Current Concepts of CKD-MBD

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2019-05-24
Objectives

• 1. Mineral and hormonal disruption
  – Ca, InP, PTH, vit-D, FGF23/Klotho
  – Calcification – vascular and soft tissue
    • Nutritional vit-D
      – Renal outcome

• 2. Nutritional vitamin-D in SHPT
  – PTG: 1α-Hydroxylase/24-Hydroxylase/DBP
  – Cholecalciferol for SHPT

• 3. Bone disorders
  • Uremic Osteoporosis
  • Renal osteodystrophy
Pathogenesis of secondary hyperparathyroidism in CKD

Chronic kidney disease

Phosphate retention

↑ FGF23

↓ 1,25(OH)₂D

↓ 25(OH)D

↓ Ca²⁺

↑ PTH

↑ Pi excretion in the urine

↑ Ca²⁺  ↑ Pi  ↑ FGF23
FGF23, an endocrine nexus between hormones and mineral ions

Cholecalciferol supplementation increases FGF23 in PD dialysis patients with hypovitaminosis D

A Serum 25D

B Serum intact FGF23

C Serum 1,25D

D Serum Corrected Calcium

E Serum Phosphorus

F Serum intact PTH

4800 IU/daily

J Nephrol. 2019 Mar 19
Treatment of abnormal PTH levels in CKD-MBD
2017 KDIGO guideline

- **4.2.1:** In patients with CKD G3a-G5 not on dialysis, the optimal PTH level is not known. However, we suggest that patients with levels of intact PTH progressively rising or persistently above the upper normal limit for the assay be evaluated for modifiable factors, including hyperphosphatemia, hypocalcemia, high phosphate intake, and vitamin D deficiency (2C).

- **4.2.4:** In patients with CKD G5D requiring PTH-lowering therapy, we suggest calcimimetics, calcitriol, or vitamin D analogs, or a combination of calcimimetics with calcitriol or vitamin D analogs (2B).
Vitamin D metabolism and nomenclature

Native Nutritional
h1/2: 2 months

Active
h1/2: ½ day

UV-B radiation
Skin
7-dehydrocholesterol
Cholecalciferol Vitamin D₃
Liver Calcifediol or calcidiol
25-hydroxyvitamin D₃
Kidneys Calcitriol
1,25-dihydroxyvitamin D₃

### Atmospheric pollution and vitamin D status

<table>
<thead>
<tr>
<th>Environmental factor</th>
<th>Mori Gate High pollution area n=26</th>
<th>Gurgaon Low pollution area n=31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>16 (4.1)</td>
<td>15.9 (3.8)</td>
</tr>
<tr>
<td>Haze score</td>
<td>2.1 (0.5)</td>
<td>2.7 (0.4)*</td>
</tr>
<tr>
<td>Gender</td>
<td>15 males, 11 females</td>
<td>15 males, 16 females</td>
</tr>
<tr>
<td>Ca (mg %)</td>
<td>9.7 (0.9)</td>
<td>9.6 (0.8)</td>
</tr>
<tr>
<td>ALP (IU/l), median (range)</td>
<td>498 (116–3739)</td>
<td>398* (196–780)</td>
</tr>
<tr>
<td>25(OH)D₃ (ng/ml)</td>
<td>11.7 (7)</td>
<td>27.1 (7)***</td>
</tr>
<tr>
<td>25(OH)D₂ (ng/ml)</td>
<td>2.4 (0.6) (n=5)</td>
<td>0</td>
</tr>
<tr>
<td>Total 25(OH)D (ng/ml)</td>
<td>12.4 (7)</td>
<td>27.1 (7)***</td>
</tr>
<tr>
<td>1,25(OH)₂D (pg/ml)</td>
<td>73.7 (30)</td>
<td>65 (19)</td>
</tr>
<tr>
<td>PTH (pg/ml), median (range)</td>
<td>25 (5–284)</td>
<td>13.1** (1.6–37)</td>
</tr>
</tbody>
</table>

**Haze scores**, regarded as a surrogate marker of solar UVB radiation reaching ground level.

A filter that only allowed UVB radiation (285-310 nm) to be detected by the sensor’s light detecting diode.
**Natural sources of vitamin D**

<table>
<thead>
<tr>
<th>Source</th>
<th>Content in vitamin D*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild salmon</td>
<td>600–1000 IU</td>
</tr>
<tr>
<td>Farmed salmon</td>
<td>100–250 IU</td>
</tr>
<tr>
<td>Sardines (canned)</td>
<td>300–600 IU</td>
</tr>
<tr>
<td>Mackerel (canned)</td>
<td>250 IU</td>
</tr>
<tr>
<td>Tuna (canned)</td>
<td>236 IU</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>400–1000 IU per tablespoon</td>
</tr>
<tr>
<td>Shiitake mushrooms (fresh)</td>
<td>100 IU</td>
</tr>
<tr>
<td>Shiitake mushrooms (dried)</td>
<td>1600 IU</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>20 IU per yolk</td>
</tr>
<tr>
<td>Fresh mushrooms</td>
<td>Vegetable are grown by fertilizer</td>
</tr>
<tr>
<td>Cheese (e.g., Emmental)</td>
<td>44 IU</td>
</tr>
</tbody>
</table>

*Per 100 grams, unless otherwise specified.*
High Prevalence of vitamin D deficiency/insufficiency in general population worldwide

Nutrients 2017; 9: 651
Mechanisms of inhibition of vascular calcification by active vitamin D

- Active vitamin D inhibits phosphate uptake by vascular smooth muscle cells.
- Inhibits crystal growth and promoting active regression.

Kidney International (2012) 82, 1248–1250
Role of Local Versus Systemic Vitamin D Receptors in Vascular Calcification

活性vit-D 易引发血管钙化

Arterioscler Thromb Vasc Biol 2014; 34: 146-151
Coronary artery calcification (CAC) & valve calcification in young adults with childhood onset of ESRD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Heidelberg Oh et al. [2]</th>
<th>Berlin Briese et al. [1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with CAC</td>
<td>34/37 (92%)</td>
<td>4/40 (10%)</td>
</tr>
<tr>
<td>With cardiac valve calcification</td>
<td>12/37 (32%)</td>
<td>0/40 (0%)</td>
</tr>
<tr>
<td>Nb of dialysis/transplanted</td>
<td>13/26</td>
<td>9/31</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>25/14</td>
<td>22/18</td>
</tr>
<tr>
<td>Duration of ESRD (years)</td>
<td>13.5</td>
<td>12.1</td>
</tr>
<tr>
<td>Time on dialysis (yet)</td>
<td>5</td>
<td>2.9</td>
</tr>
<tr>
<td>Time on transplant</td>
<td>7.8</td>
<td>9.2</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>BMI</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>108</td>
<td>97</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>109</td>
<td>109</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>6.2</td>
<td>17</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>43</td>
<td>41</td>
</tr>
<tr>
<td>Homocysteine (µmol/l)</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>iPTH (pg/ml)</td>
<td>364</td>
<td>155</td>
</tr>
<tr>
<td>Ca × P product (mmol²/l²)</td>
<td>3.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Cumulative Ca × OPB dose (kg); (per year)</td>
<td>27; (2)</td>
<td>4; (0.8)</td>
</tr>
<tr>
<td>Cumulative 1α OH vit D dose (µg); (per year)</td>
<td>13, 300; (1)</td>
<td>383; (130)</td>
</tr>
<tr>
<td>Cumulative Cholecalciferol dose (10³IU); (per year)</td>
<td>?</td>
<td>13; (2.1)</td>
</tr>
</tbody>
</table>

Early use native vit-D may prevent vascular calcification

Factors related to the osteogenic transdifferentiation of vascular smooth muscle cells in CKD

What kind of VSMC will proceed the osteogenic transdifferentiation?

Lu et al. Int Journal Nephrol, 2011 OA
Lu et al. TSWJ 2014
Vit-D deficiency contribute to vascular calcification through increase Gli1+ expression in CKD

Cell Stem Cell 2016; 19: 1-15
Components of calciprotein particles (CPPs)

CPP1
- Amorphous CaPi
- Fetuin-A
- Matrix Gla Protein (MGP)
- Apolipoprotein

 CPP1:
- 60-75 nm
- ↑ Fetuin-A
- Amorphous CaPi predominant
- ↓ Apo A4, ApoE, Apo C3
- Higher surface charge

CPP2
- Crystalline CaPi predominant
- ↓ Fetuin-A
- Crystalline CaPi predominant
- ↑ Apo A4, ApoE, Apo C3
- Low surface charge
- ↑ Lipid

Fetuin-A: binding with calcium phosphate as well as prevent its growth, aggregation, and precipitation

Lu et al. Nutrients 2019 Jan; 11(1). pii: E152.1
Calcifying EVs and Gli1+ Progentior Cells

Platelet-VSMC

1. VSMC-derived EVs
2. Macrophage
3. Platelets

Myofibroblast
Gli1+ Progenitor
Intimal calcification

Medial calcification

Platelet-derived EVs

Osteoblast-like cells
Runx2+

Contractile VSMCs
Calponin+ aSMA+ Sm22a+

Synthetic VSMCs
Tpm4, vimentin, nmMLC1B
Role of vit-K in CPP & MV of CKD

vit-K

Carboxylation
Matrix Gla Protein

Primary Calciprotein
CPP1

Endothelial Cells

Matrix Vesicle
MV (EV)

Vsacular SM cells

Macrophage/Phagocytic cells

Edited by Dr. Lu 2018/09
Phosphorylation and carboxylation process of MGP

Lu et al. Nutrients 2019 Jan; 11(1). pii: E152.1
Vascular smooth muscle cells and osteoblasts are able to synthesize Matrix-Gla-Protein (MGP) and Osteocalcin (OC), respectively.
Supplementary nutrients for prevention of vascular calcification in patients with CKD

- Retardation of renal progression
- Dietary restriction: low protein or very low protein diet
- Vitamin D deficiency
- PTH increase
- Malnutrition
- Inflammation Oxidative stress
- Use active vitamin D
- Ergocalciferol or cholecalciferol supplements
- Calcium phosphate loads increase
- Intimal arterial calcification
- Medial arterial calcification
- Phenotype of vascular aging
Vascular calcification during cholecalciferol overload in mice
400,000 IU/kg/IM x 3 days
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  – Cholecalciferol for SHPT

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  • Uremic Osteoporosis
  • Renal osteodystrophy
Comparison of constitutive gene expression in hMSCs from a hemodialysis subject and a control subject
Bone Loss in CKD

- Chronic Kidney Disease
- IS/PCS
- Uremic Toxins ↑ (Indoxyl Sulphate)
- PTH ↑
- Direct toxicity to OBs/OCs
- Vitamin D Deficiency
- Wnt Antagonist ↑ (SOST/DKK1)
- Low Bone Turnover Bone Loss
- Renal Function More Worsen
- High Bone Turnover or Low Bone Turnover Bone Loss

Progression of CKD

Klotho deficiency
Aluminum intoxication
Metabolic Acidosis
Others
1,25-Dihydroxyvitamin D₃ Inhibits the Hepatic Production of 25-Hydroxyvitamin D₃

**Diagram:**
- Conversion of 7-dehydrocholesterol (7-DHC) to pre-vitamin D₃ by UV-B radiation.
- Pre-vitamin D₃ is converted to vitamin D₃ by heat.
- Vitamin D₃ is converted to 25-hydroxyvitamin D₃ (25(OH)D₃) by CYP 2R1.
- Vitamin D₃ is converted to 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) by CYP 27B1.

**Graph:**
- Serum 25-OHD levels over days 1 and 5 show a decrease in 25-Hydroxylase and an increase in 24-Hydroxylase.

**Reference:**
J Clin Invest 1984; 74(4):1540-1544
Active vitamin D
↓ 1α-hydroxylase
↓ 25-hydroxylase
↑ 24-hydroxylase

Skin, Pancreas, Prostate
Colon, Breast, Brain, Lung
WBC, Bone, PTG, Placenta

Lu, et al.
Clinica Chimica Acta
2016; 453: 1-12
Parathyroid Tissue in *Normal* and *CKD* subjects

**Normal subject**

**CKD patient**

主细胞

嗜酸细胞

Kidney International 2017; 92: 1217-1222
Oxyphil cell hyperplasia and proportion of oxyphil cells to total cells (O/T ratio) in parathyroid glands of uremic SHPT patients

Mild (A)                        Moderate (B)                           Severe (C)

Hyperplasia of oxyphil cells

Refractory hyperparathyroidism
Enhanced innate vit-D necessary in hyperplasia PTG

$1\alpha$-hydroxylase/GAPDH mRNA expression ratio

NVD hunger state

PTG

24-OHase

$1\alpha$-OHase $\sim x10$

24-OHase $\sim 10\%$

PTH $\uparrow$ present

$1\alpha$-hydroxylase $\uparrow x10$

24-hydroxylase $\downarrow <10\%$

J Clin Endocrinol Metab 2002; 87(6):2967–2972
J Clin Endocrinol Metab 2002; 87(12):5826–5829
Part of differential abundance *proteins* in oxyphil and chief cell nodules

<table>
<thead>
<tr>
<th>Description</th>
<th>Symbol</th>
<th>Protein IPI</th>
<th>Oxyphil / Chief</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid Hormone</td>
<td>PTH</td>
<td>IPI00000940</td>
<td>0.47</td>
</tr>
<tr>
<td>Vitamin D-Binding Protein Precursor</td>
<td>GC</td>
<td>IPI00742696</td>
<td>0.32</td>
</tr>
<tr>
<td>Extracellular Calcium-Sensing Receptor</td>
<td>CaSR</td>
<td>IPI00216479</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Downregulation of DBP in *oxyphil nodules* may reduce the vitamin D transport, therefore participate in calcitriol resistance. Should we use large dose of active vit-D?
Pathophysiology of secondary hyperparathyroidism in CKD
Revised by Dr. Lu

Progression of PTG hyperplasia proceed the ↓ of VDR & CaSR

Acidosis
Low calcium
High phosphorus
Low 1α,25(OH)2D
↑ FGF23

Oxyphil
← VDR
↓ CaSR
↓ fGf R1/3c
↓ α-klotho

Oxyphil ↑
↓↓ VDR
↓↓ CaSR
↓↓ fGf R1/3c
↓↓ α-klotho

Polyclonal proliferation
Normal gland

Diffuse hyperplasia
Early nodularity

Monoclonal proliferation
Nodular hyperplasia

Single nodular gland

Vit-D hunger in PTG of SHPT
1α-hydroxylase ~ x 10
24-hydroxylase ~ x 1/10

Cholecalciferol
25(OH)D
Calcifediol

1α hydroxylase
1α hydroxylase
1α hydroxylase
1α hydroxylase
1α hydroxylase
1α hydroxylase
1α hydroxylase
1α hydroxylase
1α hydroxylase
1α hydroxylase
1α hydroxylase
1α hydroxylase

1,25(OH)D
1,25(OH)D
1,25(OH)D
1,25(OH)D
1,25(OH)D
1,25(OH)D
1,25(OH)D
1,25(OH)D
1,25(OH)D
1,25(OH)D
1,25(OH)D
1,25(OH)D

CaSR
DBP
DBP
DBP
DBP

Intracrine
Paracrine

↓PTH
↑CaSR

Edited by Dr. Lu, 2015 Dec
Direct upregulation of parathyroid CaSR and VDR by calcimimetics in uremic rats

Add on Effects: VDRA

Calcimimetics directly increase CaSR and VDR expression by hyperplastic parathyroid glands.

Am J Physiol Renal Physiol 2009; 296: F605-F613
Cinacalcet on parathyroid cell proliferation in 5/6 Nx rats

(x4 weeks)

In human parathyroid cells

Effects of cAMP or FGF-23 on 1α-OHase mRNA

Effect of the cinacalcet on 1α-OHase and PTH mRNA

Cinacalcet

↑1α-OHase 75%

↓PTH mRNA
Means for different variables before and after 12 weeks on treatment with cholecalciferol (8,000 IU/day) or placebo

<table>
<thead>
<tr>
<th></th>
<th>Cholecalciferol</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 12</td>
<td>Baseline</td>
</tr>
<tr>
<td>25(OH)D (nmol/L)</td>
<td>57.5 ± 22</td>
<td>161.6 ± 49</td>
<td>56.8 ± 22</td>
</tr>
<tr>
<td>iPTH (pmol/L)</td>
<td>10.9 ± 5</td>
<td>10.5 ± 5</td>
<td>13.1 ± 9</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.24 ± 0.14</td>
<td>2.23 ± 0.13</td>
<td>2.27 ± 0.12</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.2 ± 0.28</td>
<td>1.3 ± 0.28</td>
<td>1.2 ± 0.27</td>
</tr>
<tr>
<td>1,25(OH)D (pmol/L)</td>
<td>64 ± 43</td>
<td>102 ± 54</td>
<td>64 ± 40</td>
</tr>
<tr>
<td>FGF23 (pg/mL)</td>
<td>132 ± 115</td>
<td>160 ± 131</td>
<td>138 ± 87</td>
</tr>
<tr>
<td>Urinary phosphate (mmol/L)</td>
<td>14.7 ± 9.6</td>
<td>15.8 ± 9.3</td>
<td>16.3 ± 7.8</td>
</tr>
<tr>
<td>Hand grip strength (kg)</td>
<td>26.5 ± 11</td>
<td>27.7 ± 12</td>
<td>33.2 ± 13</td>
</tr>
<tr>
<td>Fatigue score, total</td>
<td>14.7 ± 5</td>
<td>12.3 ± 5</td>
<td>13.7 ± 4</td>
</tr>
<tr>
<td>Physical</td>
<td>9.6 ± 4</td>
<td>8 ± 4</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Mental</td>
<td>5 ± 2</td>
<td>4.2 ± 2</td>
<td>4.7 ± 2</td>
</tr>
<tr>
<td>VAS (mm)</td>
<td>42.6 ± 26</td>
<td>36.0 ± 23</td>
<td>38.8 ± 25</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. P denotes difference in mean change between groups based on ANCOVA with baseline value as covariate. VAS, visual analogue scale.

High doses of cholecalciferol alleviate the progression of hyperparathyroidism in patients with CKD Stages 3-4: results of a 12-week double-blind, randomized, controlled study.
Combination therapy
The effect of lowering serum parathyroid hormone
Native + Active vit-D, n=60

<table>
<thead>
<tr>
<th>Primary Outcome</th>
<th>4th Week</th>
<th>8th Week</th>
<th>12th Week</th>
<th>16th Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPTH ≤ 300 pg/mL</td>
<td>n/30 (%)</td>
<td>n/30 (%)</td>
<td>n/30 (%)</td>
<td>n/30 (%)</td>
</tr>
<tr>
<td>Paricalcitol</td>
<td>2/30 (6.7)</td>
<td>7/30 (23)</td>
<td>12/30 (40)</td>
<td>15/30 (50)</td>
</tr>
<tr>
<td>Paricalcitol + Cholecalciferol</td>
<td>2/30 (6.7)</td>
<td>7/30 (23)</td>
<td>18/30 (60)</td>
<td>23/30 (76.7)</td>
</tr>
<tr>
<td>p = 1.000</td>
<td>p = 1.000</td>
<td>p = 1.000</td>
<td>p = 0.121</td>
<td>p = 0.032</td>
</tr>
</tbody>
</table>

% change of iPTH

P<0.05

Lu et al. Nutrients 2016; 8: 708
Cholecalciferol additively reduces serum parathyroid hormone levels in severe SHPT treated with calcitriol and cinacalcet in HD patients.

Lu et al. Nutrients 2018; 10(2): 196
### Reduce VDRA dose

<table>
<thead>
<tr>
<th>Week</th>
<th>CCC (n = 27)</th>
<th>CCP (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>3 μg/week</td>
<td>3 μg/week</td>
</tr>
<tr>
<td>4th week</td>
<td>3 μg/week</td>
<td>3 μg/week</td>
</tr>
<tr>
<td>8th week</td>
<td>3 μg/week</td>
<td>3 μg/week</td>
</tr>
<tr>
<td>12th week</td>
<td>2.67 μg/week</td>
<td>3 μg/week</td>
</tr>
<tr>
<td>16th week</td>
<td>2.22 μg/week</td>
<td>2.79 μg/week</td>
</tr>
<tr>
<td>20th week</td>
<td>1.44 μg/week</td>
<td>2.57 μg/week</td>
</tr>
<tr>
<td>24th week</td>
<td>0.56 μg/week</td>
<td>2.25 μg/week</td>
</tr>
</tbody>
</table>

Mean dose of intravenous calcitriol (μg/week) during the course of treatment

<table>
<thead>
<tr>
<th>Primary Outcome</th>
<th>12th Week</th>
<th>16th Week</th>
<th>20th Week</th>
<th>24th Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPPTH ≤ 300 pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCC (n = 27)</td>
<td>3/27 (11.1%)</td>
<td>8/27 (29.6%)</td>
<td>15/27 (55.6%)</td>
<td>22/27 (81.5%)</td>
</tr>
<tr>
<td>CCP (n = 28)</td>
<td>2/28 (7.1%)</td>
<td>3/28 (10.7%)</td>
<td>4/28 (14.3%)</td>
<td>7/28 (25%)</td>
</tr>
<tr>
<td>p = 1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary Outcome</td>
<td>12th Week</td>
<td>16th Week</td>
<td>20th Week</td>
<td>24th Week</td>
</tr>
<tr>
<td>25(OH)D₃ ≥ 30 ng/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCC (n = 27)</td>
<td>21/27 (77.8%)</td>
<td></td>
<td></td>
<td>24/27 (88.9%)</td>
</tr>
<tr>
<td>CCP (n = 28)</td>
<td>2/28 (7.1%)</td>
<td></td>
<td></td>
<td>3/28 (10.7%)</td>
</tr>
<tr>
<td>p = 0.001</td>
<td></td>
<td></td>
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<tr>
<td>↑ FN-BMD &gt; 10%</td>
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<tr>
<td>CCC (n = 27)</td>
<td></td>
<td></td>
<td>13/27 (40%)</td>
<td></td>
</tr>
<tr>
<td>CCP (n = 28)</td>
<td></td>
<td>5/28 (6.7%)</td>
<td></td>
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<tr>
<td>p = 0.150</td>
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</table>
Therapeutic role of cholecalciferol in CKD: A multidisciplinary-based opinion

25(OH) vitamin D <30 ng/mL

Cholecalciferol “starting” dose of ≥1000-2000 IU/day

Add active Vit D if target not reached

Target
- 25(OH) vitamin D: 30 ng/mL
- Normal PTH
- Proteinuria: <0.5 g/24h
- Hb 11-12 g/dl with low dose EPO
Changes in serum 25-(OH)D, 1,25-(OH)₂D, and plasma iPTH by treatment group and CKD stage

Extended Release Calcifediol (ERC) 25(OH)D

Reevaluation of Target Concentrations of Serum 25-Hydroxyvitamin D in Secondary Hyperparathyroidism

• PTH levels were suppressed significantly only in the group that achieved average concentrations of 25D of 50.8 ng/mL or greater.

Am J Nephrol 2019; 49: 281-283
Active Vitamin D in CKD: Getting Right Back Where We Started from?

- **Nephrologists** need to use an integrated approach that
  - *avoids* excessive use of VDRA
  - *ensures* replenishment of vitamin D stores
  - *avoids* hypercalcemia and hyperphosphatemia

Kidney Dis 2019; 5: 59-68
Objectives

• 1. Mineral and hormonal disruption
  – Ca, InP, PTH, vit-D, FGF23/Klotho
  – Calcification – vascular and soft tissue
    • Nutritional vit-D
      – Renal outcome

• 2. Nutritional vitamin-D in SHPT
  – PTG: 1α-Hydroxylase/24-Hydroxylase/DBP
  – Cholecalciferol for SHPT

• 3. Bone disorders
  • Uremic Osteoporosis
  • Renal osteodystrophy
Overview of the Wnt signalling pathway in OB

A  Wnt 4, Wnt 5a, Wnt 10b, Wnt 16

B  CKD, VC, MP, GIOP

C  Antibodies

Lancet Diabetes Endocrinol 2017 Jul 6
Evidence of low-turnover bone disease in early, as compared to late, chronic kidney disease.
Early CKD

- Kidney Wnt inhibitor
  - DKK1

- Uremic Toxins
  - IS, PCS

- Suppress OB & OC
- Decrease BF & BR
- Low bone turnover
- Altered bone architecture

Bone loss: Quality + Quantity

Bone loss: Quality

- CKD-MBD
- ROD (renal osteodystrophy)
- (TMV changes)
- Uremic Osteoporosis (Decrease elasticity)

Bone loss: Quantity


dKd1, SOST, SFRP

Low PTH
- RANKL ↓
- Bone formation ↓↓↓
- Bone resorption ↓↓

High PTH
- RANKL ↑↑
- Bone formation ↑↑
- Bone resorption ↑↑↑

Bone loss: Quality + Quantity

Bone loss (BL) & Vascular Calcification (VC) in CKD

Lu et al. Bone 2016; 87 : 57–70
Effects of active vitamin D compounds in inhibiting bone resorption

Suppression of RANKL expression

Active vitamin D compounds

Long-term exposure to active vitamin D

Altering in the calcium endocrine system

Long-term effects

pg v.s. µg

X 100,000倍

RANKL

Osteoblastic cells

Hematopoietic cells

No effect

QOPs

Osteoblastic cells

Osteoclasts

Changes in the cellularity of osteoblastic cells

Active vitamin D compounds

Daily administration of active vitamin D compounds

Mesenchymal progenitors

BoneKEy Reports 3, Article number: 495 (2014)

JBMR 2017 Jul; 32(7): 1406-1420
Calcitriol inhibits osteoclast commitment from monocyte via the BMP-Smad1-\(\gamma\)B\(\alpha\)-NF\(\gamma\)B-NFATc1 axis

Pharmacologic dose of VDRA

JBMR 2017 Jul; 32(7): 1406-1420
Clastokines after vitamin D₃ treatment

Therapeutic dosage of active vit-D

Fig 1. TRAP stain analysis in osteoclast derived from RANKL-stimulated monocytes. Calcitriol inhibit clastogenesis effect of osteoclast precursor cells. Bar = 50 µm.

Fig 4. Confocal analysis of immunofluorescent labeling of Wnt10b (green) in calcitriol-treated osteoclast; Actin was labeled with Cy3 (red). Bar = 20 µm.

Fig 2. Wnt10b expression in osteoclasts. Osteoclasts were treated with different concentration of calcitriol and Wnt10b was significant increased as the dose dependent manner.
Bone quality is improved under calcitriol treatment in CKD mice model

All experiments in this figure use mice in the C57BL/6 background.
(* p<0.05, n=3)
Wnt 5a protein level is increased in calcitriol-treated osteoblasts

Data are means ± SD. (* p<0.05 versus control group, n=3)
Short-Term Effect of Calcitriol on Bone Cells in CKD

Mesenchymal Stem Cell

- Promote differentiation

Calcitriol

- Wnt 5a (OB) Wnt 10b (OC)
- Stimulate mineralization
- Inhibit differentiation

Hematological Stem cell

- Inhibit differentiation

osteoblast

osteoblast

osteoclast

osteoclast

osteoclast

osteoclast

Bone formation (von Kossa / P1NP)

Bone mass

Bone resorption (Trap stain)

2019 KSN, poster
Uremic Osteoporosis

Indoxy sulfate, P-Cresol sulfate

Quality↓

- Measurement point DMA
- Analysis point of chemical composition

Measurement area of BMD (diaphysis)

Measurement area of BMD (distal metaphysis)
Indoxyl sulfate induces PTH resistance in CKD

1. Downregulation of PTH1R
2. Reduction in intracellular cAMP production
3. Competitive inhibition between PTH and PTH fragments to PTH1R
4. Increase in cellular oxidative stress
5. Osteoprotegerin /SOST↑ and Bone Morphogenetic Protein-7↓

Expression of OAT3 mRNA in osteoblasts, bone marrow macrophages, and RAW264.7 cells

<table>
<thead>
<tr>
<th></th>
<th>POB</th>
<th>BMM</th>
<th>RAW264.7</th>
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<tbody>
<tr>
<td>OAT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAT3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
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</tbody>
</table>

Primary osteoblasts  
Bone marrow macrophages  
Osteoclasts cell line
Aryl hydrocarbon receptor (AHR) signaling

Effect of indoxyl glucuronide (IG) on aryl hydrocarbon receptor (AHR) express

<table>
<thead>
<tr>
<th></th>
<th>CoCl₂</th>
<th>IG (100 μM)</th>
<th>IS (100 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IG + CoCl₂</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IS + CoCl₂</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>IS + IG</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Expressions of AHR and Lamin B1 proteins in nuclear protein extracts were detected by immunoblot.

Biochem Biophys Res Commun. 2018 Oct 2; 504(2): 538-544
The effects of IS on bone formation and the expression of bone formation-related genes in osteoblasts

Indoxyl sulfate (IS) and p-cresyl sulfate (PCS) promote calcification in the aorta and peripheral arteries.

CKD rats exposed to vehicle, IS, or PCS.
**Osteoclast differentiation** (**NFATc1**)

**Figure 1.** Indoxyl sulfate has varying effects on AhR and NFATc1 levels depending on dose and time. (A and B) On day 3, the highest expression of intranuclear AhR and intracellular NFATc1 occurred at 100 micromolar indoxyl sulfate. (C and D) On day 6, the expression of AhR and NFATc1 have an inverse relationship with the dose of indoxyl sulfate. (nuclear factor of activated T cells (NFAT) c1)
Figure 2. The effects of indoxyl sulfate on the proliferation of osteoclasts at 48 hours. From using a fluorenced based CCK-8 kit, increasing indoxyl sulfate will lead to a dose-dependent increase in proliferation of osteoclasts until 100 micromolar at 48 hours before developing a negative correlation. Addition of CH223191 (AhR antagonist) will abolish the original increase in the proliferation of osteoclasts observed with indoxyl sulfate. * P < 0.05
**Osteoclast activity**

A. Day 3

![Bar chart showing TRAP activity levels on day 3 for different IS concentrations with and without CH223191.](image)

B. Day 5

![Bar chart showing TRAP activity levels on day 5 for different IS concentrations with and without CH223191.](image)

**Figure 3.** The effects of indoxyl sulfate on TRAP activity of osteoclasts are *dose-dependent* and *time-dependent*. (A and B) On day 3, the highest TRAP activity of osteoclasts occurred at 250 micromolar IS. However, the addition of CH223191 will decrease the activity of TRAP activity in all IS concentrations. (C and D) On day 6, the highest TRAP activity of osteoclasts occurred at 100 micromolar IS, and similarly, addition of CH223191 will decrease TRAP activity. The staining images were observed with randomly different fields under the microscope to calculate the densitometric sum and area sum of TRAP+ cells in each group by Axio Imager with Axio cam MRc (Carl Zeiss, Germany).

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2019 KSN, poster
Impact of Indoxyl Sulfate and p-Cresol Sulfate on CKD and Mitigating Effects of AST-120

Protein bound uremic toxins (PBUTs)

Liu, Tominos & Lu
Toxins (Basel), 2018 Sep 11; 10(9)
Vit-D deficiency

Low serum $25\text{(OH)}D_3$

Quantity $\downarrow$ & Quality $\downarrow$
25-OH-D₃ increase mineralization front (Osteoblast) and osteoclast in renal osteodystrophy

Kidney International 1979; 15: 196-204
Treatment with 25-OH-D$_3$ for 86 wks

endosteal fibrosis↓ osteoid↓

active bone (High turnover-SHPT)

增加類骨質鈣化 减低纖維變性
降低骨吸收

Resorption of woven bone is necessary

Lamellar Bone

Woven Bone (SHPT)

吸收編織骨 製造優良骨
Treatment with 25-OH-D$_3$ for 86 wks
mineral appositional rate↑, osteoid mineralization↑
inactive bone (Low turnover-ABD)

Rescue osteoblast viability is necessary

Mineralized bone

Osteoid

Mineralized bone
Native vitamin D and bone turnover disorders

Lu, et al.
Clinica Chimica Acta
2016 Jan; 453: 1-12
Trends in Hip Fracture Rates in US HD Patients
1993-2010 Medicare data

Unadjusted fracture rates by site, age, and treatment group

Cinacalcet ↓ Fractures in Hemodialysis

Sharon M. Moe et al. JASN 2015; 26: 1466-1475
PTH, Ca, and Pi Concentrations in HD Patients Receiving Cinacalcet or Etelcalcetide

- **A** Parathyroid hormone concentrations
- **B** Calcium concentrations
- **C** Phosphate concentrations
- **D** Parathyroid hormone concentrations change from baseline

*JAMA* 2017; 317(2): 156-164
Discovery of Evocalcet

Cinacalcet hydrochloride (1)
Human CaSR (Ca^{2+}) EC_{50}: 0.18 \mu M
CYP2D6 IC_{50}: <0.1 \mu M
Adverse effects on the gastrointestinal tract
(e.g., nausea and vomiting)

Evocalcet

Addition of steric bulk around the NH group

(S)-pyrrolidine moiety

Nature 2016 Jan 14; 529(7585): 195-9
Evocalcet v.s. Cinacalcet in Japanese HD pts with SHPT

**GastroIntestinal-related adverse events**

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Percent</th>
<th>(95% CI)</th>
<th>Favors evocalcet</th>
<th>Favors cinacalcet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal-related adverse events</td>
<td>-14.2</td>
<td>(-20.9, -7.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>-6.9</td>
<td>(-11.7, -2.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>-3.2</td>
<td>(-7.8, 1.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>-6.6</td>
<td>(-10.7, -2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>-0.9</td>
<td>(-3.6, 1.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal distension</td>
<td>-1.6</td>
<td>(-3.4, 0.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Evocalcet suppresses the parathyroid cell function with little effect on the GI tract or CYP isozymes

- Evocalcet showed no significant inhibitory effects on the specific activities of any CYP isozymes other than CYP2D6.
Effects of a calcimimetic (CM) in bone histomorphometric parameters of nephrectomized (Nx) rats and Nx rats with parathyroidectomy (PTX) and PTH infusion 6-fold the usual replacement dose

Kidney Int. 2019 May; 95(5): 1064-1078
The administration of a calcimimetic (CM) triggered Erk1/2 phosphorylation (P-ERK1/2) of osteoblasts

OB proliferation 6-fold the usual replacement dose

Kidney Int. 2019 May; 95(5): 1064-1078
The severe secondary hyperparathyroidism of CKD is mainly characterized by skeletal resistance to its action.

Direct bone effects of calcimimetics in chronic kidney disease?
Cinacalcet improves both trabecular and cortical bone microarchitecture (CKD mice)
Cinacalcet increases femoral bone structural properties in 5/6 nephrectomy CKD mice.
Cinacalcet inhibits osteoclast survival, increases osteoclast Wnt 10b expression
Cinacalcet increases osteoclast Wnt 10b expression and improves mineralization

Figure 5.
Toluidine blue staining of CKD mice femur with and without cinacalcet treatment

Int J Mol Sci. 2019 under revise
TRPV6 mediated $\text{Ca}^{2+}$ absorption is attenuated by CaSR activation.

**A**

[Graph showing net $J_{\text{Ca}^{2+}}$ (nmol/h/cm$^2$) for TRPV6WT/WT and TRPV6D541A/D541A mice before and after Cinacalcet treatment.]

**B**

[Graph showing net $J_{\text{Ca}^{2+}}$ (nmol/h/cm$^2$) for TRPV6WT/WT and TRPV6D541A/D541A mice under high and low Ca$^{2+}$ conditions.]

* JCI Insight. 2019 Apr 23; 5
Early CKD
Bone formation ↓↓
Bone resorption ↓
Non-Ca PB, Renamizeine
Bone formation ↑↑
Bone resorption ↑
Elasticity↑, BMD ↔↑
DKK1/SOST Nab
Bone formation ↑
Bone resorption ↓
± HCO₃⁻

Progress CKD
Bone formation ↓↓
Bone resorption ↑
Normonatremia
Bone resorption ↓
Hcy, ± HCO₃⁻
Bone formation ↑
Bone resorption ↓

Low PTH
iPTH < 100~150 pg/mL + BAP ↓L1/4
Bone formation ↓↓↓
Bone resorption ↓↓
Native Vit-D₃/25-D₃
Bone formation ↑
Bone resorption ↑/↓
Teriparatide
Bone formation ↑↑
Bone resorption ↑
Calcilytics (MK-5442)
Bone formation ↑↑
Bone resorption ↑
Adequate dialysis
Biocompatible dialyzer

High PTH
iPTH ↑x6 ULN + BAP↑
Bone formation ↑↑
Bone resorption ↑↑
N/A Vit-D₃/25-D₃
Bone formation ↑
Bone resorption ↓/↑
Cinacalcet
Bone formation ↑
Bone resorption ↓
Bisphosphonate
Bone formation ↓
Bone resorption ↓
Denosumab
Bone formation ↓/↔
Bone resorption ↓

SERM
Bone formation ↓
Bone resorption ↓↓
Odanacatib
Bone formation ↓
Bone resorption ↓↓
Sarcatinib
Bone formation ↓
Bone resorption ↓↓

Treatment of Bone loss in CKD

Lu et al. Bone 2016; 87: 57-70
Fu-Jen Catholic University Hospital
New Taipei City, Taiwan

Thanks for your attention.