Inc., Santee, California

High Prevalence of Vertebral Fractures Assessed by Quantitative Morphometry in Hemodialysis Patients, Strongly Associated with Vascular Calcifications

Marzia Fasce, Giovanni Tripepi, Macimena Noso, Nicola Volante, Mario Pianini, Martina Zaninotto, Giuseppe Guglielmi, Diego Miotti, Luca Dalle Carbonare, Angela D'Angelo, Daniele Ciardino, Riccarda Puggia, Davide Miozzo, Sandro Giannini, Maurizio Gallieni

Inverse association between bone microarchitecture assessed by HR-pQCT and coronary artery calcification in patients with end-stage renal disease

Daniel Cejka a,‡ Michael Weber a,§ Daniella Diarra a,‡ Thomas Reiter a,‡ Franz Kainberger a,‡ Martin Haas a,‡

Vertebral bone density associates with coronary artery calcification and is an independent predictor of poor outcome in end-stage renal disease patients

Zhimin Chen a,‡ Abdul Rashid Qureshi a,‡ Jonaz Ripsweden a,‡ Lars Wennberg a,‡ Olof Heimburger a,‡ Bengt Lindholm a,‡ Peter Barany a,‡ Mathias Haarhaus a,‡ Torkel R. Brismar a,‡ Peter Steeninkum a,‡
This concept of Bone-Vascular axis is also suggested by basic research.
Vascular calcification: from pathophysiology to biomarkers

Séverine Evrard a, Pierre Delanaye b, Said Kamel c,d, Jean-Paul Cristol e, Etienne Cavalier a,*
On behalf of the SFB/C/SN joined working group on vascular calcifications
J. Arnaud 1, Ph. Zaoui 1, M.C. Carlier 2, M. Laville 2, D. Fouque 3, E. Cavalier 3, P. Delanaye 3, J.P. Cristol 4,
A.S. Bargnoux 4, S. Kamel 5,6, Z. Massy 7,8, D. Prie 6, P. Urena-Torres 8, J.C. Souberbielle 8, A. Boutten 5,10,

- FGF-23/Klotho
- Fetuin-A
- Matrix Gla protein
- Bone Morphogenic Protein
- Osteoprotegerin/RANKL
- Osteopontin
- Osteonectin
- Osteocalcin
- Pyrophosphate
- Sclerostin

Still not fit for clinical purpose!
Matrix Gla Protein and Sclerostin

• Recent
• Clinical research with VC is active
• Potentially linked to future therapy
• Specific challenges in Clinical Chemistry
Matrix Gla protein (MGP)

• 11 kD protein, 84 amino acids
• Secreted by chondrocytes and vascular smooth muscle cells (VSMC)
• Act as a local calcification inhibitor
  ▪ Directly inhibiting calcium precipitation and crystallization (fetuin A)
  ▪ Antagonizing BMP-2 which promote osteoblastic differentiation of VSMC
• MGP knockout mice
  ▪ Extensive arterial and cartilage calcification
  ▪ Death within 2 months due to rupture of calcified aorta
Matrix Gla protein (MGP)

Two post-translational modifications

- **Carboxylation of glutamate residues**
  - Binding of Ca-ions and crystals
- **Phosphorylation of serine residues**
  - Function? Regulation of secretion of MGP into the extracellular matrix.
  - Binding of MGP to sites of calcification

Carboxylation of glutamate residues is highly dependent on Vitamin K
Circulating MGP

Carboxylation and phosphorylation of MGP are not always fully exerted →

- Results in **different MGP species** in the circulation (ELISA, VitaK, Maastricht)
- Very different results according to species
- The first commercially available automate (IDS, Boldon, UK) measures the inactive form: **dp-ucMGP**
MGP and Vascular Calcification

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

Leon J. Schurgers,* Daniela V. Barreto,†† Felype C. Barreto,†† Sophie Liabeuf,†† Cédric Renard,§ Elke J. Magdeleyns,* Cees Vermeer,* Gabriel Choukroun,†† and Ziad A. Massy††

*Cardiovascular Research Institute Maastricht and VitaK, University of Maastricht, Maastricht, the Netherlands; ††Institut National de la Santé et de la Recherche Médicale ERI-12 (EA 4292), Amiens, France; ‡Division of Clinical Pharmacology, Clinical Research Centre, Amiens University Hospital and the Jules Verne University of Picardie, Amiens, France; and Divisions of §Radiology and ‡Nephrology, Amiens University Hospital, Amiens, France


- 107 CKD (40 HD)
- CAC (n=101)
Table 3. Univariate linear regression analysis: variables associated with the aortic calcification score on CT (logarithmic normalized)

<table>
<thead>
<tr>
<th>Variable</th>
<th>β (95% CI)</th>
<th>r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.057 (0.039 to 0.075)</td>
<td>0.290</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>[dp-ucMGP]</td>
<td>0.001 (0.000 to 0.001)</td>
<td>0.143</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Previous CVD</td>
<td>0.570 (0.016 to 1.124)</td>
<td>0.040</td>
<td>0.044</td>
</tr>
<tr>
<td>CKD stage</td>
<td>0.173 (−0.019 to 0.365)</td>
<td>0.144</td>
<td>0.076</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Variable</th>
<th>β (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.050 (0.032 to 0.068)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>[dp-ucMGP]</td>
<td>0.000 (0.000 to 0.001)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

n = 101 patients. CI, confidence interval.

Figure 4. Kaplan-Meier estimates of overall mortality as a function of the median plasma dp-ucMGP level.
Circulating Nonphosphorylated Carboxylated Matrix Gla Protein Predicts Survival in ESRD

Georg Schlieper,* Ralf Westenfeld,† Thilo Krüger,* Ellen C. Cranenburg,* Elke J. Magdeleyns,‡ Vincent M. Brandenburg,§ Zivka Djuric,‖ Tatjana Damjanovic,‖ Markus Ketteler,¶ Cees Vermeer,* Nada Dimkovic,‖ Jürgen Floege,* and Leon J. Schurgers‡


• 188 HD
• Adragao score for VC + FAV
Dephosphorylated-uncarboxylated Matrix Gla protein concentration is predictive of vitamin K status and is correlated with vascular calcification in a cohort of hemodialysis patients

Pierre Delanaye¹, Jean-Marie Krzesinski¹, Xavier Warling², Martial Moonen², Nicole Smelten³, Laurent Médart⁴, Hans Potter⁵ and Etienne Cavalier⁶

- 160 HD
- Kauppila score for VC
Figure 2 Univariate regression between the calcification score and dp-ucMGP (in pmol/L) in patients not treated with VKA (n = 137) ($r^2 = 0.02850$, $p = 0.049$).

Table 2 Variables associated with dp-ucMGP concentrations in the multivariate model

<table>
<thead>
<tr>
<th>Variable</th>
<th>$r$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index</td>
<td>0.17</td>
<td>0.0032</td>
</tr>
<tr>
<td>Albumin</td>
<td>-0.24</td>
<td>0.0368</td>
</tr>
<tr>
<td>FGF-23</td>
<td>0.28</td>
<td>0.0032</td>
</tr>
<tr>
<td>CRP</td>
<td>0.33</td>
<td>0.0012</td>
</tr>
<tr>
<td>Calcification score</td>
<td>0.19</td>
<td>0.0206</td>
</tr>
</tbody>
</table>

Note: $r$ is the zero order correlation coefficient for the variable in the univariate analysis. $p$ is the $p$ value of the variable in the multivariate analysis. CRP, C-reactive protein; FGF, Fibroblast Growth Factor.
Vascular calcification in chronic kidney disease: are biomarkers useful for probing the pathobiology and the health risks of this process in the clinical scenario?

Sophie Liabeuf4,2, Hirokazu Okazaki1, Lucie Desjardins1,2, Danilo Fliser3, David Goldsmith4, Adrian Covic5, Andrzej Wiecik6, Alberto Ortiz7, Alberto Martinez-Castelao8, Bengt Lindholm9, Gultekin Suleymanlar10, Francesca Mallamaci11, Carmine Zoccali11, Gerard London12 and Ziad A. Massy1,13

• N=131 CKD (45 HD)
• Aorta CT
Table 1. Correlation between studied biomarkers and aortic calcification/coronary calcification

<table>
<thead>
<tr>
<th></th>
<th>Aortic calcification (Ln)</th>
<th>Coronary calcification (Ln)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r (P)</td>
<td>$r^2 \times 100$</td>
</tr>
<tr>
<td>Phosphate (Ln)</td>
<td>0.009 (0.921)</td>
<td>0.008</td>
</tr>
<tr>
<td>FGF23 (Ln)</td>
<td>0.20 (0.019)</td>
<td>4.2</td>
</tr>
<tr>
<td>OPN</td>
<td>0.06597 (0.545)</td>
<td>0.4</td>
</tr>
<tr>
<td>OPG (Ln)</td>
<td>0.21 (0.03)</td>
<td>4.4</td>
</tr>
<tr>
<td><strong>dp-ucMGP</strong></td>
<td><strong>0.401 (&lt;0.0001)</strong></td>
<td><strong>16.1</strong></td>
</tr>
<tr>
<td>Fetuin A</td>
<td>0.001 (0.995)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

$r$, Pearson’s coefficient; FGF23, fibroblast growth factor 23; OPN, osteopontin; OPG, osteoprotegerin; dp-ucMGP, dephosphorylated uncarboxylated Matrix Gla protein; Ln, log normalized.

Bold value represent significance.
Limitation

- Observation
- Not the same calcification scores
- Added value at the individual level to quantify or detect the calcifications?

**FIGURE 1**: ROC curves for risk factors for aortic calcification. The areas under the ROC curves are 0.87 [95% confidence interval (CI), 0.81–0.95, P < 0.0001], 0.76 (95% CI, 0.64–0.88, P < 0.0001) and 0.64 (95% CI, 0.52–0.75, P = 0.02) for age, uncarboxylated, dephosphorylated Matrix Gla protein (dp-ucMGP) and fibroblast growth factor 23 (FGF23), respectively. Phosphate, OPN, fetuin-A and OPG levels were not found to be potential predictors of aortic calcification.
But Inactive MGP is representative of the vitamin K status

• Low vitamin K status in dialysis patients  
  
  Cranenburg E, Thromb Haemostase, 2010, p811
MGP and anti-vitamin K in dialysis patients

Figure 1 Median concentration of dp-ucMGP in patients treated with antivitamin K (VKA) (n = 23) and in patients not treated with VKA (n = 137) (5604 [3758; 7836] vs. 1939 [1419; 2841] pmol/L, p <0.0001).
MGP and anti-vitamin K in dialysis patients

*Figure 1*: Evolution of dp-ucMGP concentrations after stopping VKA in 7 HD patients. In red, median and P25-P75 values. The thick dark line corresponds to the time when AVK therapy is stopped.

*Delanaye P, Clin Chem Lab Med, 2015, pe191*
Vitamin K and inactive MGP

Caluwe R, NDT, 2014, p1385
Inhibiting the progression of arterial calcification with vitamin K in HemoDialysis patients (iPACK-HD) trial: rationale and study design for a randomized trial of vitamin K in patients with end stage kidney disease

Rachel M Holden\textsuperscript{1,2,3}, Sarah L Booth\textsuperscript{4}, Andrew G Day\textsuperscript{2}, Catherine M Clase\textsuperscript{5}, Deborah Zimmerman\textsuperscript{6}, Louise Moist\textsuperscript{7}, M Kyla Shea\textsuperscript{2}, Kristin M McCabe\textsuperscript{2}, Sophie A Jama\textsuperscript{9}, Sheldon Tobe\textsuperscript{9}, Jordan Weinstein\textsuperscript{9}, Rao Madhumathi\textsuperscript{10}, Michael A Adams\textsuperscript{2} and Daren K Heyland\textsuperscript{14}
Sclerostin and VC

• 22 kDa, produced by osteocytes and inhibitor of bone formation if lack of mechanical stimulation
• Sclerostin is higher in CKD
Sclerostin and VC

• Sclerostin could be involved in VC
• Anti-sclerostin antibody (romosozumab)
But the clinical results are highly discrepant...

Fig. 2. Aortic calcification score according to the serum sclerostin levels tertiles. * p < 0.05.
Because we don’t know what we measure...

<table>
<thead>
<tr>
<th></th>
<th>BM</th>
<th>TE</th>
<th>RD</th>
<th>MSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>All n=121</td>
<td>1209 [889]</td>
<td>698 [452]</td>
<td>157 [99]</td>
<td>32 [21]</td>
</tr>
<tr>
<td>Iohexol GFR &gt; 60 mL/min (group A) n=50</td>
<td>904 [613]</td>
<td>609 [181]</td>
<td>156 [55]</td>
<td>36 [17]</td>
</tr>
<tr>
<td>Iohexol GFR &lt; 60 mL/min (group B) n=32</td>
<td>1137 [743]</td>
<td>745 [377]</td>
<td>140 [121]</td>
<td>35 [21]</td>
</tr>
</tbody>
</table>

Median [IQR] concentrations of sclerostin. CKD: chronic kidney disease. All results expressed in pg/mL

Delanaye P, 2017, submitted
Group A: Iohexol GFR > 60 mL/min (n=50)
Group B: Iohexol GFR < 60 mL/min (n=32)
Group C: Dialysis patients (n=39)
Bone Biomarkers and vascular calcifications

• Solid pathophysiological basis
• The exact role must still be defined: Detection? Quantification? Therapy effect?
• Focus on biomarkers with a potential therapeutic implication
• Need for improvements in measurements
• Still much work for clinicians and labs!
Thank you for your attention