METABOLIC BONE DISEASE SATELLITE MEETING
EUROMEDLAB 2017

Bone Markers and Vascular Calcification in CKD-MBD

Pierre Delanaye, MD, PhD
Department of Nephrology, Dialysis, Transplantation
CHU Sart Tilman
University of Liège
BELGIUM

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Conflict of interest

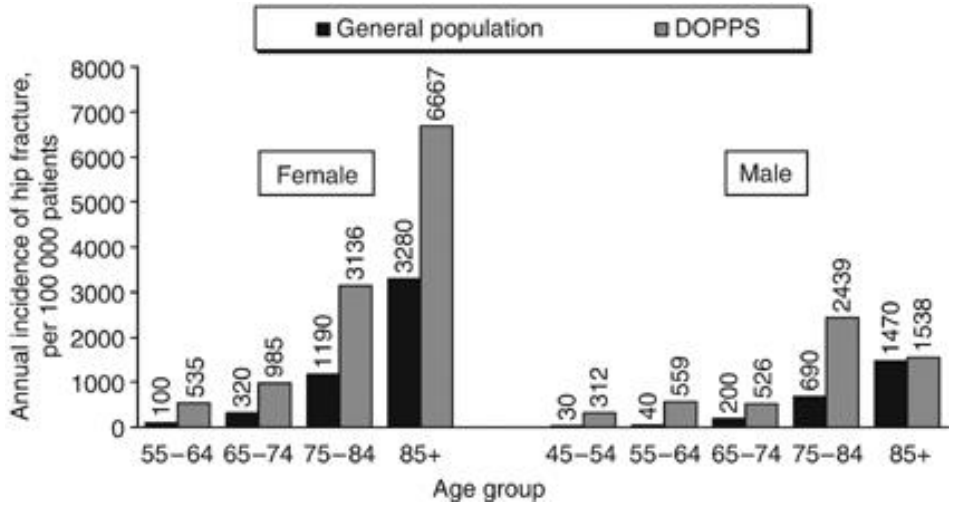
- Honorarium (speaker or travel grant) :

Fresenius, Menarini, Sanofi, Amgen, Roche

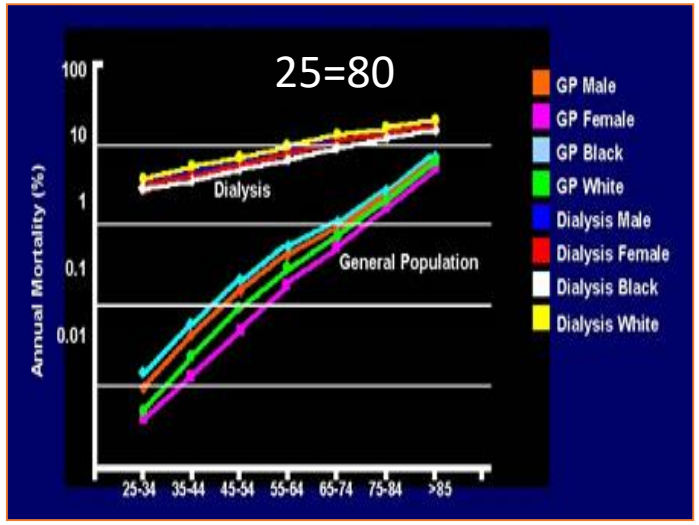
- Consultancy:

Immunodiagnostic Systems limited

Two facts in dialysis patients…



Jadoul M, Kidney Int ,2006, p1358



Foley RN, Am J Kidney Dis, 1998, S112

Coronary calcifications in dialysis patients:

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

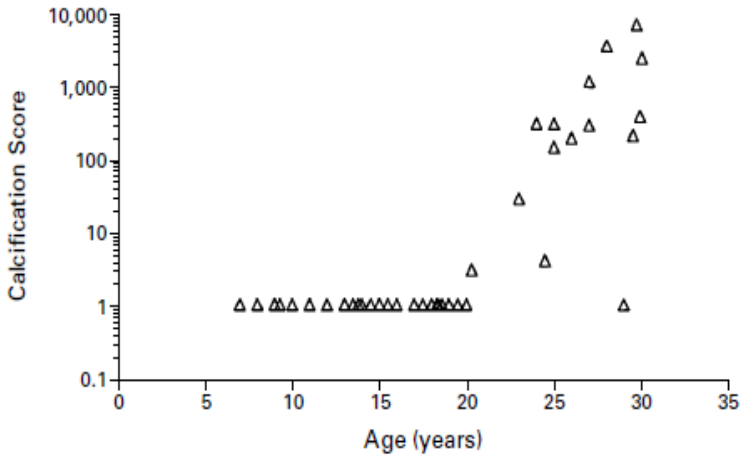


Figure 1. Coronary-Artery Calcification Scores in 39 Children and Young Adults with End-Stage Renal Disease Who Were Treated by Dialysis, According to Age.

Coronary-artery calcification was assessed by electron-beam computed tomography. The scale on the y axis is logarithmic.

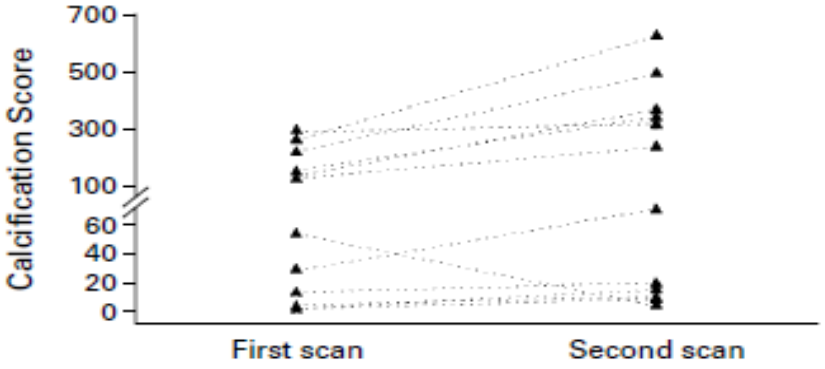


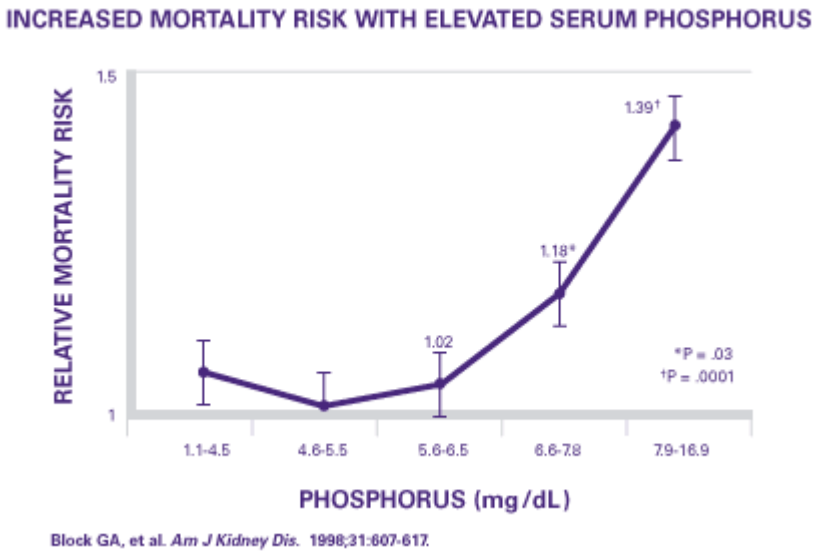
Figure 3. Coronary-Artery Calcification Scores in 10 Patients with Evidence of Coronary-Artery Calcification on the Initial Scan and in 2 Patients in Whom Calcification Was Detected during Follow-up.

Coronary-artery calcification was assessed by electron-beam computed tomography. The mean interval between the scans was 20 months (range, 12 to 41). All patients underwent regular dialysis, and all were 20 to 30 years of age at the time of the first scan.

Goodman WG, N Engl J Med, 2000, p1478

Relationship between

- Cardiovascular mortality and mineral metabolism markers (P, Ca, and PTH)



Block GA, et al. *Am J Kidney Dis.* 1998;31:607-617.

Relationship between

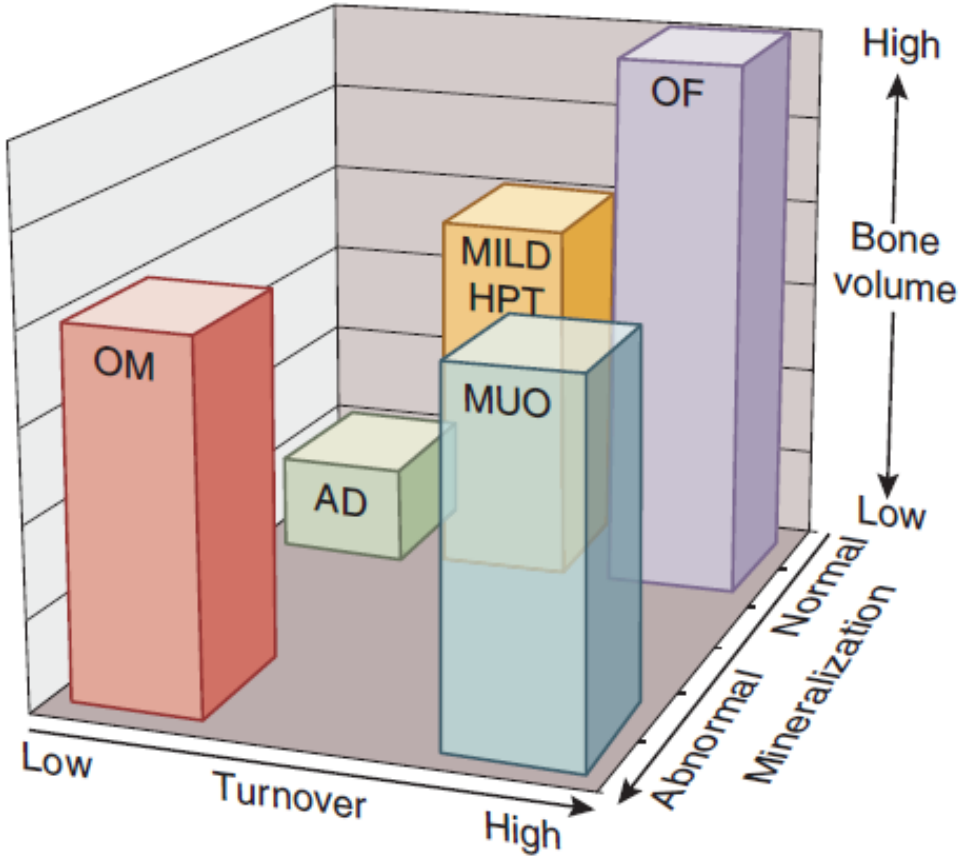
- Several mineral metabolism markers and VC

Relationship between

- VC and cardiovascular mortality

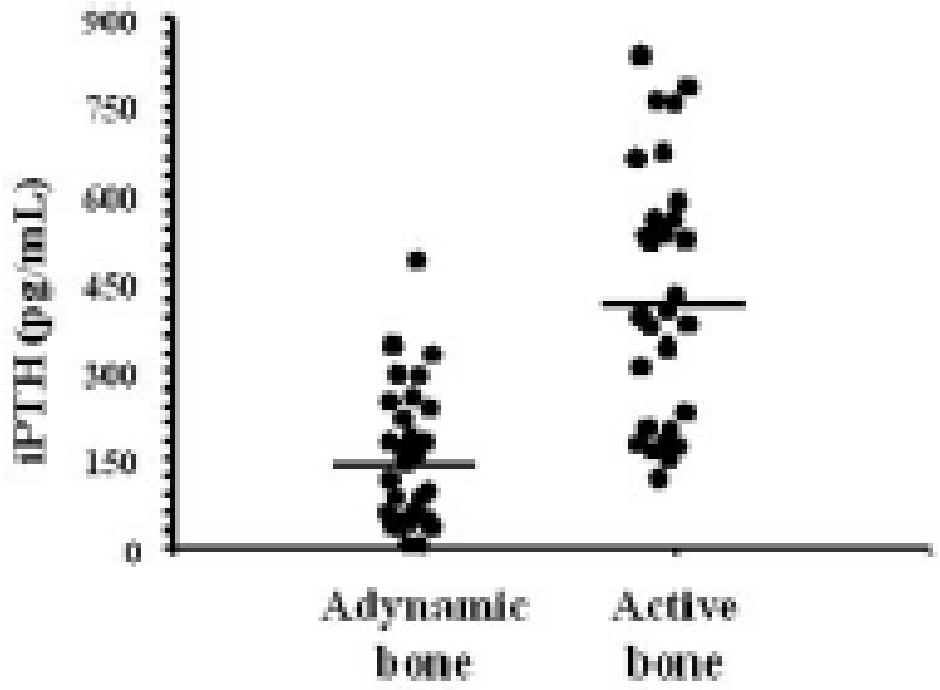
Is it causal?

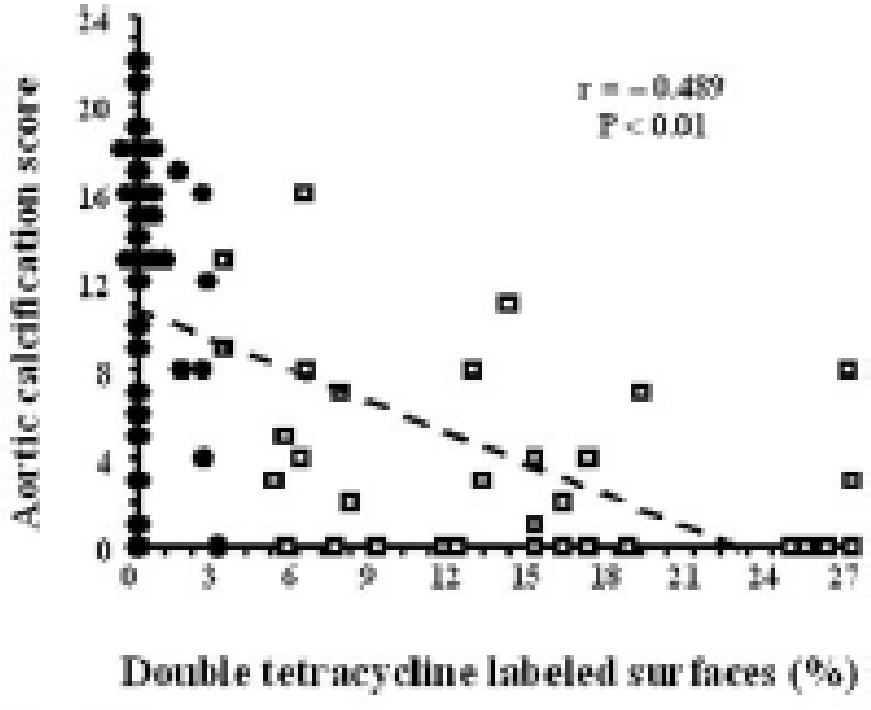
Bone health in CKD patient
(turnover versus volume versus mineralization)



Moorthi N, Kidney Int, 2013, p886

Bone turnover is associated with VC





London GM, J Am Soc Nephrol, 2008, p1827

Osteoporosis is associated with VC

CLINICAL RESEARCH www.jasn.org

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 L. Cantor,‡ and Daniel L. Davenport§

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

Calcif Tissue Int (2013) 93:39–47
DOI 10.1007/s00223-013-9722-x

ORIGINAL RESEARCH

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

Maria Fusaro · Giovanni Tripepi · Marianna Noale · Nicola Vajente · Mario Plebani · Martina Zaninotto · Giuseppe Guglielmi · Diego Miotto · Luca Dalle Carbonare · Angela D'Angelo · Daniele Ciurlino · Riccarda Puggia · Davide Miozzo · Sandro Giannini · Maurizio Gallieni

Bone 64 (2014) 33–38

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Original Full Length Article

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

Daniel Cejka [a,b], Michael Weber [b,c], Danielle Diarra [a,b], Thomas Reiter [a,b], Franz Kainberger [b,d], Martin Haas [a,b,*]

[a] Division of Nephrology and Dialysis, Department of Medicine III, Medical University Vienna, Vienna, Austria
[b] Medical University Vienna, Vienna, Austria
[c] Department of Radiology, Medical University Vienna, Vienna, Austria
[d] Division of Neuroradiology and Musculoskeletal Radiology, Department of Radiology, Medical University Vienna, Vienna, Austria

Bone 92 (2016) 50–57

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

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,b], Abdul Rashid Qureshi [a], Jonaz Ripsweden [c,d], Lars Wennberg [e], Olof Heimburger [a], Bengt Lindholm [a], Peter Barany [a], Mathias Haarhaus [a], Torkel B. Brismar [c,d,1], Peter Stenvinkel [a,*,1]

[a] Division of Renal Medicine and Baxter Novum, Department of Clinical Sciences, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden
[b] Kidney Disease Center, 1st Affiliated Hospital College of Medicine, Zhejiang University, Hangzhou, China
[c] Division of Medical Imaging and Technology, Department of Clinical Sciences, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden
[d] Department of Radiology, Karolinska University Hospital, Huddinge, Sweden
[e] Division of Transplantation Surgery, Department of Clinical Sciences, Intervention and Technology, Karolinska Institutet, Stockholm, Sweden

This concept of Bone-Vascular axis is also suggested by basic research

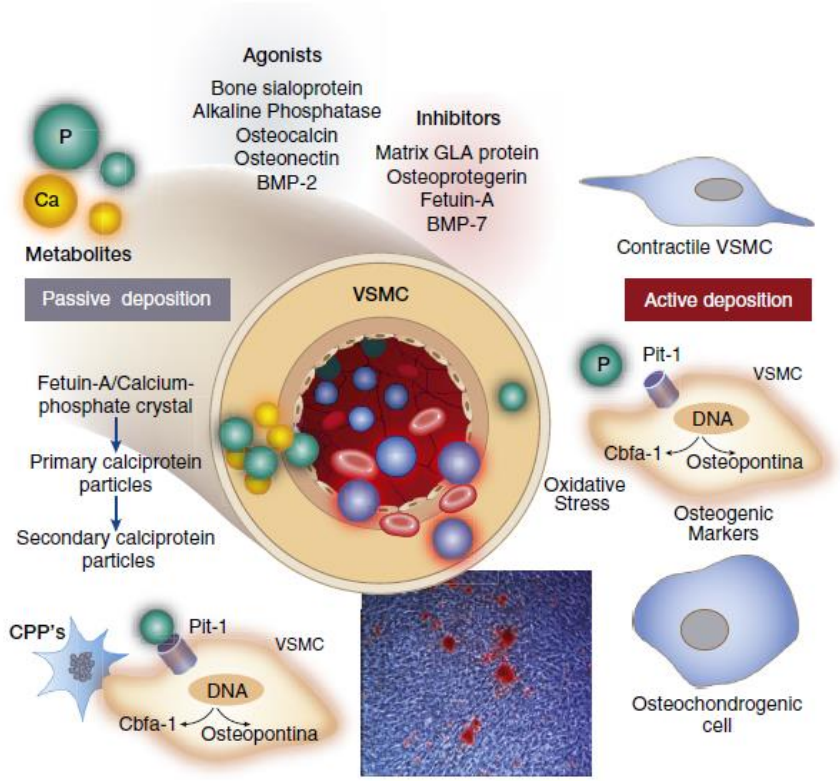


Figure 1 | Summary of several key aspects of medial calcification. In the center a schematic diagram of a medium-sized artery is shown, transporting red blood cells (biconcave red shapes) and leucocytes (blue cells). The latter are involved in both intimal and medial calcification. A key role in initiating and propagation is for calcium ions in yellow and phosphate ions in green. Several factors are involved in promoting and inhibiting vascular calcification, and these are listed on top of the vessel diagram. Below the diagram a typical histological image is shown of an arterial wall affected by medial layer calcification, shown as dark pink areas, surrounded by non-affected segment (light-pink). On the left the primarily passive formation of calciprotein particle is depicted. Initially small calciumphosphate can be scavenged by fetuin-A, which eventually can be overwhelmed leading to primary calciprotein particles that can evolve secondary particle. On the lower left and midright the entrance of phosphate into vascular smooth muscle cells through Pit-1 is shown, which can drive transdifferentiation of these cells by increased expression of core binding factor A1 (core-binding factor alpha-1 or RunX2) and osteopontin. BMP, bone morphogenetic protein; Cbfa, core binding factor A1; CPP, calciprotein particle; VSMC, vascular smooth muscle cell.

Vervloet M, Kidney Int, 2017, p808

Clinica Chimica Acta 438 (2015) 401–414

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Invited critical review

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 SFBC/SN joined working group on vascular calcifications
J. Arnaud [1], Ph. Zaoui [1], M.C. Carlier [2], M. Laville [2], D. Fouque [2], E. Cavalier [3], P. Delanaye [3], J.P. Cristol [4], A.S. Bargnoux [4], S. Kamel [5,6], Z. Massy [6,7], D. Prié [8], P. Urena-Torres [8], J.C. Souberbielle [8], A. Boutten [9,10], A. Guérin [11], T. Hannedouche [12], G. Jean [13], M.H. Lafage-Proust [14], G. London [15], L. Mercadal [16], L. Pieroni [17]

[1] CHU Grenoble, France
[2] CHU Lyon, France
[3] CHU Sart-Tilman, Liège, Belgium
[4] CHU Montpellier, France
[5] INSERM U1088, France
[6] CHU Amiens, France
[7] CHU Ambroise Paré, France
[8] Inserm/Université U 845, Paris, France
[9] APHP, France
[10] GH Paris Nord Val de Seine, France
[11] CH de Manhes, France
[12] CHU Strasbourg, Ph Brunet CHU Marseille, France
[13] Nephrocare Tassin-Charcot, Lyon, France
[14] CHU St Etienne, France
[15] INSERM U970, Paris, France
[16] Pitié Salpétriere, Paris, France
[17] CH Avignon, France

[a] Department of Clinical Chemistry, University of Liège, CHU Sart-Tilman, Liège, Belgium
[b] Department of Nephrology, Dialysis and Hypertension, University of Liège, CHU Sart-Tilman, Liège, Belgium
[c] Laboratoire de Biochimie, CHU Amiens, Amiens, France
[d] INSERM U1088, Université de Picardie Jules-Verne, Amiens, France
[e] Laboratoire de Biochimie, CHRU de Montpellier, Hôpital Lapeyronie, Montpellier, France

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

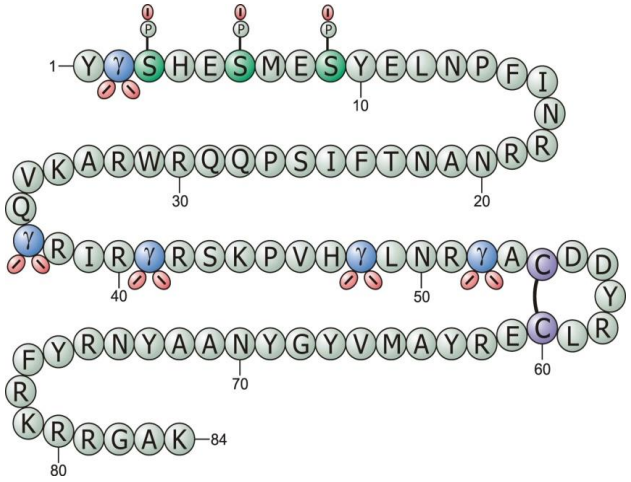
Still not fit for clinical purpose !

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 osteablastic 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

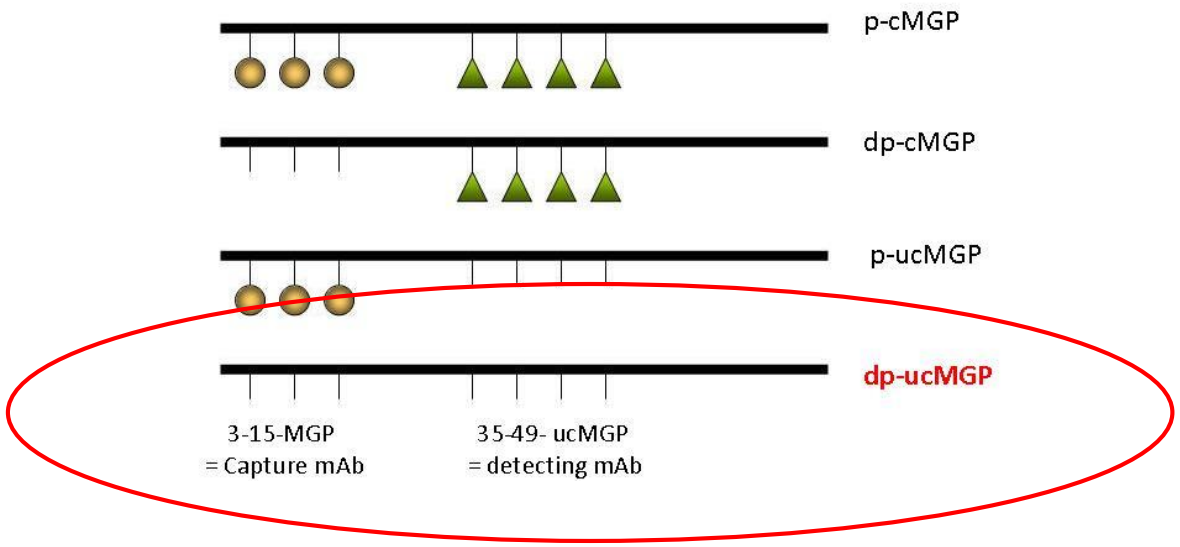
- **Carboxylation of glutamate residues**
  - Binding of Ca-ions and crystals
- **Phosphorylation of serine residues**
  - Function? Regulation of secretion of MGP into the extracellular matrix.
  - Binding of MGP to sites of calcification

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,†‡ Fellype 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*

Clin J Am Soc Nephrol 5: 568–575, 2010.

- 107 CKD (40 HD)
- CAC (n=101)

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.

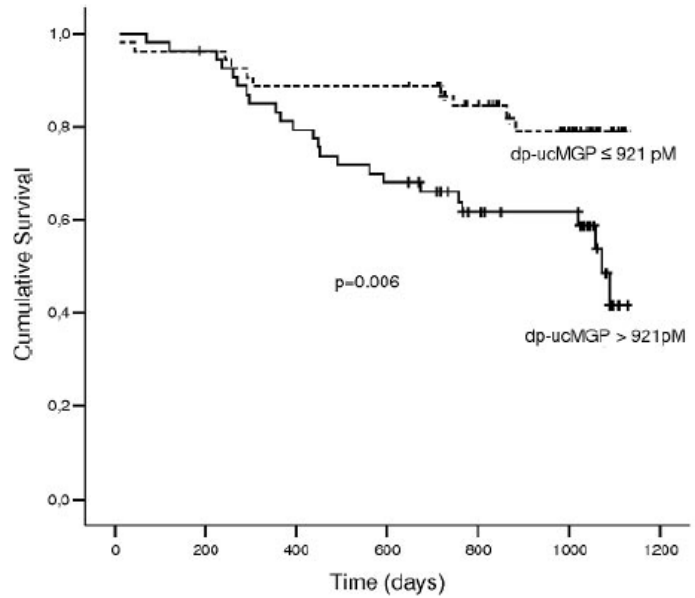


Figure 4. Kaplan-Meier estimates of overall mortality as a function of the median plasma dp-ucMGP level.

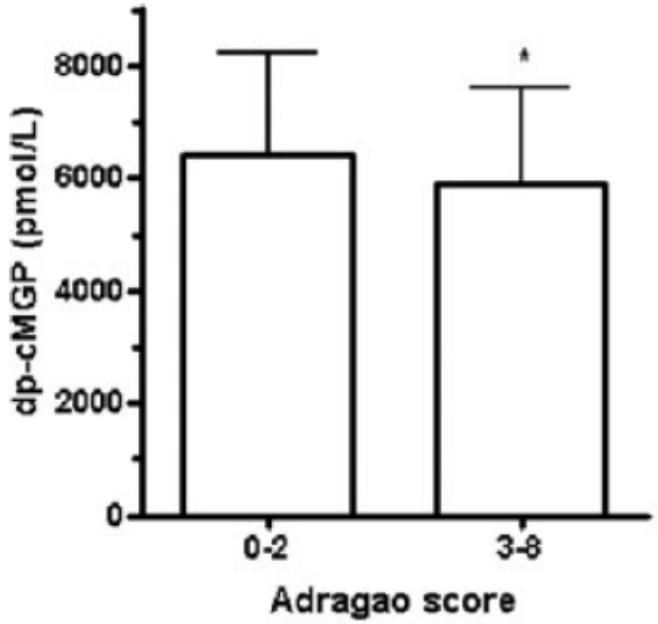
CLINICAL RESEARCH www.jasn.org

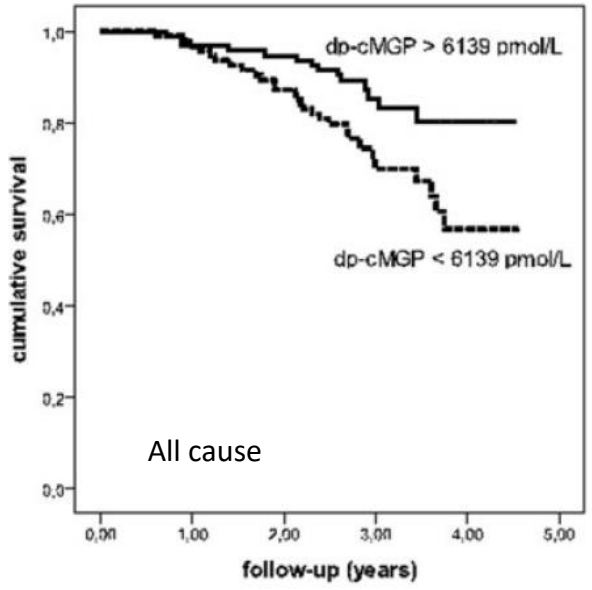
Circulating Nonphosphorylated Carboxylated Matrix Gla Protein Predicts Survival in ESRD

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‡

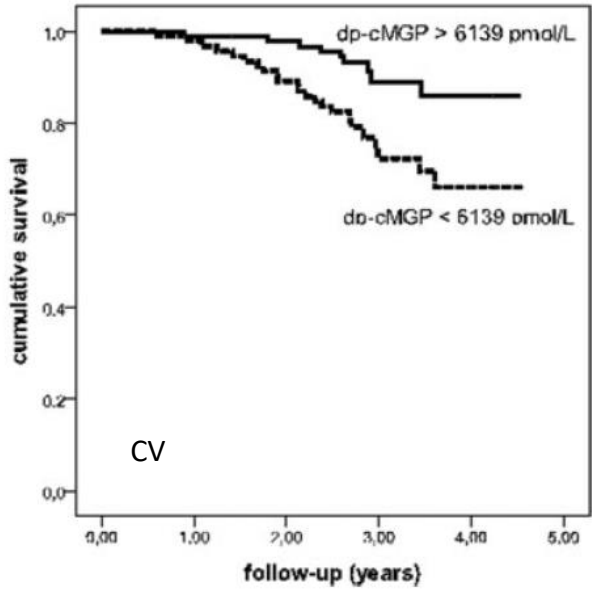
J Am Soc Nephrol 22: 387–395, 2011

- 188 HD
- Adragao score for VC + FAV


dp-cMGP (pmol/L)
8000
6000
4000
2000
0
0-2
3-8
Adragao score


dp-cMGP > 6139 pmol/L
dp-cMGP < 6139 pmol/L
cumulative survival
follow-up (years)

All cause


dp-cMGP > 6139 pmol/L
dp-cMGP < 6139 pmol/L
cumulative survival
follow-up (years)

CV

Delanaye *et al. BMC Nephrology* 2014, **15**:145
http://www.biomedcentral.com/1471-2369/15/145

BMC Nephrology

RESEARCH ARTICLE Open Access

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 Delanaye[1*], Jean-Marie Krzesinski[1], Xavier Warling[2], Martial Moonen[2], Nicole Smelten[3], Laurent Médart[4], Hans Pottel[5] and Etienne Cavalier[6]

- 160 HD
- Kauppila score for VC

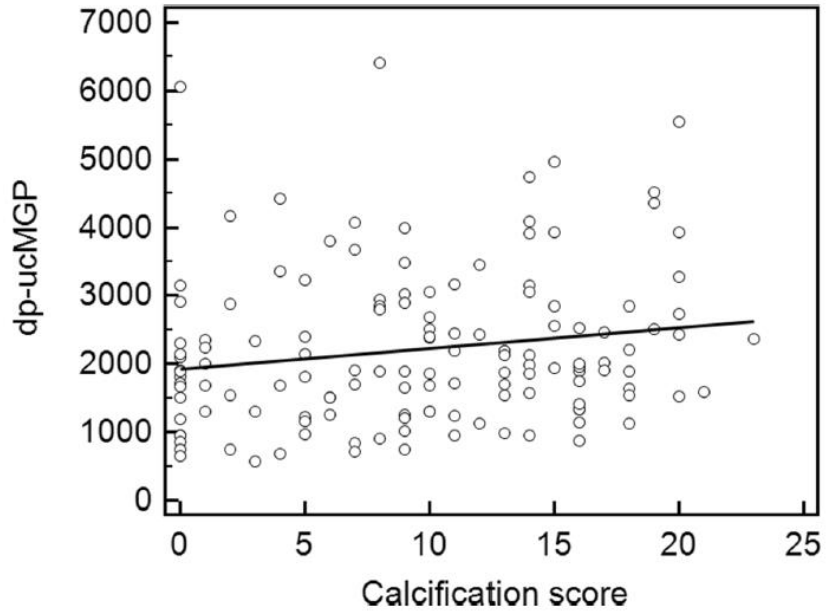


Figure 2 Univariate regression between the calcification score and dp-ucMGP (in pmol/L) in patients not treated with VKA (n = 137) ($r^2 = 0.02850$, p = 0.049).

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

	r	p
Body mass index	0.17	0.0032
Albumin	−0.24	0.0368
FGF-23	0.28	0.002
CRP	0.33	0.0012
Calcification score	0.19	0.0206

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.

Nephrology Dialysis Transplantation

NDT Perspectives

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 Liabeuf[1,2], Hirokazu Okazaki[1], Lucie Desjardins[1,2], Danilo Fliser[3], David Goldsmith[4], Adrian Covic[5], Andrzej Wiecek[6], Alberto Ortiz[7], Alberto Martinez-Castelao[8], Bengt Lindholm[9], Gultekin Suleymanlar[10], Francesca Mallamaci[11], Carmine Zoccali[11], Gerard London[12] and Ziad A. Massy[1,13]

- N=131 CKD (45 HD)
- Aorta CT

Table 1. Correlation between studied biomarkers and aortic calcification/coronary calcification

	Aortic calcification (Ln)		Coronary calcification (Ln)	
	r (P)	$r^2 \times 100$	*r* (P)	$r^2 \times 100$
Phosphate (Ln)	0.009 (0.921)	0.008	0.091 (0.427)	0.8
FGF23 (Ln)	**0.20 (0.019)**	**4.2**	**0.20 (0.031)**	**4**
OPN	0.06597 (0.545)	0.4	−0.036 (0.80)	0.1
OPG (Ln)	**0.21 (0.03)**	**4.4**	**0.278 (0.03)**	7.7
dp-ucMGP	**0.401 (<0.0001)**	**16.1**	**0.320 (0.02)**	**10.2**
Fetuin A	0.001 (0.995)	0.0001	0.026 (0.823)	0.07

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.

Limitatio

- Observation
- Not the sam
- Added value
  calcification

tect the

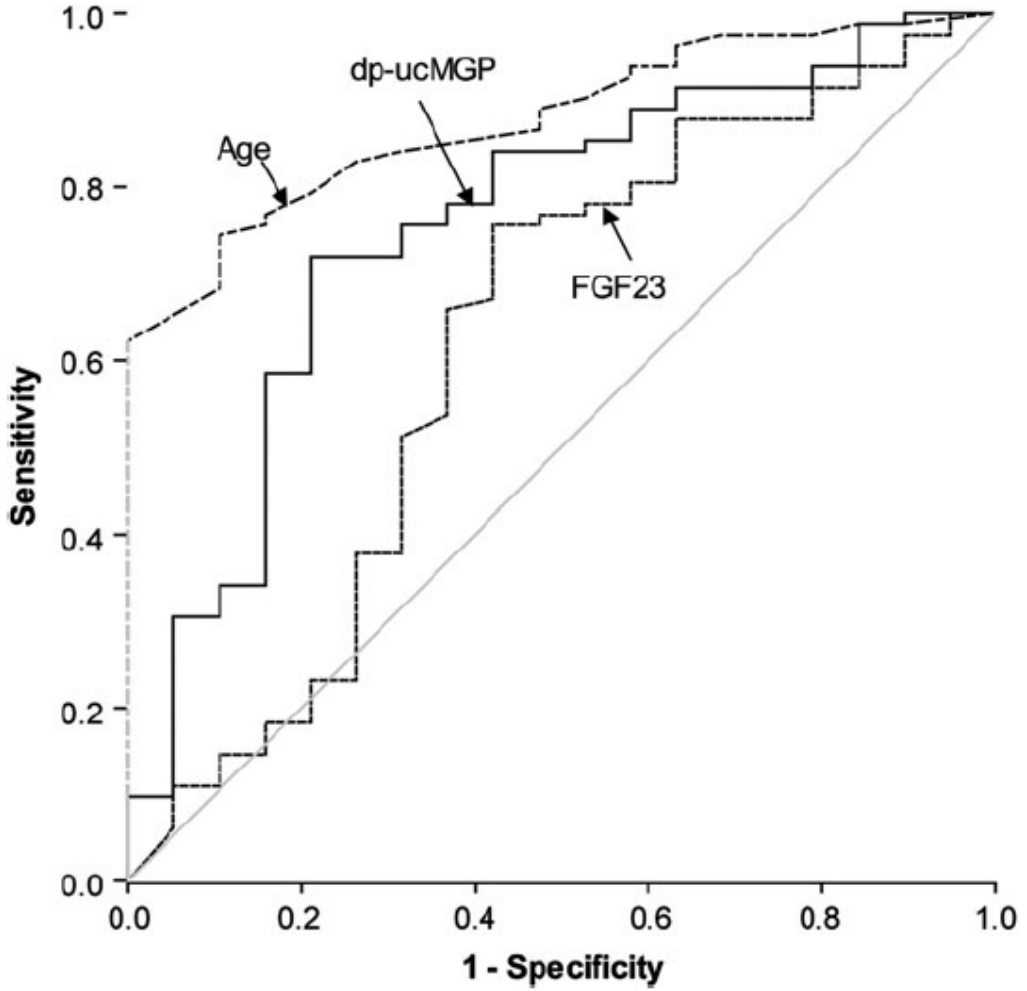
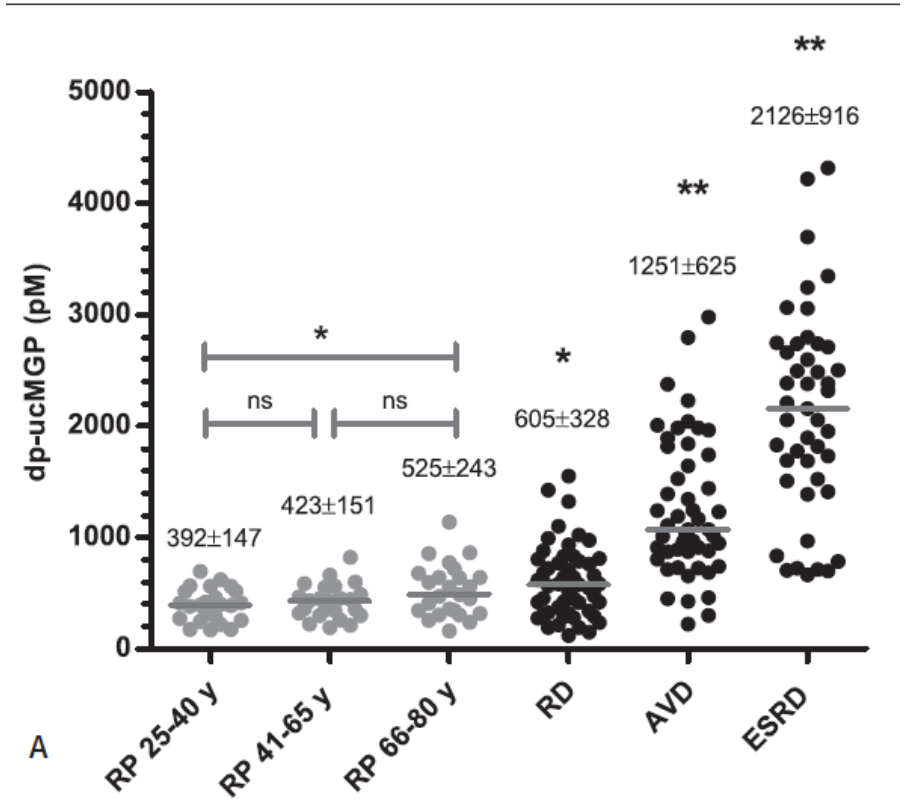


FIGURE 1: ROC curves for risk factors for aortic calcification. The areas under the ROC curves are 0.87 [95% confidence interval (CI), 0.81–0.95, P < 0.0001), 0.76 (95% CI, 0.64–0.88, P < 0.0001) and 0.64 (95% CI, 0.52–0.75, P = 0.02) for age, uncarboxylated, dephosphorylated Matrix Gla protein (dp-ucMGP) 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

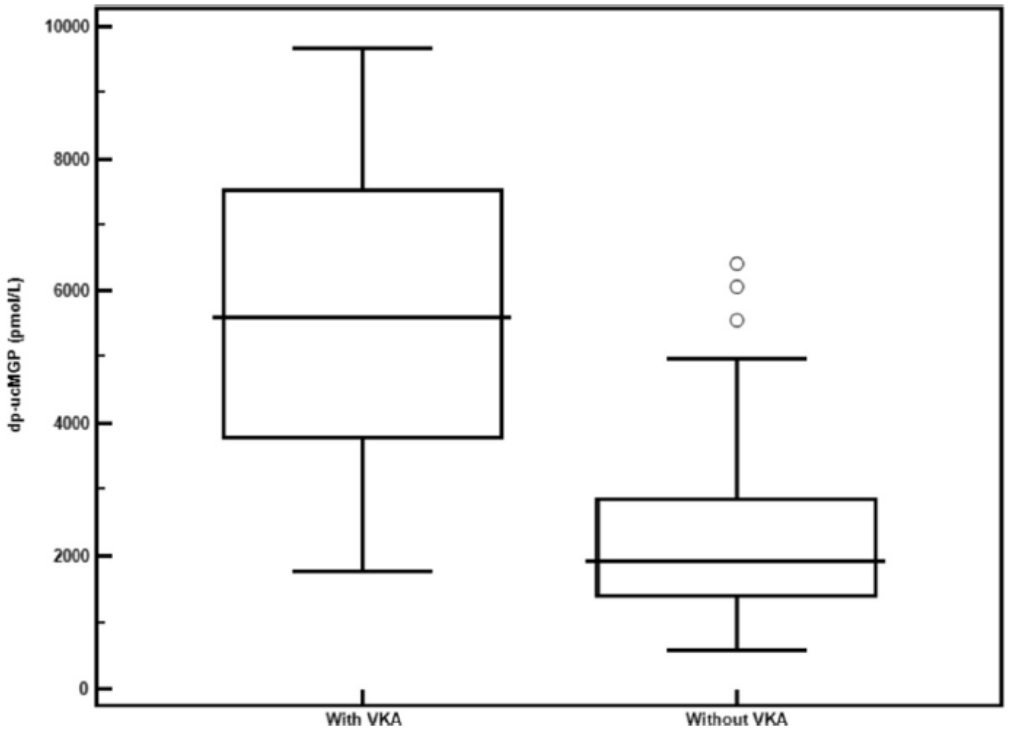


Figure 1 Median concentration of dp-ucMGP in patients treated with antivitamin K (VKA) (n = 23) and in patients not treated with VKA (n = 137) (5604 [3758; 7836] vs. 1939 [1419; 2841] pmol/L, p <0.0001).

Delanaye P, BMC Nephrol, 2014, p145

MGP and anti-vitamin K in dialysis patients

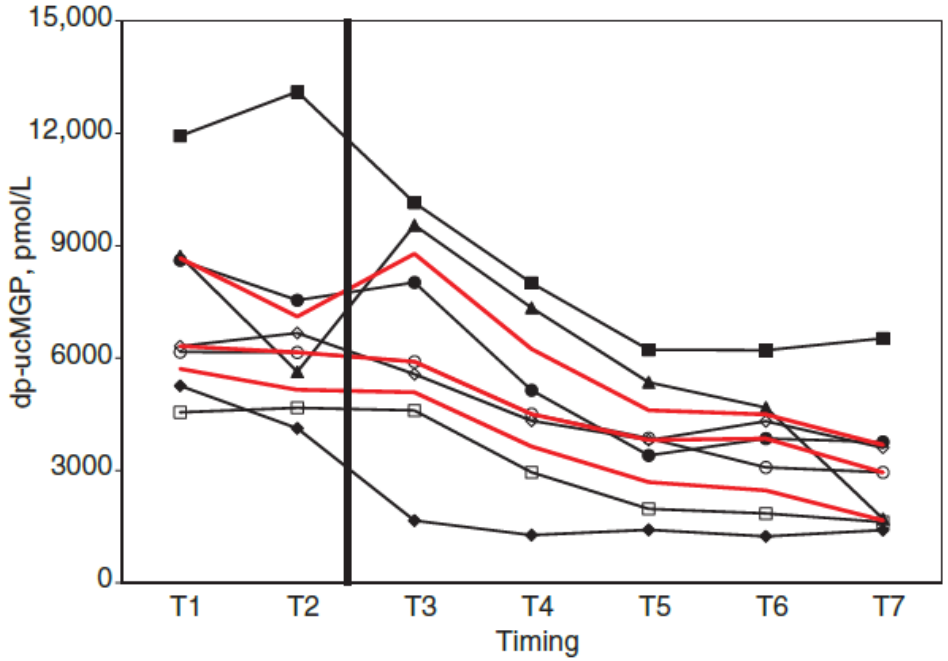
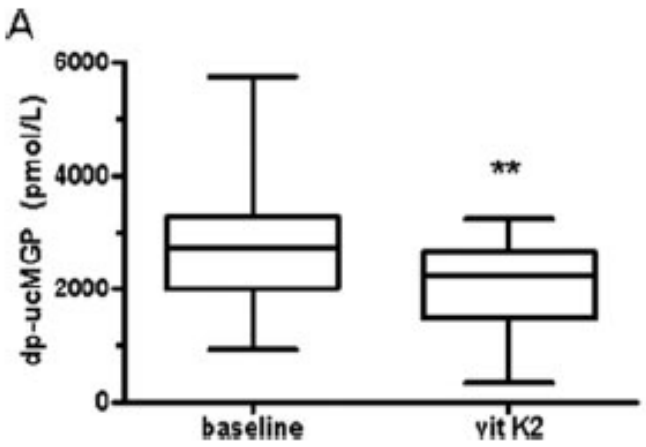


Figure 1: Evolution of dp-ucMGP concentrations after stopping VKA in 7 HD patients.
In red, median and P25-P75 values. The thick dark line corresponds to the time when AVK therapy is stopped.

Delanaye P, Clin Chem Lab Med, 2015, pe191

Vitamin K and inactive MGP

- 

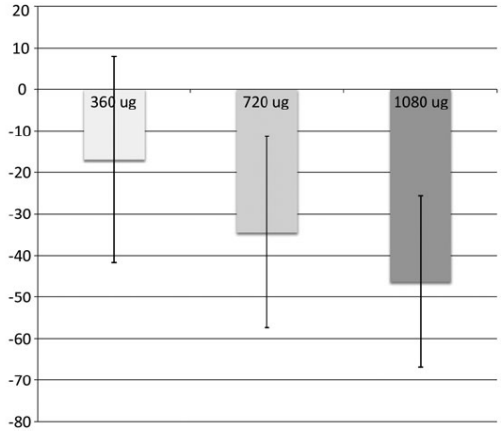


FIGURE 2: Relative decrease (%) in circulating dp-uc-MGP levels after 8 weeks of supplementation with different doses of MK-7. Data represent mean ± standard deviation. The decrease was statistically significant in every treatment group ($P < 0.001$).

Schlieper G, J Am Soc Nephrol, 2011, 22, 387-395
Westenfeld R, Am J kidney Dis, 2012, 59, 186-195
Caluwe R, NDT, 2014, p1385

Nephrol Dial Transplant (2014) 29: 1633–1638
doi: 10.1093/ndt/gft459
Advance Access publication 26 November 2013

ndt
Nephrology Dialysis Transplantation

NDT Perspectives

Vitamin K1 to slow vascular calcification in haemodialysis patients (VitaVasK trial): a rationale and study protocol

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Holden *et al. Canadian Journal of Kidney Health and Disease* (2015) 2:17
DOI 10.1186/s40697-015-0053-x

Canadian Society of Nephrology/
Société canadienne de néphrologie
CSN/SCN

CANADIAN JOURNAL OF
KIDNEY HEALTH AND DISEASE
Journal canadien de la santé et de la maladie rénale

STUDY PROTOCOL **Open Access**

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

N=448

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Sclerostin and VC

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

Pelletier S, *Clin J Am Soc Nephrol* 8: 819–823, 2013.

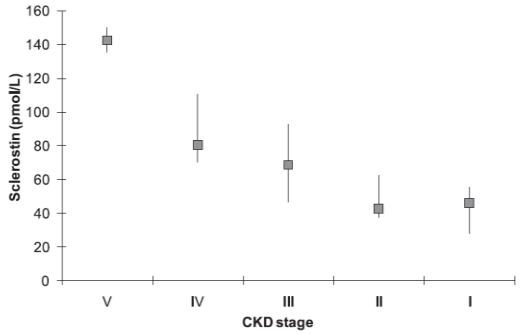


Figure 1. | Serum sclerostin as a function of CKD stage based on GFR measured by inulin clearance. *n*=90; results are expressed as median (interquartile range).

Sclerostin and VC

- Sclerostin could be involved in VC
- Anti-sclerostin antibody (romosozumab)

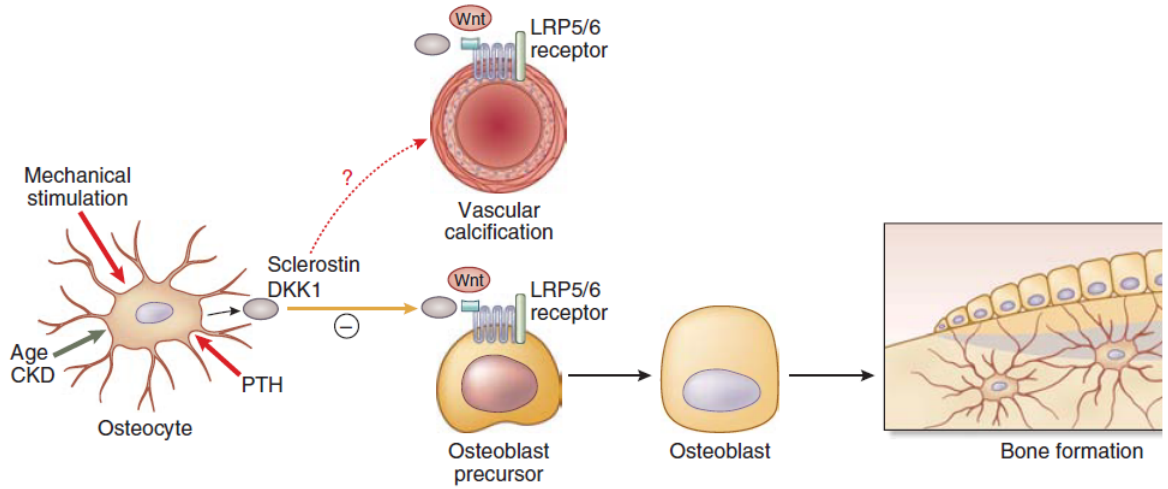


Figure 1 | Sclerostin: regulation, bone effect, and (hypothetical) link with vascular calcifications. The absence of mechanical stimulation induces sclerostin secretion by osteocytes. Sclerostin inhibits the Wnt receptor (LRP5/6), inducing inhibition of differentiation and proliferation of osteoblast precursors into mature osteoblasts. Age and CKD increase sclerostin secretion. Parathyroid hormone (PTH) decreases sclerostin production. Green arrow: Promotion of sclerostin production by osteocytes. Red solid line: Inhibition of sclerostin secretion by osteocytes. Yellow line: Inhibition of the Wnt pathway by sclerostin in bones through the LRP5/6 receptor. Black arrow: Regular way of bone formation. The link between sclerostin and vascular calcifications remains hypothetical (red dotted line). Red solid line: Inhibition of sclerostin secretion by osteocytes. Grey solid line: Stimulation of sclerostin secretion by osteocytes. Yellow line: Inhibition of the Wnt pathway by sclerostin in bones through the LRP5/6 receptor. Black arrow: Regular way of bone formation. The link between sclerostin and vascular calcifications remains hypothetical (red dotted line).

Delanaye P, Kidney Int, 2015, p1221

But the clinical results are highly discrepant…

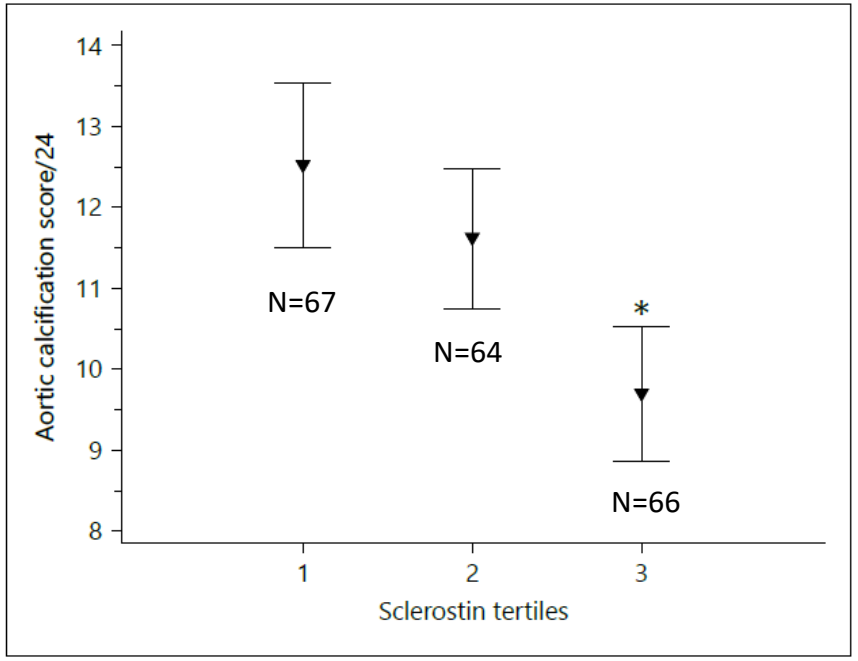
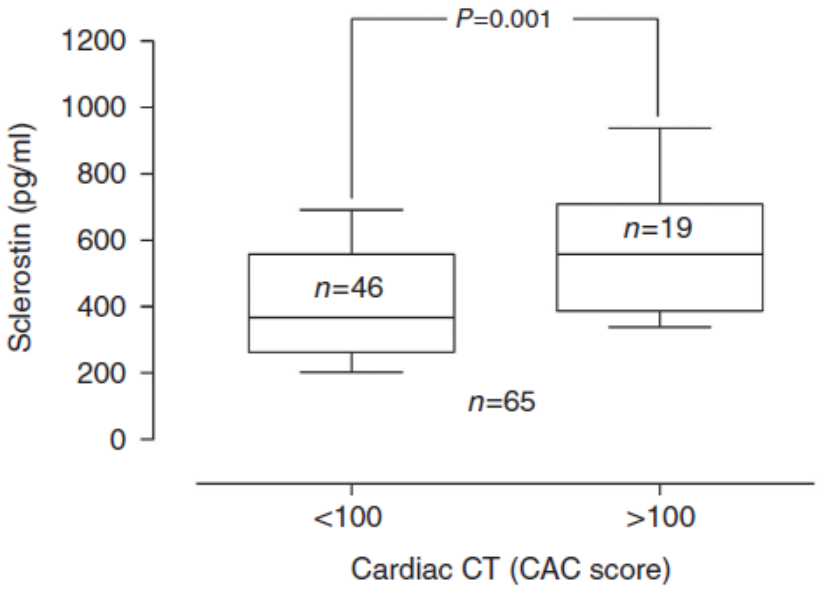


Fig. 2. Aortic calcification score according to the serum sclerostin levels tertiles. * $p < 0.05$.

Jean G, Nephron, 2016, p181



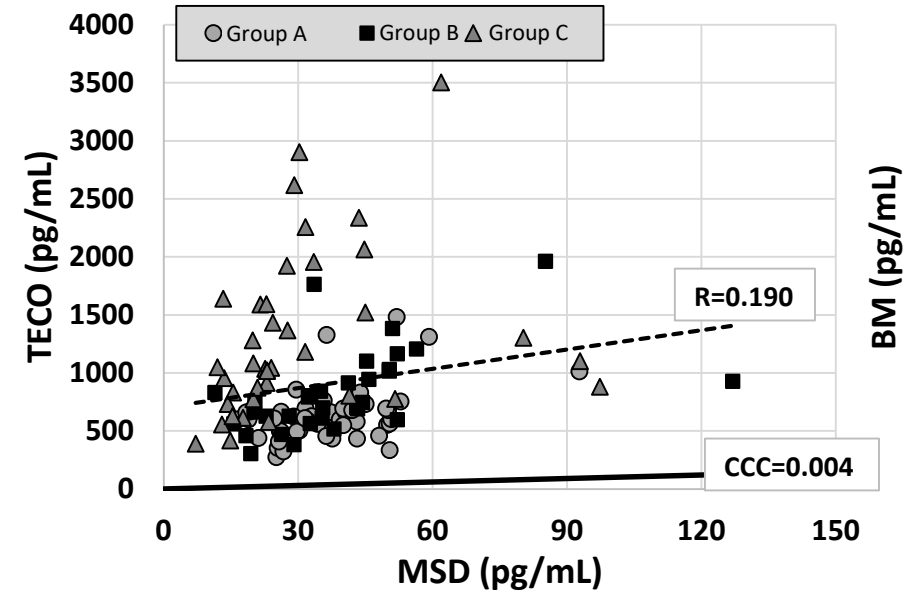
Qureshi AR, Kidney Int, 2015, p1356

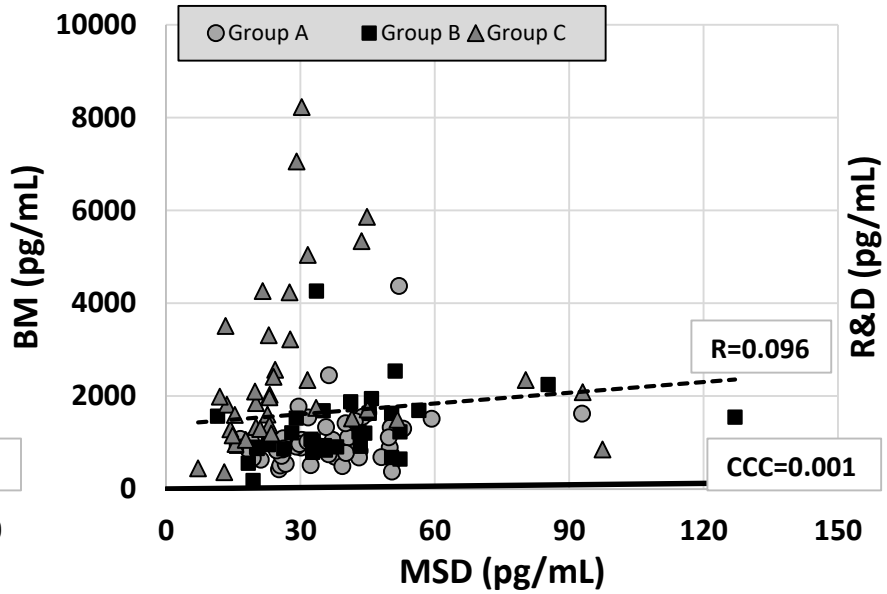
Because we don’t know what we measure…

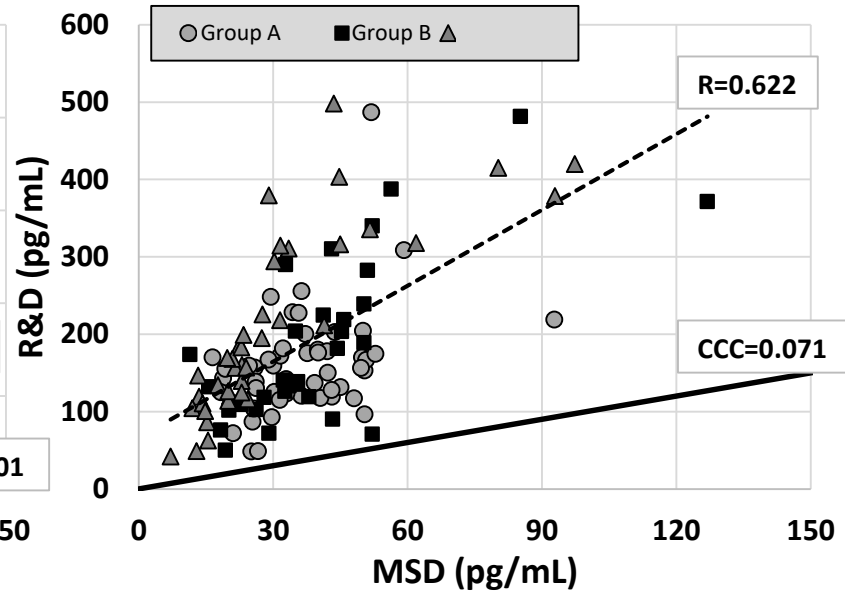
	BM	TE	RD	MSD
All n=121	1209 [889]	698 [452]	157 [99]	32 [21]
All non-dialysis n=82	984 [648]	629 [237]	154 [84]	36 [19]
Iohexol GFR > 60 mL/min (group A) n=50	904 [613]	609 [181]	156 [55]	36 [17]
Iohexol GFR < 60 mL/min (group B) n=32	1137 [743]	745 [377]	140 [121]	35 [21]
Dialysis n=39 (group C)	1976 [1972]	1050 [788]	169 [195]	23 [16]

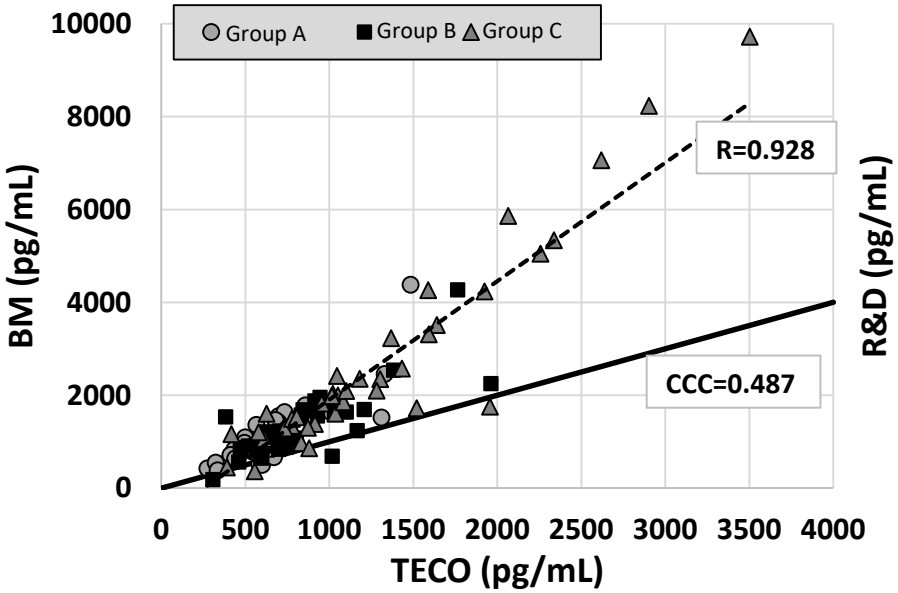
Median [IQR] concentrations of sclerostin. CKD: chronic kidney disease. All results expressed in pg/mL

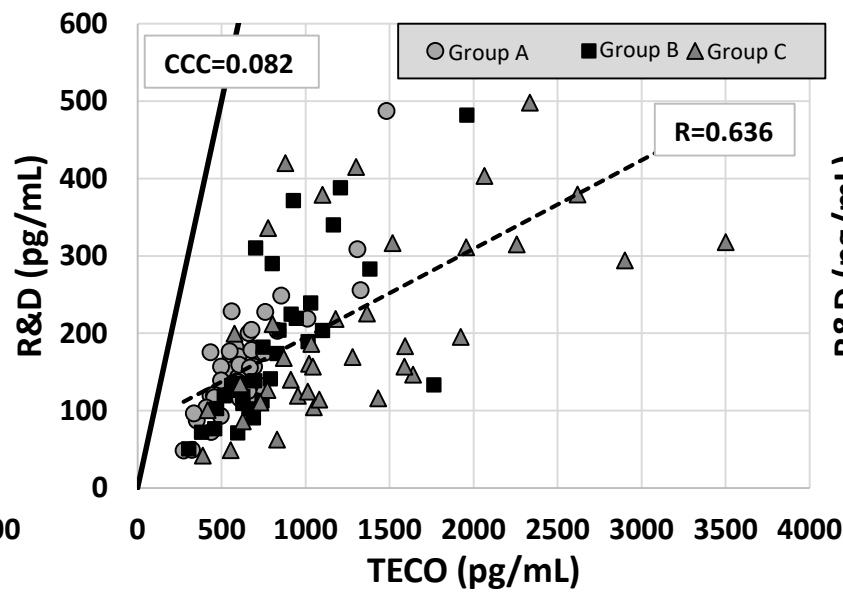
Delanaye P, 2017, submitted

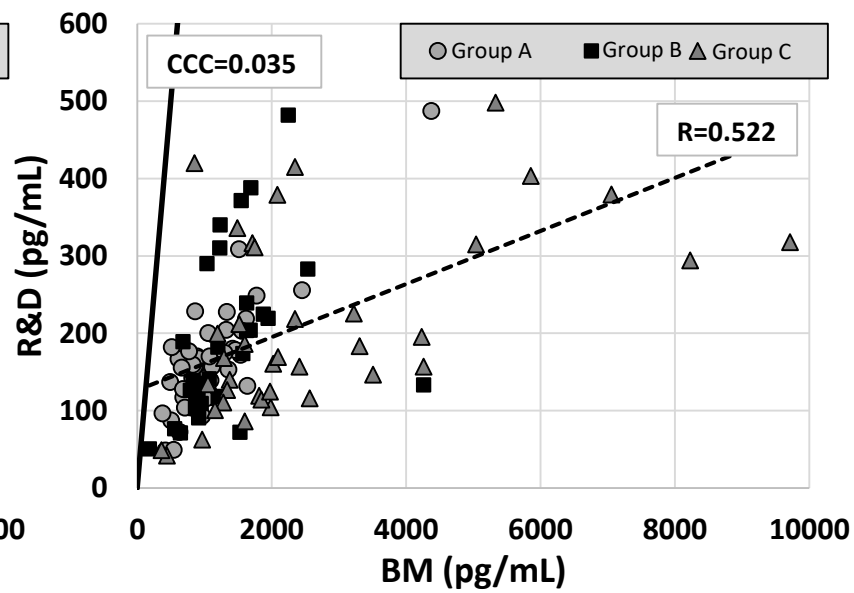






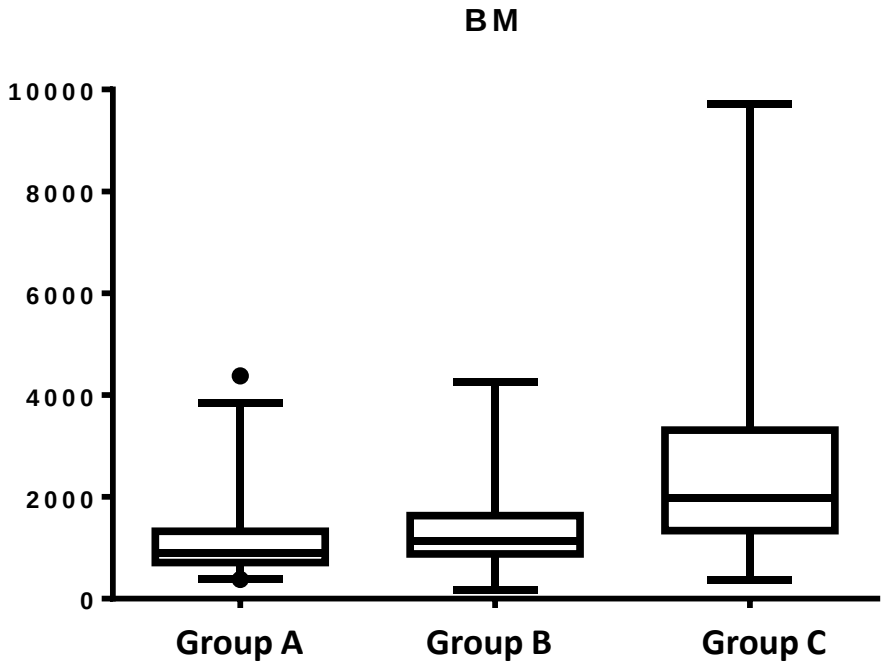


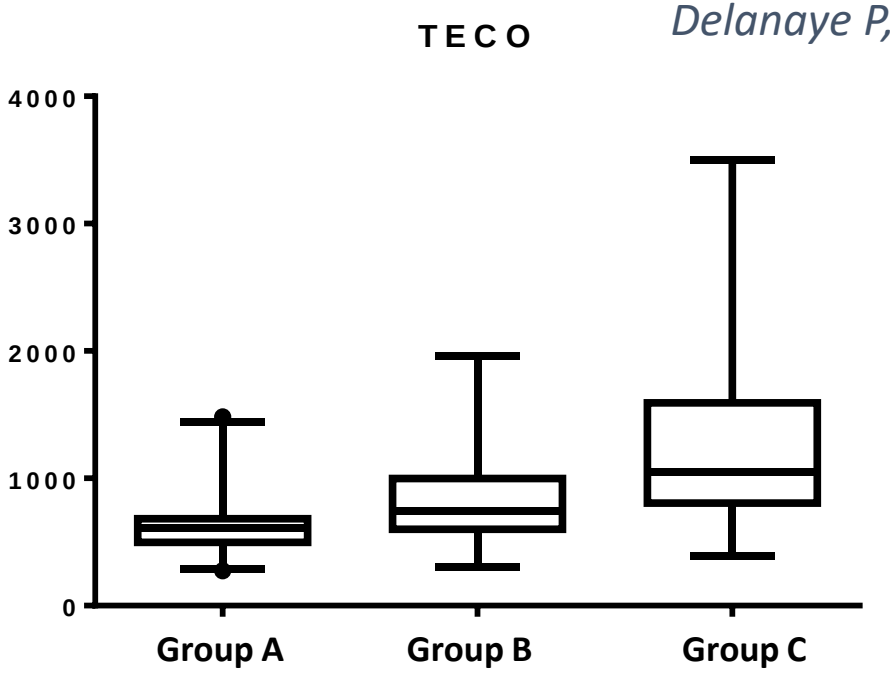


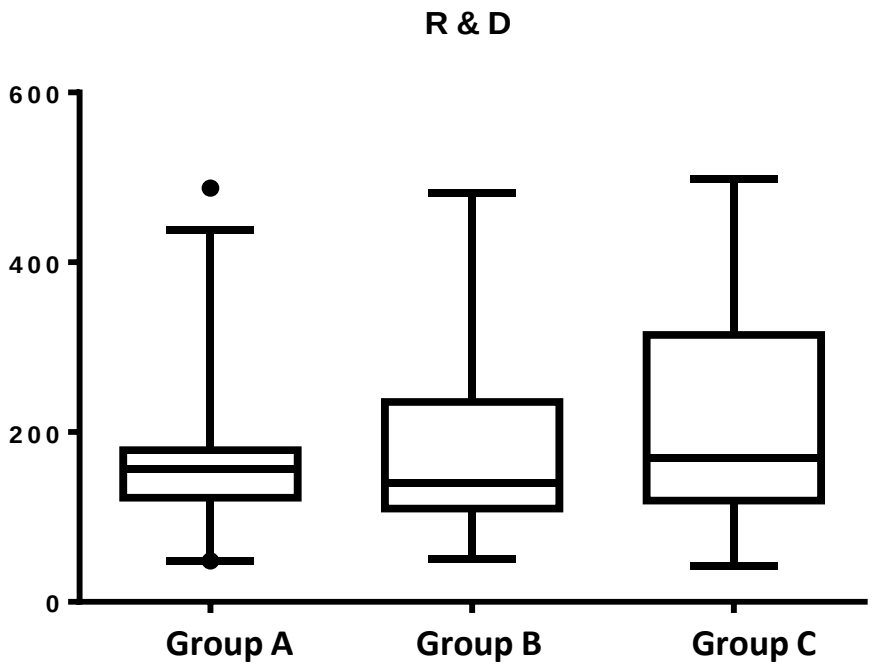


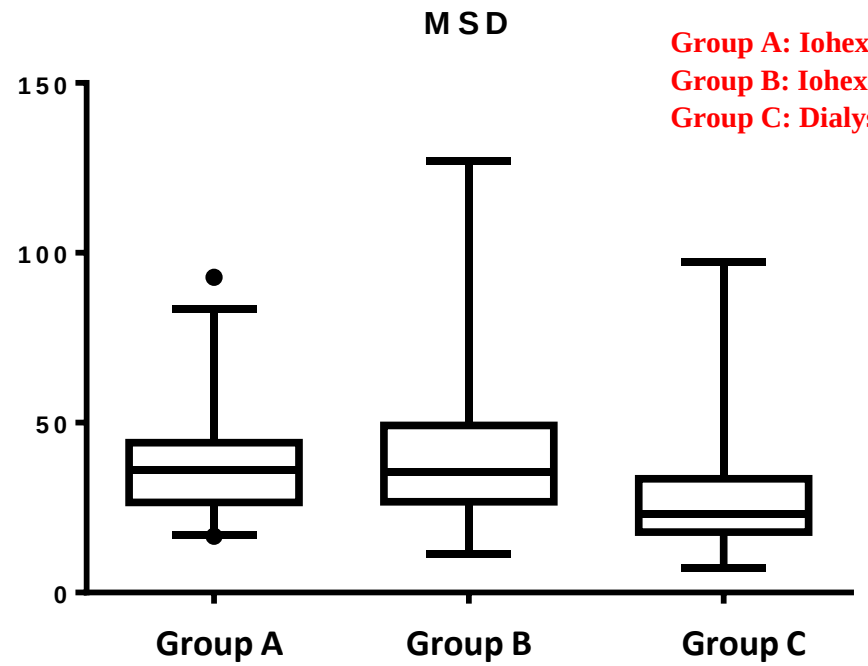
Delanaye P, 2017, submitted

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