

Paricalcitol for Secondary Hyperparathyroidism in Renal Transplantation

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ABSTRACT

Secondary hyperparathyroidism contributes to post-transplant CKD mineral and bone disorder. Paricalcitol, a selective vitamin D receptor activator, decreased serum parathyroid hormone levels and proteinuria in patients with secondary hyperparathyroidism. This single-center, prospective, randomized, crossover, open-label study compared the effect of 6-month treatment with paricalcitol (1 μ g/d for 3 months and then uptitrated to 2 μ g/d if tolerated) or nonparicalcitol therapy on serum parathyroid hormone levels (primary outcome), mineral metabolism, and proteinuria in 43 consenting recipients of renal transplants with secondary hyperparathyroidism. Participants were randomized 1:1 according to a computer-generated sequence. Compared with baseline, median (interquartile range) serum parathyroid hormone levels significantly declined on paricalcitol from 115.6 (94.8–152.0) to 63.3 (52.0–79.7) pg/ml ($P<0.001$) but not on nonparicalcitol therapy. At 6 months, levels significantly differed between treatments ($P<0.001$ by analysis of covariance). Serum bone-specific alkaline phosphatase and osteocalcin decreased on paricalcitol therapy only and significantly differed between treatments at 6 months ($P<0.001$ for all comparisons). At 6 months, urinary deoxypyridinoline-to-creatinine ratio and 24-hour proteinuria level decreased only on paricalcitol ($P<0.05$). L3 and L4 vertebral mineral bone density, assessed by dual-energy x-ray absorption, significantly improved with paricalcitol at 6 months ($P<0.05$ for both densities). Paricalcitol was well tolerated. Overall, 6-month paricalcitol supplementation reduced parathyroid hormone levels and proteinuria, attenuated bone remodeling and mineral loss, and reduced eGFR in renal transplant recipients with secondary hyperparathyroidism. Long-term studies are needed to monitor directly measured GFR, ensure that the bone remodeling and mineral effects are sustained, and determine if the reduction in proteinuria improves renal and cardiovascular outcomes.

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Most functional and metabolic abnormalities of uremia that are only marginally affected by chronic dialysis therapy may substantially improve with successful kidney transplantation. However, there are exceptions. CKD mineral and bone disorders (MBDs) may even worsen post-transplant, despite restoring near-normal renal function.^{1,2} Bone mineral density (BMD) rapidly decreases in the early post-transplant period, and bone demineralization may eventually progress over years, albeit at a lower rate. This result largely explains why, in recipients of kidney transplants, the incidence of fractures is

4-fold higher than in the general population and even exceeds the incidence observed in patients on hemodialysis.^{3–5}

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Several factors involved in the pathogenesis of post-transplant CKD-MBD include immunosuppressive therapy, especially corticosteroids,⁶ hormonal disturbances, and progressive deterioration of graft function.⁷ Secondary hyperparathyroidism (SHPT) has been consistently reported to play a central role in post-transplant CKD-MBD.^{8–11} Most of the clinical and laboratory abnormalities of SHPT seem to be largely explained by enhanced intact parathyroid hormone (iPTH) production.¹² Thus, reducing iPTH levels by, for instance, supplementation of calcitriol, the active metabolite of vitamin D, has been explored as a rational approach to prevent or ameliorate SHPT.¹³ However, in patients with preterminal CKD,¹³ calcitriol therapy was found to increase serum calcium and phosphate concentrations, an effect that, in the long term, translated into accelerated vascular calcification and increased cardiovascular mortality.¹⁴ Because of these major side effects, the idea to test the expected benefits of supplementation for post-transplant CKD-MBD with natural or active forms of vitamin D on mineral metabolism was progressively abandoned.

The enthusiasm for this line of research was revived by the availability for clinical use of paricalcitol, a selective activator of vitamin D receptor, that, in patients on hemodialysis, was found to achieve a faster reduction in serum iPTH levels with less hypercalcemia compared with calcitriol,¹⁵ an effect that even associated with improved patient survival.¹⁶ Moreover, in patients with diabetic and nondiabetic chronic nephropathies, treatment with oral paricalcitol, in addition to improving mineral metabolism, was found to significantly reduce urinary protein excretion, an effect that, in the long term, is expected to translate into slower progression toward ESRD and need for RRT.^{17,18}

Thus, in this prospective, randomized, crossover study, we aimed to assess whether paricalcitol treatment may reduce iPTH levels and ameliorate the clinical and laboratory features of SHPT in recipients of renal transplant. Secondarily, we evaluated whether and to what extent this effect associated with reduced protein excretion in this population.

RESULTS

Baseline Clinical Characteristics

Of 60 screened participants, 13 participants were excluded because of iPTH < 80 pg/mL, 1 participant was excluded for hypercalcemia, and three participants were excluded when they withdrew consent before randomization. Thus, 43 participants (30 men) were randomized from September of 2009 to June of 2011 (Figure 1): 22 patients to paricalcitol followed by nonparicalcitol

therapy and 21 patients to nonparicalcitol therapy followed by paricalcitol treatment. Baseline characteristics of the two sequence groups were similar (Table 1). All participants were on cyclosporin-based immunosuppression that included mycophenolate mofetil or azathioprine. Of them, 25 participants were also on steroid therapy and similarly distributed between the two treatment arms (Table 1). No change in the immunosuppressive regimen was introduced throughout the whole study period. Sixteen patients were on renin-angiotensin system inhibitor therapy at baseline and continued their treatment without changes during the whole study period; 10 participants were receiving statins, 3 participants were on polyunsaturated fatty acids, and 17 participants were on a combination of both medications. Lipid-lowering therapy was not modified during the study. Two of seven patients who were being treated with phosphate oral supplementations at the time of screening reduced the drug dose to target serum phosphorous levels after randomization. No patient stopped or was given calcium- or noncalcium-based phosphate binders *ex novo* during the study. Two patients per sequence group withdrew from the study, all of them during the paricalcitol treatment period: two patients because of consent withdrawal and two patients because of adverse events (pulmonary carcinoma and severe anemia) considered as not

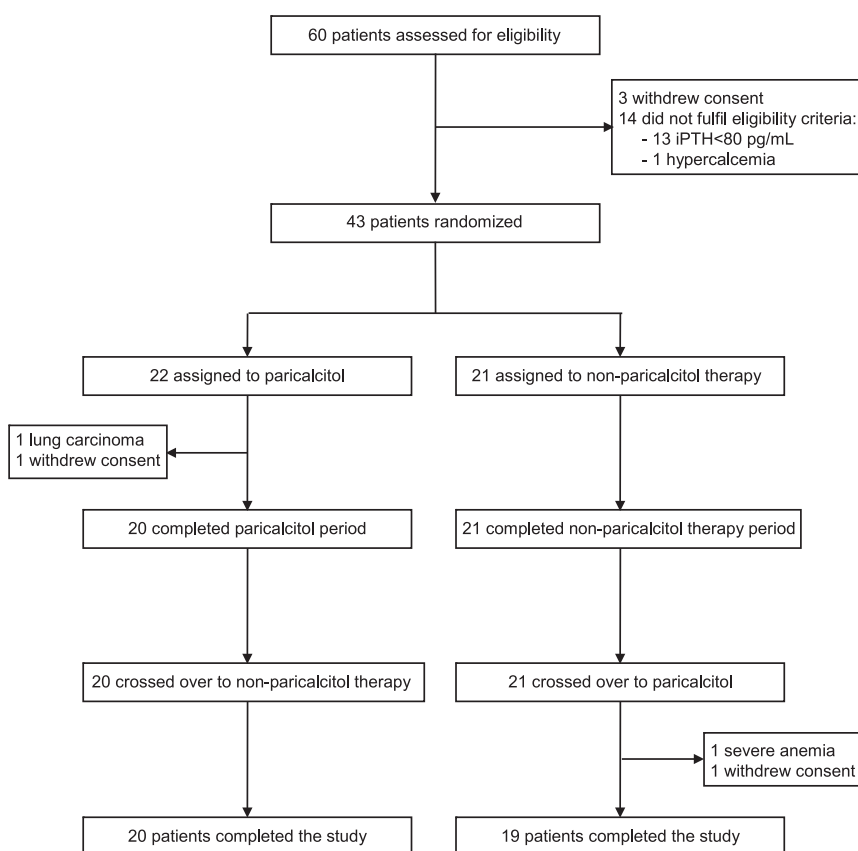


Figure 1. Study flow chart. Sixty kidney transplant recipients with secondary hyperparathyroidism and stable renal function were assessed for eligibility. Forty-three of the 60 participants were randomly assigned to paricalcitol ($n=22$) or nonparicalcitol ($n=21$) therapy.

Table 1. Baseline patient characteristics in the study group as a whole (overall) and according to treatment sequence

Parameters	Overall (n=43)	Paricalcitol to Nonparicalcitol Therapy (n=22)	Nonparicalcitol to Paricalcitol Therapy (n=21)
Age (yr)	52.3 (9.2)	53.4 (8.7)	51.2 (9.8)
Sex (men/women)	30/13	17/5	13/8
Body mass index (kg/m ²)	25.75 (4.34)	26.27 (5.06)	25.21 (3.47)
Systolic BP (mmHg)	143.72 (17.12)	145.95 (17.44)	141.38 (16.88)
Diastolic BP (mmHg)	84.01 (9.69)	82.94 (7.75)	85.13 (11.46)
Mean BP (mmHg)	103.91 (10.47)	103.94 (8.76)	103.88 (12.23)
Time since transplant (mo)	92.22 (60.90–203.63)	84.54 (59.70–198.67)	93.47 (68.01–212.63)
Serum iPTH (pg/ml)	115.60 [94.80–152.00]	104.65 [94.80–152.00]	121.40 [103.3–148.90]
Serum 25-hydroxy vitamin D (ng/ml)	17.30 [10.20–27.20]	13.90 [9.30–22.80]	20.70 [13.30–31.50]
Serum calcium (mg/dl)	9.55 (0.50)	9.59 (0.46)	9.50 (0.55)
Serum phosphate (mg/dl)	3.27 (0.57)	3.11 (0.45)	3.42 (0.64)
Serum creatinine (mg/dl)	1.39 (0.33)	1.42 (0.28)	1.37 (0.38)
Creatinine clearance (ml/min per 1.73 m ²)	65.37 (50.05–79.89)	62.44 (46.15–69.68)	72.60 (59.26–85.29)
Proteinuria (g/24 h)	0.27 (0.13–0.60)	0.25 (0.13–0.44)	0.28 (0.13–0.80)
Steroid therapy N (%)	25 (58.14)	14 (63.64)	11 (52.38)
ACEi/ARBs therapy N (%)	16 (37.21)	8 (36.36)	8 (38.10)
Diabetes mellitus N (%)	8 (18.60)	3 (13.64)	5 (23.81)

Data are mean (standard deviation) or median [interquartile range] for continuous variables and numbers (percentages) for dichotomous variables. ACEi/ARBs, angiotensin-converting enzyme inhibitors/angiotensin II type 1 receptor antagonists.

related to paricalcitol treatment (Figure 1). Thus, 39 patients completed both treatment periods.

Serum PTH Concentrations

Serum iPTH levels significantly declined during the first 3-month treatment with 1 μ g/d paricalcitol ($P<0.001$ versus baseline) and further decreased after drug dose up-titration to 2 μ g/d during the next 3 months ($P<0.001$ versus baseline) (Table 2). By contrast, iPTH concentrations did not change appreciably over 6 months of nonparicalcitol therapy. Serum iPTH levels were significantly lower on paricalcitol compared with nonparicalcitol therapy at both 3 ($P<0.001$) and 6 ($P<0.001$) months of follow-up (Figure 2). Three- and six-month differences between paricalcitol and nonparicalcitol therapies ($P=0.03$ and $P<0.001$, respectively) were confirmed by analysis of covariance restricted to the first treatment period before patient crossover. Serum iPTH levels decreased in 36 (95%) and 19 (46%) patients during 6-month paricalcitol and nonparicalcitol therapies, respectively ($P<0.001$). Twenty-nine patients had their serum iPTH levels reduced to <80 pg/ml at the end of the paricalcitol treatment period, and nine patients had their serum iPTH levels reduced to <80 pg/ml at the end of the nonparicalcitol treatment period ($P<0.001$).

Mineral Metabolism Biomarkers and Bone Remodeling

Bone Formation

As shown in Table 2, bone alkaline phosphatase (BAP) and osteocalcin serum levels significantly decreased during the first 3-month paricalcitol treatment and further declined over the next 3 months ($P<0.05$ and $P<0.001$ versus baseline for both parameters, respectively). Conversely, during the nonparicalcitol treatment period, BAP serum levels gradually increased, and the

increase approached statistical significance at 6 months ($P=0.06$) versus baseline, whereas osteocalcin levels did not change appreciably. Serum BAP and osteocalcin levels decreased in 34 (89%) and 32 (82%) patients during 6-month paricalcitol therapy and 13 (33%) and 16 (39%) patients during 6-month nonparicalcitol therapy, respectively ($P<0.001$ and $P<0.001$, respectively). Both BAP and osteocalcin concentrations were significantly lower on paricalcitol than nonparicalcitol therapy both at 3 (BAP: $P<0.005$; osteocalcin: $P<0.05$) and 6 ($P<0.001$ for both parameters) months.

Bone Reabsorption

Urine deoxypyridinoline-to-creatinine ratio progressively declined during paricalcitol treatment, and the ratio was significantly lower ($P<0.05$) at both 3 and 6 months than at baseline, whereas it did not change appreciably over the nonparicalcitol treatment period (Table 2).

Serum Concentrations of 25-Hydroxy Vitamin D, Calcium, and Phosphate

Serum levels of these parameters did not change appreciably during both treatment periods and were all comparable between treatment periods at both 3 and 6 months (Table 2).

BMD

Compared with baseline, BMD of L3 vertebra increased on paricalcitol, and the increase approximated the statistical significance ($P=0.06$) at the end of the 6-month treatment period. At variance, it did not appreciably change during nonparicalcitol therapy. Thus, at 6 months, BMD values were significantly higher on paricalcitol than nonparicalcitol therapy (1.054 ± 0.225 versus 1.010 ± 0.185 g/cm², respectively; $P=0.04$). At 6 months, L4 vertebra

Table 2. Clinical and laboratory parameters of study patients at baseline and 3 and 6 months of treatment with paricalcitol or nonparicalcitol therapy

Parameters	Baseline		3 mo		6 mo	
	Paricalcitol Yes	Paricalcitol No	Paricalcitol Yes	Paricalcitol No	Paricalcitol Yes	Paricalcitol No
iPTH and other mineral metabolism biomarkers						
Serum iPTH (pg/ml)	115.60 [94.80–152.00]		77.90 [60.50–106.50] ^{a,b}	116.45 [84.50–158.10]	63.25 [52.00–79.70] ^{a,b}	128.00 [94.00–166.40]
Serum BAP (μg/L)	12.85 (7.12)		10.23 (3.72) ^{c,d}	13.46 (7.07)	9.58 (4.42) ^{a,b}	15.00 (8.01)
Serum osteocalcin (ng/ml)	24.80 [15.40–33.20]		20.20 [14.20–36.60] ^{c,e}	22.15 [15.50–37.55]	17.60 [10.30–26.70] ^{a,b}	25.50 [15.70–38.50]
Urine deoxypyridinoline-to-creatinine ratio (nM/mM)	5.50 (2.89)		4.53 (2.37) ^c	5.08 (2.80)	4.15 (3.19) ^c	4.36 (3.25)
Serum 25-hydroxy vitamin D (ng/ml)	17.30 [10.20–27.20]		16.80 [10.60–23.60]	17.30 [9.10–28.00]	16.30 [10.30–21.00]	15.30 [8.30–24.10]
Serum calcium (mg/dl)	9.55 (0.50)		9.59 (0.44)	9.53 (0.50)	9.61 (1.14)	9.56 (0.47)
Serum phosphate (mg/dl)	3.27 (0.57)		3.34 (0.62) ^e	3.20 (0.62)	3.42 (0.71)	3.23 (0.62)
BP (mmHg)						
Systolic BP	143.72 (17.12)		141.85 (17.23)	140.37 (17.06)	139.32 (17.07)	140.85 (17.64)
Diastolic BP	84.01 (9.69)		82.78 (9.38)	83.34 (9.31)	82.26 (8.53)	83.08 (8.35)
Mean BP	103.91 (10.47)		102.46 (9.82)	102.35 (10.39)	101.28 (9.92)	102.33 (9.70)
Kidney function parameters						
Serum creatinine (mg/dl)	1.39 (0.33)		1.43 (0.34) ^e	1.36 (0.35)	1.48 (0.45) ^{c,d}	1.36 (0.33)
Creatinine clearance (ml/min per 1.73 m ²)	65.37 [50.05–79.89]		65.07 [47.79–81.09]	65.71 [50.90–87.76]	60.38 [44.10–79.24] ^{c,e}	68.11 [52.75–87.04]
Proteinuria (g/24 h)	0.27 [0.13–0.60]		0.20 [0.13–0.48]	0.22 [0.14–0.74]	0.14 [0.10–0.41] ^c	0.23 [0.10–0.54]

Data are presented as mean (standard deviation) or median [interquartile range], as appropriate.

^a*p*<0.001 versus baseline.^b*p*<0.001 versus paricalcitol No at the same time-point.^c*p*<0.05 versus baseline.^d*p*<0.01 versus paricalcitol No at the same time-point.^e*p*<0.05 versus paricalcitol No at the same time-point.

BMD showed a nonsignificant trend to increase during paricalcitol (1.046 ± 0.222 g/cm²) and decrease during nonparicalcitol therapy (1.004 ± 0.177 g/cm²) compared with baseline (1.016 ± 0.2082 g/cm²). Thus, at 6 months, BMD was significantly different between paricalcitol and nonparicalcitol treatment periods (*P*=0.05). Consistently, BMD of L2 vertebra and femoral neck also showed a significant trend to increase on paricalcitol and decrease during nonparicalcitol therapy (data not shown).

BP, Serum Creatinine, and Proteinuria

Systolic BP showed a trend to decrease during paricalcitol therapy. However, no significant changes in systolic and diastolic BPs were observed throughout both treatment periods (Table 2). Serum creatinine slightly but significantly increased and creatinine clearance decreased over the 6-month treatment with paricalcitol compared with baseline, whereas both parameters did not change appreciably during the nonparicalcitol treatment period (Table 2).

Compared with baseline, proteinuria showed a trend to decrease at month 3 of paricalcitol treatment versus baseline, and the reduction achieved nominal significance at 6 months (Figure 3, Table 2). Urinary protein did not change appreciably during the nonparicalcitol treatment period. Among 38 patients with available data throughout both 6-month treatment periods, proteinuria decreased in 25 (65.8%) and 20 (52.6%) patients and increased in 13 (34.2%) and 18 (47.4%) patients during paricalcitol and nonparicalcitol therapy, respectively (Supplemental Figure 1).

Safety

Twenty-seven (62.8%) participants had at least one adverse event during paricalcitol. Twenty-one (48.8%) participants had at least one adverse event during nonparicalcitol treatment (Table 3). Most side effects were nonserious during both periods. No serious adverse event was considered to be treatment-related. Despite the trend to more events on paricalcitol, there was no significant difference between treatments in the incidence of total, serious (including cardiovascular), and nonserious events considered as a whole or according to the involved apparatus. Hypercalcemia was observed at one single occasion in two patients on paricalcitol dose up-titration to 2 μg/d and no patients on nonparicalcitol therapy. Both events recovered with dose backtitration to 1 μg/d. Hyperphosphatemia was never observed over both treatment periods. Lung carcinoma and bilateral renal carcinoma were diagnosed in two participants during paricalcitol treatment. No acute rejection episode or pathologic bone fracture was observed throughout the study.

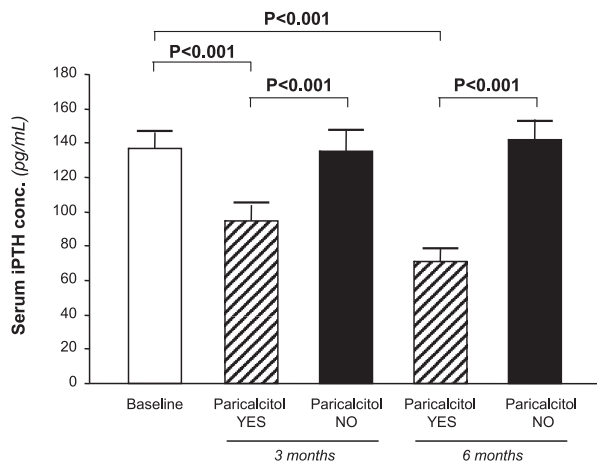


Figure 2. iPTH levels significantly decreased at 3 and 6 months of treatment with paricalcitol compared with baseline but did not change appreciably during nonparicalcitol therapy. At 3 and 6 months, PTH levels were significantly lower on paricalcitol than nonparicalcitol therapy. Data are means \pm SEMs. conc., Concentration.

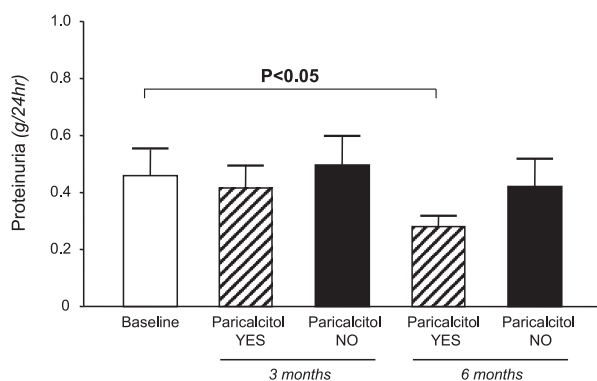


Figure 3. Effect of paricalcitol on urinary protein excretion. Proteinuria significantly decreased at 6 months of treatment with paricalcitol compared with baseline but did not change appreciably during nonparicalcitol therapy. Data are means \pm SEMs.

DISCUSSION

In this prospective, randomized, controlled clinical trial, we found that 6-month treatment with oral paricalcitol achieved a prompt and significant reduction in serum iPTH levels in recipients of renal transplants with persistent SHPT and normal or slightly reduced renal function. Treatment effect was already apparent after 3 months of treatment with 1 μ g/d paricalcitol and further increased during the subsequent 3-month treatment period after up-titration to the target dose of 2 μ g/d. Amelioration of SHPT was associated with biologic evidence of slower bone remodeling, radiologic findings suggestive for prevented bone mass loss, and reduced urinary protein excretion. Overall, treatment was well tolerated.

Despite the trend to more serious and nonserious adverse events during paricalcitol therapy (Table 3), all treatment-related adverse events were nonserious and generally mild, and patients fully recovered after treatment withdrawal.

To the best of our knowledge, this study is the first formal demonstration in the context of a prospective trial that paricalcitol therapy may ameliorate already established SHPT—and related mineral and bone abnormalities—in patients with a long-term functioning kidney graft. The idea of the trial germinated from a retrospective, uncontrolled observation that paricalcitol therapy associated with amelioration of SHPT in recipients of long-term renal allografts¹⁹ and prospective data that paricalcitol supplementation started early post-transplant reduced the 1-year prevalence of hyperparathyroidism compared with no additional therapy.²⁰ Of interest, our data extend previous evidence that paricalcitol may effectively suppress PTH production in patients with diabetic renal disease to recipients of renal transplants.²¹

Finding that controlled PTH production associated with reduced serum levels of biomarkers of bone formation, such as osteocalcin and BAP, and at the same time, reduced urinary excretion of a biomarker of osteoclastic-mediated bone reabsorption, such as deoxypyridinoline,²² provides consistent evidence that paricalcitol therapy may effectively suppress the high-turnover bone disease that characterizes SHPT. This effect could explain the improved BMD, particularly of lumbar vertebrae, documented by dual-energy x-ray absorptiometry at the end of the paricalcitol treatment period compared with nonparicalcitol therapy period. Although a confounding effect of concomitant changes in abdominal vessels calcifications on lumbar vertebral mineral density cannot be definitely excluded, particularly without evidence of concomitant improvement of femoral neck calcification, altogether, the above findings can be taken to suggest that, in the long term, paricalcitol supplementation might help prevent progressive bone mass loss and excess risk of pathologic bone fractures that invariably associate with SHPT, particularly in recipients of kidney transplants.^{3–5} Moreover, experimental data suggest that reduced osteocalcin and BAP production could help prevent vascular calcifications in this population. Indeed, osteocalcin modulates osteochondrogenic differentiation of pathologically mineralizing vascular smooth muscle cells, a crucial process in the progression of vascular calcification,²³ and in mice with arterial calcifications, BAP activity of aortic tissue correlated with the area of calcified lesion.²⁴ These findings may have clinical implications, because elevated BAP levels have been associated with increased cardiovascular morbidity and mortality in patients with preterminal or terminal CKD.^{25,26}

An ancillary finding was that amelioration of hyperparathyroidism and mineral bone metabolism was already apparent with 1- μ g daily doses of paricalcitol. This result may have clinical implications, because patients who fail to tolerate the target 2- μ g daily dose might be safely maintained on the lower dose to avoid renouncing to the potential benefit of long-term paricalcitol exposure.

Table 3. Serious and nonserious adverse events with paricalcitol or nonparicalcitol therapy during each treatment period considered separately

Adverse Events	Overall	First 6-mo Treatment Period		Second 6-mo Treatment Period	
		Paricalcitol Yes	Paricalcitol No	Paricalcitol Yes	Paricalcitol No
Total	105	50	27	16	12
Serious	17	9	4	2	2
Cardiac and vascular					
Stroke	2	2	0	0	0
TIA	1	1	0	0	0
Atrial fibrillation	1	1	0	0	0
Ischemic heart disease	1	0	0	0	1
Other vascular	3	1	0	1	1
Liver and gastrointestinal					
Hiatus hernia and gastric reflux	2	0	2	0	0
Tubulovillous adenoma	1	1	0	0	0
Neoplasm					
Renal cancer	1	1	0	0	0
Lung neoplasm	1	1	0	0	0
Metabolism and allergic					
Anemia	1	0	0	1	0
Hyperpirexia	2	1	1	0	0
Respiratory					
Pneumonia	1	0	1	0	0
Nonserious	88	41	23	14	10
Cardiac and vascular	14	9	3	0	2
Liver and gastrointestinal	5	3	0	1	1
Metabolism and endocrine	17	4	7	4	2
Nervous system	8	3	5	0	0
Respiratory	8	5	1	2	0
Skin, subcutaneous, musculoskeletal, and trauma	30	13	7	6	4
Allergic and nonspecific infection	6	4	0	1	1

TIA, transient ischemic attack.

Another message of our study is that paricalcitol supplementation may reduce urinary protein excretion in recipients of renal transplants with SHPT, whereas nonparicalcitol therapy is devoid of any specific antiproteinuric effect in this context. Although the relatively small sample size and the inclusion of patients with no or subclinical, proteinuria reduced the power to detect significant differences in urinary protein excretion between the two treatment periods, this finding seems to reflect a genuine effect of treatment for at least three reasons. (1) Solid experimental evidence shows that defective vitamin D receptor associates with albuminuria and glomerulosclerosis,²⁷ whereas receptor activation by paricalcitol supplementation is antiproteinuric and nephroprotective²⁸ through several mechanisms²⁹ that include suppressed renin transcription,^{30,31} antiproliferative and antifibrotic effects, inhibited transforming growth factor production,³² nephrin upregulation, and reduced NF- κ B activity.³³ (2) There is evidence of a specific antiproteinuric effect of paricalcitol supplementation in subjects with stage 3 or 4 CKD¹⁸ or diabetes and albuminuria.¹⁷ (3) The extent of proteinuria reduction prospectively observed in our series is consistent with that retrospectively observed in a previous uncontrolled series of recipients of renal transplants with SHPT.¹⁹ Altogether, the above findings converge to indicate

that paricalcitol may have a specific antiproteinuric effect, even in human transplantation. This effect seems to be clinically relevant, because even low-protein excretion rates ranging from 150 to 500 mg/d predict poor long-term graft survival in recipients of kidney transplants.^{34–36} Long-term controlled clinical trials are needed to assess whether paricalcitol-induced reductions in proteinuria as well as serum iPTH and BAP²⁵ levels may translate into a protective effect against renal and cardiovascular events in the long term.

Safety

Six-month treatment with oral paricalcitol was well tolerated. Although the treatment period was too short to definitely establish the safety of chronic paricalcitol therapy, finding that treatment-related adverse events were mild in nature and always reversible was reassuring. Finding that only two transient and reversible episodes of hypercalcemia were observed in two patients on paricalcitol dose up-titration to 2 μ g/d was consistent with evidence from randomized trials that, unlike supplementation with nonselective vitamin D receptor activators, such as calcitriol,¹⁴ paricalcitol therapy did not associate with an excess risk of hypercalcemia^{15,37,38} in patients on hemodialysis with SHPT. These findings are most likely explained by less

stimulation of intestinal calcium (and phosphate) absorption with paricalcitol compared with calcitriol supplementation,³⁹ an effect that, in the long term, might contribute to reduced risk for coronary artery calcification,⁴⁰ an independent cardiovascular risk factor in the kidney transplant population.⁴¹

Consistent with previous investigations in patients with CKD,^{17,42} we found that paricalcitol treatment was associated with a slight increase in serum creatinine. This finding was most likely explained by decreased creatinine tubular secretion, increased creatinine generation, or both. Worsening of kidney function was unlikely, because comparative analyses showed that activators of vitamin D receptors do not affect inulin clearance—the gold standard for the measurement of GFR.^{42–44} Thus, on the basis of the above findings, paricalcitol should not be misperceived as a potentially nephrotoxic drug, and changes in serum creatinine levels or creatinine clearance should not be taken as an indication to stop paricalcitol supplementation.

A fully unexpected finding was the diagnosis of lung carcinoma and native kidney cancer in two patients during paricalcitol therapy. We are prone to consider these findings as casual findings, because cancer has never been described as a possible paricalcitol-related side effect, and actually, experimental and epidemiologic evidence is available that vitamin D and even paricalcitol may exert specific anticancer effects.⁴⁵ Moreover, it is extremely unlikely that paricalcitol might have independently induced these neoplasms and sustained their growth to the point of becoming clinically apparent over only 6 months of exposure.

Limitations and Strengths

Major limitations of the study were the small sample size and the short-term follow-up, which do not allow us to draw definite conclusions on the risk/benefit profile of paricalcitol therapy in this clinical context. These limitations reflect the explorative nature of the trial and in part, were dictated by the resource restriction that almost invariably stifles the finalization of academic internally funded studies. Given the proof-of-concept nature of the study, paricalcitol treatment was not tested against the active metabolite of vitamin D calcitriol. Data, however, were robust enough to suggest that previous benefits of paricalcitol therapy observed in patients on chronic dialysis¹⁶ or with diabetes and albuminuria¹⁷ can be extended to recipients of renal transplants with SHPT. However, adequately powered long-term randomized clinical trials are needed to assess whether chronic paricalcitol therapy may improve the outcome of CKD-MBD in this population and is safe.

Major strengths were the prospective randomized design, the rigorous monitoring of study participants, and the generalizability of the results to a large part of the renal transplant population.

Conclusions

In recipients of renal transplants with SHPT, 6-month paricalcitol supplementation reduced PTH levels and proteinuria, attenuated bone remodeling and mineral loss, and also, reduced eGFR.

Long-term studies are needed to monitor directly measured GFR,⁴⁶ ensure that the bone remodeling and mineral effects are sustained, and determine if the reduction in proteinuria improves renal and cardiovascular outcomes.

CONCISE METHODS

Study participants were identified among >18-year-old recipients of kidney transplants referred to the Outpatient Clinic of the Unit of Nephrology at the Azienda Ospedaliera Papa Giovanni XXIII who, at baseline evaluation, fulfilled the following selection criteria: serum iPTH levels >80 pg/ml (after at least a 1-month washout period in those with previous treatment with natural vitamin D or analogs), serum calcium ≤10.2 mg/dl, serum creatinine <2 mg/dl, maintenance immunosuppressive therapy with calcineurin inhibitors and mycophenolate mofetil or azathioprine, no ongoing therapy with vitamin D analogs, and no evidence of active hepatitis C or B virus or HIV infection or drug or alcohol abuse. Patients on previous treatment with natural vitamin D or analogs were eligible if, at 1 month after treatment withdrawal and at baseline evaluation, their serum iPTH levels were >80 pg/ml (a prospective, pilot, crossover study to assess the efficacy of paricalcitol in reducing parathyroid hormone levels and ameliorating markers of bone remodeling in renal transplant recipients with secondary hyperparathyroidism [APPLE Study], ClinicalTrials.gov identifier NCT01220050, date of registration October 11, 2010). Patients with previous history of hypersensitivity, allergy, or intolerance to the study drug; changes in serum creatinine >30% or acute rejection episodes over the last 6 months; or chronic clinical conditions expected to affect completion of the study or jeopardize data interpretation as well as pregnant, lactating, or fertile women without adequate contraception were excluded. The study conformed to the principles of the Declaration of Helsinki and the Declaration of Istanbul as outlined in the “Declaration of Istanbul on Organ Trafficking and Transplant Tourism.” The hospital’s Ethics Committee approved the protocol, and all participants provided a written informed consent. The study was coordinated and monitored by the Department of Renal Medicine of the Clinical Research Center for Rare Diseases “Aldo e Cele Daccò,” Istituto di Ricerche Farmacologiche Mario Negri.

Baseline Evaluations

BP was recorded after a 5-minute rest in the sitting position as the mean of three readings 2 minutes apart. Blood was sampled in the morning after overnight fast for laboratory measurements, including routine hematochemistry, renal and liver function tests, and serum levels of iPTH, 25-hydroxy vitamin D, calcium, phosphate, and bone remodeling biomarkers osteocalcin and BAP. Twenty-four-hour urine collections were sampled for protein excretion, whereas spot morning urine samples were analyzed for the level of the index of bone reabsorption deoxypyridinoline. BMDs of lumbar spine and femoral neck were determined by dual-energy x-ray absorptiometry.

Treatment and Monitoring

Patients off therapy with any form of vitamin D for at least 1 month who fulfilled the selection criteria were randomized on a 1:1 basis to two

treatment sequences by an independent investigator (A.P.) at the coordinating center according to a computer-generated randomization list: 6-month treatment with oral paricalcitol (Zemplar; Abbvie) followed by 6 months of nonparicalcitol therapy, or 6 months of nonparicalcitol therapy followed by 6 months of paricalcitol therapy. Paricalcitol was started at 1 $\mu\text{g}/\text{d}$ and if tolerated (calcium ≤ 10.2 and phosphate ≤ 5.1 mg/dl) for 3 months, uptitrated to the target dose of 2 $\mu\text{g}/\text{d}$. To prevent the risk of adynamic bone disease, the dosage had to be backtitrated or treatment was withdrawn in case of iPTH level reduction to <50 pg/ml.⁴⁷ If serum calcium or phosphate levels exceeded 10.2 or 5.1 mg/dl, respectively, paricalcitol was reduced or withdrawn until level normalization and then restarted at 1 $\mu\text{g}/\text{d}$. Participants on oral calcium-based phosphate binders or calcium and phosphate supplementation during the screening period were maintained on their treatment without systematic changes throughout the study period. However, adjustments were allowed to maintain serum calcium and phosphorous within predefined normal range (8.7–10.2 and 2.3–5.1 mg/dl, respectively). Serum calcium and phosphate levels were also measured at 1 week after the administration of the first paricalcitol dose and after paricalcitol dose up titration. The same clinical and laboratory parameters evaluated at baseline were assessed every 3 months during both treatment periods up to study end, and any intercurrent adverse event was recorded at the same time points. BMD measurement was repeated at 6 months and the end of the study. All serious and nonserious adverse events observed during the whole study period were reported and described in detail by the investigators in patient case report forms. Data were obtained by direct interview of the patient at each study visit and revision of the clinical records. All adverse events were monitored up to their complete resolution, even after study conclusion, by the Data Monitoring Group of the Clinical Research Center, and specific queries had to be adequately addressed by the investigators whenever causes, treatments, and outcomes of any observed event were not exhaustively reported.

Measurements

Serum iPTH concentrations were measured by Immulite 2000 Intact PTH Assay (Siemens Healthcare Diagnostics), serum BAP and osteocalcin were measured by Liaison BAP Ostase and Liaison Osteocalcin assays, respectively (CLIA Assay, Saluggia, Italy), and urinary deoxypyridinoline was measured by a CLIA immunoassay and normalized to urinary creatinine concentration. BMD was measured from L2 to L4 vertebral bodies and the femoral neck and expressed in grams per square centimeter.

Sample Size Estimation

The main prespecified efficacy variable of the study was the change in iPTH levels during the 6-month paricalcitol therapy compared with the change observed during the 6-month nonparicalcitol therapy. By analogy, with patients on chronic hemodialysis, we predicted a mean 20% ($\pm 42.8\%$) reduction in serum iPTH levels during the 6-month paricalcitol therapy and no change on nonparicalcitol therapy. To show a significant difference ($\alpha=0.05$, paired t test, two-sided test) in serum iPTH changes during the two treatment periods with 80% power, 38 patients were needed for comparative analysis. To account,

conservatively, for a 10% dropout rate before study completion, we planned to include 42 patients.

Statistical Analyses

Normally and not normally distributed data are reported as means \pm SDs or medians and interquartile ranges, respectively. Binary data are summarized by counts and percentages. Primary and secondary efficacy outcomes were evaluated in the modified intention-to-treat population consisting of all randomly assigned patients who took at least one dose of study drug and had at least one efficacy measurement after the first dose of study drug, irrespective of protocol violations. The differences between treatments were assessed by repeated measures ANOVA. A mixed model ANOVA with treatment and period as fixed factors and participant as a random factor was also performed. This model had no baseline covariates, because we assumed that baseline variation was accounted for by adjustment for period and participant effects and that all effects of carryover had disappeared by the time of baseline for the second treatment period. Carryover effects were assessed by visual inspection. The robustness of the results was assessed by analysis of covariance (which included baseline values) of the primary efficacy variable restricted to the first period only before treatment crossover. Safety data were assessed in all randomly assigned patients who took at least one dose of study drug. Categorical variables were compared by McNemar test. $P<0.05$ was considered statistically significant. All statistical analyses were performed using SAS (version 9.1).

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DISCLOSURES

None.

REFERENCES

1. Pichette V, Bonnardeaux A, Prudhomme L, Gagné M, Cardinal J, Ouimet D: Long-term bone loss in kidney transplant recipients: A cross-sectional and longitudinal study. *Am J Kidney Dis* 28: 105–114, 1996
2. Julian BA, Quarles LD, Niemann KM: Musculoskeletal complications after renal transplantation: Pathogenesis and treatment. *Am J Kidney Dis* 19: 99–120, 1992

3. Grotz WH, Mundinger FA, Gugel B, Exner V, Kirste G, Schollmeyer PJ: Bone fracture and osteodensitometry with dual energy X-ray absorptiometry in kidney transplant recipients. *Transplantation* 58: 912–915, 1994
4. Ball AM, Gillen DL, Sherrard D, Weiss NS, Emerson SS, Seliger SL, Kestenbaum BR, Stehman-Breen C: Risk of hip fracture among dialysis and renal transplant recipients. *JAMA* 288: 3014–3018, 2002
5. Abbott KC, Oglesby RJ, Hypolite IO, Kirk AD, Ko CW, Welch PG, Agodoa LY, Duncan WE: Hospitalizations for fractures after renal transplantation in the United States. *Ann Epidemiol* 11: 450–457, 2001
6. Nikkel LE, Mohan S, Zhang A, McMahon DJ, Boutroy S, Dube G, Tanriover B, Cohen D, Ratner L, Hollenbeak CS, Leonard MB, Shane E, Nickolas TL: Reduced fracture risk with early corticosteroid withdrawal after kidney transplant. *Am J Transplant* 12: 649–659, 2012
7. Weisinger JR, Carlini RG, Rojas E, Bellorin-Font E: Bone disease after renal transplantation. *Clin J Am Soc Nephrol* 1: 1300–1313, 2006
8. Akaberi S, Lindergrård B, Simonsen O, Nyberg G: Impact of parathyroid hormone on bone density in long-term renal transplant patients with good graft function. *Transplantation* 82: 749–752, 2006
9. Heaf JG: Bone disease after renal transplantation. *Transplantation* 75: 315–325, 2003
10. Setterberg L, Sandberg J, Elinder CG, Nordenström J: Bone demineralization after renal transplantation: Contribution of secondary hyperparathyroidism manifested by hypercalcaemia. *Nephrol Dial Transplant* 11: 1825–1828, 1996
11. Roe SD, Porter CJ, Godber IM, Hosking DJ, Cassidy MJ: Reduced bone mineral density in male renal transplant recipients: Evidence for persisting hyperparathyroidism. *Osteoporos Int* 16: 142–148, 2005
12. Torres A, Lorenzo V, Salido E: Calcium metabolism and skeletal problems after transplantation. *J Am Soc Nephrol* 13: 551–558, 2002
13. Baker LR, Abrams L, Roe CJ, Faugere MC, Fanti P, Subayti Y, Malluche HH: 1,25(OH)₂D₃ administration in moderate renal failure: A prospective double-blind trial. *Kidney Int* 35: 661–669, 1989
14. Moe SM, Drüeke TB: Management of secondary hyperparathyroidism: The importance and the challenge of controlling parathyroid hormone levels without elevating calcium, phosphorus, and calcium-phosphorus product. *Am J Nephrol* 23: 369–379, 2003
15. Sprague SM, Llach F, Amdahl M, Taccetta C, Battle D: Paricalcitol versus calcitriol in the treatment of secondary hyperparathyroidism. *Kidney Int* 63: 1483–1490, 2003
16. Teng M, Wolf M, Lowrie E, Ofsthun N, Lazarus JM, Thadhani R: Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. *N Engl J Med* 349: 446–456, 2003
17. de Zeeuw D, Agarwal R, Amdahl M, Audhya P, Coyne D, Garimella T, Parving HH, Pritchett Y, Remuzzi G, Ritz E, Andress D: Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): A randomised controlled trial. *Lancet* 376: 1543–1551, 2010
18. Agarwal R, Acharya M, Tian J, Hippensteel RL, Melnick JZ, Qiu P, Williams L, Battle D: Antiproteinuric effect of oral paricalcitol in chronic kidney disease. *Kidney Int* 68: 2823–2828, 2005
19. Gonzalez E, Rojas-Rivera J, Polanco N, Morales E, Morales JM, Egido J, Amado A, Praga M: Effects of oral paricalcitol on secondary hyperparathyroidism and proteinuria of kidney transplant patients. *Transplantation* 95: e49–e52, 2013
20. Amer H, Griffin MD, Stegall MD, Cosio FG, Park WD, Kremers WK, Heilman RL, Mazur MJ, Hamawi K, Larson TS, Kumar R: Oral paricalcitol reduces the prevalence of posttransplant hyperparathyroidism: Results of an open label randomized trial. *Am J Transplant* 13: 1576–1585, 2013
21. Coyne DW, Andress DL, Amdahl MJ, Ritz E, de Zeeuw D: Effects of paricalcitol on calcium and phosphate metabolism and markers of bone health in patients with diabetic nephropathy: Results of the VITAL study. *Nephrol Dial Transplant* 28: 2260–2268, 2013
22. Srivastava AK, Vliet EL, Lewiecki EM, Maricic M, Abdelmalek A, Gluck O, Baylink DJ: Clinical use of serum and urine bone markers in the management of osteoporosis. *Curr Med Res Opin* 21: 1015–1026, 2005
23. Idelevich A, Rais Y, Monsonego-Ornan E: Bone Gla protein increases HIF-1 α -dependent glucose metabolism and induces cartilage and vascular calcification. *Arterioscler Thromb Vasc Biol* 31: e55–e71, 2011
24. Orita Y, Yamamoto H, Kohno N, Sugihara M, Honda H, Kawamata S, Mito S, Soe NN, Yoshizumi M: Role of osteoprotegerin in arterial calcification: Development of new animal model. *Arterioscler Thromb Vasc Biol* 27: 2058–2064, 2007
25. Fahrleitner-Pammer A, Herberth J, Browning SR, Obermayer-Pietsch B, Wirmsberger G, Holzer H, Dobnig H, Malluche HH: Bone markers predict cardiovascular events in chronic kidney disease. *J Bone Miner Res* 23: 1850–1858, 2008
26. Drechsler C, Verduijn M, Pilz S, Krediet RT, Dekker FW, Wanner C, Ketteler M, Boeschoten EW, Brandenburg V, NECOSAD Study Group: Bone alkaline phosphatase and mortality in dialysis patients. *Clin J Am Soc Nephrol* 6: 1752–1759, 2011
27. Zhang Z, Sun L, Wang Y, Ning G, Minto AW, Kong J, Quigg RJ, Li YC: Renoprotective role of the vitamin D receptor in diabetic nephropathy. *Kidney Int* 73: 163–171, 2008
28. Mizobuchi M, Morrissey J, Finch JL, Martin DR, Liapis H, Akizawa T, Slatopolsky E: Combination therapy with an angiotensin-converting enzyme inhibitor and a vitamin D analog suppresses the progression of renal insufficiency in uremic rats. *J Am Soc Nephrol* 18: 1796–1806, 2007
29. Pérez-Gómez MV, Ortiz-Ardúan A, Lorenzo-Sellares V: Vitamin D and proteinuria: A critical review of molecular bases and clinical experience. *Nefrologia* 33: 716–726, 2013
30. Freundlich M, Quiroz Y, Zhang Z, Zhang Y, Bravo Y, Weisinger JR, Li YC, Rodriguez-Iturbe B: Suppression of renin-angiotensin gene expression in the kidney by paricalcitol. *Kidney Int* 74: 1394–1402, 2008
31. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP: 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* 110: 229–238, 2002
32. Tan X, Li Y, Liu Y: Paricalcitol attenuates renal interstitial fibrosis in obstructive nephropathy. *J Am Soc Nephrol* 17: 3382–3393, 2006
33. Tan X, Wen X, Liu Y: Paricalcitol inhibits renal inflammation by promoting vitamin D receptor-mediated sequestration of NF- κ B signaling. *J Am Soc Nephrol* 19: 1741–1752, 2008
34. Amer H, Cosio FG: Significance and management of proteinuria in kidney transplant recipients. *J Am Soc Nephrol* 20: 2490–2492, 2009
35. Amer H, Fidler ME, Myslak M, Morales P, Kremers WK, Larson TS, Stegall MD, Cosio FG: Proteinuria after kidney transplantation, relationship to allograft histology and survival. *Am J Transplant* 7: 2748–2756, 2007
36. Halimi JM: Low-grade proteinuria and microalbuminuria in renal transplantation. *Transplantation* 96: 121–130, 2013
37. Ross EA, Tian J, Abboud H, Hippensteel R, Melnick JZ, Pradhan RS, Williams LA, Hamm LL, Sprague SM: Oral paricalcitol for the treatment of secondary hyperparathyroidism in patients on hemodialysis or peritoneal dialysis. *Am J Nephrol* 28: 97–106, 2008
38. Martin KJ, González EA, Gellens M, Hamm LL, Abboud H, Lindberg J: 19-Nor-1- α -25-dihydroxyvitamin D₂ (Paricalcitol) safely and effectively reduces the levels of intact parathyroid hormone in patients on hemodialysis. *J Am Soc Nephrol* 9: 1427–1432, 1998
39. Brown AJ, Finch J, Slatopolsky E: Differential effects of 19-nor-1,25-dihydroxyvitamin D(2) and 1,25-dihydroxyvitamin D(3) on intestinal calcium and phosphate transport. *J Lab Clin Med* 139: 279–284, 2002
40. Mazzaferro S, Pasquali M, Taggi F, Baldinelli M, Conte C, Muci ML, Pirozzi N, Carbone I, Francone M, Pugliese F: Progression of coronary artery calcification in renal transplantation and the role of secondary hyperparathyroidism and inflammation. *Clin J Am Soc Nephrol* 4: 685–690, 2009

41. Roe P, Wolfe M, Joffe M, Rosas SE: Inflammation, coronary artery calcification and cardiovascular events in incident renal transplant recipients. *Atherosclerosis* 212: 589–594, 2010
 42. Agarwal R, Hynson JE, Hecht TJ, Light RP, Sinha AD: Short-term vitamin D receptor activation increases serum creatinine due to increased production with no effect on the glomerular filtration rate. *Kidney Int* 80: 1073–1079, 2011
 43. Perez A, Raab R, Chen TC, Turner A, Holick MF: Safety and efficacy of oral calcitriol (1,25-dihydroxyvitamin D3) for the treatment of psoriasis. *Br J Dermatol* 134: 1070–1078, 1996
 44. Bertoli M, Luisetto G, Ruffatti A, Urso M, Romagnoli G: Renal function during calcitriol therapy in chronic renal failure. *Clin Nephrol* 33: 98–102, 1990
 45. Ali MM, Vaidya V: Vitamin D and cancer. *J Cancer Res Ther* 3: 225–230, 2007
 46. Gaspari F, Perico N, Ruggerenti P, Mosconi L, Amuchastegui CS, Guerini E, Daina E, Remuzzi G: Plasma clearance of nonradioactive iothexol as a measure of glomerular filtration rate. *J Am Soc Nephrol* 6: 257–263, 1995
 47. Brandenburg VM, Westenfeld R, Ketteler M: The fate of bone after renal transplantation. *J Nephrol* 17: 190–204, 2004
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