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Serum phosphate is associated with increased risk of bone fragility fractures in haemodialysis patients

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ABSTRACT

Background. Bone fragility fractures are associated with high morbidity and mortality. This study analysed the association between the current biochemical parameters of chronic kidney disease–mineral and bone disorders (CKD-MBD) and bone fragility fractures in the COSMOS (Current management Of Secondary hyperparathyroidism: a Multicentre Observational Study) project.

Methods. COSMOS is a 3-year, multicentre, open cohort, prospective, observational study carried out in 6797 haemodialysis patients (227 centres from 20 European countries). The association of bone fragility fractures (outcome) with serum calcium, phosphate and parathyroid hormone (PTH) (exposure), was assessed using standard Cox proportional hazards regression and Cox proportional hazards regression for recurrent events. Additional analyses were performed considering all-cause mortality as a competitive event for bone fragility fracture occurrence. Multivariable models were used in all strategies, with the fully adjusted model including a total of 24 variables.

Results. During a median follow-up of 24 months, 252 (4%) patients experienced at least one bone fragility fracture (incident bone fragility fracture rate 28.5 per 1000 patient-years). In the fractured and non-fractured patients, the percentage of men was 43.7% and 61.4%, mean age 68.1 and 63.8 years and a haemodialysis vintage of 55.9 and 38.3 months, respectively. Baseline serum phosphate >6.1 mg/dL (reference value 4.3–6.1 mg/dL) was significantly associated with a higher bone fragility fracture risk in both regression models {hazard ratio (HR) 1.53 [95% confidence interval (CI) 1.10–2.13] and HR 1.44 (95% CI 1.02–2.05)}. The significant association persisted after competitive risk analysis [subHR 1.42 (95% CI 1.02–1.98)] but the finding was not confirmed when serum phosphate was considered as a continuous variable. Baseline serum calcium showed no association with bone fragility fracture risk in any regression model. Baseline serum PTH >800 pg/mL was significantly associated with a higher bone fragility fracture risk in both regression models, but the association disappeared after a competitive risk analysis.

Conclusions. Hyperphosphatemia was independently and consistently associated with an increased bone fracture risk, suggesting serum phosphate could be a novel risk factor for bone fractures in haemodialysis patients.

Keywords: bone fragility fractures, CKD, chronic kidney disease–mineral and bone disorders (CKD-MBD), haemodialysis, serum phosphate

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KEY LEARNING POINTS

What was known:

- Bone fragility fractures are a frequent clinical disorder among chronic kidney disease (CKD) patients and are associated with high morbidity and mortality.
- Bone fragility fracture risk factors in CKD patients are the same as those in the general population but they may also include other specific factors such as serum phosphate, calcium and parathyroid hormone disorders.
- Hyperphosphatemia has been suggested as a risk factor for bone fragility fracture. However, to date, there is no solid published evidence of the association between high serum phosphate and bone fractures in haemodialysis patients.

This study adds:

- The present large study is the first to describe an association between high serum phosphate and the risk of incident bone fractures in haemodialysis patients.

Potential impact:

- Hyperphosphatemia is a prevalent and treatable condition among haemodialysis patients. The identification of the association between elevated serum phosphate and bone fractures emphasizes the importance of prospective studies to identify whether lowering phosphate towards the normal range reduces fracture risk.

INTRODUCTION

Bone fragility fractures, a major health problem and economic burden for public health systems [1, 2], have been related to high morbidity and mortality in the general population and more recently in chronic kidney disease (CKD) patients [3–6]. Bone fragility fracture is an important component of the CKD–mineral and bone disorders (CKD-MBD) constellation. The age-dependent low bone mass, the abnormalities of bone turnover and microarchitecture found in the progression of CKD are the main factors responsible for the decrease in bone quality and strength leading to high bone fragility.

In CKD patients, long-term disturbances in the main classical factors involved in the regulation of bone turnover, bone mass, bone quality and strength, namely serum calcium, phosphate, parathyroid hormone (PTH) and alkaline phosphatase, have been individually and collectively implicated, along with ageing, as responsible for the increased bone fragility [1, 7]. Maintained exposure to secondary hyperparathyroidism in CKD patients is a well-established risk factor for reduced bone resistance and increased fracture susceptibility. The relationship between serum PTH and bone fractures appears to be U-shaped in haemodialysis patients: lower PTH levels are also associated with higher fracture risk, possibly because of its association with poorer nutritional status and inflammation [8].

Serum PTH has been reported to discriminate between low and non-low 'bone-formation rate/bone surface' patterns of bone turnover among haemodialysis patients [9]. Other factors such as serum phosphate and serum calcium have not been well analysed as risk factors for bone fragility fractures in haemodialysis patients.

This study aimed to investigate the association between serum phosphate, calcium and PTH, and bone fragility fractures in a large pan-European multinational prospective cohort of haemodialysis patients.

MATERIALS AND METHODS

Study design and population

COSMOS (Current management Of Secondary hyperparathyroidism: a Multicentre Observational Study) is a 3-year, multicentre, open cohort, prospective, observational study including data from adult patients on haemodialysis, not previously transplanted, from 227 centres in 20 European countries. The number of patients recruited per country was proportional to the haemodialysis population. Centres were randomly selected from a full list of haemodialysis centres across the participating countries. Within each centre, 20 patients were randomly selected to obtain a sample of 4500 patients who were followed for 3 years. To maintain a stable number of patients during follow-up, 2297 additional patients with a dialysis vintage <1 year were recruited to replace those leaving the study for any reason. The total number of recruited patients was 6797.

The study was approved by the Ethics Committee of University Hospital Doctor Peset (Approval number 05/054, Valencia, Spain). All patients provided informed consent for participation in the study, which was conducted thoroughly following the principles of the Declaration of Helsinki. Recruitment began in February 2005 and finished in July 2007; data collection ended in July 2010. Details on the design of the study and data collection were already published elsewhere [10–13]. At baseline and every 6 months, a patient-specific form was completed following study protocol, including demographics, comorbidities, treatments and monthly biochemical parameters of the previous 6 months, including serum phosphate, PTH, calcium, albumin and haemoglobin. Laboratory values were obtained from medical records [10]. Mean values of the previous 6 months were calculated for each biochemical parameter at baseline and every 6 months. The presence of symptomatic, non-traumatic and non-metastatic bone fractures was collected from medical records in the previous 12 months at baseline (previous fractures) and every 6 months (incident fractures) during the whole period of follow-up. The site of bone fracture was also collected (vertebral, hip, radial and others).

Statistical analysis

A descriptive analysis compared patients who suffered at least one bone fracture during follow-up with those who did not fracture. Numerical variables were compared using Student's *t*-test or the Mann–Witney U-test for normal and non-normal distribution, respectively. Chi-squared test was used to compare categorical variables.

Two distinct strategies were implemented to assess the consistency of the association between bone fractures (outcome) and serum phosphate, calcium and PTH (exposure): (i) standard Cox

proportional hazards regression—the dependent variable was the time to first bone fracture, each individual considered at risk until first bone fracture occurrence; and (ii) Cox proportional hazards regression for recurrent events—the dependent variable was the time to bone fracture (and refracture), each individual considered at risk during the whole follow-up period.

In both strategies, the exposure was the baseline values of serum calcium, phosphate or PTH which were categorized as follows; serum calcium: ≤8.6, 8.6–9.5 and >9.5 mg/dL; serum phosphate: ≤4.3, 4.3–6.1 and >6.1 mg/dL; and serum PTH: ≤300, 300–800 and >800 pg/mL. Serum PTH cut-off values were selected according to previous publications assessing the association between bone fractures and serum PTH [6, 14]. Serum calcium and phosphate values were separated into quartiles, and quartile 1 (low values) and quartile 4 (high values) were compared with the combined quartiles 2 and 3 (reference).

Time-dependent values of serum phosphate, calcium and PTH were also analysed using the same categories. Competitive risk analyses were additionally performed as a sensitivity analysis considering all-cause mortality as a competitive event for bone fracture occurrence.

Univariate and progressive multivariable models were used in all strategies for adjustments with different covariates. A total of 27 variables were collected in COSMOS [10]. Some of them, such as the dose of the drugs used, were not included in multivariate analyses. Every other variable was included in the fully adjusted models, with no selection of variables. Model 1 included demographic variables and comorbidities (11 variables): age, sex, body mass index, aetiology of CKD, time on haemodialysis, smoking habit, diabetes, cardiovascular disease history, bone fracture history in the previous 12 months, and vascular or valvular calcification and parathyroidectomy. Model 2 included variables from Model 1 plus treatments (eight variables): dialysis type, calcium concentration in the dialysate, hours of haemodialysis per week, treatment with erythropoietin-stimulating agents, prescription of vitamin D metabolites/analogues (calcitriol, alfacalcidol or paricalcitol), native vitamin D or calcidiol, phosphate-binding agents (calcium-containing, sevelamer, aluminium-containing, lanthanum carbonate or other) and calcimimetics. Model 3 included all previous variables plus five biochemical parameters: haemoglobin, albumin, serum calcium, PTH and phosphate. The fully adjusted model included 24 variables: the variable under study plus 23 additional covariates.

Additional analyses were conducted separately for vertebral and non-vertebral fractures, as well as considering serum PTH, calcium and phosphorus as continuous variables.

All the statistical analyses were performed using R software for statistical computing and graphics (version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The present study included 6274 patients (92.3% of the COSMOS cohort) with follow-up data and non-missing information regarding bone fractures at baseline and during follow-up, of whom 252 (4%) suffered at least one bone fracture during the 3-year follow-up period, making a total of 350 bone fractures: 110 (31.4%) clinical vertebral, 86 (24.6%) hip, 29 (8.3%) radius and 125 (35.7%) others. The incident rate for bone fracture was 28.5 bone fractures of any type per 1000 patient-years: 8.9 clinical vertebral fractures per 1000 patient-years, 7 hip fractures per 1000 patient-years, 2.4 radius fractures per 1000 patient-years and 10.2 bone fractures of other types per 1000 patient-years. Among fractured patients, 196 (77.7%) suffered only one incident fracture; 41 (16.3%) suffered two; 12 (4.8%) suffered three; and 3 patients (1.2%) suffered four or more bone fractures. A total of 2581 patients were lost to follow-up (87 fractured and 2494 non-fractured): 1632 died (69 fractured and 1563 non-fractured), 639 were transplanted (4 fractured and 635 non-fractured), 236 were referred to other haemodialysis units (12 fractured and 224 non-fractured), 23 changed to peritoneal dialysis (0 fractured and 23 non-fractured) and 51 left the study for other reasons (2 fractured and 49 non-fractured). The mean time of follow-up was 23.5 (median 24) months.

Table 1 depicts the baseline characteristics of the patients who fractured and did not fracture during the follow-up. Patients who suffered at least one bone fracture during follow-up (fractured), showed a higher prevalence of previous fractures, a higher percentage of women, older age, more background of cardiovascular disease, longer time on dialysis, lower haemodialysis dose per week, higher serum calcium and lower serum albumin, among others. Supplementary data, Table S1 depicts the baseline characteristics of the patients who fractured and not fractured in the 12 months before the start of follow-up.

Multivariate Cox regression showed that bone fractures in the previous 12 months {hazard ratio (HR) 7.33 [95% confidence interval (CI) 4.78–11.24], $P < .001$}, female sex [HR 1.57 (95% CI 1.18–2.10), $P = .002$], age [HR 1.03 (95% CI 1.02–1.04), $P < .001$, per 1 year], haemodialysis vintage [HR 1.00 (95% CI 1.00–1.01), $P = .029$, per 1 month] and serum albumin [HR 0.66 (95% CI 0.49–0.89), $P < .007$] were associated with a higher incidence of bone fractures.

No association was found between baseline serum calcium >9.5 mg/dL and the incidence of bone fractures after full adjustment (Model 3) in any of the two regression models used (standard Cox proportional hazards regression and Cox proportional hazards regression for recurrent events) [HR 1.14 (95% CI 0.83–1.57) and HR 1.10 (95% CI 0.78–1.55); Fig. 1]. Baseline serum PTH >800 pg/mL was associated with a higher risk of bone fractures (reference 300–800 pg/mL) in both regression models [HR 1.61 (95% CI 1.01–2.58) and HR 2.01 (95% CI 1.23–3.29); Fig. 2].

Baseline serum phosphate >6.1 mg/dL was significantly associated with a higher risk of bone fractures, using as reference serum phosphate levels between 4.3 and 6.1 mg/dL (Fig. 3) in both regression models in the fully adjusted analysis [HR 1.53 (95% CI 1.01–2.13) and HR 1.44 (95% CI 1.02–2.05), respectively; Fig. 3].

Additional analyses were performed including adjustment in the Cox regression models by the individual phosphate binder type (calcium, magnesium, lanthanum, polyanionic gels, aluminium and others) instead of the phosphate binder use (as a dichotomic variable). None of the phosphate binder types was significantly associated with the incidence of bone fractures. The HR (95% CI) for calcium-containing phosphate binders was 0.85 (0.63–1.14) in the fully adjusted model. The association between serum phosphate >6.1 mg/dL and the incidence of bone fractures remained statistically significant in this model [HR 1.57 (95% CI 1.13–2.19)].

The percentage of patients treated with phosphate binders increased according to the serum phosphate level increase: 73.4% of patients with baseline serum phosphate <4.3 mg/dL, 85.9% of patients with baseline serum phosphate 4.3–6.1 mg/dL and 94.0% of patients with baseline serum phosphate >6.1 mg/dL ($P < .001$). For the treatment with vitamin D receptor activators, the percentages are as follows: 44.3%, 49.2% and 48.3% for serum phosphate <4.3, 4.3–6.1 and >6.1 mg/dL, respectively ($P < .01$).

The fully adjusted model found no significant association between baseline serum phosphate ≤4.3 mg/dL and bone fractures during the follow-up.

Table 1: Baseline characteristics of fractured and non-fractured patients during follow-up.

	All patients (N = 6274)	Non-fractured (N = 6022)	Fractured (N = 252)	P-value
Previous fractures = yes (%)	128 (2.0)	92 (1.5)	36 (14.3)	<.001
Sex = male (%)	3810 (60.7)	3700 (61.4)	110 (43.7)	<.001
Age (years) [mean (SD)]	64.0 (14.4)	63.8 (14.4)	68.1 (12.9)	<.001
BMI (kg/m^2) [mean (SD)]	25.3 (5.1)	25.3 (5.0)	24.9 (5.6)	.197
Current smoker = yes (%)	872 (13.9)	838 (13.9)	34 (13.5)	.922
Diabetes = yes (%)	1925 (30.7)	1852 (30.8)	73 (29.0)	.594
CVD history = yes (%)	4520 (72.0)	4320 (71.7)	200 (79.4)	.010
Parathyroidectomy = yes (%)	307 (4.9)	291 (4.8)	16 (6.3)	.345
Months on HD [mean (SD)]	39.0 (49.6)	38.3 (48.8)	55.9 (64.0)	<.001
Hours of dialysis per week [mean (SD)]	12.0 (2.1)	12.0 (2.1)	11.7 (1.9)	.012
Dialysis technique (%)				.889
Low-flux	3387 (54.0)	3250 (54.0)	137 (54.4)	
High-flux	2336 (37.2)	2241 (37.2)	95 (37.7)	
Others	551 (8.8)	531 (8.8)	20 (7.9)	
Calcium concentration in dialysate (%)				.482
2.5 mEq/L	1670 (29.8)	1600 (29.7)	70 (32.4)	
3.0 mEq/L	2848 (50.8)	2747 (50.9)	101 (46.8)	
3.5 mEq/L	1091 (19.5)	1046 (19.4)	45 (20.8)	
Patients prescribed PBAs = yes (%)	5336 (85.1)	5132 (85.3)	204 (81.0)	.071
Patients prescribed VDRAs = yes (%)	2979 (47.5)	2860 (47.5)	119 (47.2)	.970
Patients prescribed calcimimetics = yes (%)	384 (6.2)	363 (6.1)	21 (8.3)	.184
Patients treated with ESAs = yes (%)	5576 (90.6)	5348 (90.6)	228 (91.6)	.681
Calcium (mg/dL) [mean (SD)]	9.1 (0.7)	9.1 (0.7)	9.2 (0.8)	.099
PTH (pg/mL) [median (IQR)]	210.0 (108.0, 375.0)	209.3 (107.9, 373.1)	228.5 (114.7, 431.0)	.025
Phosphorus (mg/dL) [mean (SD)]	5.4 (1.4)	5.4 (1.4)	5.2 (1.5)	.071
Albumin (g/dL) [mean (SD)]	3.8 (0.5)	3.8 (0.5)	3.7 (0.5)	.004
Haemoglobin (g/dL) [mean (SD)]	11.4 (1.4)	11.4 (1.4)	11.4 (1.3)	.841

Biochemical parameters (PTH, calcium, phosphorus, albumin and haemoglobin) are expressed as the mean of the last 6 months before the start of the follow-up period.
BMI: body mass index; CVD: cardiovascular disease; HD: haemodialysis; PBAs: phosphate-binding agents; VDRAs: vitamin D receptor activators; ESAs: erythropoietin-stimulating agent; SD: standard deviation; IQR: interquartile range.

Time-dependent serum calcium values >9.5 mg/dL and phosphate >6.1 mg/dL did not correlate with bone fracture risk in the two Cox regression models (Supplementary data, Tables S2 and S4). However, time-dependent serum PTH >800 pg/mL was associated with a higher incidence of bone fractures in both, standard Cox regression and Cox regression for recurrent events (Supplementary data, Table S3).

After competitive risk analysis, the association between the incidence of bone fractures and phosphate >6.1 mg/dL remained statistically significant in the fully adjusted model [subHR 1.42 (95% CI 1.02–1.98)]. However, serum calcium above 9.5 mg/dL and serum PTH >800 pg/mL were not significant [subHR 1.06 (95% CI 0.77–1.46) and 1.49 (95% CI 0.94–2.34), respectively; Supplementary data, Table S5].

Baseline serum phosphate >6.1 mg/dL was significantly associated with an increased relative risk in non-vertebral fractures [HR 1.76 (95% CI 1.22–2.53)] but not in symptomatic vertebral fractures [HR1.01 (95% CI 0.50–2.04)] in the fully adjusted Cox regression model.

In the linear approach analysis, only serum PTH showed a significant association with the incidence of bone fractures [HR 1.04 (95% CI 1.01–1.08) per 100 pg/mL].

DISCUSSION

In haemodialysis patients, bone fragility fractures are frequent adverse events associated with increased morbidity and mortality. Prospective studies evaluating bone fractures in this population are scarce and have agreed to describe an association between its occurrence and elevated serum alkaline phosphatase [15, 16]. In the present study, in addition to the well-known classical risk factors for bone fractures, high serum phosphate was consistently associated with increased risk for bone fractures, regardless of other factors such as previous fractures, age, sex, time on haemodialysis, serum calcium and PTH.

Among the known risk factors for bone fractures, an important finding of the present study was the strong association between a previous bone fracture in the 12 months before the start of the study and the incidence of new fractures [HR 7.33 (95% CI 4.78–11.24)], a phenomenon also described both in the general population and in haemodialysis patients, allowing us to speculate that appropriate preventive measures should be initiated in a timely manner to prevent new fractures after the first event [17–20].

The relationship between the incidence of bone fractures and serum calcium or PTH is worth mentioning. The evidence in this area is scarce [21]. A previous study of haemodialysis patients from the Dialysis Outcomes and Practice Patterns Study (DOPPS), found that serum calcium ≥10.2 mg/dL showed increased odds of previous hip fracture [20]. Nevertheless, in line with the results described in the present study, no association was found between serum calcium and the risk of new fractures of any type in adjusted logistic regression models. Similarly, in the Dialysis Morbidity and Mortality Study (DMMS) waves 1 to 4, no association between serum calcium and bone fractures was found in dialysis patients after adjustment by several covariates [14]. The same study together with a more recent retrospective study from

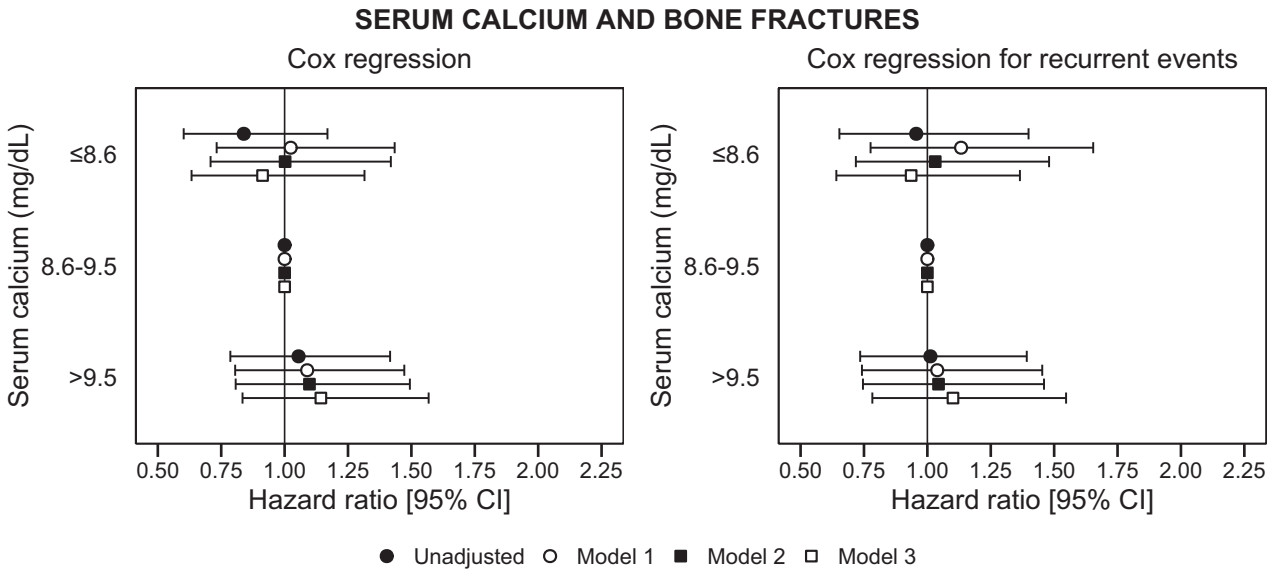

Figure 1: Association between serum calcium and the incidence of bone fractures. Multivariate adjustments; Model 1: age, sex, body mass index, aetiology of CKD, time on haemodialysis, smoking habit, diabetes, cardiovascular disease history, bone fracture history in the previous 12 months, vascular or valvular calcification, and parathyroidectomy. Model 2: Model 1 plus dialysis type, calcium concentration in the dialysate, hours of haemodialysis per week, treatment with erythropoietin-stimulating agents (ESAs), prescription of vitamin D metabolites/analogues, native vitamin D or calcidiol, phosphate-binding agents. Model 3: Model 2 plus haemoglobin, albumin, phosphate and PTH.

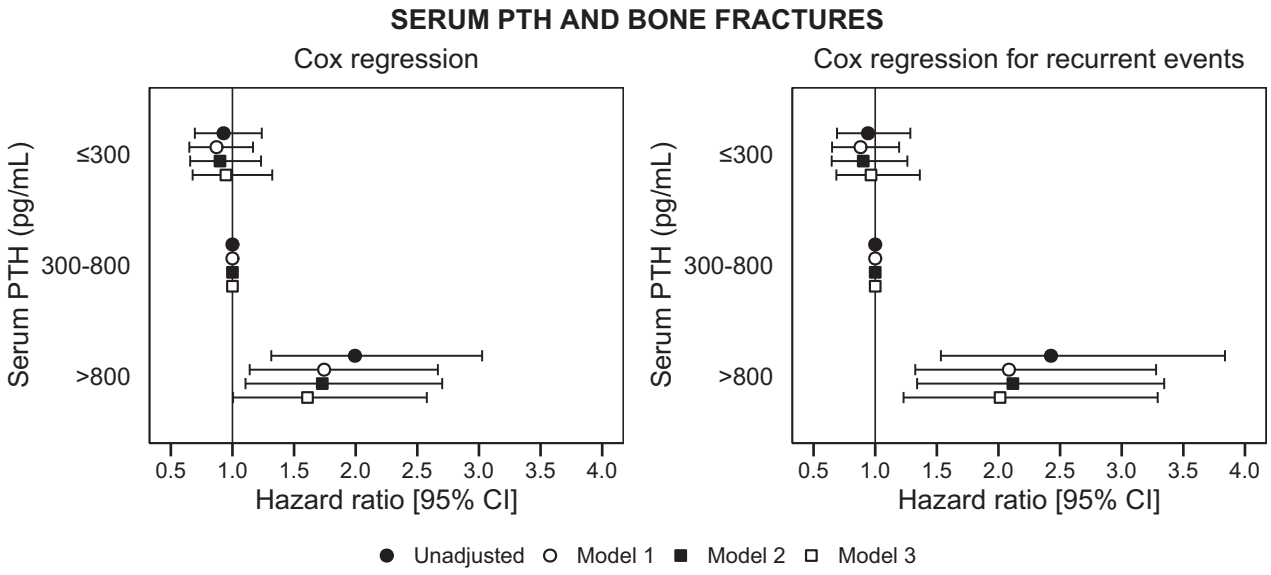

Figure 2: Association between serum PTH and the incidence of bone fractures. Multivariate adjustments; Model 1: age, sex, body mass index, aetiology of CKD, time on haemodialysis, smoking habit, diabetes, cardiovascular disease history, bone fracture history in the previous 12 months, vascular or valvular calcification, and parathyroidectomy. Model 2: Model 1 plus dialysis type, calcium concentration in the dialysate, hours of haemodialysis per week, treatment with erythropoietin-stimulating agents (ESAs), prescription of vitamin D metabolites/analogues, native vitamin D or calcidol, phosphate-binding agents. Model 3: Model 2 plus haemoglobin, albumin, phosphate and calcium.

Matias *et al.* including 341 dialysis patients reported a U-shaped association between bone fracture risk and serum PTH [6]. In both studies, a serum PTH <300 pg/mL or >800 pg/mL showed an association with bone fractures after multivariate adjustment. These data partially agree with the present study, where values of PTH >800 pg/mL were associated with higher fracture risk, but not serum PTH ≤300 pg/mL.

It is well known that serum phosphate increases in the late stages of CKD, and it has been associated with a higher mortality risk [22]. However, the possible causal relationship between hyperphosphatemia and the increased incidence of bone fractures has scarcely been evaluated [23, 24].

The possible relationship between serum phosphate and bone fractures in haemodialysis was described several years ago [22]; it was reported that the relative risk of fracture-related hospitalization increased by 12% per mg/dL increase in serum phosphate after adjustment for a total of 15 potentially confounding variables including age, gender, ethnicity and serum albumin, among

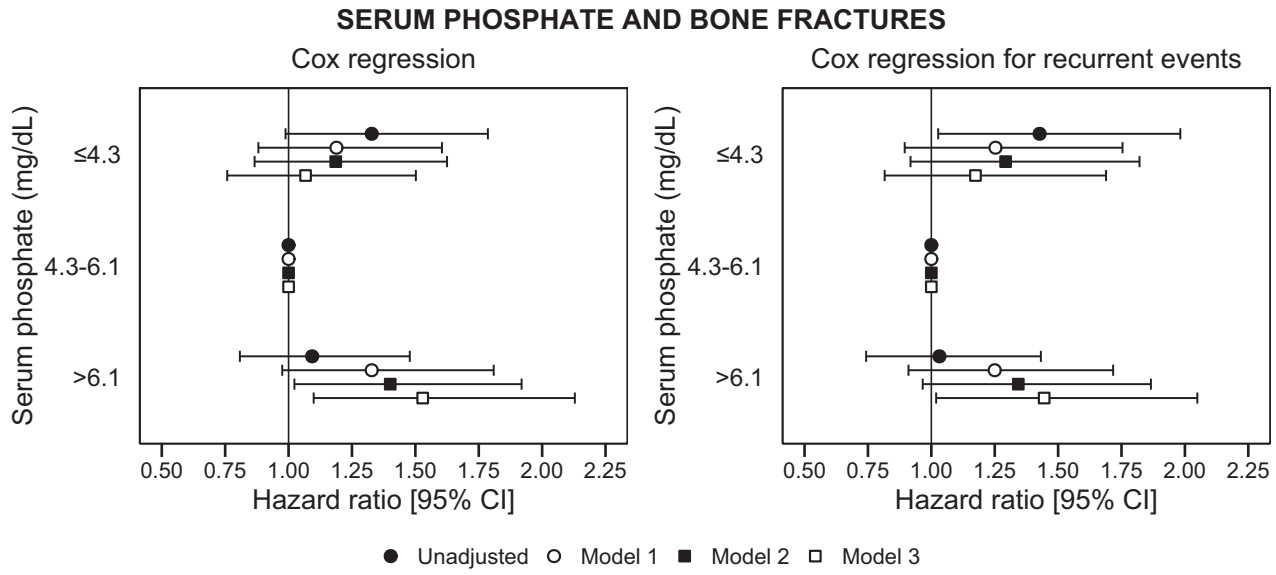

Figure 3: Association between serum phosphate and the incidence of bone fractures. Multivariate adjustments; Model 1: age, sex, body mass index, aetiology of CKD, time on haemodialysis, smoking habit, diabetes, cardiovascular disease history, bone fracture history in the previous 12 months, vascular or valvular calcification, and parathyroidectomy. Model 2: Model 1 plus dialysis type, calcium concentration in the dialysate, hours of haemodialysis per week, treatment with erythropoietin-stimulating agents (ESAs), prescription of vitamin D metabolites/analogues, native vitamin D or calcidiol, phosphate-binding agents. Model 3: Model 2 plus haemoglobin, albumin, PTH and calcium.

others. However, unlike in the present study, only fractures requiring hospitalization were included in the analysis. A more recent study found that high serum phosphate was associated with bone fracture risk in the general population and men with CKD (glomerular filtration rate ≤58 mL/min/1.73 m^2) [24]. The association between serum phosphate and bone fractures persisted after adjustment for several potential confounders [including fibroblast growth factor 23 (FGF-23) and PTH], which agrees with the present study, suggesting that the effect of hyperphosphatemia is at least partially independent of other underlying hormonal abnormalities.

The association between serum PTH >800 pg/mL and bone fractures observed in this study during the follow-up is worth mentioning, but not novel. Despite the known relationship between serum phosphate and PTH, the association between serum phosphate >6.1 mg/dL and bone fractures was independent of serum PTH, as the latter was included in the multivariate models as a covariate.

Hyperphosphatemia treatment regimens have also been associated with bone fracture occurrence among haemodialysis patients. For example, a recent observational study including 13 427 dialysis patients showed that patients not treated with phosphate-binding agents presented a 20% higher fracture risk compared with those treated with phosphate binders [25].

Sevelamer (non-calcium-containing phosphate binder) treatment has been associated with higher vertebral fracture risk among haemodialysis patients with total bone Gla proteins (or osteocalcin) <150 µg/L [26]. Oral calcitriol use has been associated with lower vertebral fracture risk among haemodialysis patients without increased vascular calcification [27].

Another study from DOPPS in haemodialysis patients found no association between serum phosphate and the risk of new fractures [20]. The discrepancy between the latter and the present study could be attributed to the fact that COSMOS is a study specifically designed to represent the European dialysis population, and its results may not be applicable to other populations.

To our knowledge, the present study is the first to describe an association between high serum phosphate and the risk of incident bone fractures in a large population of haemodialysis patients. Such association was consistently found across the different statistical strategies used (Cox regression, Cox regression for recurrent events and competitive risk regression).

The time-dependent analysis did not show an association between serum phosphate and the incidence of bone fractures. A time-dependent Cox analysis may address relatively short-term effects, meanwhile a traditional Cox analysis using baseline risk factors addresses the relatively long-term effects [28]. In the current study, the outcome of interest is bone fracture, which can occur as result of the effect of many factors that negatively affect the process of bone remodelling which may take several months. In healthy adults, the complete turnover of the skeleton may take between 7 and 10 years. Therefore, it is expected that any risk factor for bone fracture would be of a long-term nature, such as the case of serum phosphate.

Vertebral fractures are especially prevalent among haemodialysis patients. Unlike peripheral fractures, vertebral fractures are asymptomatic in a high percentage of patients, but a study assessing them by quantitative morphometry described a prevalence as high as 55.3% in a sample of 387 haemodialysis patients [28]. Vertebral fractures have been associated with increased vascular calcification among haemodialysis patients and increased mortality among CKD patients, whether in haemodialysis or not [28, 29].

Considering bone fracture localization, high serum phosphate was significantly associated selectively with an increased risk of peripheral bone fractures during follow-up, but not with vertebral fractures. It is described that secondary hyperparathyroidism in CKD patients preferentially affects the cortical bone compartment associated with peripheral bone fracture occurrence [8]. Nonetheless, like in previous studies, the association between serum phosphate and peripheral bone fractures persisted after adjustment for potential confounders such as serum PTH

and serum calcium [24]. These results suggest that high serum phosphate is associated with cortical bone compartment compromise and peripheral bone fractures at least partially independently of underlying serum PTH abnormalities. Our results confront others describing an association between lower serum phosphate levels and prevalent vertebral fractures in non-dialysis-dependent (stages 3–5) CKD patients [28]. It is also worth mentioning that an underpower of our results evaluating vertebral fractures cannot be ruled out due to the lack of systematic radiological screening for non-symptomatic fractures. This limitation would affect vertebral and non-vertebral (peripheral) fractures differently since the latter are rarely asymptomatic or undiagnosed.

Bone fracture was not significantly associated with serum phosphate when it was considered a continuous variable (linear relationship). Figure 3 shows higher HRs for both low and high serum phosphate compared with the reference values (4.3–6.1 mg/dL), although the differences were not significant for low levels of serum phosphate (≤4.3 mg/dL). This suggests that the relationship between serum phosphate and bone fracture may not be linear (U-shaped) and would explain the lack of association when serum phosphate is used as a continuous variable.

Direct and indirect mechanisms have been hypothesized to explain the link between serum phosphate and fractures, but the findings of epidemiological studies are controversial [20, 22, 25, 30]. The possible mechanisms by which serum phosphate may affect bone quality and strength are based on *in vitro* experiments. Phosphate may affect both bone formation and resorption. Regarding bone formation, inorganic phosphate may stimulate several regulatory molecules (fos-related antigen 1, osteopontin, insulin-like growth factor-I and sclerostin), which would inhibit Wnt/Beta-catenin and osteoblast proliferation [31–33]. Inorganic phosphate also affects bone resorption by limiting osteoclast survival and differentiation, inducing changes in the expression of RANKL, miR-223 and osteoprotegerin [34–38].

The main limitation of the present study is its observational nature which does not allow causality to be established. Despite the different statistical approaches implemented and multivariate adjustments, residual confounding cannot be discarded. Another limitation is that the COSMOS data were collected more than 12 years ago and may not fully reflect the current situation of haemodialysis patients. Also, the COSMOS population is considered representative of European haemodialysis patients and results may not be extrapolated to other populations. In addition, other biochemical markers and drugs related to bone metabolism, such as FGF-23, alkaline phosphatase and vitamin D metabolites, oral anticoagulants or proton pump inhibitors, were not available for the adjustments. Finally, no systematic radiological screening for non-symptomatic fractures was carried out during follow-up, leading to a plausible underpower of the results. Undetected incident fractures may have occurred during follow-up, especially considering vertebral fractures, as they are asymptomatic in a high percentage of patients. The prevalence of asymptomatic vertebral fractures among CKD patients has been estimated between 2% and 28%; a study assessing them by quantitative morphometry described a prevalence of 55.3% among haemodialysis patients [39, 40].

The strengths of the study are the large size of the population (6274 patients) and its randomized prospective design representative of the European haemodialysis population.

In summary, high serum phosphate was independently and consistently associated with an increased risk of bone fragility fractures in haemodialysis patients, suggesting that serum phosphate might be a novel risk factor or a marker for bone fractures in this population [41]. Randomized long-term clinical trials are necessary to confirm the role of phosphate in the incidence of bone fractures. There is a registered ongoing clinical trial to study the effect of phosphate binder treatment on incident bone fractures that may shed some additional light on the relationship between phosphate and bone fractures [42].

SUPPLEMENTARY DATA

Supplementary data are available at *ndt* online.

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COSMOS participating centres are listed in the Supplementary Appendix.

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AUTHORS' CONTRIBUTIONS

Conception and study design: M.R.-G., C.Z., F.L., M.C.-S., J.F., M.A.F., M.K., G.M.L., J.L.G.-T., J.B.C.-A. and J.L.F.-M.; analysis design: P.B.-B., C.Z., F.L., M.C.-S., J.F., M.A.F., M.K., G.M.L., J.L.G.-T., E.S.-A., C.A.-M., J.B.C.-A. and J.L.F.-M.; statistical analysis: P.B.-B., E.R.-R., L.G.-L., B.M.-C., C.A.-M. and J.L.F.-M.; interpretation of results: P.B.-B., M.R.-G., E.R.-R., L.G.-L., A.O., C.Z., F.L., M.C.-S., M.A.H.-S., J.M.F.-G., C.A.-M., J.B.C.-A. and J.L.F.-M.; draft writing: P.B.-B., C.G.-A., J.B.C.-A.

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DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

F.L. is a member of an advisory board and speaker at meetings supported by Amgen. A.O. has received grants from Sanofi and consultancy or speaker fees or travel support from Advicciene, Astellas, AstraZeneca, Amicus, Amgen, Fresenius Medical Care, GSK, Bayer, Sanofi-Genzyme, Menarini, Mundipharma, Kyowa Kirin, Alexion, Freeline, Idorsia, Chiesi, Otsuka, Novo-Nordisk, Sysmex and Vifor Fresenius Medical Care Renal Pharma, and is Director of the Catedra Mundipharma-UAM of diabetic kidney disease and the Catedra Astrazeneca-UAM of chronic kidney disease and electrolytes. J.F. has received honoraria from Amgen, AstraZeneca, Bayer, Boehringer, Fresenius, Novartis and Vifor, and serves on the data safety monitoring board of Novo Nordisk and Visterra. J.B.C.-A. has received grants or consultancy, speaker fees and travel support from Amgen, Kyowa Kirin and Vifor Fresenius Medical Care Renal Pharma. The remaining authors declare no conflict of interest concerning the work. The results presented in this paper have not been published previously in whole or part, except in abstract format. The authors are not aware of any additional relationships, funding or financial holdings that might be perceived as affecting the objectivity of this study.

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