Warfarin use in chronic kidney disease: a risk factor for vascular calcification?

Sharon Nessim, MD, MSc, FRCPC
Division of Nephrology, Jewish General Hospital
Assistant Professor, McGill University
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Case

- 76M
- PMHx
  - Diabetes Mellitus
  - Hyperlipidemia
  - Ischemic cardiomyopathy with R>L heart failure
  - Atrial fibrillation (CHADS 3)
  - Stage 4-5 CKD
A Case

**Meds**
- Furosemide
- Atorvastatin
- Bisoprolol
- Valsartan
- Amiodarone
- Allopurinol
- Ferrous sulfate
- Lanthanum carbonate
- Sodium bicarbonate
- Vitamin D
- **Warfarin**
A Case

- Presented for CKD follow-up
- eGFR 12 ml/min
- Patient’s wife: “Can I show you something on his legs?”
A case

- Diagnosis: Calciphylaxis
  - AKA calcific uremic arteriolopathy
  - syndrome of ischemic skin ulceration and necrosis that occurs in tissues supplied by blood vessels with dense medial calcification
  - Incidence ~1-4% among patients with ESRD
A case

Risk factors for calciphylaxis
- Hypercalcemia
- Hyperphosphatemia
- Hyperparathyroidism
- Inadequate dialysis
- Warfarin (11.4-fold increased risk!)

What does warfarin have to do with vascular calcification?

Hayashi et al, NDT 2012
Objectives

- Discuss the prevalence and mediators of vascular calcification among CKD patients
- Discuss the role of vitamin K deficiency and warfarin use in promoting vascular calcification
- Discuss ongoing studies of the effect of vitamin K2 supplementation on reducing vascular calcification
Vascular calcification

- Intimal vs. medial

MEDIAL CALCIFICATION
INTIMAL CALCIFICATION
Vascular calcification

Intima and media calcifications: light microscopy aspects

a) intima calcification

b) media calcification
Vascular calcification

- Medial calcification can be seen in non-CKD patients (eg. diabetic), but is most common in patients with CKD and ESRD
What is different about CKD patients?

- Traditional risk factors
  - Hypertension
  - Diabetes
  - Hyperlipidemia
  - Family history
  - Age
  - Smoking

- Non-traditional risk factors
  - Altered mineral metabolism
  - Uremia
  - Proteinuria
  - Anemia
  - Inflammation
Why is medial calcification so bad?

- Causes stiffening of the vascular wall, reducing arterial compliance
- Contributes to LVH and decreased coronary perfusion, which are associated with increased CV mortality

Blacher et al, Hypertension 2000
Relationship between eGFR and cardiovascular disease

Go et al, NEJM 2004
Why do patients develop vascular calcification?

- Extent of vascular calcification depends on the balance between factors that favour calcification and those that inhibit it.
Promoters of vascular calcification

- Uremia
- Hyperphosphatemia
- Hypercalcemia/calcium load
- PTH
- Age
- Diabetes
- Inflammation
Uremic serum promotes vascular calcification

- Incubated bovine vascular smooth muscle cells with calcification media
- Cultured in the presence of control serum or uremic serum
- Measured calcium deposition
- Measured osteopontin expression

Chen et al, KI 2002
Phosphate promotes vascular calcification

- Incubated bovine vascular smooth muscle cells with increasing concentrations of phosphate
- Measured osteopontin expression

Chen et al, KI 2002
Phosphate promotes vascular calcification

Hyperphosphatemia

Downregulation of VSMC genes

Upregulation of osteogenic genes

![Image of vascular tissue]
Do phosphate levels matter in the CKD/ESRD population?

Block et al, JASN 2004
Do phosphate levels matter in the non-CKD population?

CARE trial

- Post-hoc analysis
- Divided into 4 groups by serum phosphate (mmol/L):
  - <0.83
  - 0.83-1.1
  - 1.1-1.3
  - >1.3

Tonelli et al, Circulation 2005
Mediators of vascular calcification

- Uremia
- Phosphate
- Others

Diagram:
- Calcification promoters
- Calcification inhibitors

??
Inhibitors of calcification

- Matrix Gla protein
- Pyrophosphate
- Fetuin-A
- Osteoprotegerin
Inhibitors of vascular calcification

- **Matrix Gla protein (MGP)**
  - The major local calcification inhibitor in the arterial media
  - Inhibits calcium crystal formation
  - A vitamin K-dependent protein
  - Requires $\gamma$-carboxylation to its active form

If MGP inhibits medial calcification, does dietary vitamin K deficiency or vitamin K antagonism (warfarin) induce vascular calcification?
How important is MGP?

Luo et al, Nature 1997
How important is MGP?

- MGP -/- mice
  - Develop severe medial calcification
  - Die of aortic rupture

Luo et al, Nature 1997
How important is MGP?

- Keutel Syndrome
  - Autosomal recessive
  - Non-functional MGP
  - Patients have pulmonary stenosis and abnormal cartilage calcification
  - Extensive medial calcification seen at autopsy

Munroe et al, Nat Genet 1999
Mediators of vascular calcification

- Uremia
- Hyperphosphatemia
- Hypercalcemia/calcium load
- PTH
- Age
- Diabetes
- Inflammation

Calcification promoters

Calcification inhibitors

MGP
  - Pyrophosphate
  - Fetuin-A
  - Osteoprotegerin
Objectives

- Discuss the prevalence and mediators of vascular calcification among CKD patients
- Discuss the role of vitamin K deficiency and warfarin use in promoting vascular calcification
- Discuss ongoing studies of the effect of vitamin K2 supplementation on reducing vascular calcification
Vitamin K subtypes

- **Vitamin K1** (phylloquinones)
  - from green leafy vegetables
  - poor oral absorption
  - accumulates predominantly in liver

- **Vitamin K2** (menaquinones)
  - from fermented cheeses, soy beans (~10% of intake)
  - excellent absorption
  - accumulates predominantly in extra-hepatic tissue, including arterial vessel wall
Natto!
Vitamin K subtypes

<table>
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<th>Vitamin K1 (phyloquinones)</th>
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Vitamin K

- High doses of vitamin K1 can be converted in extrahepatic tissues to vitamin K2 (MK-4)
  - Warfarin blocks this conversion
Vitamin K

- What are the major vitamin K dependent proteins?
  - Coagulation factors
  - Osteocalcin (involved in bone turnover)
  - Matrix-Gla protein (inhibitor of vascular calcification)
Vitamin K

- Recommended intake = 1µg/kg/d
  - But recommendations are based on hepatic requirements for synthesis of clotting factors
  - At low vitamin K intakes, liver is main site of tissue uptake
  - At high vitamin K intakes, there is increased accumulation in other tissues
  - ie. recommended intake for extrahepatic effects should be higher
Ways to measure vitamin K2 sufficiency

- (1) Measure dietary intake of vitamin K2
- (2) Measure levels of uncarboxylated MGP or osteocalcin (ELISA)
Prevalence of low vitamin K levels

- Pilkey et al, AJKD 2007
  - Measured vitamin K levels in 142 HD patients
  - Results
    - 29% had low K1 levels
    - 93% had significant amounts (at least 20%) of uncarboxylated osteocalcin
Prevalence of low vitamin K levels

- Cranenburg, KI 2012
  - 40 hemodialysis patients
  - Measured vitamin K1 and K2 intake (by food record)
  - Measured vitamin K status

- Results
  - Intake of K1 and K2 lower than general population
  - High levels of uncarboxylated bone and coagulation proteins in 33/40 patients
  - Very high uncarboxylated MGP levels in all patients
Effect of low Vitamin K intake

- Rotterdam trial
  - 4807 elderly patients observed over time
  - No history of CAD at baseline
  - Divided into tertiles of vitamin K intake
  - Low vitamin K2 intake associated with higher incidence of severe vascular calcification and CV mortality
  - No effect of vitamin K1 intake

Geleijnse et al, J Nutrition 2004
Effect of low Vitamin K intake

- Schlieper et al, JASN 2011
- 188 hemodialysis patients compared with 98 age-matched controls
- Low levels of carboxylated MGP were associated with
  - higher all-cause mortality (HR 2.2)
  - higher CV mortality (HR 2.7)
  - higher vascular calcification scores
Warfarin: mechanism of action
Effect of Warfarin

- Observational study of 157 patients with A-fib but without significant CV disease
- 71 (45%) on warfarin
- No difference in clinical characteristics between patients taking or not taking warfarin

Weijs et al, Er Heart J 2011
Effect of Warfarin

- No VKA (n=86)
- VKA 6–60 months (n=53)
- VKA >60 months (n=18)

- Agatston score 0
- Agatston score 1–100
- Agatston score 101–400
- Agatston score >400

Weijs et al, Er Heart J 2011
Effect of Warfarin

- Observational study of 83 patients with calcific aortic valve disease
- 23/83 on warfarin (mean duration 88 months)
- Compared coronary and aortic calcification scores

Table 3. Valvular and coronary calcium scores assessed by multislice spiral computed tomography stratified by anticoagulation status

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oral Anticoagulants</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n = 23)</td>
<td>No (n = 63)</td>
</tr>
<tr>
<td>Valvular Agatston score</td>
<td>$2,409.9 \pm 1,758.5$</td>
<td>$1,070.1 \pm 1,084.6$</td>
</tr>
<tr>
<td>Coronary Agatston score</td>
<td>$1,561.3 \pm 1,140.5$</td>
<td>$738.2 \pm 977.5$</td>
</tr>
</tbody>
</table>

Koos et al, American J Cardiol 2005
Effect of Warfarin

- 108 hemodialysis patients, 18 of whom were on warfarin for >18 months

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds ratio (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;18 months warfarin*</td>
<td>3.77 (0.97-14.7)</td>
<td>0.055</td>
</tr>
<tr>
<td>Age (10-year increase)</td>
<td>1.69 (1.12-2.55)</td>
<td>0.012</td>
</tr>
<tr>
<td>Dialysis vintage (1-year increase)</td>
<td>1.13 (0.98-1.30)</td>
<td>0.089</td>
</tr>
<tr>
<td>Calcium (500-mg increments)</td>
<td>1.41 (1.03-1.91)</td>
<td>0.027</td>
</tr>
<tr>
<td>Calcitriol (0.25-μg increments)</td>
<td>1.73 (0.88-3.41)</td>
<td>0.113</td>
</tr>
</tbody>
</table>

The model accounted for 23.3% of the variance (Cox and Snell r²).
*Reference group was <18 months of warfarin exposure.

Holden et al, J Nephrol 2007
Effect of Warfarin: Caveat

Most of these studies are observational, so one cannot infer causality

- Possibility #1: Warfarin causes vascular calcification
- Possibility #2: Warfarin is associated with vascular calcification because patients on warfarin are sicker
Effect of Warfarin

- Rat model of warfarin-induced low K2
  - Rats given warfarin + vitamin K1 supplementation (to prevent bleeding)
    - ie. adequate levels of K1, inadequate levels of K2
  - Induced rapid calcification of rat arteries and heart valves

Effect of Warfarin

- Rat model of warfarin in CKD vs. normal renal function

McCabe KM et al, KI 2013
Effect of Warfarin

- Rat model of warfarin in CKD vs. normal renal function
  - CKD rats showed
    - 3x increased calcium concentration in thoracic aorta
    - 8x increase in abdominal aorta
    - 4x increase in renal artery
    - 20x increase in carotid artery
    - Increased pulse pressure and pulse wave velocity

McCabe KM et al, KI 2013
Summary so far

- MGP is a potent inhibitor of vascular calcification
- MGP is dependent on vitamin K to $\gamma$-carboxylate it to its active form
- Vitamin K2 is more important than vitamin K1 for $\gamma$-carboxylation of extra-hepatic proteins
- Low vitamin K2 levels and warfarin use are associated with increased vascular calcification
Objectives

- Discuss the prevalence and mediators of vascular calcification among CKD patients
- Discuss the role of vitamin K deficiency and warfarin use in promoting vascular calcification
- Discuss ongoing studies of the effect of vitamin K2 supplementation on reducing vascular calcification
How to supplement Vitamin K2?

**OPTION #1**

**OPTION #2**
Vitamin K2 Supplementation

- Westenfield et al, AJKD 2012
  - Randomized non-placebo controlled trial
  - 53 hemodialysis patients vs. with 50 age-matched controls
  - 3 parallel groups
    - Vitamin K2 at 45 ug, 135 ug or 360 ug/d for 6 weeks
Vitamin K2 Supplementation

- Westenfield et al, AJKD 2012
  - At baseline, hemodialysis patients had
    - 4.5x higher uncarboxylated MGP
    - 8.4x higher uncarboxylated osteocalcin
    - (suggests that most HD patients have a functional vitamin K deficiency)
  - Vitamin K2 induced a dose-dependent and time-dependent decrease in uncarboxylated MGP and osteocalcin
Can Vitamin K2 prevent calcification?

- Rat model comparing
  - Control
  - Warfarin + K1
  - Warfarin + K2

Spronk et al, J Vasc Res 2003
Conclusion: Administering vitamin K2 at the same time as warfarin can prevent vascular calcification.
Can Vitamin K2 regress calcification?

Schurgers et al, Blood 2007
Conclusion: Administering vitamin K2 can regress warfarin-induced vascular calcification
# Registered trials on Vitamin K2

<table>
<thead>
<tr>
<th>Rank</th>
<th>Status</th>
<th>Study</th>
<th>Conditions</th>
<th>Interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Completed</td>
<td><strong>Comparison of Efficacy of Different Dosages Vitamin K2</strong></td>
<td>Carboxylation Level: Vitamin K-dependent Proteins</td>
<td>Dietary Supplement: placebo; Dietary Supplement: vitamin K1; Dietary Supplement: vitamin K2</td>
</tr>
<tr>
<td>2</td>
<td>Completed</td>
<td><strong>Dose Defining Study for the Administration of Vitamin K2 Supplements in Hemodialysis Patients</strong></td>
<td>Vascular Calcification</td>
<td>Dietary Supplement: Vitamin K2 supplementation</td>
</tr>
<tr>
<td>3</td>
<td>Recruiting</td>
<td><strong>Vitamin K2 Intervention in Patients With Vitamin K Antagonists</strong></td>
<td>Thrombosis</td>
<td>Dietary Supplement: Vitamin K2</td>
</tr>
<tr>
<td>4</td>
<td>Recruiting</td>
<td><strong>The Effects of Vitamin K2 Supplementation on the Progression of Coronary Artery Calcification</strong></td>
<td>Coronary Artery Disease</td>
<td>Dietary Supplement: Menaquinone-7 (Vitamin K2); Other: Placebo capsules</td>
</tr>
<tr>
<td>5</td>
<td>Unknown†</td>
<td><strong>Vitamin K2 and Vessel Calcification In Chronic Kidney Disease Patients</strong></td>
<td>Kidney Diseases; Coronary Artery Calcification</td>
<td>Drug: Vitamin K2+10µg cholecalcifer; Drug: Vitamin D</td>
</tr>
<tr>
<td>6</td>
<td>Completed</td>
<td><strong>Comparison of Absorption of Vitamin K2</strong></td>
<td></td>
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“Vitamin K2 and vessel calcification in CKD patients”

- Randomized double-blind study
- N=60 patients with CKD (GFR 15-60 ml/min) with calcium score >10 (Agatston scoring system)
- Randomized to vitamin K2 + Vit D3, or Vit D3 alone
- Primary endpoint – the degree of vessel calcification by imaging at 9 months
“The effects of vitamin K2 supplementation on the progression of coronary calcification”
- Randomized, double-blind, placebo-controlled trial
- N=180 patients with established coronary artery calcification
- Objective: to investigate whether daily supplementation of Vitamin K2 will lead to a decreased progression-rate of CAC after 24 months of follow-up as compared with placebo
“Vitamin K2 intervention in patients with Vitamin K antagonists”
- Randomized placebo-controlled trial
- N=52 patients on coumadin
- To receive Vitamin K2 (75 ug/d) or placebo for 4 months
- Primary objective: to determine whether Vitamin K2 supplementation upsets the balance of anticoagulant treatment
- Secondary objective: to evaluate if regular consumption of vitamin K2 improves the markers of bone mineralization
“Effect of low-dose vitamin K2 on the stability of oral anticoagulant treatment”

- 18 healthy volunteers given warfarin x 4 weeks
  - Found to have increased levels of UC-MGP
- Then warfarin continued and given increasing doses of MK-7 x 6 weeks
  - Highest dose decreased INR by 40%
  - Lower doses caused clinically relevant lowering of INR in 40-60% of patients
Back to the case…

- Started on daily dialysis to optimize uremic clearance and phosphate removal
- Warfarin stopped
- Started on Vitamin K2
- Developed infection of wound, which persisted despite broad spectrum antibiotics
- Died 1 month later
Summary

- MGP is the major inhibitor of calcification in the blood vessel wall
- MGP is activated (carboxylated) by vitamin K
- MGP may be inactive in the presence of vitamin K2 deficiency or warfarin
- There may be a role for vitamin K2 in the prevention of vascular calcification