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ORIGINAL ARTICLE

Disorders in bone-mineral parameters and the risk of death in persons with chronic kidney disease stages 4 and 5: the PECERA study

Pablo Molina[1,2] · Mariola D. Molina[3] · Luis M. Pallardó[1,2] · Javier Torralba[4] · Verónica Escudero[1] · Luis Álvarez[5] · Ana Peris[6] · Pilar Sánchez-Pérez[6] · Miguel González-Rico[7] · María J. Puchades[7] · José E. Fernández-Nájera[8] · Elena Giménez-Civera[7] · Luis D'Marco[7] · Juan J. Carrero[9] · José L. Górriz[2,7]

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Abstract

Background Abnormalities of bone mineral parameters are associated with increased mortality in patients on dialysis, but their effects and the optimal range of these biomarkers are less well characterized in non-dialysis chronic kidney disease (CKD).

Methods PECERA (Collaborative Study Project in Patients with Advanced CKD) is a 3-year, prospective multicenter, open-cohort study of 966 adult patients with non-dialyzed CKD stages 4–5 enrolled from 12 centers in Spain. Associations between levels of serum calcium (Ca) (corrected for albumin), phosphate (P), and intact parathyroid hormone (iPTH) with all-cause mortality (primary outcome) and cardiovascular mortality (secondary outcome) were examined using time-dependent Cox proportional hazards models and penalized splines analysis adjusted by demographics and comorbidities, treatments and biochemical values collected every 6 months for 3 years.

Results After a median follow-up of 29 months (IQR: 13–36 months) there were 181 deaths (19%). The association of calcium with all-cause mortality was J-shaped, with an increased risk for all-cause mortality at levels > 10.5 mg/dL. For phosphate and iPTH levels, the association was U-shaped. The serum values associated with the minimum risk of mortality were 3.8 mg/dL for phosphate and 70 pg/mL for iPTH, being the lowest risk ranges between 2.8 and 5.0 mg/dL, and between 38 and 112 pg/mL for phosphate and iPTH, respectively.

Conclusions Our study provides evidence on the non-linear association of serum calcium, phosphate and iPTH levels with mortality in stage 4 and 5 CKD patients, and suggests potential survival benefits for controlling bone mineral parameters in this population, as previously reported for dialysis patients.

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✉ Pablo Molina
molina_pab@gva.es

[1] Department of Nephrology, Hospital Universitari Dr Peset, FISABIO, Avda. Gaspar Aguilar, 90, 46017 Valencia, Spain

[2] Department of Medicine, Universitat de València, Valencia, Spain

[3] Department of Mathematics, Universidad de Alicante, Alicante, Spain

[4] Department of Nephrology, Hospital General Universitario, Alicante, Spain

[5] Section of Nephrology, Hospital Virgen de Los Lirios, Alcoi, Spain

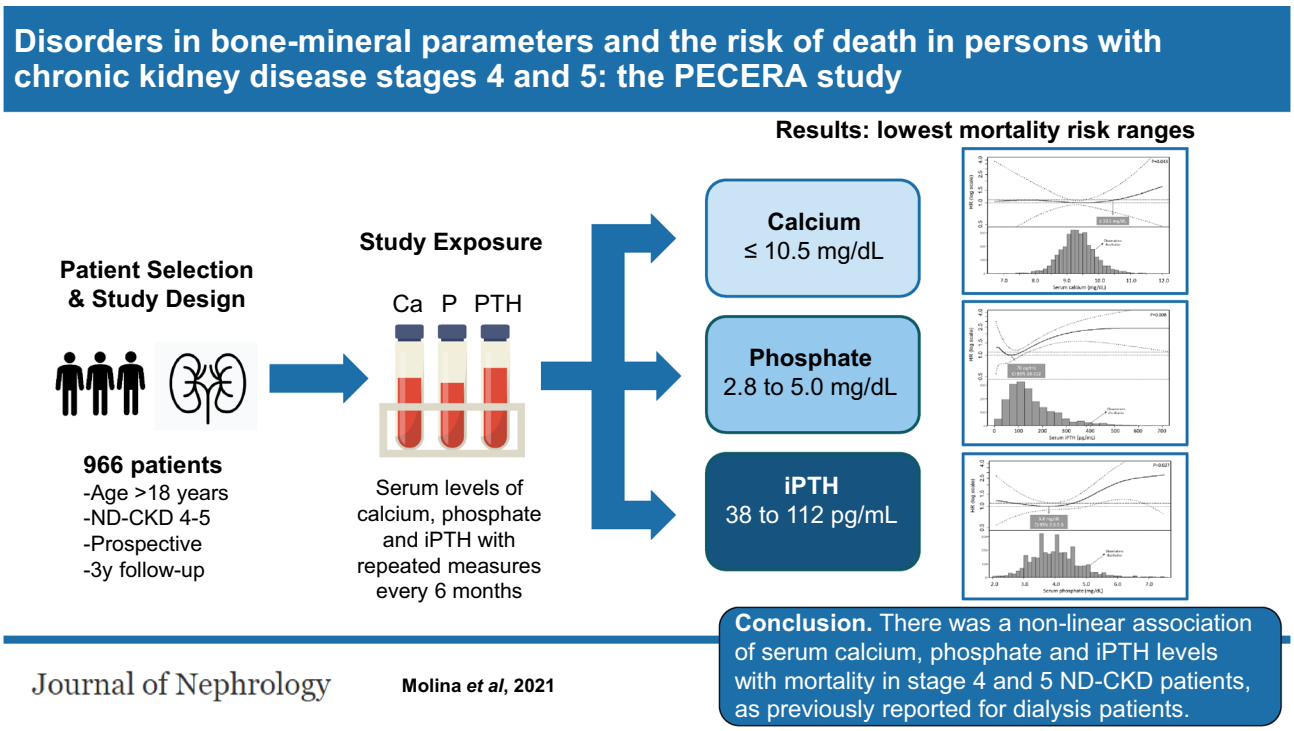
[6] Department of Nephrology, Hospital Universitario y Politécnico La Fe, Valencia, Spain

[7] Department of Nephrology, Hospital Clínico Universitario, INCLIVA, Valencia, Spain

[8] Department of Nephrology, Hospital de Manises, Valencia, Spain

[9] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

Graphic abstract



Keywords Calcium · Chronic kidney disease · Hyperparathyroidism · Phosphate · Survival analysis

Introduction

Abnormalities in serum calcium (Ca), phosphate (P) and parathyroid hormone (PTH), bony derangements and vascular calcification are progressively more common in patients with chronic kidney disease (CKD) stages 4 and 5 and are collectively referred to as CKD-mineral and bone disorders (CKD-MBD) [1–3].

Epidemiological studies associate these disturbances with the risk of both all-cause and cardiovascular mortality of patients with CKD [4–7]. Accordingly, the international Kidney Disease: Improving Global Outcomes (KDIGO) guidelines for CKD-MBD suggest (2C) maintaining serum calcium, phosphate and PTH within different target ranges for each CKD stage, making the assessment and control of theses biochemical parameters an integral component of the routine care of these patients [8].

These recommendations have been challenged and criticized for being based on observational studies [9–11]. However, it is unlikely that large clinical trials evaluating target ranges of CKD-MBD biomarkers will be conducted. Prospective, well-designed observational studies may instead contribute to provide the strength of evidence needed to inform practice [12]. While some prospective cohorts in dialysis have described "optimal" risk ranges for calcium, phosphate and PTH levels based on mortality risk prediction [13, 14], no evidence on the matter exists for persons with non-dialysis-dependent (NDD) CKD stages 4 and 5 [8]. Using data from the PECERA study, we prospectively investigated the longitudinal associations of serum CKD-MBD biomarkers with mortality in patients with CKD stages 4–5 undergoing routine clinical care, and evaluated clinical ranges associated to lowest mortality risks.

Methods

Study design and population

The PECERA (Collaborative Study Project In patients with Advanced Renal Failure) is a multi-center, prospective, observational cohort of NDD patients with CKD stages 4–5 undergoing repeated clinical assessments during a maximum of 3 years. Patients were enrolled from 12 centers in the Valencian Community of Spain. All centers were ascribed to the Spanish National Healthcare system, providing universal and government-subsidized healthcare. The study was

conducted in accordance with the Declaration of Helsinki, and the Hospital Ethics Committees approved the study.

Patient inclusion and data collection

Prospective enrollment occurred between January 2007 and August 2009. All adult patients (equal to or older than 18 years), who were referred to nephrologist-care and had Stage 4 or 5 CKD not receiving dialysis therapy were eligible. After signing informed consent, all patients underwent a baseline examination, and were re-examined after 6, 12, 24 and 36 months.

At each examination, a thorough clinical assessment and routine testing of laboratory markers was performed. The clinical assessment included the recording of demographics, smoking and body size, comorbidity history and ongoing medication. During the follow-up examinations, patient status and hospitalizations were also recorded. Follow-up continued until death, commencement of dialysis or pre-emptive transplantation, loss to follow-up or 36 months from study entry. Patients who started dialysis therapy or were transplanted or lost to follow-up were included in analyses to the point of study discontinuation. As per protocol, all patients starting chronic renal replacement therapy were followed over the first 30 days of dialysis initiation, recalculating their final status as "death" if it occurred during that period.

Standardized data collection was ensured and conducted by nephrologists using internet-based electronic case report forms. The data collected in each center was anonymized and then centralized by the study coordinators. When inconsistencies were identified, the coordinators contacted the reporting centers for verification and/or correction. Each investigator was advised to adhere strictly to best practice guidelines and clinical recommendations.

Study exposure

The study exposure was the serum levels of calcium, phosphate and intact PTH (iPTH), both at baseline and as a time-dependent exposure with repeated measures every 6 months. Calcium levels were corrected using the following formula: Corrected calcium (mg/dL) = measured total calcium (mg/dL) + 0.8 [4.0 − serum albumin (g/dL)], according to KDIGO guideline recommendations [8]. All laboratory evaluations were performed according to the routine methods of the laboratory departments at each center. iPTH levels were assessed by an automated 2nd generation electrochemiluminescence immunoassay. The Elecsys assay (Roche Diagnostic) was used in 10 of the 12 centers, accounting for 91% (n = 866) of the patients included in the study. The Advia Centaur (Bayer) and the Immulite 2500 Intact PTH (Siemens) assays were used in two centers, which enrolled 6% (n = 55) and 3% (n = 28) of patients, respectively.

Study outcomes

The primary outcome was all-cause mortality. The secondary outcome was cardiovascular mortality, defined as death attributable to myocardial ischemia and infarction, heart failure, cardiac arrest because of other or unknown cause, cerebrovascular disease (stroke), or peripheral vascular disease.

Study covariates

Other study covariates were age, sex, comorbidities, ongoing medications, anthropometric measurements, and laboratory tests. Comorbidities registered in our study forms were diabetes mellitus, smoking status, and cardiovascular disease history prior to enrollment. Current medication recorded during each study visit included phosphate binding agents (PBAs), vitamin D compounds, erythropoiesis stimulating agents (ESAs), oral or i.v. iron, and antihypertensives. Other retrieved laboratory parameters were: serum levels of creatinine, sodium, potassium, bicarbonate, albumin, C reactive protein (CRP), and blood cell count. 24-h urine tests included urinary volume, urea, creatinine, sodium, and protein. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula [15].

Statistics

According to the information obtained from the literature, annual mortality in patients with non-dialysis CKD stages 4 and 5 is around 8% [16]. Approximately 45% of this population is estimated to show hyperphosphatemia, defined by phosphate levels higher than 4.6 mg/dL [17]. Several studies have analyzed the influence of phosphate levels on the mortality of non-dialysis CKD patients, showing an 80% increase in mortality in the group of patients with hyperphosphatemia [18]. With a minimum follow-up of three years, assuming losses of 20% and considering an error of beta = 0.8, it is estimated that the initial inclusion of at least 918 patients is required in order to find significant mortality differences between groups with and without hyperphosphatemia.

Categorical variables are presented as percentage and continuous variables as mean ± SD or median (interquartile range), where appropriate. Missing values were replaced by the last measured value (last observation carried forward) [14]. Differences in the characteristics of patients were assessed with one-way analysis of variance for continuous variables and chi-squared test for categorical variables.

Time-dependent Cox proportional hazards regressions were used to assess the association between Ca, P and PTH on all-cause and cardiovascular mortality. The exposure variables (serum levels of phosphate, calcium and PTH at each visit) were introduced in the Cox models

as continuous variables and fitted by using non-linear p-splines [19]. All Cox models were stratified by center. The optimal degree of smoothness was selected using the Akaike Information Criterion.

Three different multivariate models were used for adjustment: Model 0 (crude) included each of the parameters, calcium, phosphate and iPTH, separately. Model 1 included adjustment for demographic, anthropometric characteristics and co-morbidities: age, sex, BMI, waist circumference, cardiovascular comorbidity, diabetes, and blood pressure. Model 2 included the variables of Model 1 plus medications: prescription of vitamin D, PBAs, iron and ESAs. Similarly, Model 3 (full model) was adjusted for all previous variables plus laboratory parameters: serum levels of calcium, phosphate, iPTH, eGFR, albumin, hemoglobin, CRP and potassium, and proteinuria. All variables included were modeled as time-varying throughout every six-month patient visits, including the patient identifier as a cluster variable to account for correlated observations within each patient.

The serum values of calcium, phosphate and PTH with the minimum log hazard ratio (HR) were used as reference (HR = 1.0). Following previous studies [13, 14], the lowest mortality risk ranges were estimated as serum ranges with a hazard ratio ≤ 1.1 (≤ 10% increase in the relative risk of mortality). Afterwards, the serum values of all patients were categorized as below, within and above the lowest mortality risk ranges, and the association of these categories with mortality was assessed. Serum categories were used as time-varying variables, and the reference (HR = 1.0) was the serum values within the estimated ranges. Crude and adjusted relative mortality risks were calculated by using the same three multivariate models described above.

Finally, taking the baseline risk ranges categories as reference, we additionally evaluated the potential survival benefits of remaining or moving across risk ranges categories during follow-up. To this end, categorical changes from the minimal risk range at baseline to another risk category (moving to below or above the minimal risk range) were analyzed in the same manner as above. Because of the limited number of patients with phosphate levels above minimal risk range at baseline (n = 76), analysis of phosphate category changes was only adjusted for age, using a restricted adjustment model [14]. Due to the low number of patients with phosphate (n = 25) and iPTH (n = 34) below minimal risk range at baseline, we did not perform analyses of changes from these categories.

A p value of < 0.05 was considered significant. All statistical analyses were done using R Statistical Software version 3.0.1 with the "survival" packages (R Foundation for Statistical Computing, Vienna, Austria) [19].

Results

Patient characteristics at enrollment, overall and by CKD-MBD biomarker categories

Of the 995 patients enrolled, 966 had non-missing values for demographics, comorbidity history, ongoing medication, and at least one available value for serum phosphate, total calcium and iPTH, and were included in the analysis (Supplementary Figure S1). No differences between included and excluded patients were observed at baseline (Supplementary Table S1). At inclusion, 707 (73%) patients had CKD Stage 4 and 259 (27%) had CKD Stage 5. Mean age was 69.6 ± 13.7 years, and 61% were men. The baseline characteristics by CKD stage are summarized in Table 1.

Supplementary Tables S2–S4 show the characteristics of patients categorized into terciles of distribution according to baseline serum calcium, phosphate and iPTH levels. Patients in the highest tercile of calcium were older, had lower body mass index (BMI) and albumin levels compared to patients in the lowest tercile, who showed the lowest levels of iPTH. The proportion of patients treated with vitamin D and calcium-free PBAs was higher among patients in the highest calcium tercile (Supplementary Table S2). Patients in the highest tercile of phosphate showed lower eGFR and hemoglobin levels, with a higher proportion of diabetes and treatment with PBAs and ESAs (Supplementary Table S3). Many baseline comorbidities and laboratory parameters varied among the different terciles of serum iPTH (Supplementary Table S4). Patients in the highest tercile were older and showed lower eGFR, albumin and hemoglobin levels, with higher proteinuria. proportion of diabetes and treatment with PBAs and ESAs. The proportion of patients treated with active vitamin D and calcium-free PBAs was higher among patients in the highest iPTH tercile.

Follow-up and outcomes

During median follow-up of 29 months (interquartile range: 13–36 months), a total of 3,183 subsequent 6-month patient visits were recorded (mean: 3.3 visits per patient), together with 181 deaths (19%) and 305 (32%) events of kidney replacement therapy initiation. Most deaths were attributed to cardiovascular causes (n = 95; 53%), followed by infections (n = 25; 14%), tumors (n = 20; 11%), renal death (n = 13; 7%), other (n = 10; 6%) and unknown causes (n = 18; 10%). 297 (31%) patients initiated chronic dialysis and 8 (1%) underwent pre-emptive kidney transplantation. Only 67 (7%) patients were lost to follow-up, and censored at the time point of discontinuation.

Table 1 Baseline patient characteristics (n = 966)

Characteristics	N_{base}	All (n = 966)	CKD stage 4 (n = 707)	CKD stage 5 (n = 259)
Age (years)	966	69.6 ± 13.7	69.5 ± 13.9	69.9 ± 12.9
Sex (%)	966			
Men		586 (61%)	447 (63%)	139 (54%)
Women		380 (39%)	260 (37%)	120 (46%)
CKD stage (%)	966			
4		707 (73%)	707 (100%)	–
5		259 (27%)	–	259 (100%)
Cause of CKD (%)	966			
Nephrosclerosis		401 (42%)	302 (43%)	99 (38%)
Diabetic nephropathy		124 (13%)	92 (13%)	32 (13%)
Interstitial		103 (11%)	72 (10%)	31 (12%)
Glomerular		61 (6%)	48 (7%)	13 (5%)
Polycystic kidney disease		44 (5%)	25 (3%)	19 (7%)
Other causes		120 (12%)	91 (13%)	29 (11%)
Not specified		113 (12%)	77 (11%)	36 (14%)
CVD history	966	191 (20%)	141 (20%)	50 (19%)
Diabetes mellitus (%)	966	346 (36%)	266 (38%)	80 (31%)
Smoking (%)	966			
Never		531 (55%)	379 (54%)	152 (59%)
Ex-smoker		330 (34%)	255 (36%)	75 (29%)
Active		105 (11%)	73 (10%)	32 (12%)
BMI (Kg/m^2)	965	28.3 ± 5.3	28.2 ± 5.4	28.4 ± 5.1
Waist circumference (cm)	963	100.9 ± 15.3	100.7 ± 15.4	101.5 ± 14.9
Blood pressure (mmHg)	966			
Systolic		132.7 ± 17.3	132.0 ± 17.1	134.6 ± 17.6
Diastolic		72.8 ± 9.4	72.6 ± 9.5	73.2 ± 8.9
Ca_{alb} (mg/dL)	966	9.3 ± 0.5	9.3 ± 0.5	9.3 ± 0.6
Phosphate (mg/dL)	965	4.0 ± 0.7	3.9 ± 0.7	4.4 ± 0.7
iPTH (pg/mL)[a]	936	133 (85–208)	123 (79–186)	178 (111–275)
eGFR (CKD-EPI, mL/min/$1.73m^2$)	966	18.6 ± 5.0	20.9 ± 3.6	12.4 ± 1.8
24 h Urine Proteinuria (g/24 h)	933	0.66 (0.23–1.65)	0.51 (0.20–1.50)	1.07 (0.46–2.07)
Potassium (mEq/L)	966	4.9 ± 0.6	4.9 ± 0.6	5.0 ± 0.6
Bicarbonate (mEq/L)	807	24.1 ± 3.5	24.3 ± 3.5	23.6 ± 3.4
Albumin(g/dL)	942	4.1 ± 0.4	4.1 ± 0.4	4.0 ± 0.4
CRP (mg/dL)[a]	685	2.0 (0.5–5.1)	2.0 (0.6–5.6)	1.5 (0.4–4.1)
Hemoglobin (g/L)	964	12.3 ± 1.4	12.4 ± 1.5	12.1 ± 1.3
Any CKD–MBD-directed therapy (%)	966			
Native vitamin D		65 (7%)	42 (6%)	23 (9%)
Active vitamin D		311 (32%)	176 (25%)	135 (52%)
Calcium-based phosphate binder		336 (35%)	219 (31%)	117 (45%)
Calcium-free phosphate binder		101 (11%)	46 (7%)	55 (21%)
Antihypertensive drugs (%)	966			
RAAS inhibition (%)		709 (73%)	542 (77%)	167 (64%)
Diuretic (%)		647 (67%)	459 (65%)	188 (73%)
Beta blockers (%)		269 (28%)	199 (28%)	70 (27%)
Other treatments (%)	966			
ESA		427 (44%)	274 (39%)	153 (59%)
Iron		516 (53%)	352 (50%)	164 (63%)

$Ca_{alb,}$ calcium adjusted for albumin levels, *CRP* C reactive protein, *CVD* cardiovascular disease, *eGFR* estimated glomerular filtration rate, *iPTH* intact parathyroid hormone, *CKD-EPI* chronic kidney disease epidemiology collaboration, *n* number of patients, *N_{base}* information available at baseline, *RAAS* renin–angiotensin–aldosterone system.

[a]Skewed values are presented as median (inter-quartile range). If not indicated otherwise, results are presented as mean ± SD

Association between calcium levels and risk of mortality

In both crude and minimally adjusted analyses (models 1 and 2), high and low values of calcium were associated with a higher risk for death (Supplementary Figure S2). In the fully adjusted models, however, the association of calcium with all-cause mortality was J-shaped, and only high values were significantly associated with the risk of mortality (Fig. 1a).

The calcium levels associated with the minimum relative risk of mortality (HR = 1.0) was found at 9.5 mg/dL, being ≤ 10.5 mg/dL the calcium range associated with the lowest risk (HR ≤ 1.1) in the fully adjusted model (p = 0.043 for the joint significance of the polynomial terms) (Table 2). The 95% CI for high serum calcium was wide (Fig. 1a), and only 2% of patients were above the lowest risk range for calcium (Fig. 2), not allowing to detect a significant association between survival and calcium values above the > 10.5 mg/dL threshold (Table 2). For cardiovascular mortality, the lowest mortality risk in the fully adjusted model was 9.3 mg/dL, with the lowest risk ranging from 8.5 to 10.0 mg/dL (p = 0.007), although values below or above this threshold only tended to be associated with a higher cardiovascular mortality risk (Supplementary Table S5).

Association between phosphate levels and risk of mortality

In both crude and adjusted models, the association between phosphate levels and mortality was U-shaped (Fig. 1b and Supplementary Figure S3). The serum values associated with the minimum risk of mortality were 3.8 mg/dL, being the lowest risk range between 2.8 and 5.0 mg/dL (p = 0.027) (Table 2). At baseline, 3%, 89% and 8% of patients were found to be below, within and above this range, respectively (Fig. 2). Phosphate values above the lowest risk range were associated with a higher relative risk of mortality, whereas serum levels below this range did not reach statistical significance (Table 2). For cardiovascular mortality, the phosphate levels associated with the minimum relative risk were reduced to 2.1 mg/dL, being the lowest risk range between 2.1 and 4.4 mg/dL (p < 0.001) in the fully adjusted model. As shown with all-cause mortality, only phosphate values above these ranges were associated with a higher relative risk of cardiovascular mortality (Supplementary Table S5).

Association between iPTH levels and risk of mortality

In both crude and adjusted models, high and low values of iPTH were associated with a higher risk for all-cause mortality (Fig. 1c and Supplementary Figure S4). The

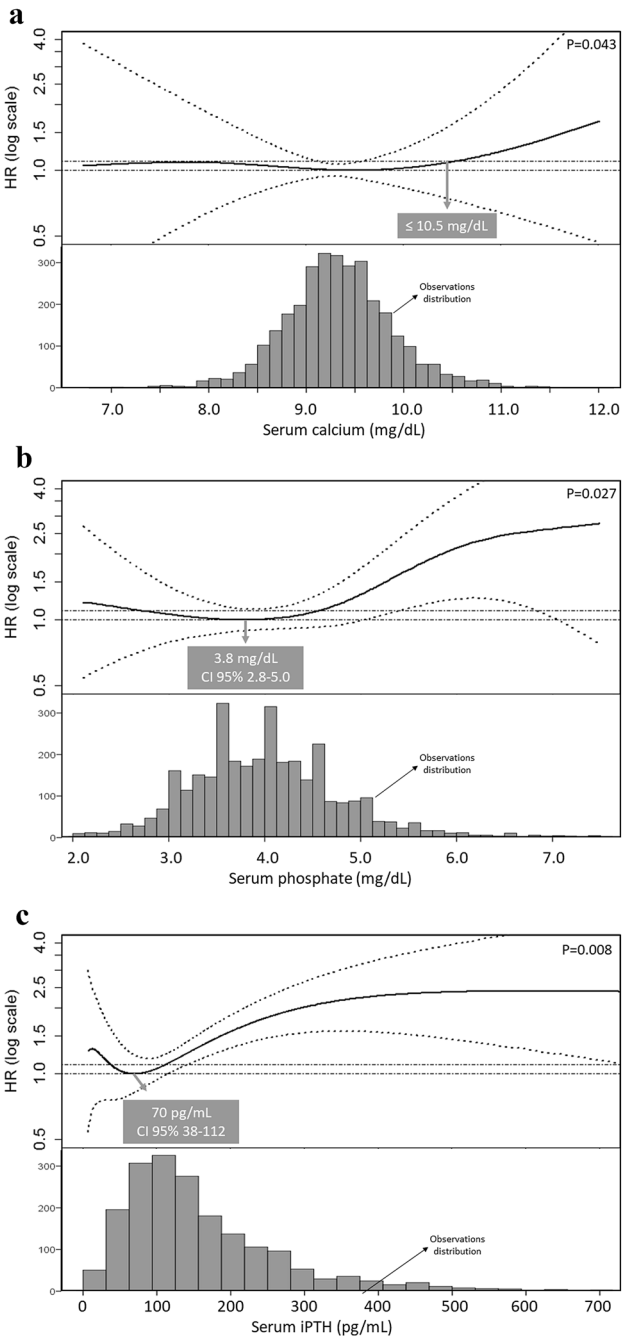


Fig. 1 Hazard ratios (HR) and 95% confidence intervals (CI) for the risk of mortality associated with time-dependent measurements of **a** albumin-corrected calcium, **b** phosphate and **c** iPTH in patients with CKD stages 4–5. The upper panels at each graph graphically depict the output of a restricted cubic spline analysis with time-dependent exposures. Multivariable adjustment considered demographic and anthropometric characteristics, co-morbidities, medications, and laboratory parameters at each repeated patient visit (full list in the legend of Table 2). Grey boxes show the serum concentration (with 95% CI) associated with the lowest risk of mortality. Horizontal dashed lines indicate the minimal risk range of mortality (HR ≤ 1.1). The lower panels show the distribution of calcium, phosphate and iPTH measurements

Table 2 Minimal risk ranges (HR ≤ 1.1) for all-cause mortality for levels of calcium, phosphate and iPTH

Observed minimal risk ranges (HR ≤ 1.1) that predict all-cause mortality					
					Minimal risk range
Calcium (mg/dL)					≤ 10.5
Phosphate (mg/dL)					2.8–5.0
iPTH (pg/mL)					38–112
Association between deviations from the minimal risk range and all-cause mortality					
Biochemical parameter	Below minimal risk range		Within minimal risk range	Above minimal risk range	
	HR (95% CI)	p		HR (95% CI)	p
Calcium (mg/dL)			REF	0.92 (0.41–2.04)	0.83
Phosphate (mg/dL)	1.06 (0.36–3.12)	0.91	REF	2.12 (1.26–3.57)	0.005
iPTH (pg/mL)	1.56 (0.75–3.26)	0.23	REF	1.64 (1.08–2.49)	0.020

The same covariates were included in all the fully adjusted analyses: age, sex, BMI, waist circumference, cardiovascular comorbidity, diabetes, blood pressure, prescription of vitamin D, PBAs, ESAs and iron treatments, serum levels of calcium, phosphate, iPTH, eGFR, albumin, hemoglobin, CRP and potassium, and proteinuria

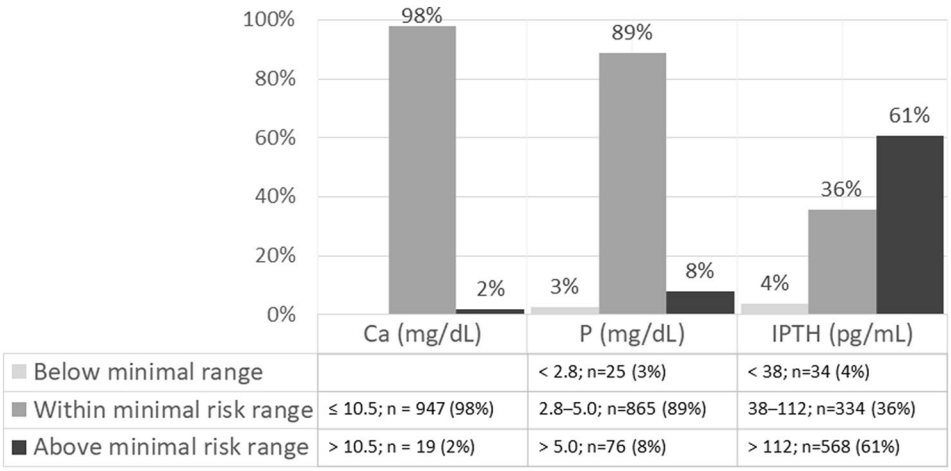


Fig. 2 Percentage of patients within the lowest mortality risk ranges for each serum biochemical parameter at baseline

minimum risk of mortality was found at 70 pg/mL, with the lowest mortality risk ranging from 38 to 112 pg/mL (p = 0.008) (Table 2). At baseline, 4%, 36% and 61% of patients were found to be below, within and above this range, respectively (Fig. 2). iPTH values above the lowest risk range were associated with a higher relative risk of mortality (Table 2). An increased risk was also observed for iPTH values below the lowest risk range, but this did not reach statistical significance.

Lowest risk ranges for cardiovascular mortality did not differ markedly, being 62 pg/mL the iPTH value associated with the minimum relative risk of mortality, with a lowest range between 34 and 109 pg/mL (p = 0.008) in the fully adjusted model. As for all-cause mortality, only iPTH values above these ranges were associated with a higher relative risk of cardiovascular mortality (Supplementary Table S5).

Association between changes in serum calcium, phosphate and iPTH categories and risk of mortality

Figure 2 summarizes the percentage of patients within the lowest mortality risk ranges for each serum biochemical parameter at baseline. Maintaining optimal ranges for both phosphate and PTH levels was related to better survival. In patients with baseline serum phosphate and iPTH values above the lowest mortality risk ranges (> 5.0 mg/dL for serum phosphate, > 112 mg/dL for iPTH levels), the reduction in serum phosphate or iPTH towards the lowest range (2.8–5.0 mg/dL for serum phosphate, 38–112 pg/mL for iPTH levels), was associated with a subsequent lower risk of mortality (Table 3). Conversely, in patients with baseline serum phosphate or iPTH values within the lowest mortality range, increases in the phosphate or iPTH levels were associated with a higher risk of mortality, although statistical

Table 3 All-cause mortality risk associated with changing within risk categories of calcium, phosphate and iPTH from baseline, using a fully adjusted model

Risk categories at baseline	Move below minimal risk range			Move within minimal risk range			Move above minimal risk range		
	HR	95% CI	p	HR	95% CI	p	HR	95% CI	p
Calcium within minimal risk range (≤ 10.5 mg/dL)				1.00	–	–	0.96	0.32–2.88	0.95
Calcium above minimal risk range (> 10.5 mg/dL)[a]				1.46	0.22–9.51	0.69	1.00	–	–
Phosphate within minimal risk range (2.8–5.0 mg/dL)	1.51	0.46–4.89	0.49	1.00	–	–	2.01	1.01–4.02	0.049
Phosphate above minimal risk range (> 5.0 mg/dL)[a]	NA[b]	–	–	0.14	0.03–0.69	0.016	1.00	–	–
iPTH within minimal risk range (38–112 pg/mL)	1.08	0.16–7.29	0.93	1.00	–	–	1.03	0.55–1.93	0.92
iPTH above minimal risk range (> 112 pg/mL)	0.95	0.22–4.21	0.95	0.41	0.18–0.95	0.039	1.00	–	–

Reference with HR = 1.00: staying within the group, the patient was categorized at baseline. Number of patients with phosphate and iPTH below minimal risk range at baseline was too low to perform this survival analysis. The same covariates were included in all the fully adjusted analyses: age, sex, BMI, waist circumference, cardiovascular comorbidity, diabetes, systolic blood pressure, prescription of vitamin D, PBAs, ESAs and iron treatments, serum levels of calcium, phosphate, iPTH, eGFR, albumin, hemoglobin, CRP and potassium, and proteinuria

[a]Only adjusted for age due to the limited sample size

[b]None of the patients with phosphate levels at baseline above minimal risk range changed to below minimal risk range during follow-up

significance was not reached for iPTH. The proportion of patients outside the minimal risk range for calcium at baseline (> 10.5 mg/dL) was very low (Fig. 2), and no survival associations were observed when moving to lower calcium categories (Table 3). Similarly, in patients with baseline serum calcium within the lowest mortality range (≤ 10.5 mg/dL), increases in serum calcium did not show significant association with the mortality risk.

Discussion

We believe this is the largest and longest prospective study evaluating the association between CKD-MBD parameters and survival in patients with non-dialysis dependent CKD stages 4–5. In line with what has been reported for patients on dialysis [13, 14], we found that high calcium levels, as well as low and high phosphate and iPTH levels, were associated with the risk of mortality. We report ranges in which the lowest mortality risk was observed, and that transitioning to a higher or a lower risk range over time consequently predicted the risk of mortality. In the absence of trial evidence, our results may inform clinical decisions on when to initiate or intensify therapeutic strategies to control CKD-MBD disorders in advanced CKD.

The lowest risk value and minimal risk range observed for serum calcium (9.5 mg/dL, range ≤ 10.5 mg/dL) agree with those suggested by KDIGO guidelines [8], where avoidance of hypocalcemia is advocated, and with previous studies. In a historic cohort of 1,243 men with NDD-CKD, Kovesdi et al. observed non-linear associations between time-averaged serum calcium and mortality, with higher risk seen with higher calcium levels. [20]. However, most of the patients included in the study (67%) were in CKD stages 1–3 and hypercalcemia is typically uncommon at that early stage, limiting their inferences on risk associations at high calcium ranges.

Our observed lower risk value and minimal risk range for phosphate (3.8 mg/dL, range 2.8–5.0 mg/dL) are also consistent with KDIGO recommendations of lowering serum phosphate "towards normal" (i.e. 2.8–4.7 mg/dL) [8], while they largely confirm previous studies on phosphate as an independent risk factor for mortality in NDD-CKD patients [4–7, 21, 22].

This said, the observed lower risk value and minimal risk range for iPTH (70 pg/mL, range 38–112 pg/mL) are novel. Due to a dearth of data on clinical outcomes, current KDIGO guidelines indicate that the optimal PTH level in patients with CKD 4–5 not on dialysis is not known, and suggest, somewhat ambiguously, that patients with levels of iPTH progressively rising or persistently above the upper normal limit for the assay be evaluated for modifiable factors. However, they do suggest maintaining iPTH levels in the range of approximately 2–9 times the upper normal limit for the assay in dialysis patients [8], whereas we know that higher iPTH levels are associated with cardiovascular events in the general population, in those whose optimal iPTH levels should be below the upper limit of normal (in the laboratory) [23]. Thus, somewhere around CKD stages 3 to 5 there should be a transition point between these recommendations [24]. Our data can contribute to fill this void of evidence and they correspond well with the ranges recommended by other guidelines [25, 26], which conclude that a modest degree of hyperparathyroidism represents an appropriate adaptive response to declining kidney function, due to its phosphaturic effects and increasing bone resistance to PTH to maintain a normal bone remodeling rate in the setting of CKD. The association between higher iPTH levels and mortality

agrees with a recent cohort study of 536 CKD patients with eGFR between 89 and 15 ml/min/1.73 m^2 [27]. In contrast, Fouque et al. failed to show a relationship between serum iPTH and mortality in a prospective study which included 719 adult patients with non-dialysis Stage 4 or 5 CKD [28], which we attribute to the fact that those analyses assumed the association to be linear. Because of that, we regard our use of penalized splines smoothing analyses as a strength in our report [29].

Another key finding of our study is the independent association between maintaining biochemical parameters towards lowest risk ranges during the 3-year follow-up with a lower relative risk of mortality. It was especially demonstrated for the group of patients above the lowest risk ranges for serum phosphate and iPTH above ranges, which also represented the two conditions associated with a higher risk of mortality. Although no survival benefit could be observed when patients with hypercalcemia at baseline moved into the minimal risk category, we speculate that the limited number of subjects in this group of patients may have reduced the statistical power for detecting significant associations with mortality, as previously noted [13, 14]. Taken together, our data illustrate the potential benefits of controlling these three main CKD-MBD biochemical parameters in patients with NDD-CKD, and provide clinicians with a rationale to guide their routine clinical practice in the absence of randomized clinical trials which may be unlikely to be performed [10].

The assessment of CKD-MBD as a factor for cardiovascular mortality was a secondary aim of this study. Lowest risk ranges for this outcome did not differ markedly for all three parameters, showing a mild reduction in the levels associated with the minimum relative mortality risk. Several mechanisms might explain the increased mortality associated with abnormalities in CKD-MBD biochemical markers. High calcium levels have been linked to vascular calcification, adynamic bone disease and immobility [17, 30, 31]. In addition to the calcification-inducing effects on the vessels [4, 32, 33], phosphate could increase mortality by other mechanisms, such as through fibroblast Growth Factor-23 that has been implicated in the pathogenesis of atherosclerosis and myocardial hypertrophy [34, 35]. Lastly, abnormal levels of iPTH have been implicated in the pathogenesis of left ventricular myocardial function, cardiac fibrosis, vascular calcification, and bone remodeling [36].

The strengths of PECERA reside in the relatively large sample size, prospective data collection and long period of follow-up, with high events rate and minimal loss of follow-up. However, we are limited by the observational nature of our analysis and may still be unpowered for evaluating minimal risk ranges. Thus, confirmation in other independent and preferably geographically and ethnically diverse cohorts is necessary to increase generalizability. The low proportion of patients not receiving CKD-MBD related medications prevented us from investigating the association between spontaneous changes in the laboratory parameters and mortality. Although laboratory parameters were evaluated at each center, most iPTH levels were assessed by the same assay, improving the comparability of the results of the study.

To conclude, we found a non-linear association of serum calcium, phosphate and iPTH levels with mortality in stage 4 and 5 CKD patients. Whereas the ranges of calcium and phosphate associated with the lowest mortality in the study were consistent with the current KDIGO targets, our observed PTH thresholds were lower than currently recommended. All these data suggest potential survival benefits for controlling bone mineral parameters in thispopulation, as previously reported for dialysis patients.

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Compliance with ethical standards

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Ethics approval The study was conducted in agreement with the Declaration of Helsinki, and the Hospital Ethics Committees approved the study.

Consent to participate All patients provided informed consent.

Consent for publication All authors consent for publication.

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