Causes and Consequences of Vascular Pathologies in CKD

Catherine M. Shanahan
Professor of Cellular Signalling

King’s College London
Cardiovascular Division
Cardiovascular mortality in CKD patients

Adolescents and young adult with CKD:
- structural and functional abnormalities in the large vessels
- present even in the second decade of life
- linked to disorders in mineral metabolism

Adapted from Foley et al. Am J Kid Dis 1998

Goodman, NEJM, 2000; Litwin, JASN, 2005;
Mitsneses, JASN 2005; Goldsmith, NDT, 2006
Is Vascular Calcification A Major Cause Of Cardiovascular Mortality in Renal Failure Patients?
early medial calcification along elastic lamellae

late medial and intimal calcification

medial bone formation

medial bone formation

lens confined to intima

MEDIAL CALCIFICATION
organised along elastic lamellae
bone formation common
VSMCs only
little lipid
Ageing
Uremia
Diabetes

INTIMA

INTIMAL CALCIFICATION
punctate, disorganised
bone formation uncommon
macrophages + VSMCs
lipid always present
Atherosclerosis
Vascular calcification occurs at two sites:

**MEDIAL**
- Diabetes
- Renal Disease
- Ageing
- Stiffness

**INTIMAL**
- Atherosclerosis
- Plaque Rupture

- Major cause of cardiovascular mortality in CKD
- Increased risk of myocardial infarction and all cause mortality
- Surgical complications and amputations
- Valve calcification
Vascular Calcification is a Regulated Process similar to bone calcification.

So Reflects Disease Processes?
Renal osteodystrophy

Disease
- ↑Calcium
- ↑Phosphate
- ↑Ca x PO4
- Oxidized lipids
- Inflammation
- Hypertension
- PTH
- Advanced glycation end-products

Vascular insults

Treatment
- Vitamin D
- Calcium-based phosphate binders
- Warfarin

VSMC Damage

- Time on dialysis
- Pre-existing vascular calcification (once present rapidly progresses)
Vessel Rings from Children *in vivo* and *ex vivo*

Studied vessels from children on dialysis who develop rapid medial vascular calcification

- pristine vessels - no atherosclerosis

- Intact - vascular matrix structure maintained

Measured: CALCIUM LOAD
VESSEL HISTOLOGY

Correlated with: VASCULAR MEASURES
BIOCHEMICAL DATA

*KDIGO*
Children on Dialysis develop rapid medial calcification

High Circulating Phosphate Levels, Transient Hypercalcemia?
Calcification correlates with VSMC loss via apoptosis

![Graph showing the relationship between calcification and number of VSMCs/unit area across different stages of dialysis.

- Normal: 125 VSMCs/unit area, p < 0.001
- Pre-dialysis: 75 VSMCs/unit area
- Dialysis: 50 VSMCs/unit area

- α-SM actin and TUNEL staining images for Normal and Dialysis conditions.

KDIGO
Loss of Calcification Inhibitors
Non-functional Glu-MGP predominates in Dialysis vessels
Dialysis vessels show increased osteogenic differentiation

(Shroff et al 2008, Circulation)
Ca load is associated with increased vesicle deposition by VSMCs

(Shroff et al 2008, Circulation)
Mechanisms of Vascular Smooth Muscle Cell Calcification

CKD  
Diabetes  
Ageing

ROS?

Elevated P  
Elevated Ca

MGP  
Fetuin A  
Pyrophosphate

Contractile VSMCs  
(regulate tone)

Synthetic VSMCs  
(adaptation/repair)

Runx2

Loss of endogenous inhibitors

Increased osteogenesis  
(Alkaline phosphatase)

Apoptosis  
Necrosis

Nanocrystals deposited

Endocytosis of nanocrystals

Osteochondrocytic VSMCs  
(mal-adaptation/calcification)

Shanahan, C. M. (2013)
Why is Calcification Important Clinically?
The Clinical Consequence
Medial Calcification is Arterial Stiffening
Impact on all-cause and CV mortality of arterial Calcification in CKD

Diabetes is Associated with a high Prevalence of Vascular Calcification in Peripheral Arteries

Peripheral Artery Calcification in Diabetes

Associated with increased CV mortality, amputation and ulcers, surgical complications
Calciphylaxis in CKD

Medial calcification of small arterioles

Von Kossa

α-SM actin

Progressive Gangrene
High Mortality
Is there medial calcification in the coronary arteries of patients with ESRD?

Detailed analysis of calcified areas using the Kossa stain showed that calcification of the coronary lesions was predominantly located in the intima, i.e. the arterial plaque, whereas we could hardly find any media calcification in the coronary arteries (Fig. 1).

N=25 CKD patients  
Watcher et al, Histol Histopathol, 2018
Vascular Calcification is Associated with an increased risk of Plaque Rupture.

Does calcification cause plaque rupture?

Is the type of calcification Important?
Small Calcium deposits are associated with plaque instability

Spotty Calcification Typifies the Culprit Plaque in Patients With Acute Myocardial Infarction
An Intravascular Ultrasound Study

Shoichi Ebara, MD; Yoshiki Kobayashi, MD; Minoru Yoshiyama, MD; Kenei Shimada, MD; Yoshihisa Shimada, MD; Daiku Fukuda, MD; Yasuhiro Nakamura, MD; Hajime Yamashita, MD; Hironori Yamashita, MD; Kazuhide Takeuchi, MD; Takahiko Naruko, MD; Kazuo Haze, MD; Anton E. Becker, MD; Junichi Yoshikawa, MD; Makiko Imai, MD

Background—Calcification is a common finding in human coronary arteries; however, the relationship between calcification patterns, plaque morphology, and patterns of remodeling of culprit lesions in a comparison of patients with acute coronary syndromes (ACS) and those with stable conditions has not been documented.

Methods and Results—Preinterventional intravascular ultrasound (IVUS) angiography of 112 patients were studied, 61 with acute myocardial infarction (AMI), 70 with unstable angina patients (UAP), and 47 with stable angina pectoris (SAP). The frequency of calcium deposits within an area of less than 90° for calcified areas was significantly different in culprit lesions of patients with AMI, UAP, and SAP (P<0.0001). Moreover, the average number of calcium deposits within an area of 30° per patient was significantly higher in AMI than in SAP (P=0.0002; mean±SD, AMI 4±3.3, SAP 0.5±0.8). Conversely, calcium deposits were significantly longer in SAP patients (P<0.0001; mean±SD, AMI 2.2±1.6, UAP 1.3±0.8, and SAP 4.3±3.5 mm). In AMI patients, the typical pattern was spotty calcification, associated with a fibrofatty plaque and positive remodeling. In ACS patients showing negative remodeling, no calcification was the most frequent observation. Conversely, SAP patients had the highest frequency of extensive calcification.

Conclusion—Our observations show that IVUS allows the identification of vulnerable plaques in coronary arteries, not only by identifying a fibrofatty plaque and positive remodeling, but also by identifying a spotty pattern of calcification.
(Circulation. 2004;110:3424-3429.)
Calcium Crystals Cause VSMC Death and Inflammation and Plaque Rupture?

- Nanocrystals induce VSMC death
  (Ewence et al Circ Res 2008)

- Nanocrystals cause macrophage Inflammation
  (Nadra et al Circ Res 2006)

- Changes in plaque response to mechanical forces
  (Richardson et al, Lancet 1989)

- Nanocrystals cause rupture of the fibrous cap
  (Kelly-Arnould, et al Weinbaum, PNAS 2013)
Plaque Rupture is Associated with Thinning of the FC but Plaque Erosion can also occur
Nano-crystals from plaques induce VSMC death. Small crystals most potent.

Small crystals also activate the Infammasome and IL1a signalling.

Proudfoot et al 2018

VSMC death is induced by intracellular Ca overload due to phagocytosis and lysosomal degradation of nano-crystals.
Nano-crystals activate inflammatory NfκB signalling in macrophages
Micro CT shows microcalcifications in the Fibrous Cap

Plaque Erosion?
Vermanni

Maldonado et al 2012
Plaques Rupture at Sites of Micro-calcifications

Why?
Microcalcifications are associated with Voids in the Extracellular Matrix

Exosomes (matrix vesicles) contain Matrix metalloproteinases that can degrade collagen – create a void.

Aikawa Lab
Modelling of Material Properties of Mineral/Matrix Interface

Predicts Material Stress at these Sites.

Sheldon Weinbaum
Maldonado et al 2012
The Holy Grail of Atherosclerosis Research!

How can Unstable Atherosclerotic Plaque Be Detected?
**18**F-fluoride positron emission tomography for identification of ruptured and high-risk coronary atherosclerotic plaques: a prospective clinical trial

Nikhil V Joshi, Alex T Vesey, Michelle C Williams, Anoop S V Shah, Patrick A Calvert, Felicity H M Craighead, Su Ern Yeoh, William Wallace, Donald Salter, Alison M Fletcher, Edwin J R van Beek, Andrew D Flapan, Neal G Uren, Miles W H Behan, Nicholas L M Cruden, Nicholas L Mills, Keith A A Fox, James H F Rudd, Marc R Dweck*, David E Newby*

Figure 2: ¹⁸F-fluoride and ¹⁸F-fluorodeoxyglucose uptake in patients with myocardial infarction
1⁸F-fluoride activity (maximum tissue-to-background ratio) was increased in the culprit plaque (red) compared with the maximum uptake in any of the non-culprit plaques (blue). By contrast, there was no difference in the activity of ¹⁸F-fluorodeoxyglucose between these regions.

Figure 3: Carotid ¹⁸F-fluoride uptake and carotid plaque rupture
In vitro (A and B) and ex vivo (C, D) positron emission computed tomograms showing colocalisation of ¹⁸F-fluoride (¹⁸F NaF) uptake (yellow-orange) to the site of plaque rupture with adherent thrombus on excised carotid endarterectomy tissue (E and F). Histology of the ¹⁸F-NaF positive region shows a large necrotic core (Movat's pentachrome, magnification 4×, G), within which increased staining for tissue non-specific alkaline phosphatase can be seen as a marker of calcification activity on immunohistochemistry (magnification 4×, H, magnification 10×, I).
Can and Should Calcification be Treated?
What is the Treatment Window

Aikawa and Otto, Circulation 2012
Impact of Statins on Serial Coronary Calcification During Atheroma Progression and Regression

Rishi Puri, MBBS, PhD, Stephen J. Nicholls, MBBS, PhD, Mingyuan Shao, MS, Yu Kataoka, MD
Kiyoko Uno, MD, PhD, Samir R. Kapadia, MD, E. Murat Tuzcu, MD, Steven E. Nissen, MD

CENTRAL ILLUSTRATION
Plaque Calcification in the Setting of No-Statin Therapy or High-Intensity Statin Therapy

STATINS - The Gold Standard for Treating CAD
INCREASE calcification

Are statins effective in calcified renal patients?
Wanner et al NEJM 2005
What is the Nature of CAD in Renal Patients?

• Is the calcification medial or intimal?
• Is the calcification micro or macro?
• Are the lesions different from those seen in the ‘general’ population?
• Lipid, Inflammation?
Vascular Calcification – A Degenerative Unmodifiable Risk Factor that Predicts Disease and Death?

A man is as old as his arteries.
(Thomas Sydenham)

British Physician 1624-1689
Incidence of Vascular Calcification with Age

\[ \text{Ca (mmol/kg)} \]

\[ \text{AGE (years)} \]


Aorta (r=0.84)

Internal Iliac (r=0.75)
Ageing is the Strongest Risk Factor for Defects in Kidney-Bone-Vascular Axis Tissues

- Elevated Phosphate/FGF23/Klotho
- Low Vitamin D
- DNA damage
- Oxidative Stress
- Systemic Inflammation

KDIGO
Multiple Pathways Regulate Vascular Calcification

Mouse gene knockouts develop vascular calcification and bone defects (e.g., osteoporosis).

- MGP (matrix Gla protein)
- Fetuin**
- Osteoprotegerin
- Klotho/FGF23** - Phosphate and Vit D metabolism
- Pyrophosphate metabolism (ENPP1)
- Carbonic anhydrase
- Smad 6

Human single gene defects
- Keutel Syndrome (MGP null)
- Idiopathic calcification of newborn (ENPP1)

Develop vascular calcification

Genetic Component

Hutchinson-Gilford Progeria Syndrome (HGPS)

- VSMC loss
- Premature Atherosclerosis
- Calcification
- Vascular Stiffening
- Osteoporosis
- Death before age 16 of MI or stroke

Due to accumulation of mutant or unprocessed Prelamin A

LMNA or FACE1 mutations

Protein selectively accumulates in MSC populations
Is there evidence for this pathway in dialysis patients?

Children on dialysis show prelamin A accumulation and increased levels of p16 positive cells.

Liu et al Circ Res 2013
Ageing is Associated with Increased Inflammation
Senescence Associated Secretory Phenotype (SASP)

Shanahan, C. M. (2013)
SMCs overexpressing prelamin A show osteogenic paracrine effects on surrounding cells \textit{in vitro}.
Array analysis shows VSMCs secrete pro-osteogenic cytokines in response to prelamin A accumulation.

**Table: SASP factors**

<table>
<thead>
<tr>
<th>Category</th>
<th>SASP factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukins and chemokines</td>
<td>IL6, IL8, GRO, GROα, MCP-1,-2,-3, ENA78, GCP-2</td>
</tr>
<tr>
<td>Growth factors and regulators</td>
<td>Angiogenin, IGFBP-4,-6, VEGF, BMP2</td>
</tr>
<tr>
<td>Proteases</td>
<td>TIMP-1,-2</td>
</tr>
<tr>
<td>Soluble or shed receptors or ligands</td>
<td>OPG, Fas, uPAR, ICAM-1</td>
</tr>
</tbody>
</table>

**Liu et al Circ Res 2013**

**Same Inflammatory Profile seen in VSMCs from Children on Dialysis**
Is Inflammation the Key?

Monoclonal antibody to Interleukin-1β
CANTOS trial
Calcium phosphate particles stimulate interleukin-1β release from human vascular smooth muscle cells: A role for spleen tyrosine kinase and exosome release

Tissue ageing is driven by DNA damage and inflammatory mediators released from senescent tissues.
1. Calcification is a cell mediated process that reflects a disease process.

2. Calcification occurs at two sites with different clinical outcomes.

3. Calcification can be used to predict clinical events.

4. There are no treatments for vascular calcification.

5. The status of calcification in plaque stability remains controversial.

6. Inflammation may be a key process in CAD in renal failure

7. THE NATURE OF CAD IN RENAL PATIENTS REQUIRES FURTHER BASIC KNOWLEDGE