Association between CKD-MBD biomarkers and symptom burden in older patients with advanced CKD: Results from the EQUAL study

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Key points

- In non-dialysis patients with advanced CKD, mild-to-moderately increased PTH is associated with lower levels of reported symptoms.
- Phosphate and calcium are not independently associated with overall symptom burden.
- Patients with both severe hyperphosphatemia and severe hyperparathyroidism had the highest symptom burden.

Abstract

Background: Patients with advanced chronic kidney disease (CKD) develop numerous symptoms, with a multifactorial origin. Evidence linking mineral disorders (CKD-MBD) and uremic symptoms is scant and mostly limited to dialysis patients. Here we aim to assess the association between CKD-MBD and symptom burden in non-dialysis CKD patients.

Methods: We used data from the European Quality study, which includes patients aged ≥65 with eGFR ≤20 ml/min/1.73m^2 from six European countries, followed up to five years. We used generalized linear mixed-effect models to determine the association between repeated measurements of parathyroid hormone (PTH), phosphate and calcium with the overall symptom number (0-33), the overall symptom severity (0-165), and the presence of 33 CKD-related symptoms. We also analyzed subgroups by sex, age, and diabetes mellitus, and assessed effect mediation and joint effects between mineral biomarkers.

Results: The 1396 patients included in the study had a mean of 13±6 symptoms at baseline, with a median overall severity score of 32 (IQR 19-50). The association between PTH levels and symptom burden appeared U-shaped with a lower symptom burden found for mild-to-moderately increased PTH levels. Phosphate and calcium were not independently associated with overall symptom burden. The highest symptom burden was found in patients with a combination of both severe hyperparathyroidism and severe hyperphosphatemia (+2.44 symptoms (0.50, 4.38), *P*=0.01). The association of both hypocalcemia and hyperphosphatemia with symptom burden seemed to differ by sex and age.

Conclusions: In older patients with advanced CKD not on dialysis, mild-to-moderately increased PTH was associated with a lower symptom burden, although the effect size was relatively small (less than one symptom). Neither phosphate nor calcium were associated with

the overall symptom burden, except for the combination of severe hyperphosphatemia and severe hyperparathyroidism which was associated with an increased number of symptoms.

Introduction

Patients with advanced chronic kidney disease (CKD) typically develop a constellation of symptoms defined as uremic syndrome. These may include fatigue, gastrointestinal disorders, muscle cramps, pruritus, and mental status changes[1-7]. Symptom burden affects patients' well-being and health-related quality of life (HRQOL)[8] to such an extent that CKD patients often consider it more important than survival[9]. Moreover, the presence of symptoms forms one of the most pertinent reasons for initiating dialysis[10,11], although it may fail to resolve some symptoms[12] and generate additional ones. A deeper understanding of the mechanisms underlying symptom development is important to identify targeted interventions aimed at their reduction and HRQOL improvement. Several factors are thought to be involved, including multimorbidity and polypharmacy, accumulation of uremic solutes, acid-base imbalances, anaemia, fluid retention, and also mineral disorders.

CKD-Mineral and Bone Disorder (CKD-MBD) is a common complication of CKD, characterized by abnormalities in calcium, phosphate, parathyroid hormone (PTH), or vitamin D metabolism, which lead to bone and cardiovascular (CV) disease[13]. In dialysis patients, elevated PTH has been associated with weight loss[14], impaired appetite[15], pain[16-18], and sleep disorders[19,20]. Hyperphosphatemia has been associated with muscle pain[21], pruritus[21-23], restless legs syndrome[24,25], and sleep disorders[26,27]. Additionally, hypercalcemia has been associated with xerosis and pruritus[21-23,28], and with chronic pain[16], while increasing calcium levels have been inversely associated with the severity of restless legs syndrome[24]. However, the impact of CKD-MBD on symptoms in the larger population of patients with CKD not on dialysis remains largely unknown.

In the present study, we aim to comprehensively assess the association between longitudinal CKD-MBD biomarkers (PTH, phosphate and calcium) and the overall symptom burden in a European cohort of older patients with CKD stages 4-5 not on dialysis in up to

five years of follow up. Secondly, we seek to explore whether mineral biomarkers are associated with the presence of 33 individual CKD-related symptoms.

Materials and methods

Study design and population

The European Quality (EQUAL) study is a prospective cohort study on CKD patients from Germany, Italy, the Netherlands, Poland, Sweden, and the UK, which started in 2012, and is fully described elsewhere[29]. Patients aged ≥65 were included when their estimated glomerular filtration rate (eGFR) dropped to ≤20 mL/min/1.73m^2 and excluded if their eGFR drop was due to an acute event, or if they previously had kidney replacement therapy. For the current study, we selected EQUAL participants with at least one symptoms assessment and at least one measurement of either PTH, phosphate or calcium at the same time (Figure 1). Patients were followed up to five years or until death, start of kidney replacement therapy, refusal for further participation, or loss to follow-up. The study received approval by the Medical Ethics Committee or Institutional Review Boards of all participating countries. Written informed consent was obtained from all patients.

Data collection

Data on demographics, medical history, and medications were collected at baseline. Data on laboratory measurements and symptoms assessment were collected at baseline and updated at each study visit, scheduled at three to six-month intervals. eGFR was calculated using the CKD Epidemiology Collaboration (CKD-EPI) 2009 equation. The measurement of mineral biomarkers was performed according to local practice and not standardized across centres. PTH levels were classified in five predefined groups: <100, 100-200, 200-300, 300-400, and >400 pg/mL. Phosphate levels were classified as hypophosphatemia (<3 mg/dL),

normophosphatemia (3-4 mg/dL), and mild, moderate and severe hyperphosphatemia (respectively 4-5, 5-6, and >6 mg/dL). Albumin-corrected calcium levels were classified as hypocalcemia (<8.5 mg/dL), normal-low calcium levels (8.5-9.5 mg/dL), normal-high calcium levels (9.5-10.5 mg/dL), and hypercalcemia (>10.5 mg/dL). Data on symptom burden were collected through questionnaires, investigating the presence of 33 CKD-related signs and symptoms including the 30 Dialysis Symptom Index (DSI) items[30], and loss of weight, bleeding, and loss of strength, resulting in a total symptom number ranging from 0 to 33. For each symptom present, patients rated symptom severity on a five-point scale ranging from 1 "not bothering at all" to 5 "very much bothering". Absent symptoms were assigned a score of 0, resulting in an overall symptom severity score ranging from 0 to 165. Symptom burden in the EQUAL cohort has already been described elsewhere[8,12,31,32].

Statistical analysis

Patient characteristics were reported as frequencies, means and standard deviation or medians and interquartile range (IQR), as appropriate, and compared between EQUAL participants included and excluded from the current study to assess any selection bias. The associations between repeated measurements of CKD-MBD biomarkers and the overall symptom number and severity, as well as the probability of reporting each symptom, were assessed through generalized linear mixed-effect models (GLMMs) using random intercepts for individuals, clustered by countries, sequentially adjusted for potential confounders. The use of GLMMs is advantageous because they allow patients to have irregular measurement times and are able to handle various patterns of missing data using maximum likelihood estimation, including cases in which some participants have more missing data than others. Missing data at baseline and over time are reported in Supplementary Table 1 and Supplementary Figure 1. Non-linearity was assessed using natural splines. Subgroup analyses

were conducted for sex, age group (65-75 vs. >75 years old), and diabetes mellitus status by the inclusion of interaction terms in GLMMs models. In the analysis of secondary outcomes, to reduce the probability of observing a significant effect by chance because of multiple testing, P values were adjusted using the Benjamini–Hochberg procedure for false discovery rates[33]. As sensitivity analyses, first we assessed effect mediation between different biomarkers by including possible mediators as covariates in the models and comparing effect sizes between the adjusted and unadjusted models, as described by Baron and Kelly[34]. Second, we tested joint effects of different biomarkers by including interaction terms between them in the models. Third, due to the high proportion of missing data in the last year of follow up, we repeated the analysis censoring patients at Year 4. All analyses were performed with R version 4.1.1.

Results

Patient characteristics

At baseline (Table 1), the 1396 patients included in the study had a mean age of 76±7 years and 66% were male. The median Charlson Comorbidity Index (CCI) was 7 (IQR 6-8) and median eGFR 17 ml/min/1.73m^2 (IQR 14-20). Median PTH was high (144 pg/mL, IQR 89-223), whereas median and mean levels of phosphate and calcium were in the normal range (respectively, 3.9 mg/dL, IQR 3.4-4.5, and 9.4±0.3 mg/dL). We did not find clinically relevant differences in baseline characteristics of EQUAL participants included and excluded from the current study (Supplementary Table 2).

Symptom burden

At baseline, patients reported a mean of 13±6 symptoms out of 33, with a median overall severity score of 32 (IQR 19-50) out of 165.

As shown in Figure 2, the most prevalent symptom at baseline was fatigue (72%), followed by musculoskeletal symptoms such as loss of strength (59%), muscle cramps (58%), and bone or joint pain (55%), and skin and mucosal disorders (dry skin, dry mouth and pruritus: 55, 52 and 51%, respectively). Other frequently reported symptoms were decreased interest in sex (53%), difficulty becoming sexually aroused (50%), and swelling in legs (52%).

CKD-MBD biomarkers and overall symptom burden

Both PTH and phosphate levels showed U-shaped associations with the overall symptom burden (Supplementary Figure 2). The lowest symptom burden was observed for mild-to-moderately increased PTH levelsand for normal phosphate levels. When using categories (Figure 3 and Supplementary Table 3), compared to PTH <100 pg/mL, mildly increased levels (100-200 pg/mL) were significantly associated with a reduction in symptom number and severity (respectively, -0.54 (-1.03, -0.05), *P*=0.03, and -1.79 (-3.45, -0.13), *P*=0.04). Hyperphosphatemia tended to be associated with a higher symptom burden in the unadjusted models, but this association was lost after adjustment for kidney function (Supplementary Table 3). Calcium levels were not associated with the overall symptom burden.

CKD-MBD biomarkers and specific CKD-related signs and symptoms

The associations between CKD-MBD biomarkers and the probability of reporting each symptom are shown in Figure 4 and Supplementary Table 4. Compared to the reference category, mild-to-moderately increased PTH seemed associated with a lower probability of bone or joint pain, skin and mucosal disorders and trouble falling asleep. On the other hand, PTH levels seemed to be positively associated with the probability of reporting diarrhea and

shortness of breath. Phosphate levels seemed associated with gastrointestinal symptoms. In particular, hyperphosphatemia seemed to be associated with a higher probability of reporting decreased appetite and diarrhea, and a lower probability of vomiting and constipation. Compared to calcium in the range 8.5-9.5 mg/dL, higher levels seemed to be associated with less frequent muscle cramps, chest pain, and difficulty becoming sexually aroused, whereas hypocalcemia seemed associated with a lower probability of dry mouth.

However, after adjustment for multiple testing none of the above-described associations remained statistically significant.

Subgroup analysis

The observed U-shaped association between PTH and symptom burden was more evident in men and to a lesser extent in women (Figure 5 and Supplementary Table 5). Similarly, higher PTH levels seemed to be more strongly associated with a lower symptom burden in patients older than 75 years compared to younger patients. Severe hyperphosphatemia seemed to have a stronger association with increased symptom burden in females compared to males and in patients aged 65-75 years compared to older patients, although not statistically significant. The association between hypocalcemia and the overall symptom burden differed by sex and age (respectively, *P* for interaction 0.02 and 0.01). Women with hypocalcemia had a lower symptom number (-1.72 (-3.28, -0.16), *P*=0.03) and severity (-6.04 (-11.73, -0.35), *P*=0.04) compared to women with calcium in the reference category, while no effect was found in males. Similarly, in the presence of hypocalcemia, patients older than 75 years had a lower symptom number (-1.10 (-2.14, -0.06), *P*=0.04), while patients aged 65-75 had a higher symptom number (+1.02 (0, 2.04), *P*=0.05), both compared to the reference group. The associations between CKD-MBD biomarkers and individual symptoms in sex and age subgroups are presented in Supplementary Tables 6-7.

The association between CKD-MBD biomarkers and the overall symptom burden did not differ based on diabetes mellitus status (Supplementary Figure 3).

Sensitivity analyses

After adjusting for other mineral biomarkers as possible mediators, the results for PTH and calcium did not change, while severe hyperphosphatemia showed a stronger association with symptom burden, leading to an increase in the number of symptoms of +1.37 ((0.09-2.65), *P*=0.04) compared to normophosphatemia (Supplementary Table 3, models E-H).

We did not detect any joint effect between either calcium and PTH, or calcium and phosphate. PTH and phosphate, however, acted as reciprocal effect modifiers, with the highest symptom burden found in patients with a combination of both PTH >400 pg/mL and severe hyperphosphatemia (+2.44 symptoms (0.5, 4.38), *P*=0.01) compared to patients with normal PTH and phosphate levels (Supplementary Figure 4).

Finally, the high proportion of missingness in the last year of follow up had little effect on the results, as they remained largely unchanged after repeating the analysis using only the first four years of follow up (Supplementary Figure 5).

Discussion

In this study, we describe how CKD-MBD biomarkers are individually and jointly associated with uremic symptom burden in a cohort of older patients with advanced CKD not on dialysis. Interestingly, we found that mild-to-moderately increased PTH was independently associated with a lower symptom burden compared to PTH <100 pg/mL, although the effect size of this association was relatively small (less than one symptom). Phosphate and calcium were not associated with symptom burden, except for a higher burden found in those with severe hyperphosphatemia in the presence of severe hyperparathyroidism. Finally, we

analysed these associations by sex and age, and explored 33 individual CKD-related signs and symptoms to generate hypotheses for future research on CKD-MBD and patient-related outcomes.

Secondary hyperparathyroidism (SHPT) has traditionally been thought to induce both somatic and neuropsychiatric symptoms. In dialysis patients, elevated PTH was associated with anorexia[14,15], pain[16-18], and sleep disorders[19,20]. Moreover, dialysis patients with refractory SHPT have shown reduced bone and joint pain[35,36], and improved HRQOL[37,38], muscle strength[36,39], restless legs syndrome [40], and cognition[41] after parathyroidectomy or calcimimetics therapy. Results on the association between SHPT and pruritus have been conflicting[42-48]. In our cohort, the association between PTH levels and symptom burden appeared U-shaped with the lowest symptom burden found for mild-to-moderately increased PTH levels. Severe hyperparathyroidism (PTH>400 pg/mL) was associated with a significantly higher symptom burden only in the presence of severe hyperphosphatemia. In our pre-dialysis cohort, PTH levels are presumably lower than in dialysis patients, with a shorter duration of SHPT, but evidence for comparison in this particular population is scant. In a cohort of 302 patients with all stages of CKD (88% of whom were not on dialysis), Deme et al. found that PTH levels ≥100 pg/mL were associated with fewer musculoskeletal disorders compared to PTH ≤65 pg/mL (aOR 0.43 (0.21-0.87), P=0.02)[49]. In line with their results, we also found a trend for bone and joint pain reduction for relatively higher PTH levels. Current international guidelines suggest maintaining PTH levels in the range of approximately two to nine times the upper normal limit for the assay in dialysis patients[50], as these levels were associated with the lowest mortality in large observational studies. However, consensus regarding the optimal PTH range in non-dialysis CKD patients has not yet been reached[51] and research has been focusing on hard outcomes such as survival[52], cardiovascular events and fractures. Our results add a different aspect that should be also

taken into account, as we show that, in this specific population, moderate SHPT is not associated with an increased symptom burden, not even for those symptoms traditionally thought to be affected by PTH levels, such as bone pain and pruritus. Thus, from a "symptom/patient-centred" perspective, the optimal PTH levels in this population might not correspond to the normal range for the assay.

Unexpectedly, we did not find significant associations between either hypo- or hyperphosphatemia and overall symptom burden after adjustment for kidney function. However, our sensitivity analyses revealed that the association between hyperphosphatemia and symptom burden might be partially mediated - or confounded - by changes in PTH, and that patients with both severe hyperphosphatemia (>6 mg/dL) and severe hyperparathyroidism (>400 pg/mL) had more symptoms. In our cohort, hyperphosphatemia was not associated with those symptoms previously described in dialysis patients, such as muscle pain[21], pruritus[21-23], restless legs syndrome [24,25], and sleep disorders[26,27]. This difference could be attributed to the fact that our pre-dialysis cohort had lower phosphate levels than dialysis patients, with only 14% of measurements above 5 mg/dL, and a shorter exposure to hyperphosphatemia. The development of pruritus, for instance, might require a longer duration of abnormal calcium and phosphate balance, leading to abnormal distribution of calcium ions in the skin[53] or subcutaneous ectopic calcifications.

In the general population, hypercalcemia may manifest with fatigue, constipation, anorexia, nausea, anxiety, depression, and cognitive dysfunction[54]. On the other hand, hypocalcemia may cause myalgia and muscle cramps, but also emotional instability, anxiety, and depression[55]. However, few studies have investigated the relationship between calcium and symptoms in the specific setting of CKD. In dialysis patients hypercalcemia was associated with xerosis and pruritus[21-23,28], and with chronic pain[16], while increasing calcium levels have been inversely associated with the severity of restless legs syndrome [24]. In non-

dialysis CKD patients, few studies suggested a link between calcium levels and cognitive impairment[56], anxiety, depression and insomnia[57]. In contrast with these previous findings, we did not find significant associations between calcium levels and symptoms, although these findings should be interpreted with caution considering the low prevalence of both hypo- and hypercalcemia in our cohort (respectively, 7% and 5% of all measurements).

Interestingly, our subgroup analyses revealed that the association of both hypocalcemia and hyperphosphatemia with symptom burden differed by sex. Women with hypocalcemia reported a lower symptom burden compared to women with normal calcium levels, while this was not observed in males. On the contrary, women with severe hyperphosphatemia seemed to report a higher symptom burden, while this was not observed in males. It has been previously described that in the EQUAL cohort women had a substantially higher symptom burden compared to men[32], and that this disparity did not seem to be explained by different uremic toxins profiles (including baseline PTH)[58]. Our longitudinal exploration suggests a potential role for mineral biomarkers in explaining sex differences in symptom burden. Although not fully understood, a complex interplay exists between sex hormones and mineral metabolism, with evidence from experimental studies demonstrating that estrogens may affect intestinal calcium absorption[59], tubular phosphate reabsorption[60], PTH transcription[61], and bone homeostasis[62]. Further research is required to confirm the existence of an interaction between sex hormones and mineral biomarkers in symptom burden.

Major strengths of our study were the large sample size from six different European countries improving the generalizability of our results, the long follow-up time with repeated measurements of both CKD-MBD biomarkers and symptom burden, the comprehensive assessment of 33 CKD-related symptoms, and the evaluation of joint effect and mediation among biomarkers to account for their reciprocal nature. To our knowledge this is the first

study analysing the association of PTH, phosphate and calcium with a wide range of 33 CKD-related signs and symptoms in the non-dialysis CKD population. We also acknowledge some limitations. First, laboratory measurements were not centralized, and the specific assays used for biomarkers assessment in each center were unknown. This may have increased the variance in biomarker measurements. However, some of this variation was taken into account by clustering the analyses random effect by country using random effects. Moreover, ionized calcium was not available. We also acknowledge that PTH, phosphate and calcium are also connected with other biomarkers and given the complexity of the CKD-MBD syndrome, it is unlikely that our analysis could fully disentangle the individual contribution of each biomarker to symptom burden. Moreover, we only explored possible mediation phenomena through Baron and Kenny method and did not conduct a formal mediation analysis, as it was beyond the scope of the study. Another limitation is the possibility that missing data on symptoms is not missing at random, as the most ill patients might not have been able to fill in the questionnaire, and some patients could be embarrassed to report some symptoms (e.g., sexual or emotional disorders). Importantly, the observational nature of our study limits our ability to identify and control for unmeasured confounders, including vitamin D, fibroblast growth factor 23, and alkaline phosphatase. For instance, vitamin D deficiency, a known risk factor for hypocalcemia and SHPT, was shown to be associated with depressive symptoms in dialysis patients[63,64]. Even though our findings indicate that phosphate and calcium had no significant impact on the overall symptom burden, we cannot exclude the possibility that other less frequently measured CKD-MBD biomarkers might play a role in symptoms development. Finally, while this study provides valuable insights, it is primarily exploratory. We tested multiple hypotheses, including associations between three CKD-MBD biomarkers and 33 CKD-related symptoms in the whole cohort and in subgroups. The combination of a high number of symptoms tested, the low frequency of some symptoms, and the reduced

sample size in subgroups limited our ability to identify statistically robust associations. Moreover, after adjusting *P* values using the Benjamini–Hochberg procedure for false discovery rates to reduce the probability of observing a significant effect by chance due to multiple testing, none of the individual symptom associations remained significant. Thus, our results need caution in their interpretation and require future clinical trials before informing treatment approaches.

In conclusion, we found that in older patients with advanced CKD not on dialysis, mild-to-moderately increased PTH was associated with a lower symptom burden, although the effect size was small (less than one symptom). Neither phosphate nor calcium levels were associated with the overall symptom burden, except for the combination of severe hyperphosphatemia and severe hyperparathyroidism which was associated with an increased number of symptoms. Although future clinical trials are required to determine treatment approaches, our findings do not support aggressive treatment of CKD-MBD solely for symptom relief in pre-dialysis CKD. Importantly, our results could aid hypotheses generation and patient selection for future research. For instance, patients with both severe hyperphosphatemia and hyperparathyroidism could be a potential target group for further investigation in randomized clinical trials.

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Conflicts of Interest

The authors have nothing to disclose in respect to the present research. However, outside this work: MC declares advisory/lecture fees from Amgen, Abbvie, Shire, Vifor-Pharma, and Baxter; FC received honoraria from Baxter Healthcare; ME reports payment for advisory boards and lectures by Astellas pharma, Vifor Pharma and Astra Zeneca, institutional grants from Astra Zeneca and Astellas pharma.

The authors declare that the results presented in this paper have not been published previously.

Authors' Contributions

LM: conceptualization, formal analysis, investigation, writing the original draft. MC: conceptualization, review. KJ: conceptualization, funding acquisition, supervision, and review. NC: conceptualization, project administration, supervision, and review. All authors contributed to data curation, reviewed the manuscript draft, and approved the final version of the article.

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Data Availability Statement

The data underlying this article are sensitive health data and cannot be shared publicly due to privacy reasons. The data will be shared on reasonable request to the corresponding author.

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Table 1: Baseline characteristics

		Overall (N = 1396)
Socio-demographic factors	Age (years)	76.2 ± 6.7
	Sex, male	923 (66.1)
	Country	
	Germany	136 (9.7)
	Italy	323 (23.1)
	The Netherlands	206 (14.8)
	Poland	78 (5.6)
	Sweden	297 (21.3)
	United Kingdom	356 (25.5)
Medical history	Primary cause of kidney disease	
	Glomerular disease	138 (9.9)
	Tubulointerstitial disease	125 (9)
	Diabetes mellitus	282 (20.3)
	Hypertension	509 (36.6)
	Other/Unknown	335 (24.1)
	Hypertension	1195 (89.1)
	Diabetes mellitus	569 (41.4)
	Charlson comorbidity score	7.0 [6.0, 8]
Kidney function	CKD-EPI eGFR (ml/min/1.73m^2)	17.0 [13.9, 20]
	Creatinine (mg/dL)	3.15 [2.59, 3.76]
	Urea (mg/dL)	115 [92, 144]
Blood exams	PTH (pg/mL)	144 [89, 223]
	Phosphate (mg/dL)	3.9 [3.4, 4.5]
	Corrected calcium (mg/dL)	9.4 ± 0.3
	Sodium (mmol/L)	140.1 ± 3.3
	Potassium (mmol/L)	4.6 ± 0.6
	Albumin (g/dL)	3.8 ± 0.5
	Hemoglobin (g/dL)	11.6 ± 1.5
Medications	Vitamin D	
	Inactive supplements and prodrugs	235 (17)
	Active vitamin D	57 (4.1)
	Phosphate binders	202 (14.6)
	Calcimimetics	14 (1)
Symptoms	Overall symptom number (0-33)	12.6 ± 6.4
	Overall symptom severity (0-165)	32.0 [19, 50]

Notes: Data are reported as mean ± standard deviation for normal continuous variables, median [interquartile range] for non-normal continuous variables, and number (percentage) for categorical variables. For each variable the proportion of missing data is reported. eGFR was calculated using the CKD-EPI 2009 equation (race-based).
Abbreviations: CKD-EPI, chronic kidney disease epidemiology collaboration; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone.
Conversion factors for units: serum creatinine in mg/dL to µmol/L, ×88.4; urea in mg/dL to mmol/L, × 0.167; PTH in pg/mL to pmol/L, ×0.106; phosphate in mg/dL to mmol/L, ×0.323; calcium in mg/dL to mmol/L, ×0.250.

Figure 1
Title: Flow diagram of patient selection.

Figure 2
Title: Prevalence of all symptoms at baseline.
Legend: The bar plot represents the proportion of patients who reported each symptom at baseline. The grey color scale represents the severity of each symptom reported. Symptoms are divided in groups (by color).

Figure 3
Title: Associations between mineral biomarkers and overall symptom burden.
Legend: In each column, the bar plot at the bottom represents the number of measurements of each biomarker in categories; the top and central forest plots represent the association between the biomarker and the overall symptom number and the overall symptom severity, respectively. Numbers reported in the forest plots represent the median symptom number and severity in the reference category. The model represented consists of a generalized linear mixed-effect model using random intercept for individuals, clustered by countries, adjusted for age, sex, baseline Charlson Comorbidity Index, eGFR, urea, albumin, hemoglobin, and baseline medications (vitamin D, phosphate binders and calcimimetics).
Abbreviations: eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone. Conversion factors for units: PTH in pg/mL to pmol/L, ×0.106; phosphate in mg/dL to mmol/L, ×0.323; calcium in mg/dL to mmol/L, ×0.250.

Figure 4
Title: Associations between mineral biomarkers and all symptoms
Legend: Heatmap representing the association between each biomarker category and the probability of having each symptom, reported as odd ratio in each cell. All models are generalized linear mixed-effect models using random intercepts for individuals, clustered by countries, adjusted for age, sex, baseline Charlson Comorbidity Index, and eGFR. Other confounders were used for each specific outcome, as appropriate: urea, albumin, hemoglobin for general and musculoskeletal symptoms; urea, albumin, and baseline medications for gastrointestinal symptoms; albumin and hemoglobin for cardio-pulmonary symptoms; urea and baseline medications for skin and mucosal disorders; urea and hemoglobin for neurological symptoms; hemoglobin for sexual disorders. After adjustment for multiple testing none of these associations remained statistically significant.
Abbreviations: eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone. Conversion factors for units: PTH in pg/mL to pmol/L, ×0.106; phosphate in mg/dL to mmol/L, ×0.323; calcium in mg/dL to mmol/L, ×0.250.

Figure 5
Title: Associations between mineral biomarkers and overall symptom burden in subgroups based on sex and age.
Legend: Panel A shows the association between different categories of PTH (left), phosphorus (middle) and calcium (right) with the total number of symptoms (top) and total

severity (middle), in men (blue) and women (pink). The models represented consist of generalized linear mixed-effect models using random intercepts for individuals, clustered by countries, adjusted for age, kidney function, albumin, hemoglobin, baseline Charlson Comorbidity Index and baseline medications (vitamin D, phosphate binders, calcimimetics). The bar graph at the bottom shows the number of each category by gender. The asterisks represent the statistical significance of the interaction (P<0.05). The numbers in italics represent the average value in the reference category by sex.
Similarly, panel B shows the association between different categories of biomarkers with the overall symptom burden, in patients aged 65 to 75 (green) and patients older than 75 (orange). These generalized linear mixed-effect models are adjusted for sex, kidney function, albumin, hemoglobin, baseline Charlson Comorbidity Index and baseline medications (vitamin D, phosphate binders, calcimimetics).
Abbreviations: PTH, parathyroid hormone.
Conversion factors for units: PTH in pg/mL to pmol/L, ×0.106; phosphate in mg/dL to mmol/L, ×0.323; calcium in mg/dL to mmol/L, ×0.250.

Supplementary Materials
Supplementary Tables (see Excel file)
-Supplementary Table 1: Proportion of missing values at baseline.
-Supplementary Table 2: Baseline characteristics of EQUAL participants included and excluded from the current study.
-Supplementary Table 3: Associations between mineral biomarkers and overall symptom burden in sequentially adjusted models.
-Supplementary Table 4: Associations between mineral biomarkers and all symptoms.
-Supplementary Table 5: Associations between mineral biomarkers and overall symptom burden in subgroups based on sex and age.
-Supplementary Tables 6: Associations between mineral biomarkers and all symptoms in male and females.
-Supplementary Tables 7: Associations between mineral biomarkers and all symptoms in patients aged 65-75 and older than 75 years.
Supplementary Figures
-Supplementary Figure 1: Proportion of missing values in mineral biomarkers and symptom assessments over time.
-Supplementary Figure 2: Associations between continuous mineral biomarkers and the overall symptom burden.
-Supplementary Figure 3: Associations between mineral biomarkers and overall symptom burden in subgroups based on diabetes mellitus.
-Supplementary Figure 4: Interaction between parathyroid hormone and phosphate in the association with the overall symptom burden.
-Supplementary Figure 5: Sensitivity analysis on the first four years of follow up.