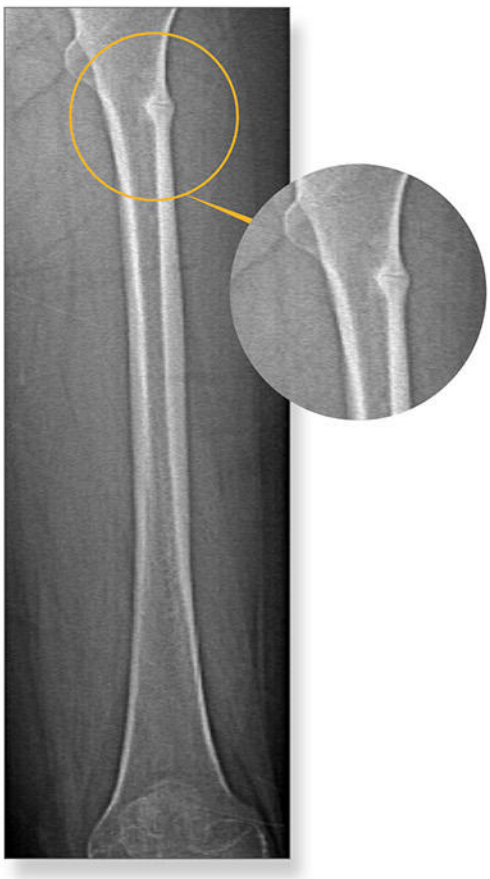
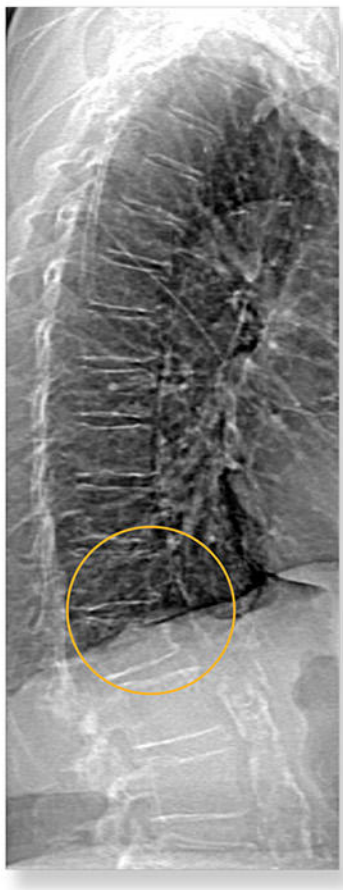
HOLOGIC®
The Science of Sure

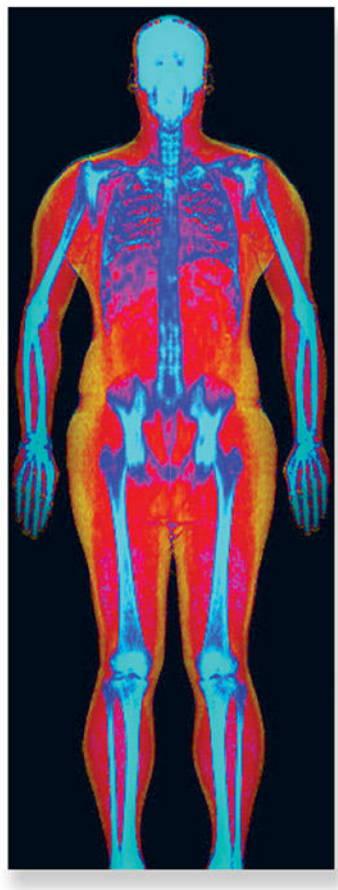
Powerful images.
Clear answers.



Manage Patient's concerns about Atypical Femur Fracture*



Vertebral Fracture Assessment – a critical part of a complete fracture risk assessment



Advanced Body Composition® Assessment – the power to see what's inside

Contact your Hologic rep today at insidesales@hologic.com

*Incomplete Atypical Femur Fractures imaged with a Hologic densitometer, courtesy of Prof. Cheung, University of Toronto

www.hologic.com | info@hologic.com | 1.781.999.7300

ORIGINAL ARTICLE

JBMR®

Poor Vitamin K Status Is Associated With Low Bone Mineral Density and Increased Fracture Risk in End-Stage Renal Disease

Pieter Evenepoel,[1,2] Kathleen Claes,[1,2] Bjorn Meijers,[1,2] Michaël Laurent,[3] Bert Bammens,[1,2] Maarten Naesens,[1,2] Ben Sprangers,[1,2] Hans Pottel,[4] Etienne Cavalier,[5] and Dirk Kuypers[1,2]

[1]Laboratory of Nephrology, Department of Microbiology and Immunology, Katholieke Universiteit (KU) Leuven, Leuven, Belgium
[2]Department of Nephrology, University Hospitals Leuven, Leuven, Belgium
[3]Centre for Metabolic Bone Diseases, KU Leuven, Leuven, Belgium
[4]Department of Public Health and Primary Care, KU Leuven Campus Kulak Kortrijk, Kortrijk, Belgium
[5]Clinical Chemistry, Université de Liège, Liège, Belgium

ABSTRACT

Chronic kidney disease and osteoporosis are major public health problems associated with an aging population. Vitamin K insufficiency is prevalent among patients with end-stage renal disease (ESRD). Preliminary data indicate that poor vitamin K status may compromise bone health and that increased inflammation may be in the causal pathway. We performed an ancillary analysis of data collected in the frame of prospective observational cohort studies exploring various aspects of bone health in de novo renal transplant recipients to investigate the association between vitamin K status, inflammation, bone mineral density, and incident clinical fractures. Parameters of mineral metabolism (including biointact PTH and FGF23, sclerostin, calcidiol, calcitriol) and inflammation (CRP and IL-6), osteoprotegerin, bone turnover markers (P1NP, BsAP, and TRAP5B), and dephosphorylated-uncarboxylated Matrix Gla Protein (dp-ucMGP) were assessed on blood samples collected immediately prior to kidney transplantation in 468 patients. Areal bone mineral density (aBMD) was measured at the lumbar spine and femoral neck by dual-energy X-ray absorptiometry within 14 days posttransplant. Poor vitamin K status, defined by dp-ucMGP >500 nmol/L, was highly prevalent (90%). High dp-ucMGP levels independently associated with elevated inflammatory markers and low aBMD. No associations were observed between vitamin K status and bone turnover markers. During a median follow-up of 5.1 years, 33 patients sustained a fragility fracture. In Cox-proportional hazards analysis, a dp-ucMGP above median associated with incident fractures, independent of classical determinants, including age, gender, history of fracture, and aBMD (HR 2.21; 95% CI, 1.00 to 4.91; $p < 0.05$). In conclusion, poor vitamin K status associates with inflammation and low aBMD in patients with ESRD and confers an increased risk of incident fractures in de novo renal transplant recipients. © 2018 American Society for Bone and Mineral Research.

KEY WORDS: DXA ANALYSIS/QUANTITATION OF BONE; BIOCHEMICAL MARKERS OF BONE TURNOVER; BONE MODELING AND REMODELING; OSTEOPOROSIS; DISEASES AND DISORDERS OF/RELATED TO BONE

Introduction

Chronic kidney disease (CKD) and osteoporosis are major public health problems associated with an aging population. Patients with CKD suffer an excessively high fracture risk. The fracture risk steadily increases along with the progression of renal disease to become four times as high in end-stage renal disease (ESRD) patients as compared to healthy controls.[1] The relative risk increases by another 30% during the first 3 years after transplantation.[2] Traditional risk factors largely fail to explain the excessive risk. This points to the existence of CKD-specific risk factors, including disturbances of mineral metabolism, uremic toxins, hypogonadism, and metabolic acidosis. We hypothesize that vitamin K insufficiency may be another candidate CKD-related risk factor for fractures.

Vitamin K is a fat soluble vitamin that is not only vital for blood coagulation, but also may play an important role in maintaining bone health. Indeed, an increasing number of studies associate poor vitamin K status with compromised bone health.[3,4] A positive association between dietary intake of vitamin K and areal bone mineral density (aBMD), as assessed by dual-energy X-ray absorptiometry (DXA), has moreover been observed in several cohort studies in the general population.[4,5] Finally, low

Received in original form June 29, 2018; revised form September 4, 2018; accepted October 6, 2018. Accepted manuscript online Month 00, 2018.
Address correspondence to: Pieter Evenepoel, MD, PhD, Division of Nephrology, University Hospitals Leuven, Herestraat 49, B-3000 Leuven, Belgium. e-mail: Pieter.Evenepoel@uz.kuleuven.ac.be
Additional Supporting Information may be found in the online version of this article.
Journal of Bone and Mineral Research, Vol. xx, No. xx, Month 2018, pp 1–8
DOI: 10.1002/jbmr.3608

intake of vitamin K has been associated with an increase in the risk of hip fracture, and treatment with vitamin K may reduce the relative risk for hip fractures.[6]

CKD is increasingly recognized as a state of (severe) vitamin K insufficiency.[7–11] Data on the association between vitamin K status and bone health in CKD patients are limited. In a recent cohort study in dialysis patients, vitamin K insufficiency was identified as the strongest predictor of vertebral fractures.[12,13]

Systematic vitamin K status can be determined either by measuring circulating vitamin K1 (phylloquinone) and K2 (menaquinones) levels (direct assay) or by measuring circulating inactive forms of vitamin K–dependent proteins, including osteocalcin (OC) and matrix Gla protein (MGP) (indirect assay). Because measuring vitamin K in plasma is hampered by low circulating levels and interference of lipids and smoking status, indirect methods to assess vitamin K status are generally preferred. The proportion of circulating OC that is not γ-carboxylated may not be an ideal circulating marker of vitamin K status in advanced CKD, because of bias inferred by variable bone resorption that occurs with underlying secondary hyperparathyroidism and the potential for retention of OC fragments in the setting of reduced kidney function.[8] A recent comparison of the four different MGP assays available (dephosphorylated-uncarboxylated Matrix Gla Protein [dp-ucMGP], dephosphorylated-carboxylated MGP [dp-cMGP], total ucMGP, and total dpMGP) indicated that circulating dp-ucMGP levels may clinically be the most relevant.[14]

The mechanisms by which vitamin K might protect against bone fracture remain ill-defined. It is suggested that vitamin K may preserve both bone quantity and quality, eg, by suppressing bone turnover, but available evidence is controversial.[15] Data from large epidemiological studies, moreover, indicate that inflammation may be in the causal pathway between poor vitamin K status and increased fracture risk.[16,17]

The present study was designed to test the hypothesis that vitamin K insufficiency confers an increased fracture risk in patients with ESRD referred for renal transplantation. A secondary objective was to investigate the association between vitamin K status, inflammation, bone turnover, and aBMD in ESRD.

Patients and Methods

Design

This is an ancillary analysis of data collected in the frame of other prospective observational cohort studies exploring various aspects of bone health in de novo renal transplant recipients (NCT00547040, NCT01886950).

Study population

Adult patients (age >18 years) with ESRD referred for single kidney transplant at the University Hospital Leuven between April 23, 2006, and December 21, 2013, and who consented to participate in our renal allograft protocol biopsy program, were eligible for inclusion in this cross-sectional observational cohort study. Only patients with available DXA scan within 2 weeks following transplantation and available archived, unthawed serum (at least 1 mL aliquot) were eligible for inclusion in this study (Supporting Fig. 1). The cohort entry date (index date) was the date an individual received their kidney transplant. Standard maintenance immunosuppressive therapy consisted of corticosteroids, a calcineurin inhibitor and an antimetabolite (mycophenolate mofetil), with corticosteroids halted at 3 months in patients with low immunologic risk and absence of signs of acute rejection on protocol allograft biopsy. Supporting Table 1 shows the exposure to immunosuppressive drugs (including glucocorticoids) and mineral metabolism therapy at the time of transplantation and up to 5 years. Cumulative methylprednisolone exposure amounted to 2.3 ± 0.9 and 6.0 ± 3.5 g, at 1 and 5 years posttransplant.

The study adhered to the principles of the Declaration of Helsinki and was approved by the ethical committee of the KU Leuven. All patients provided written informed consent.

Clinical data

Relevant demographics and comorbidities, mineral metabolism therapy, and routine biochemistry were extracted from electronic files. Data obtained after graft failure were censored. Several general risk factors for fracture (eg, age, sex, and prior fragility fracture) as well as transplant-specific risk factors were assessed including length of time on dialysis prior to transplant, cause of ESRD, and pretransplant dialysis modality.

Laboratory measurements

Blood samples were collected at the time of admission for the renal transplant procedure (random, non-fasted). Samples were stored for <2 hours at 5°C until centrifugation. Upon arrival at the laboratory, the blood samples were centrifuged at 1900 g for 10 min, aliquotted, and either processed immediately (standard techniques) or stored at –80°C until analysis. Creatinine, hemoglobin, total calcium, phosphate, magnesium, total alkaline phosphatase, and albumin were measured using standard laboratory techniques. $1,25(OH)_2D_3$ (calcitriol), $25(OH)D_3$ (calcidiol), and full-length (biointact) parathyroid hormone (PTH) were determined by immunoradiometric assays, as described.[18–20] Alkaline phosphatase levels were expressed as times upper normal limit to harmonize for the various assays being used for the duration of the study.

Serum sclerostin (TECO Medical, Sissach, Switzerland; reference range [RR]: 450 ± 150 pg/mL in men, 510 ± 140 pg/mL in premenopausal women, and 590 ± 130 pg/mL in postmenopausal women), biointact fibroblast growth factor 23 (FGF23) (Kainos Laboratories, Inc., Tokyo, Japan; RR: 8 to 78 pg/mL), osteoprotegerin (OPG; Biomedica, Vienna, Austria; median of a healthy population: 2.7 pmol/L), soluble receptor activator of nuclear factor kappa-B ligand (sRANKL; Biomedica, Vienna, Austria; median of a healthy population: 0.14 pmol/L) were measured according to the manufacturers' instructions. Interleukin 6 (IL-6) was measured on a MESO QuickPlex SQ120 multiplex imager (Meso Scale Discovery, Rockville, MD, USA) using a electrochemiluminescence multiplex immunoassay (Human Proinflammatory I-4plex; Meso Scale Discovery) according to the manufacturer's instructions. Bone-specific alkaline phosphatase (BsAP; RR: 7.9 to 25.5 μg/L in men, 6.1 to 22.2 μg/L in premenopausal women, and 7.1 to 23.9 μg/L in postmenopausal women), trimeric ("intact") N-terminal propeptide of type I collagen (P1NP; RR: 12.8 to 71.9 μg/L in men, 13.7 to 71.1 μg/L in premenopausal women, and <82.6 μg/L in postmenopausal women), and tartrate-resistant acid phosphatase isoform 5b (TRAP5B; RR: 1.4 to 6.1 U/L in men, 1.2 to 4.8 U/L in premenopausal women, and 1.1 to 6.9 U/L in postmenopausal women) were measured with the IDS iSYS instrument (IDS, Boldon, UK). These cutoffs are obviously method-dependent because large inter-method variations have been observed in

CKD patients.[21] All the coefficients of variation of the assays used in this study were <10%.

Vitamin K status was assessed by measuring dp-ucMGP in EDTA plasma using a commercial dual-antibody enzyme-linked immunoassay (InaKtif MGP, iSYS; IDS, Boldon, UK). In this assay, the capture antibody is directed against a nonphosphorylated MGP epitope comprising amino acids 3 to 15 and the detection antibody against an uncarboxylated MGP epitope that includes amino acids 35 to 49, as described.[22] Vitamin K insufficiency was defined as dp-ucMGP level >500 pmol/L.[14]

Because the timing of the deceased kidney transplantation is not predictable, blood samples collected at the time of admission for the renal transplant procedure were random. All samples were stored for <2 hours at 5°C until centrifugation. Upon arrival at the laboratory, the blood samples were centrifuged at 1900 g for 10 min, aliquotted, and either processed immediately (routine analyses) or stored at –80°C (additional batch analyses).

Bone densitometry

aBMD measurements were performed within 2 weeks after transplantation by DXA using a Hologic Discovery® densitometer (Hologic QDR-4500A; Hologic Inc., Marlborough, MA, USA) within 2 weeks after engraftment at the total lumbar spine (L_1 through L_4), total hip (TH), and femoral neck (FN). All DXA scans were analyzed by a single certified and highly experienced operator. Results were expressed as absolute BMD g/cm^2, as *T*-score (standard deviation [SD] relative to young healthy adults), and as *Z*-score (SD relative to age- and gender-matched controls). Osteopenia was defined as a *T*-score of –1 to –2.4 and osteoporosis as a *T*-score ≤ –2.5.

Outcomes

Kidney transplant recipients were followed from the date of transplant until first fracture, graft failure, death, or end of follow-up (defined as date of last visit prior to June 14, 2016). The primary outcome was major fragility fracture, which was defined as a composite of hip, leg, ankle, forearm, proximal humerus, rib, and clinical vertebral fractures occurring in the absence of major trauma. Fractures were identified in the electronic files using International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes for fractures, with exclusion of phalangeal and skull fractures. Fractures were adjudicated by one investigator (PE) through review of radiology, hospitalization, and/or surgery reports. Traumatic fractures were excluded. Patients were not screened for (asymptomatic) vertebral fractures by routine imaging. In the event of multiple fractures in the same patient, we considered first listing of a fracture specific ICD-9-CM code as the fracture event.

Statistical analysis

Results were expressed as mean ± SD or median (interquartile range), as appropriate. Patients were categorized according to tertiles of plasma dp-ucMGP. Differences between groups were evaluated using the Mann-Whitney *U* test as appropriate or ANOVA. Categorical data were compared between groups using chi-square test.

The femoral neck was the preferred skeletal site to investigate the association between aBMD and incident fractures because (i) it is part of routine DXA assessment, and (ii) because its measurement is less prone to bias caused by either bone deformities, vascular calcification, or presence of arteriovenous fistula.[23]

Independent determinants of dp-ucMGP were identified by linear regression analysis. Nonparametric distributed analytes, including dp-ucMGP, FGF23, PTH, and calcitriol, were ln-transformed to achieve normality for the regression analyses.

The fracture incidence rate (per 1000 person-years) was calculated censoring the observation period on the date of death, first fracture, or end of follow-up. The Cox proportional hazards model was used to assess effects of vitamin K status (both as continuous and categorical [above versus below median] variable), DXA classification (*T*-scores at FN, categorized according to WHO criteria), history of pretransplant fracture, gender, and recipient age on the hazard of the first fractures. Given the limited fracture rate, the number of variables in the final hazards model was restricted to five. Fracture-free survival was calculated according to Kaplan-Meier, with the first fracture that occurred taken as an event and with separation by the vitamin K status classification (above versus below median). SAS version 9.4 (SAS Institute, Cary, NC, USA) was used for the statistical analysis. Two-sided $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics

A total of 468 de novo renal transplant recipients were included in the present analysis. Most patients were white. Mean ± SD age was 54.7 ± 12.7 years; 60.5% were men. Mean ± SD body mass index was 24.8 ± 4.4 kg/m^2; 18.8%, and 40.0% of patients had a history of diabetes mellitus and cardiovascular disease, respectively. Primary renal diagnosis was diabetic nephropathy in 9.6%; glomerulonephritis or vasculitis in 24.8%; interstitial nephritis in 8.1%; hypertensive or large vessel disease in 3.4%; cystic, hereditary, or congenital disease in 24.2%; miscellaneous in 6.4%; and unknown or missing in 23.5% of patients; 72.9% of the patients were treated with hemodialysis, whereas 24.1% were on peritoneal dialysis. Median dialysis vintage was 32 months. Fourteen patients were scheduled for preemptive renal transplantation. Calcium and non-calcium containing phosphate binders were administered to 66.7% and 37.4% of the patients, respectively. Nutritional and active vitamin D supplements were administered to 40.6% and 46.0% of the patients, respectively. Forty-eight patients (10.4%) were treated with cinacalcet. Five percent of patients were treated with vitamin K antagonists.

Median plasma dp-ucMGP was 1150 nmol/L and 421 patients (90%) had elevated (>500 nmol/L) dp-ucMGP levels, indicating vitamin K insufficiency. Table 1 shows demographics and clinical characteristics across tertiles of dp-ucMGP. Patients in the highest tertile of dp-ucMGP were characterized by higher age and BMI and longer dialysis vintage. Of note, plasma triglycerides did not differ across dp-ucMGP tertiles.

dp-ucMGP and laboratory parameters of bone and metabolism

Patients in the highest tertile of dp-ucMGP were characterized by higher sclerostin and OPG (Table 1). Calcium, phosphate, PTH, FGF23, 25(OH)D_3, 1.25$(OH)_2D_3$, and bone turnover markers (trimeric P1NP, TRAP5B, and BsAP) did not differ across tertiles of dp-ucMGP. In univariate regression analysis, both sclerostin and OPG directly associated with dp-ucMGP. In multivariable

Table 1. Demographics, Clinical Variables, Laboratory Parameters, and Areal Bone Mineral Density in Patients Categorized According to dp-ucMGP Tertiles

	All ($n = 468$)[a]	Low Tertile 1 dp–ucMGP 300–862 ($n = 155$)	Mid Tertile 2 dp–ucMGP 864–1447 ($n = 154$)	High Tertile 3 dp–ucMGP 1465–10717 ($n = 159$)	ANOVA or χ^2
Demographics and relevant clinical variables					
Age (years)	54.7 ± 12.7	51.6 ± 14.4	54.7 ± 11.9	57.6 ± 11.0	0.002
Weight (kg)	71.9 ± 15.0 ($n = 449$)	69.1 ± 14.2	74.0 ± 15.8	72.8 ± 14.5	0.02
BMI (kg/m²)	24.9 ± 4.4 ($n = 336$)	23.9 ± 4.0	25.3 ± 4.5	25.4 ± 4.4	0.005
CVD (%)	40.0	32.5	30.5	56.0	<0.0001
DM (%)	18.8	12.3	18.2	25.8	0.01
Prevalent fracture (%)	5.8	5.2	3.3	8.2	0.2
Incident fracture (%)	7.1	4.6	6.5	10.1	0.2
Dialysis vintage (months)	31.8 [17.4–49.6]	31.5 [15.1–47.7]	25.3 [17.0–49.0]	37.1 [21.5–51.7]	0.02
Vitamin K antagonist (%)	4.8	5.3	1.3	7.6	0.03
Laboratory parameters of mineral metabolism, bone turnover, and inflammation					
Creatinine (mg/dL)	7.87 ± 2.87 ($n = 465$)	7.19 ± 2.79	8.30 ± 3.02	8.20 ± 2.70	0.004
Triglycerides (mg/dL)	185 ± 149 ($n = 461$)	185 ± 166	189 ± 121	182 ± 156	0.4
Total cholesterol (mg/dL)	172 ± 41 ($n = 462$)	179 ± 44	172 ± 39	167 ± 82	0.06
Phosphate (mg/dL)	4.4 ± 1.4 ($n = 460$)	4.3 ± 1.5	4.7 ± 1.5	4.4 ± 1.4	0.03
Calcium (mg/dL)	9.2 ± 0.8 ($n = 462$)	9.3 ± 0.7	9.1 ± 0.7	9.2 ± 0.8	0.3
Magnesium (mg/dL)	2.3 ± 0.4 ($n = 434$)	2.3 ± 0.4	2.3 ± 0.4	2.3 ± 0.4	0.3
Bicarbonate (mmol/L)	25.2 ± 3.1 ($n = 460$)	25.9 ± 3.2	24.7 ± 3.1	25.1 ± 3.0	0.02
biPTH (ng/L)	131.6 [73.3–236.6] ($n = 459$)	122.6 [68.1–230.5]	144.1 [85.1–250.8]	121.5 [73.3–219.6]	0.5
25(OH)D_3 (μg/L)	36.5 [24.6–48.9] ($n = 461$)	35.7 [24.6–48.9	35.4 [22.5–45.4]	38.6 [25.4–50.8]	0.2
1.25$(OH)_2D_3$ (ng/L)	27.2 [18.9–37.7] ($n = 432$)	28.0 [19.2–37.6]	25.9 [17.0–34.1]	26.7 [19.7–38.8]	0.3
dp-ucMGP (nmol/L)	1149.8 [747.9–1738.4]	619.8 [438.2–745.0]	1144.8 [1006.3–1292.5]	2148.1 [1714.0–3076.4]	<0.0001
FGF23 (ng/L)	1984.8 [604.7–7419.7] ($n = 465$)	1527.2 [400.7–6349.0]	2652.7 [687.1–8729.7]	1920.9 [680.9–8423.1]	0.1
Sclerostin (ng/L)	1.854 [1.310–2.638] ($n = 467$)	1.743 [1.172–2.444]	1.872 [1.342–2.586]	2.012 [1.500–2.908]	0.004
OPG (pmol/L)	10.0 [7.27–13.6] ($n = 467$)	8.65 [6.69–12.37]	10.13 [7.25–13.16]	11.09 [7.98–15.26]	0.0002
sRANKL (pmol/L)	0.092 [0.063–0.163] ($n = 467$)	0.087 [0.0653–0.160]	0.093 [0.063–0.158]	0.096 [0.063–0.172]	0.3
Total alkaline phosphatase (U/L)	0.79 [0.60–1.07] ($n = 452$)	0.76 [0.63–1.03]	0.76 [0.56–1.00]	0.83 [0.63–1.13]	0.1
BsAP (ng/mL)	21.8 [15.0–33.8] ($n = 467$)	22.4 [15.6–33.8]	20.9 [14.4–32.7]	22.8 [15.6–35.5]	0.3
P1NP (μg/L)	83.3 [52.9–134.1]	83.4 [51.9–149.7]	83.8 [54.0–124.1]	79.6 [49.7–134.1]	0.8

Table 1. (*Continued*)

	All ($n = 468$)[a]	Low Tertile 1 dp–ucMGP 300–862 ($n = 155$)	Mid Tertile 2 dp–ucMGP 864–1447 ($n = 154$)	High Tertile 3 dp–ucMGP 1465–10717 ($n = 159$)	ANOVA or χ^2
	($n = 467$)				
Trap5B (U/L)	5.4 [3.8–7.5] ($n = 467$)	5.4 [3.7–7.1]	5.6 [3.8–7.5]	5.5 [3.9–7.5]	1.0
C-reactive protein (mg/L)	7.3 ± 10.9 ($n = 461$)	4.1 ± 5.3	5.8 ± 8.3	11.8 ± 14.8	<0.0001
IL-6 (pg/mL)	1.37 [0.67–2.42] ($n = 467$)	1.05 [0.48–2.17]	1.39 [0.82–2.20]	1.69 [0.78–3.15]	0.001
Bone mineral density					
LS BMD (g/cm²)	0.935 [0.828–1.056]	0.996 [0.856–1.037]	0.939 [0.833–1.062]	0.923 [0.814–1.061]	0.2
LS *Z*-score	−0.81 [−1.77 to 0.38]	−0.84 [−1.63 to 0.08]	−0.65 [−1.68 to 0.51]	−0.84 [−1.87–0.51]	0.5
LS *T*-score	−1.78 [−2.41 to −1.05]	−1.40 [−2.27 to −0.50]	−1.54 [−2.48 to −0.38]	−1.68 [−2.70 to −0.44]	0.3
$LS_{nl/osteopenia/osteoporosis}$ (%)	41.3/37.5/21.3	39.9/41.8/18.3	40.8/34.9/24.3	33.6/39.4/27.1	0.3
FN BMD (g/cm²)	0.677 [0.592–0.764]	0.694 [0.610–0.763]	0.702 [0.621–0.791]	0.637 [0.557–0.725]	<0.0001
FN *T*-score	−1.53 [−2.47 to −0.46]	−1.62 [−2.29 to −1.10]	−1.65 [−2.23 to −0.85]	−2.15 [−2.69 to −1.36]	0.0002
FN *Z*-score	−0.94 [−1.50 to −0.21]	−0.99 [−1.5 to −0.37]	−0.70 [−1.28 to −0.07]	−1.13 [−1.68 to −0.46]	0.004
$FN_{nl/osteopenia/osteoporosis}$ (%)	30.1/56.2/13.7	21.1/59.2/19.7	29.6/56.6/13.8	16.9/51.3/31.8	0.001

Values are mean ± SD, median [interquartile range], or %, as indicated.

BMI = body mass index; biPTH = biointact PTH; FGF23 = fibroblast growth factor 23; OPG = osteoprotegerin; sRANKL = soluble receptor activator of nuclear factor kappa-B ligand; BsAP = bone-specific alkaline phosphatase; P1NP = procollagen type 1 N propeptide; TRAP5B = tartrate-resistant acid phosphatase 5b; IL-6 = interleukin 6; LS = lumbar spine; nl = normal; FN = femoral neck.

[a]Unless otherwise specified.

regression analysis, sclerostin but not OPG associated with dp-ucMGP, independent of age and BMI (Table 2).

dp-ucMGP and laboratory parameters of inflammation

Patients in the highest tertile of dp-ucMGP were characterized by higher inflammation (Table 1). Correlations are depicted in Supporting Table 2. When all variables, univariately associated at $p < 0.2$, were included in the regression model, we found that age, CRP, and sclerostin were independent determinants of dp-ucMGP (Model $R^2 = 0.14$) (Table 2). Results were similar when CRP was replaced by IL-6. Of note, plasma triglycerides did not associate with dp-ucMGP levels.

dp-ucMGP and aBMD

Bone mineral density at various skeletal sites and fracture prevalence according tertiles of dp-ucMGP are shown in Table 1.

Table 2. Factors Associated With dp-ucMGP: Univariate and Multivariate Regression Analyses Using Ln(dp-ucMGP) as the Dependent Variable

	Univariate			Multivariate		
	β coefficient	*p*	*R*	β coefficient	*p*	R^2
Demographics						
Age	0.01	<0.0001	0.18	0.0075	0.002	
BMI	0.02	0.001	0.13	–	–	
Mineral metabolism						
Ln sclerostin	0.2	0.002	0.14	0.136	0.02	
Ln OPG	0.2	0.0003	0.17	–	–	
Inflammation						
Ln IL-6	0.1	<0.0001	0.18			
CRP	0.02	<0.0001	0.33	0.02	<0.0001	
						0.14

Parameters studied: age, BMI, dialysis vintage, Ln sclerostin. Only parameters univariately associated at $p \leq 0.2$ are mentioned in the table.

Ln = natural logarithm.

Table 3. Factors Associated With FN aBMD: Univariate and Multivariate Regression Analyses Using FN aBMD as the Dependent Variable

	Univariate			Multivariate		
Factors	β coefficient	*p*	*R*	β coefficient	*p*	R^2
Demographics						
Age	−0.003	<0.0001	−0.27	−0.004	<0.0001	
Gender (female 0; male 1)	0.04	0.002	0.15	0.03	0.02	
BMI	0.01	<0.0001	0.33	0.01	<0.0001	
Inflammation						
Ln IL-6	−0.006	0.3	−0.05			
CRP	−0.0006	0.3	−0.05			
Vitamin K status						
Ln dp-ucMGP	−0.03	0.002	−0.15	−0.03	0.0006	
Bone turnover markers	–	–				
Ln BsAP	−0.05	<0.0001	−0.22	–		
Ln P1NP	−0.03	<0.0001	−0.18	−0.04	<0.0001	
Ln TRAP5B	−0.06	<0.0001	−0.23	–	–	
Overall model						0.34

Parameters studied: age, gender, BMI, dialysis vintage, Ln PTH, Ln FGF23, Ln Sclerostin, Ln BsAP, Ln P1NP, Ln TRAP5. Only parameters univariately associated at $p \leq 0.2$ are mentioned in the table. Because of colinearity, only P1NP was included in the multivariable model. Findings were similar for P1NP and TRAP5b (data not shown).

Ln = natural logarithm.

Patients in the highest tertile of dp-ucMGP were characterized by lower aBMD. Correlations are depicted in Supporting Table 2. In multivariable linear regression analysis, we identified dp-ucMGP level as determinant of femoral neck aBMD, independent of age, gender, BMI, and bone remodeling activity (Model $R^2 = 0.34$) (Table 3). Results were similar at the lumbar spine, but significance was lower (data not shown, Model $R^2 = 0.18$).

dp-ucMGP and incident fractures

During an average follow-up of 5.1 years (interquartile range, 3.3 to 6.8 years), 33 patients (7.1 %) sustained a fragility fracture; this corresponds to a fracture incidence of 13.8 fractures per 1000 person-years. Median time (interquartile range) from transplantation to first fracture was 19.8 months (interquartile range, 3.1 to 42.0 months). We previously identified history of fracture and a low aBMD (both as continuous and categorical [tertiles] variable) as independent determinants of incident fractures in de novo renal transplant recipients (Evenepoel and colleagues, unpublished data).

Circulating levels of dp-ucMGP, but not inflammatory markers, were higher in patients who sustained an incident fracture (Supporting Table 3). In Cox proportional hazards analysis, a dp-ucMGP above median associated with incident fractures, even after adjustment for classical determinants, including age, gender, previous fracture, and *T*-score at the FN (categorized according to WHO criteria) (HR 2.21; 95% CI, 1.00 to 4.91; $p < 0.05$). Kaplan-Meier analysis showed that patients with dp-ucMGP levels above the median have a significant higher risk to sustain a fracture (Fig. 1).

Discussion

Data from the present observational cohort study indicate the vitamin K insufficiency (i) is common in patients with ESRD referred for renal transplantation, and (ii) associates with inflammation and low aBMD. Furthermore, our data indicate that a poor vitamin K status at the time of renal transplantation can be considered a risk factor for incident fractures.

We evaluated vitamin K status in a substantial cohort of patients with ESRD referred for renal transplantation ($n = 468$) by quantifying circulating dp-ucMGP levels. Median plasma dp-ucMGP in the present cohort amounted to 1150 nmol/L and 421 patients (90%) presented levels exceeding 500 nmol/L, currently accepted as the threshold for diagnosing vitamin K insufficiency. These figures are almost identical to those recently reported in a large cohort of prevalent renal transplant recipients, at a median of 6 years after transplantation.[24] Because the renal transplant population represents a younger subgroup with less (cardiovascular) comorbidity, one may speculate that vitamin K insufficiency is even more prominent in the overall dialysis population. Consistent with this line of reasoning, in previous cohort studies enrolling dialysis patients, dp-ucMGP levels averaged 2000 to 3000 pmol/L.[10,14] Epidemiological evidence clearly indicates that functional vitamin K insufficiency is much more prevalent in CKD patients than in the general population.[25] Underlying causes remain incompletely understood. It is suggested that

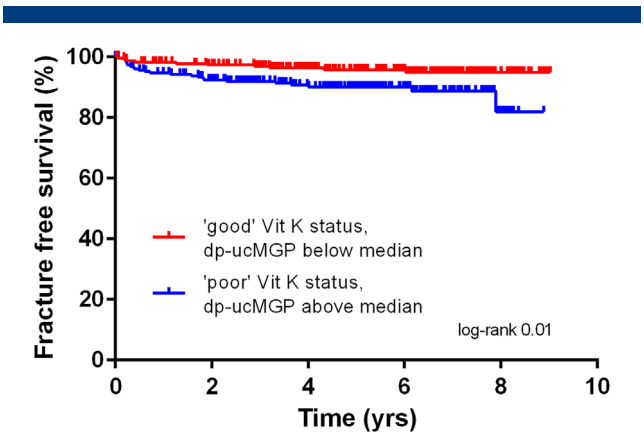


Fig. 1. Fracture-free survival according to vitamin K status (dp-ucMGP median).

both a decreased dietary intake and a decreased production by the endogenous microbiota contribute to the high prevalence of functional vitamin K insufficiency in CKD.[8,9]

In multivariable regression analyses, age, inflammation, and sclerostin were found to be independently associated with dp-ucMGP. Although associations with age and inflammatory parameters have already been reported,[26] the association between vitamin K status and circulating sclerostin levels is novel. Additional studies are required to confirm this association, and if so, to clarify its nature.

It is well established that vitamin K insufficiency plays an important role in vascular pathobiology. Indeed, experimental and clinical data show that vitamin K supplementation may attenuate the progression of vascular calcification.[27] The relationship between vitamin K and bone health has been studied less intensely. In the present study, we observed an independent association between vitamin K status and incident fractures. This observation confirms and extends data from previous studies in patients with ESRD. Kohlmeier and colleagues[13] investigated a cohort of 68 hemodialysis patient and found significantly lower vitamin K1 levels in patients with fractures (both prevalent and incident) as compared to patients free of fracture. More recently, vitamin K1 insufficiency was identified as the strongest predictor of vertebral fractures in a cohort of 387 Italian hemodialysis patients.[12] Data in the general population are not unequivocal, with some studies reporting an inverse association between vitamin K levels (or intake) and fracture risk and others reporting no association.[15] Studies evaluating the impact of vitamin K antagonists on bone health also yielded inconsistent results. According to a recent meta-analysis, vitamin K antagonists neither increase fracture risk nor reduce BMD.[28] Heterogeneity in terms of population, biomarker, and intervention (eg, vitamin K1 versus vitamin K2) or adherence issues (vitamin K antagonists) may account, at least partly, for the ongoing controversy.[15,28]

The mechanism by which vitamin K might protect against bone fracture is not known. A protective role for vitamin K has been attributed to its function as a cofactor for the posttranslational γ-carboxylation (ie, activation) of Gla containing proteins in bone, notably osteocalcin (also referred to as bone Gla protein) and MGP. The Gla residues in these vitamin K–dependent proteins are thought to confer mineral-binding properties. The specific function of these vitamin K–dependent proteins remain, however, incompletely understood and a matter of ongoing controversy.[29–31] Vitamin K may also affect bone health directly by targeting the steroid and xenobiotic receptor SXR, expressed in osteoblasts[30,32] or indirectly through the modulation of inflammation. In line with in vitro data showing that vitamin K suppresses the production of certain cytokines (including IL-6), epidemiological data revealed a negative association between vitamin K status and circulating markers of inflammation.[16] Also in the present study, high dp-ucMGP levels (indicating vitamin K insufficiency) associated directly with markers of inflammation, independent of age, BMI, and comorbidity (diabetes and cardiovascular disease). Vitamin K insufficiency may thus contribute to the microinflammatory state of ESRD and as such be involved in the pathogenesis of both vascular calcification, bone loss, and fractures.[17,33]

Few data exist on the association between vitamin K status and BMD. As in the general population,[4,34] we observed a very modest, but nevertheless significant and direct association between vitamin K status and aBMD at the central skeleton. Of interest, the association was less robust at the spine than at the femoral neck. Most plausibly, spinal osteoarthritis and aortic calcification diminish the ability to accurately assess spine BMD in the elderly and in patients with a high cardiovascular disease burden including CKD patients. This reasoning is supported by the observation of a stronger association between vitamin K and spine aBMD in a subgroup of patients free of aortic calcification (data not shown). The mechanism underlying the association between vitamin K status and aBMD remains to be clarified. As both inflammation and bone turnover (although less consistently) have been linked to BMD loss and fractures[35] and vitamin K status,[15,16] it is important to notice that the association between vitamin K and aBMD was independent of markers of inflammation and bone turnover.

A low aBMD is a predictor of fractures not only in the general population but also in patients with CKD, across stages of disease.[36] Of interest, in Cox regression analysis with incident fracture as independent variable, vitamin K status remained in the final model together with prior history of fracture. Inflammatory markers, conversely, failed to predict incident fractures. Interventional studies with vitamin K supplements, evaluating BMD or fracture as endpoint so far showed inconsistent results in the general population, and are lacking at all in the CKD population.[37,38] The latter patients may be the ideal target population given their high prevalence of (severe) vitamin K insufficiency and high fracture burden.[1]

Several strengths and limitations of the present study need to be mentioned. A major strength is the availability of DXA aBMD data together with laboratory parameters of mineral metabolism, inflammation, vitamin K status, bone turnover markers, and data on incident fractures in a substantial cohort of CKD patients. Although this study is among the largest in its kind in CKD, we acknowledge that fracture rate still is rather small, limiting statistical power and preventing subgroup analysis. Patients enrolled in the present cohort study were all renal transplant candidates and almost exclusively white. Caution is warranted when extrapolating present baseline findings to the overall ESRD population and other ethnicities. Given its observational nature, randomized trials are needed to investigate whether vitamin K supplementation can improve vitamin K status and mitigate inflammation or alter bone outcomes in CKD patients.

In conclusion, our data demonstrate that poor vitamin K status is highly prevalent among patients with ESRD and associates with inflammation and low aBMD. A poor vitamin K status at the time of renal transplantation, furthermore, can be considered a risk factor for incident fractures.

Disclosures

MRL has received lecture fees from Amgen and consultancy fees from Alexion, Kyowa Kirin, Novartis and Sandoz. PE has received lecture fees from Amgen, Vifor FMC and consultancy fees from Amgen, Medice, Vifor FMC. EC is consultant for IDS. All other authors declare no conflict of interest.

Acknowledgments

This study was financially supported by Amgen. Amgen had no role in the design of the study, nor in data analysis and data interpretation. We thank the centers of the Leuven Collaborative Group for Renal Transplantation; their clinicians, surgeons, and nursing staff; and the patients who participated in the study. We

thank A Herelixka and H Borghs for technical assistance with the data extraction.

Authors' roles: PE designed the study, collected the data, supervised the biochemical analyses and wrote the first draft of the manuscript. All co-authors contributed to the analysis of the data, and writing of the manuscript and approved the final version. In addition, EC performed part of the biochemical assays and HP assisted with the statistical analyses. PE is responsible for the integrity of the data analysis.

References

1. Jadoul M, Albert JM, Akiba T, et al. Incidence and risk factors for hip or other bone fractures among hemodialysis patients in the Dialysis Outcomes and Practice Patterns Study. Kidney Int. 2006;70(7):1358–66.
2. Ball AM, Gillen DL, Sherrard D, et al. Risk of hip fracture among dialysis and renal transplant recipients. JAMA. 2002;288(23):3014–8.
3. Shea MK, Dallal GE, Dawson-Hughes B, et al. Vitamin K, circulating cytokines, and bone mineral density in older men and women. Am J Clin Nutr. 2008;88(2):356–63.
4. Booth SL, Broe KE, Peterson JW, et al. Associations between vitamin K biochemical measures and bone mineral density in men and women. J Clin Endocrinol Metab. 2004;89(10):4904–9.
5. Bullo M, Estruch R, Salas-Salvado J. Dietary vitamin K intake is associated with bone quantitative ultrasound measurements but not with bone peripheral biochemical markers in elderly men and women. Bone. 2011;48(6):1313–8.
6. Apalset EM, Gjesdal CG, Eide GE, Tell GS. Intake of vitamin K1 and K2 and risk of hip fractures: the Hordaland Health Study. Bone. 2011;49(5):990–5.
7. Krueger T, Westenfeld R, Ketteler M, Schurgers LJ, Floege J. Vitamin K deficiency in CKD patients: a modifiable risk factor for vascular calcification? Kidney Int. 2009;76(1):18–22.
8. Holden RM, Morton AR, Garland JS, Pavlov A, Day AG, Booth SL. Vitamins K and D status in stages 3-5 chronic kidney disease. Clin J Am Soc Nephrol. 2010;5(4):590–7.
9. Cranenburg EC, Schurgers LJ, Uiterwijk HH, et al. Vitamin K intake and status are low in hemodialysis patients. Kidney Int. 2012;82(5):605–10.
10. Schlieper G, Westenfeld R, Kruger T, et al. Circulating non-phosphorylated carboxylated matrix gla protein predicts survival in ESRD. J Am Soc Nephrol. 2011;22(2):387–95.
11. Boxma PY, van den Berg E, Geleijnse JM, et al. Vitamin k intake and plasma desphospho-uncarboxylated matrix Gla-protein levels in kidney transplant recipients. PLoS One. 2012;7(10):e47991.
12. Fusaro M, Noale M, Viola V, et al. Vitamin K, vertebral fractures, vascular calcifications, and mortality: VItamin K Italian (VIKI) dialysis study. J Bone Miner Res. 2012;27(11):2271–8.
13. Kohlmeier M, Saupe J, Shearer MJ, Schaefer K, Asmus G. Bone health of adult hemodialysis patients is related to vitamin K status. Kidney Int. 1997;51(4):1218–21.
14. Cranenburg EC, Koos R, Schurgers LJ, et al. Characterisation and potential diagnostic value of circulating matrix Gla protein (MGP) species. Thromb Haemost. 2010;104(4):811–22.
15. Palermo A, Tuccinardi D, D'Onofrio L, et al. Vitamin K and osteoporosis: myth or reality? Metabolism. 2017;70:57–71.
16. Shea MK, Booth SL, Massaro JM, et al. Vitamin K and vitamin D status: associations with inflammatory markers in the Framingham Offspring Study. Am J Epidemiol. 2008;167(3):313–20.
17. Cauley JA, Barbour KE, Harrison SL, et al. Inflammatory markers and the risk of hip and vertebral fractures in men: the Osteoporotic Fractures in Men (MrOS). J Bone Miner Res. 2016;31(12):2129–38.
18. Bouillon R, Van Herck E, Jans I, Tan BK, Van Baelen H, De Moor P. Two direct (nonchromatographic) assays for 25-hydroxyvitamin D. Clin Chem. 1984;30(11):1731–6.
19. Bouillon R, De Moor P, Baggiolini EG, Uskokovic MR. A radioimmunoassay for 1,25-dihydroxycholecalciferol. Clin Chem. 1980;26(5):562–7.
20. Bouillon R, Coopmans W, Degroote DE, Radoux D, Eliard PH. Immunoradiometric assay of parathyrin with polyclonal and monoclonal region-specific antibodies. Clin Chem. 1990;36(2):271–6.
21. Cavalier E, Souberbielle JC, Gadisseur R, Dubois B, Krzesinski JM, Delanaye P. Inter-method variability in bone alkaline phosphatase measurement: clinical impact on the management of dialysis patients. Clin Biochem. 2014;47(13-14):1227–30.
22. Schurgers LJ, Barreto DV, Barreto FC, et al. The circulating inactive form of matrix gla protein is a surrogate marker for vascular calcification in chronic kidney disease: a preliminary report. Clin J Am Soc Nephrol. 2010;5(4):568–75.
23. Muxi A, Torregrosa JV, Fuster D, et al. Arteriovenous fistula affects bone mineral density measurements in end-stage renal failure patients. Clin J Am Soc Nephrol. 2009;4(9):1494–9.
24. Keyzer CA, Vermeer C, Joosten MM, et al. Vitamin K status and mortality after kidney transplantation: a cohort study. Am J Kidney Dis. 2015;65(3):474–83.
25. Riphagen IJ, Keyzer CA, Drummen NEA, et al. Prevalence and effects of functional vitamin K insufficiency: the PREVEND Study. Nutrients. 2017;9(12):1334.
26. van den Heuvel EG, van Schoor NM, Lips P, et al. Circulating uncarboxylated matrix Gla protein, a marker of vitamin K status, as a risk factor of cardiovascular disease. Maturitas. 2014;77(2):137–41.
27. Brandenburg VM, Reinartz S, Kaesler N, et al. Slower progress of aortic valve calcification with vitamin K supplementation: results from a prospective interventional proof-of-concept study. Circulation. 2017;135(21):2081–3.
28. Veronese N, Bano G, Bertozzo G, et al. Vitamin K antagonists' use and fracture risk: results from a systematic review and meta-analysis. J Thromb Haemost. 2015;13(9):1665–75.
29. Zoch ML, Clemens TL, Riddle RC. New insights into the biology of osteocalcin. Bone. 2016;82:42–9.
30. Azuma K, Shiba S, Hasegawa T, et al. Osteoblast-specific gamma-glutamyl carboxylase-deficient mice display enhanced bone formation with aberrant mineralization. J Bone Miner Res. 2015;30(7):1245–54.
31. Suzuki Y, Maruyama-Nagao A, Sakuraba K, Kawai S. Level of serum undercarboxylated osteocalcin correlates with bone quality assessed by calcaneal quantitative ultrasound sonometry in young Japanese females. Exp Ther Med. 2017;13(5):1937–43.
32. Tabb MM, Sun A, Zhou C, et al. Vitamin K2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. J Biol Chem. 2003;278(45):43919–27.
33. Viaene L, Behets GJ, Heye S, et al. Inflammation and the bone-vascular axis in end-stage renal disease. Osteoporos Int. 2016;27(2):489–97.
34. van Summeren MJ, van Coeverden SC, Schurgers LJ, et al. Vitamin K status is associated with childhood bone mineral content. Br J Nutr. 2008;100(4):852–8.
35. Panuccio V, Enia G, Tripepi R, et al. Pro-inflammatory cytokines and bone fractures in CKD patients. An exploratory single centre study. BMC Nephrol. 2012;13:134.
36. Ketteler M, Block GA, Evenepoel P, et al. Executive summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters. Kidney Int. 2017;92(1):26–36.
37. Guralp O, Erel CT. Effects of vitamin K in postmenopausal women: mini review. Maturitas. 2014;77(3):294–9.
38. Huang ZB, Wan SL, Lu YJ, Ning L, Liu C, Fan SW. Does vitamin K2 play a role in the prevention and treatment of osteoporosis for postmenopausal women: a meta-analysis of randomized controlled trials. Osteoporos Int. 2015;26(3):1175–86.