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Inflammation and elevated levels of fibroblast growth factor 23 are independent risk factors for death in chronic kidney disease

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Abstract

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Inflammation is a consequence of chronic kidney disease (CKD) and is associated with adverse outcomes in many clinical settings. Inflammation stimulates production of fibroblast growth factor 23 (FGF23), high levels of which are independently associated with mortality in CKD. Few large-scale prospective studies have examined inflammation and mortality in patients with CKD, and none tested the interrelationships between inflammation, FGF23 and risk of death. Therefore, we conducted a prospective investigation of 3875 participants in the Chronic Renal Insufficiency Cohort (CRIC) study with CKD stages 2 to 4 to test the associations of baseline plasma interleukin-6, high sensitivity C-reactive protein, and FGF23 levels with all-cause mortality, censoring at the onset of end-stage renal disease. During a median follow-up of 6.9 years, 550 participants died (20.5/1000 person-years) prior to end stage renal disease. In separate multivariable-adjusted analyses, higher levels of interleukin-6 (hazard ratio per one standard deviation increase of natural log-transformed levels) 1.35 (95% confidence interval, 1.25–1.46), C-reactive protein 1.28 (1.16–1.40), and FGF23 1.45 (1.32–1.60) were each independently associated with increased risk of death. With further adjustment for FGF23, the risks of death associated with interleukin-6 and C-reactive protein were minimally attenuated. Compared to participants in the lowest quartiles of inflammation and FGF23, the multivariable-adjusted hazard ratio of death among those in the highest quartiles of both biomarkers was 4.38 (2.65–7.23) for interleukin-6 and FGF23, and 5.54 (3.04–10.09) for C-reactive protein and FGF23. Thus, **e**levated levels of interleukin-6, C-reactive protein and FGF23 are independent risk factors for mortality in CKD.

Keywords

FGF23; Inflammation; CKD; Mortality

Introduction

Chronic kidney disease (CKD) is a growing public health problem that affects millions of people worldwide.[1] Presence of CKD is a powerful independent risk factor for death,[2, 3] but the underlying pathophysiological mechanisms remain incompletely understood. Multiple lines of evidence support a direct pathogenic role for inflammation. In experimental settings, inflammation contributes mechanistically to CKD progression, insulin resistance, oxidative stress, endothelial dysfunction, atherosclerosis, arterial calcification, and osteodystrophy.[4–12] Supportive human studies demonstrate that inflammatory markers are elevated in patients with CKD and are associated with malnutrition, atherosclerosis, coronary artery calcification, left ventricular hypertrophy, atrial fibrillation, and heart failure, each of which are risk factors for mortality.[13–21] Nonetheless, few observational studies tested the association between inflammation and mortality in CKD stages 2–4. One study reported increased risk of death in association with elevated C-reactive protein (CRP) levels in a secondary analysis of the MDRD trial of non-diabetic and predominantly Caucasian CKD patients.[22] Another study reported increased risk of mortality in association with elevated interleukin-6 (IL-6) levels in a small cohort of 125 Caucasian patients with few deaths.[23] Data are lacking from large prospective cohorts that are more broadly representative of ethnically diverse populations of CKD due to heterogeneous causes.

Elevated levels of fibroblast growth factor 23 (FGF23) are independently associated with increased risk of death in patients with CKD,[24, 25] perhaps in part, by activating fibroblast growth factor receptor 4 (FGFR4) on cardiac myocytes to promote pathological left ventricular hypertrophy and intra-myocardial fibrosis that predispose to heart failure and arrhythmia.[26–29] Elevated FGF23 levels are also independently associated with higher levels of IL-6 and CRP in patients with CKD stages 2–4.[30] Recent experimental data demonstrate that inflammation directly stimulates osteocyte production of FGF23,[31] and that elevated FGF23 can induce production of inflammatory mediators by activating FGFR4 on hepatocytes.[32] Together, these data suggest that elevated FGF23 and its consequences may be an additional novel mechanism of mortality associated with inflammation. We performed a prospective cohort study of individuals with CKD stages 2–4 who enrolled in the Chronic Renal Insufficiency Cohort (CRIC) Study to test the hypotheses that elevated levels of inflammatory markers are independent risk factors for death prior to the onset of end-stage renal disease (ESRD), and that elevated FGF23 levels mediate an important component of inflammation-associated risk of mortality.

Results

Baseline Characteristics

The study population included 3875 of the total 3939 CRIC Study participants who had baseline measurement of IL-6 and FGF23; 3873 of these participants also had baseline measurements of high-sensitivity CRP. Baseline characteristics of the study population according to IL-6 quartiles are presented in Table 1. Mean estimated glomerular filtration rate (eGFR) was 44.3 ± 15.0 mL/min/1.73 m^2, median IL-6 was 1.9 pg/ml (IQR, 1.2–3.2), median high-sensitivity CRP was 2.6 mg/l (IQR, 1.1–6.5), and median FGF23 was 145.6 RU/ml (IQR, 95.8–239.3). Compared to participants in the lowest quartile of IL-6, those with levels in the highest quartile were 4.5 years older, had 9.8 mmHg higher systolic blood pressure and 11 ml/min/1.73m^2 lower eGFR. History of diabetes and cardiovascular disease, and levels of CRP, FGF23 and urinary albumin-creatinine ratio (ACR) increased across quartiles of IL-6, while serum albumin levels decreased (Table 1). Baseline characteristics according to quartiles of FGF23 are shown in Supplemental Table 1.

Individual Effects of Inflammatory Markers and FGF23 on Mortality

During a median follow-up of 6.9 years (IQR, 4.2–8.2), 550 participants died (20.5/1000 person-years) prior to the onset of ESRD. Median levels of IL-6, CRP and FGF23 were significantly higher in participants who died compared to those who remained event-free prior to the onset of ESRD: IL-6, 2.7 (IQR, 1.7–4.6) versus 1.8 (IQR, 1.1–2.9) pg/ml, $p < .0001$; CRP, 4.0 (IQR, 1.6–8.6) versus 2.4 (IQR, 1.0–6.0) mg/l, $p < .0001$; FGF23, 192.1 (IQR, 117.7–324.9) versus 139.2 (IQR, 93.6–225.7) RU/ml, $p < .0001$.

Higher levels of IL-6 and CRP, on the continuous scale and in quartiles, were each independently associated with increased risk of death in unadjusted analyses and in multivariable models that adjusted for conventional cardiovascular risk factors, and CKD- and inflammation-related factors (Figure 1, Table 2). TNF-α was independently associated with increased mortality in crude and adjusted models, but fibrinogen was associated with

increased mortality only in unadjusted analyses of the continuous variable and in the uppermost quartile of the categorical analyses (Supplement Table 2). FGF23 was also independently associated with increased risk of death in all unadjusted and multivariable-adjusted analyses (Table 2). In their separate fully adjusted models, participants in the highest quartiles of IL-6, CRP and FGF23 had 2.65-, 1.89- and 2.48-fold higher risks of death than participants in the respective lowest quartiles of each exposure. These results were qualitatively unchanged when we substituted GFR measured directly as the clearance of iothalamate (iGFR) for eGFR in the subset of 1408 participants with available iGFR measurements (Supplement Table 3). There was no effect modification by age, sex, black race, Hispanic ethnicity, diabetes, history of cardiovascular disease, or baseline eGFR (p for interaction in all adjusted analyses ≥ 0.05).

Dual Effects of Inflammatory Markers and FGF23 on Mortality

To test whether elevated levels of inflammatory markers and FGF23 influence mortality in CKD through shared, separate or synergistic pathways, we added FGF23 to the separate crude and multivariable-adjusted IL-6 and CRP models to determine whether adjusting for FGF23 would weaken the association between the inflammatory markers and death. The point estimates that summarize the associations of IL-6, CRP and FGF23 with death were only modestly attenuated in bivariate and multivariable-adjusted models that included each individual inflammatory marker plus FGF23 on their continuous scales (Table 3). In all models, higher levels of IL-6, CRP and FGF23 remained significantly associated with increased risk of death prior to onset of ESRD. In unadjusted and adjusted tests of interaction, we found no evidence that FGF23 modified the effect of IL-6 or CRP on risk of death (p for interactions ≥ 0.05 for each).

Since frailty is common in patients with CKD and is associated with increased risk of mortality,[33] we tested whether elevated FGF23 and inflammation might share a causal pathway between frailty and mortality risk. We classified participants as frail based on their self-report of fair or poor health in response to question 1 in the Kidney Disease Quality Of Life (KDQOL) questionnaire. In an unadjusted analysis, frailty was associated with increased risk of mortality [HR 1.94 (95%CI, 1.64 – 2.30)], but the strength of association decreased following adjustments for FGF23 and IL-6 [HR 1.53 95%CI, 1.29 – 1.82], whereas FGF23 and IL6 remained leading predictors of mortality in the fully adjusted model that also included frailty (data not shown). Since infection affects inflammation, we conducted additional sensitivity analyses using leukocytosis as a surrogate of occult infection. Only 137 (3.5%) participants had leukocytosis (white blood cell [WBC] count $>10.5 \cdot 10^9$ cells/L), and the results were unchanged in models that adjusted for WBC on the continuous scale or in analyses restricted to participants without leukocytosis (data not shown).

To quantify the dual effects of inflammation and FGF23 in clinically relevant terms, we categorized participants into sixteen groups according to their quartiles of IL-6 and FGF23, and then separately, CRP and FGF23. Membership in ascending quartiles of both IL-6 and FGF23 was associated with additively increased risk of mortality (Figure 2A). Adjusted analyses in which individuals in the lowest quartiles of both IL-6 and FGF23 served as the

referent group confirmed additive effects of ascending IL-6 and FGF23 levels on risk of mortality (Table 4). We observed qualitatively similar results for parallel analyses of CRP and FGF23 (Figure 2B, and Table 5). Compared to participants in the lowest quartiles of each, those in the highest quartiles of IL-6 and FGF23 had a 4.38-fold higher adjusted risk of mortality (95%CI, 2.65–7.23), and those in the highest quartiles of CRP and FGF23 had a 5.54-fold higher adjusted risk of mortality (95%CI, 3.04–10.09).

Discussion

In this prospective cohort study of 3875 participants with CKD enrolled in the CRIC Study, elevated levels of IL-6 and CRP were each independently associated with increased risk of death prior to onset of ESRD. Similar results were observed across levels of age, gender, race, ethnicity, and CKD stage, and among individuals with or without a history of prior cardiovascular disease or diabetes. Interestingly, the strength of association between the inflammatory markers and mortality was only modestly attenuated by adjustment for FGF23, and there was no evidence that FGF23 modified the effects of the inflammatory markers. Instead, elevated levels of inflammatory markers and FGF23 independently increased risk of mortality. These data suggest that elevated levels of inflammatory markers and FGF23 increased risk of mortality mostly through seemingly distinct pathways despite their regulatory effects on one another.

Our results are broadly consistent with prior reports of increased mortality risk in association with elevated levels of inflammatory markers in patients undergoing hemodialysis,[34–36] and in earlier stages of CKD,[22, 23] but the underlying mechanisms are incompletely understood. Inflammation may increase mortality by aggravating injury of glomeruli, the tubular-interstitial compartment, and vascular endothelium that collectively accelerate CKD progression.[5, 37, 38] Indeed, transgenic mice engineered to constitutively overexpress and secrete IL-6 develop progressive glomerulosclerosis and tubular damage.[39] Epidemiological studies support an association between inflammation and CKD progression.[40–42] Alternatively, inflammation may increase mortality by accelerating atherosclerosis and arterial calcification, and increasing risks of arrhythmias and sudden death.[10, 17, 19–21] In support of a direct causal effect of inflammation on cardiovascular disease events in CKD, a recent Mendelian randomization study demonstrated that a functional polymorphism in the IL-6 promoter that is associated with increased IL-6 levels was also associated with increased risk of atherosclerotic cardiovascular events.[43]

An elevated circulating level of FGF23 has emerged as a strong risk factor for death in patients with CKD.[24, 25, 44] In a previous study of 3851 participants in the CRIC Study with CKD stages 2–4, we reported that elevated FGF23 was associated with higher levels of CRP, IL-6, TNF-α and fibrinogen, independent of renal function and other known correlates of FGF23 and inflammation.[30] Other studies corroborated these findings.[45–48] Inflammation directly induces FGF23 transcription and protein expression in osteocyte cell lines, and increases circulating FGF23 levels in animal models with and without CKD, in part, through a hypoxia inducible factor 1α-dependent mechanism.[31] Conversely, elevated FGF23 was sufficient to induce hepatic production of IL-6 and CRP via an FGFR4-dependent but α-Klotho independent mechanism.[32] Based on these experimental studies that suggest a

positive feedback loop between inflammation and elevated FGF23 levels in CKD, we hypothesized that FGF23 and inflammation would have overlapping effects on mortality that would manifest as attenuation of each exposure's individual effects when they were combined in a single multivariable model. Contrary to our hypothesis, we found independent effects of FGF23 and inflammatory markers on risk of mortality. We also found no evidence that inflammation and elevated FGF23 were dual downstream consequences of frailty or occult infection. We speculate that while FGF23 and inflammation may directly increase one another, they may have distinct downstream effects that account for their mostly additive impact on mortality. For example, FGF23 might preferentially increase risks of cardiovascular and infectious death due to consequences of left ventricular hypertrophy, heart failure, and impaired leukocyte function,[49, 50] whereas inflammation might preferentially increase risk due to occlusive atherosclerotic events.[43]

This observational study has limitations. We adjusted for numerous factors that affect inflammation, FGF23, and mortality, but cannot exclude the possibility of residual confounding by unmeasured covariates. Misclassification of true GFR by its estimation is one potential source of residual confounding, however, subgroup analyses of participants with directly measured iGFR yielded congruent results. Lack of vitamin D levels, which are modified by FGF23 and may have effects on inflammatory markers, is another limitation. The single measurements of FGF23 and inflammatory markers that we analyzed may not accurately reflect long-term exposure to these risk factors. However, since random misclassification due to biological variability will usually lead to underestimation of true associations, this limitation is unlikely to explain our findings. Furthermore, inflammation may downregulate renal expression of α-Klotho, which is the co-receptor for FGF23 in the kidney.[51] Klotho expression is reduced in CKD,[52] but in the absence of a validated assay for soluble Klotho, we could not test whether Klotho downregulation due to inflammation contributed to increased mortality associated with elevated levels of inflammatory markers and FGF23.

In conclusion, elevated levels of IL-6, CRP and FGF23 are independent risk factors for mortality in CKD. Despite the independent association between elevated levels of inflammatory markers and FGF23 in patients with CKD, FGF23 neither potentiates nor markedly attenuates the relationship between inflammation and death. Future studies should investigate the impact of FGF23-lowering interventions on inflammatory markers; the impact of anti-inflammatory therapies on FGF23 levels, for example, pentoxifylline and bariticinib, which reduce proteinuria in diabetic kidney disease possibly by anti-inflammatory mechanisms;[53, 54] and potential additive effects of combining such therapeutic strategies to improve clinical outcomes in CKD.

Methods

Study population

The CRIC Study is a prospective cohort study of risk factors for cardiovascular disease, CKD progression and mortality among individuals with moderate CKD at enrollment. Adult participants aged 21–74 years with an age-stratified eGFR of 20 – 70 ml/min/1.73m^2 at the screening visit were enrolled from seven clinical centers across the United States between

2003 and 2008.[55, 56] Participants were excluded for pregnancy, New York Heart Association class III–IV heart failure, human immunodeficiency virus, cirrhosis, myeloma, renal cancer, recent chemotherapy or immunosuppressive therapy, polycystic kidney disease, organ transplantation, or previous treatment with dialysis for at least one month. The study was approved by the institutional review boards at each participating clinical center, and all participants provided written informed consent.

Exposure and Outcomes

The primary exposures were plasma IL-6, high-sensitivity CRP and FGF23. The primary outcome was all-cause mortality prior to the onset of ESRD. Participants' follow up was censored at the time of voluntary withdrawal from the study, loss to follow-up, onset of ESRD, or database locking in mid-2013. In secondary analyses, we investigated tumor necrosis factor (TNF)-α and fibrinogen as additional inflammatory exposures.

Data collection and measurements

Data collected at the baseline visit included demographics, past medical history, smoking status, body mass index (BMI), use of medications, and laboratory test results. Using batched assays of stored plasma samples, the CRIC Study Central Laboratory at the University of Pennsylvania measured IL-6 and TNF-α by enzyme-linked immunosorbent assay (ELISA; R&D systems, Minneapolis, MN; coefficient of variation [CV] <15%); high-sensitivity CRP by particle enhanced immunonephelometry (Dade Behring – Siemens Healthcare, CV<5%); fibrinogen by immunochemical reaction (Dade Behring - Siemens Healthcare, CV<5%); second generation C-terminal FGF23 by ELISA (Immutopics, San Clemente, CA; CV <5%); and parathyroid hormone (PTH) using a total PTH assay that detects both the 1–84 molecule and 7–84 fragment (Scantibodies, Santee, CA; CV<5%). Standard assays were used to measure comprehensive metabolic panels. Glomerular filtration rate was estimated based on the CKD-Epidemiology Collaboration equation (CKD-EPI)[57] and by direct measurement of iGFR in a subgroup of 1408 participants. Albuminuria was quantified as the urinary ACR.

Statistical Analysis

We used descriptive statistics to compare clinical characteristics after categorizing participants in quartiles of baseline IL-6 levels. We chose IL-6 as the inflammatory marker for the descriptive analyses since inflammatory markers are highly inter-correlated and because IL-6 was most strongly associated with mortality and with FGF23 in prior studies of CKD populations.[23, 30, 35] Continuous variables were summarized as mean ± standard deviation (SD) or median with interquartile range (IQR). Categorical variables were expressed as frequencies and proportions. Due to their skewed distributions, we natural log (ln)-transformed IL-6, CRP, FGF23, and PTH.

We used Kaplan-Meier curves to estimate cumulative incidence of mortality according to quartiles of inflammatory markers. We used time-to-event analyses to examine ESRD-censored hazard ratios (HR) of mortality according to one SD increment in baseline ln-transformed IL-6, CRP and FGF23 levels on the continuous scale, and in quartiles, defining the lowest quartile as the reference group. We analyzed IL-6, CRP and FGF23 in separate

Cox proportional hazards models using an identical multivariable modeling strategy. These models were adjusted for conventional cardiovascular risk factors, and CKD- and inflammation-related factors. In model A, we adjusted for demographics (age, sex, race, ethnicity), CKD factors (eGFR, urinary ACR categories [< or ≥300 mg/g, or missing in 3.5% of participants], hemoglobin, serum albumin), and cardiovascular disease risk factors (diabetes, systolic blood pressure, BMI, smoking status, low-density lipoprotein, prior history of cardiovascular disease). In model B, we further adjusted for use of cardio- and reno-protective medications (aspirin, beta blockers, statins, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers), and for use of medications that may alter levels of FGF23 or inflammatory markers (nutritional vitamin D, active vitamin D, phosphate binders, corticosteroids). In model C, we adjusted for the same covariates as in model B plus serum calcium, phosphate and PTH levels.

Since inflammation is associated with sarcopenia,[58] which can reduce the reliability of eGFR as an estimate of true GFR, we performed a sensitivity analysis in which we repeated the main analyses but adjusted for directly measured iGFR in place of eGFR in the 1408 participants in whom iGFR was measured. We used the response to question 1 in the KDQOL questionnaire as a surrogate measure of frailty: In general, would you say your health is: excellent, very good, good, fair, or poor. Participants who answered fair or poor were classified as frail, and we examined models that sequentially adjusted for frailty, FGF23 and inflammation. We adjusted for WBC count and performed a sensitivity analyses that excluded participants with leukocytosis (WBC > $10.5 \cdot 10^9$ cells/L). We tested for effect modification by age, sex, black race, Hispanic ethnicity, diabetes, history of cardiovascular disease, and baseline eGFR. All models were stratified by study site to account for possible regional variability in baseline hazards. We used the same strategy in the secondary analyses of TNF-α and fibrinogen as the inflammatory exposures.

To quantify the dual effects of inflammation and FGF23 on risk of mortality in clinically interpretable terms, we categorized participants into sixteen groups defined by their quartiles of IL-6 and FGF23 (4 by 4 groups). We calculated unadjusted mortality rates per 1000-person-years of follow-up for each group, and multivariable-adjusted HRs for mortality using the same covariates as in model C. The referent group for these analyses included those participants in the lowest quartiles of IL-6 and FGF23. We repeated the analytic strategy for CRP and FGF23. Two-sided p-values <0.05 were considered statistically significant for all tests, including tests for interaction. P values were not adjusted for multiple comparisons. We analyzed the data using SAS, version 9.4 (SAS Institute, Cary, NC) and R version 3.2.2 (2015-08-14) (http://cran.r-project.org).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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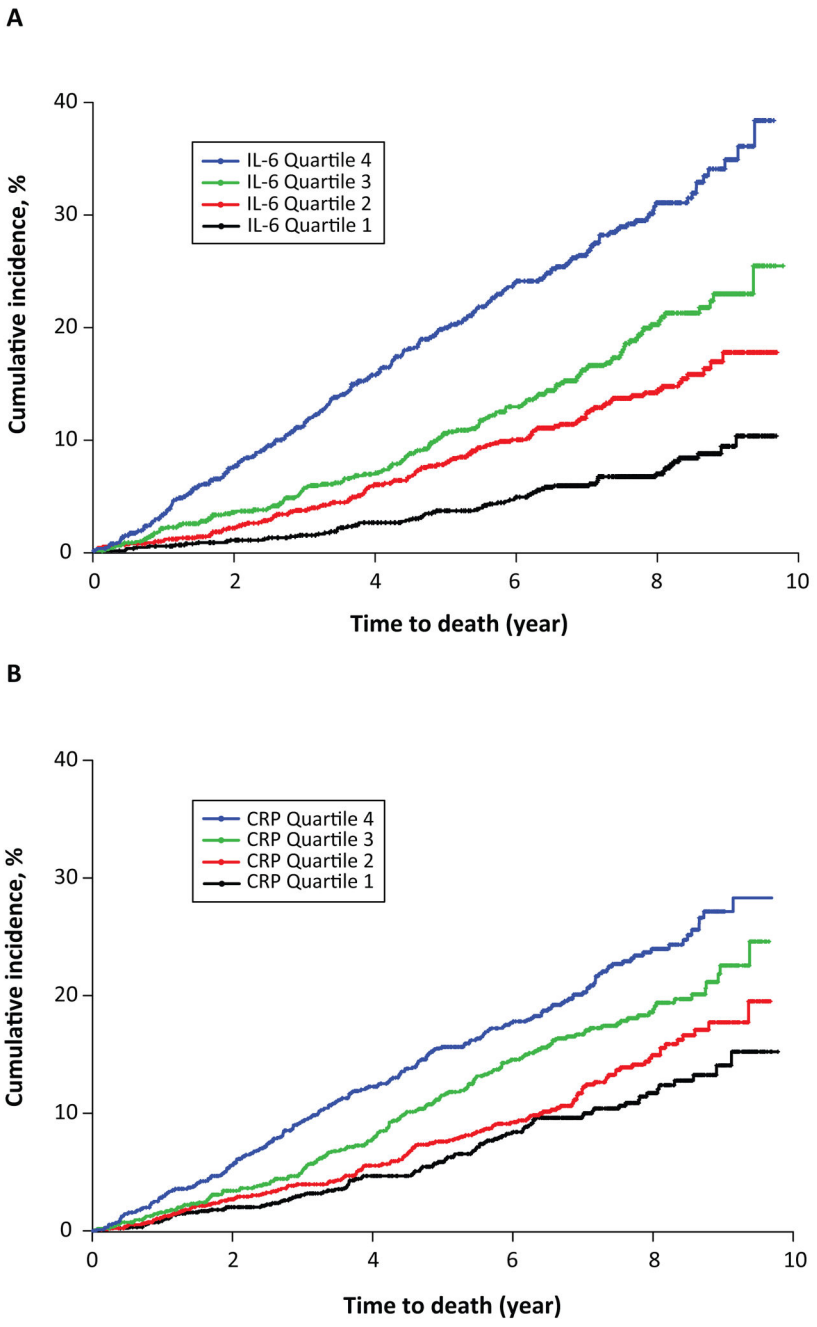
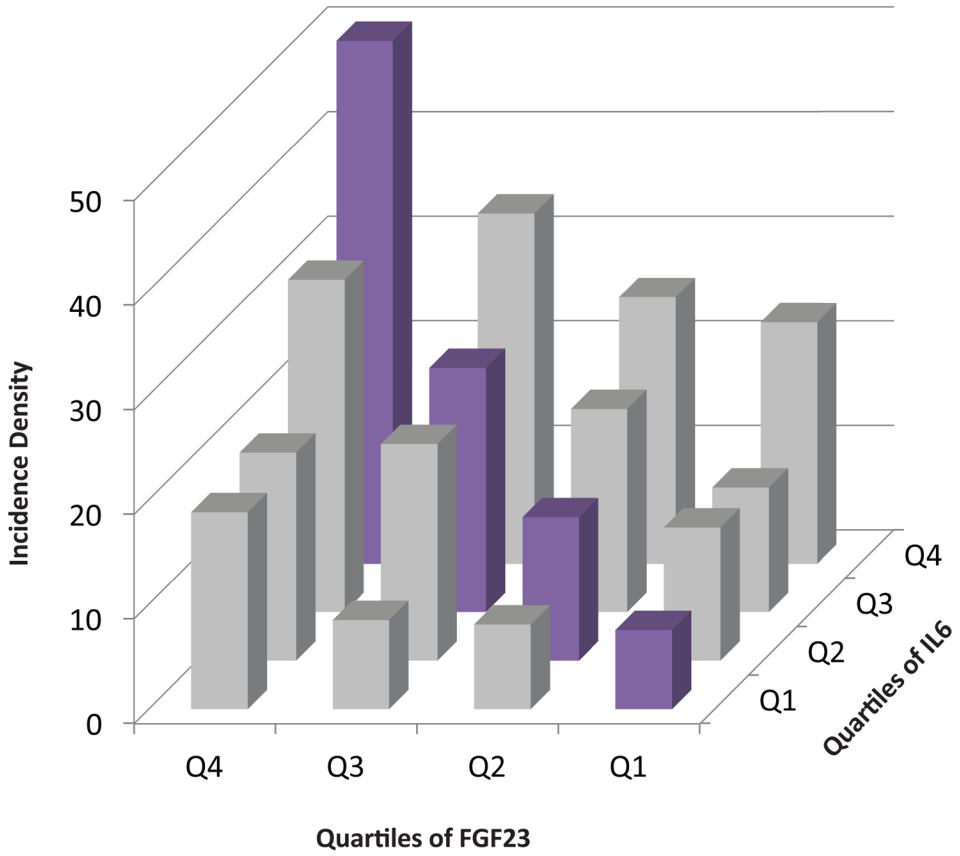


Figure 1. Kaplan–Meier analysis of cumulative incidence of mortality according to quartiles of inflammatory markers

(A) IL-6

(B) CRP



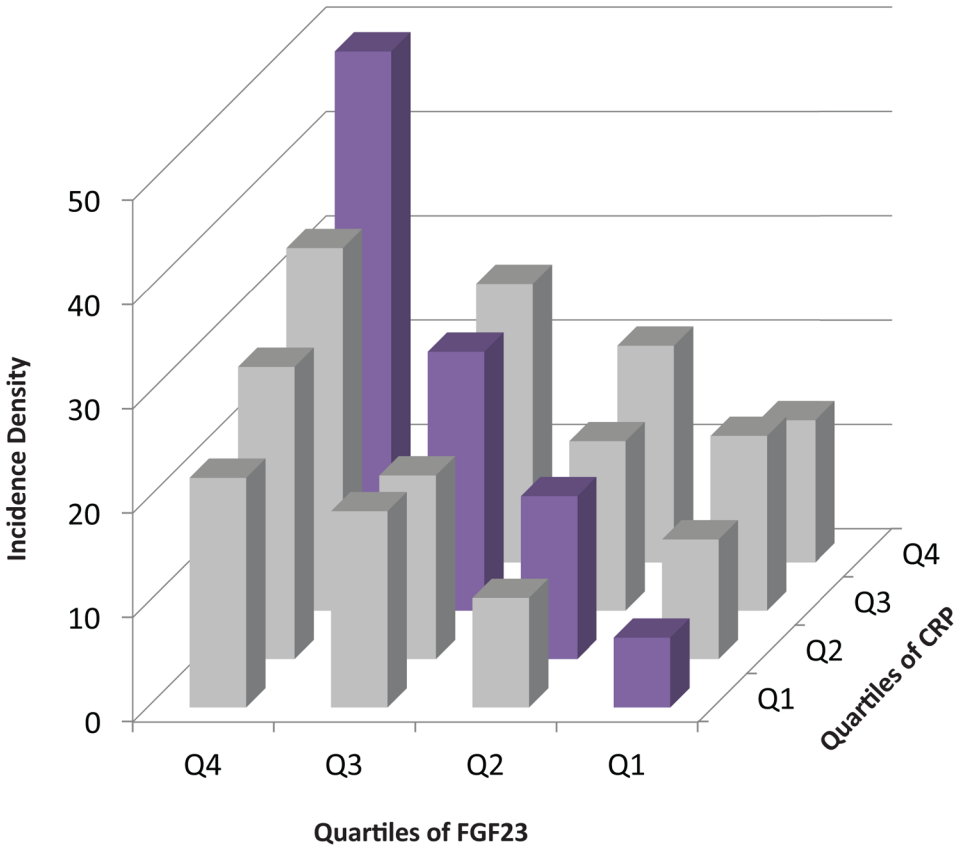


Figure 2. Additive effects of elevated levels of inflammatory markers and FGF23 on risk of mortality

(A) Crude mortality rates according to combined quartiles of IL-6 and FGF23.

(B) Crude mortality rates according to a baseline quartiles of CRP and FGF23

Table 1

Baseline characteristics of the study population according to quartiles of interleukin-6 levels

IL-6, pg/ml	Quartile 1 N = 970 ≤ 1.2	Quartile 2 N = 968 > 1.2 and ≤ 1.9	Quartile 3 N = 969 > 1.9 and ≤ 3.2	Quartile 4 N = 968 > 3.2
Demographics				
Age, years	54.5 ± 11.7	57.7 ± 11.0	59.4 ± 10.3	59.0 ± 10.3
Female, N (%)	405 (41.8)	448 (46.3)	437 (45.1)	446 (46.1)
Black, N (%)	326 (33.6)	377 (39.0)	449 (46.3)	467 (48.2)
Hispanic, N (%)	70 (7.2)	127 (13.1)	143 (14.8)	155 (16.0)
Cardiovascular risk factors				
Hypertension, N (%)	729 (75.2)	843 (87.1)	873 (90.1)	890 (91.9)
Diabetes, N (%)	305 (31.4)	463 (47.8)	555 (57.3)	556 (57.4)
Coronary artery disease, N (%)	119 (12.3)	204 (21.1)	244 (25.2)	285 (29.4)
Congestive heart failure, N (%)	38 (3.9)	75 (7.8)	109 (11.3)	154 (15.9)
Stroke, N (%)	62 (6.4)	97 (10.0)	108 (11.2)	118 (12.2)
Peripheral vascular disease, N (%)	20 (2.1)	66 (6.8)	75 (7.7)	97 (10.0)
Current smoking, N (%)	94 (9.7)	128 (13.2)	116 (12.0)	170 (17.6)
Body mass index, kg/m^2	28.9 ± 5.7	31.7 ± 7.0	33.5 ± 7.9	34.3 ± 9.1
Systolic blood pressure, mm Hg	122.1 ± 18.9	128.9 ± 21.5	131.5 ± 23.5	131.9 ± 23.3
Medication use				
Aspirin, N (%)	363 (37.6)	395 (41.2)	470 (48.9)	422 (43.8)
B -blockers, N (%)	325 (33.7)	462 (48.2)	546 (56.8)	564 (58.6)
Statins, N (%)	459 (47.6)	548 (57.2)	583 (60.7)	534 (55.5)
ACE inhibitors or ARBs, N (%)	604 (62.6)	702 (73.3)	686 (71.4)	656 (68.1)
Phosphate binders, N (%)	65 (6.7)	69 (7.2)	67 (7.0)	69 (7.2)
Active vitamin D, N (%)	22 (2.3)	22 (2.3)	34 (3.5)	46 (4.8)
Nutritional Vitamin D, N (%)	130 (13.5)	92 (9.6)	84 (8.7)	91 (9.5)
Steroids, N (%)	94 (9.7)	87 (9.1)	83 (8.6)	122 (12.7)
CKD Specific Risk Factors				
Creatinine, mg/dl	1.6 ± 0.6	1.8 ± 0.6	1.9 ± 0.6	2.0 ± 0.7
eGFR, ml/min/1.73m^2	51.0 ± 15.9	44.4 ± 14.0	41.9 ± 13.5	39.9 ± 14.2
Urinary ACR, mg/g	17.0 (5.3 – 176.2)	48.5 (8.0 – 427.7)	73.1 (11.8 – 649.0)	117.4 (17.9 – 810.5)
Hemoglobin, g/dl	13.2 ± 1.7	12.7 ±1.7	12.4 ± 1.8	12.1 ± 1.8
Albumin, g/dl	4.1 ± 0.4	4.0 ± 0.5	3.9 ± 0.5	3.8 ± 0.5
LDL, mg/dl	106.3 ± 33.5	104.1 ±35.8	101.6 ± 36.7	98.3 ± 34.9
Mineral Metabolites				
Calcium, mg/dl	9.3 ± 0.5	9.2 ± 0.5	9.1 ± 0.5	9.1 ± 0.5
Phosphate, mg/dl	3.6 ± 0.6	3.7 ± 0.7	3.8 ± 0.7	3.8 ± 0.7
Parathyroid hormone, pg/ml	41.3 (29.0 – 63.0)	52.9 (34.0 – 82.0)	58.0 (38.5 –102.4)	71.0 (43.0 – 115.6)

IL-6, pg/ml	Quartile 1 N = 970 ≤ 1.2	Quartile 2 N = 968 > 1.2 and ≤ 1.9	Quartile 3 N = 969 > 1.9 and ≤ 3.2	Quartile 4 N = 968 > 3.2
FGF23, RU/ml	103.0 (73.6 – 150.5)	141.3 (94.2 – 210.2)	160.9 (109.7 – 243.5)	217.3 (127.3 – 364.0)
Inflammatory markers				
IL-6, pg/ml	0.8 (0.6 – 1.0)	1.5 (1.3 – 1.7)	2.4 (2.1 – 2.7)	4.8 (3.7 – 7.2)
HS-CRP, mg/l	1.2 (0.6 – 2.4)	2.0 (1.0 – 4.1)	3.4 (1.4 – 7.2)	6.7 (2.8 – 13.1)
TNF-α, pg/ml	1.7 (1.2 – 2.4)	2.2 (1.5 – 3.1)	2.4 (1.7 – 3.4)	2.8 (2.0 – 4.0)
Fibrinogen, g/l	3.4 (3.0 – 4.0)	3.9 (3.4 – 4.5)	4.3 (3.6 – 5.0)	4.7 (4.0 – 5.6)

Values expressed as mean ± SD or median (interquartile range).

ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate; ACR, albumin to creatinine ratio; LDL, low-density lipoprotein; FGF23, fibroblast growth factor 23; IL-6, interleukin-6; CRP, high sensitive C-reactive protein; TNF-α, tumor necrosis factor alpha.

Table 2

Individual effects of inflammatory markers and FGF23 on all-cause mortality

For each exposure, results are presented per 1 standard deviation (SD) increase in the overall population and according to quartiles.

			Hazard Ratio (95% Confidence Interval)							
	N (total)	N (events)	Unadjusted	P*	Model A	P*	Model B	P*	Model C	P*
IL-6										
Per 1 SD lnIL-6	3875	550	1.52 (1.43–1.61)	< .0001	1.38 (1.28–1.49)	< .0001	1.38 (1.28–1.48)	< .0001	1.35 (1.25–1.46)	< .0001
Quartile 1 ≤ 1.2	970	64	Reference	< .0001	Reference	< .0001	Reference	< .0001	Reference	< .0001
Quartile 2 ≤ 1.9	968	113	2.03 (1.49–2.75)		1.43 (1.04–1.97)		1.39 (1.01–1.92)		1.34 (0.97–1.85)	
Quartile 3 ≤ 3.2	969	145	2.80 (2.08–3.75)		1.74 (1.28–2.37)		1.73 (1.27–2.36)		1.69 (1.24–2.31)	
Quartile 4 ≤ 3.2	968	228	5.00 (3.79–6.60)		2.93 (2.17–3.95)		2.83 (2.09–3.83)		2.65 (1.95–3.60)	
CRP										
Per 1 SD lnCRP	3873	550	1.39 (1.27–1.51)	< .0001	1.31 (1.19–1.43)	< .0001	1.28 (1.17–1.40)	< .0001	1.28 (1.16–1.40)	< .0001
Quartile 1 ≤ 1.1	974	91	Reference	< .0001	Reference	< .0001	Reference	< .0001	Reference	< .0001
Quartile 2 ≤ 2.6	965	115	1.26 (0.96–1.66)		1.18 (0.90–1.57)		1.19 (0.90–1.58)		1.20 (0.90–1.59)	
Quartile 3 ≤ 6.5	969	154	1.71 (1.32–2.21)		1.57 (1.20–2.04)		1.53 (1.17–2.00)		1.51 (1.15–1.97)	
Quartile 4 >6.5	965	190	2.25 (1.75–2.89)		1.97 (1.51–2.56)		1.87 (1.43–2.44)		1.89 (1.44–2.48)	
FGF23										
Per 1 SD lnFGF23	3875	550	1.64 (1.53–1.76)	< .0001	1.53 (1.40–1.67)	< .0001	1.51 (1.38–1.65)	< .0001	1.45 (1.32–1.60)	< .0001
Quartile 1 ≤ 95.8	969	83	Reference	< .0001	Reference	< .0001	Reference	< .0001	Reference	< .0001
Quartile 2 ≤ 145.6	969	107	1.41 (1.06–1.88)		1.17 (0.87–1.57)		1.16 (0.86–1.57)		1.15 (0.84–1.56)	
Quartile 3 ≤239.3	969	146	2.25 (1.72–2.95)		1.63 (1.22–2.19)		1.65 (1.23–2.22)		1.54 (1.14–2.09)	
Quartile 4>239.3	968	214	4.21 (3.26–5.43)		2.84 (2.11–3.82)		2.79 (2.07–3.77)		2.48 (1.81–3.39)	

Model A: Stratified by site and adjusted for age, sex, race, ethnicity, body mass index, diabetes, smoking status, history of cardiovascular disease, systolic blood pressure, estimated glomerular filtration rate, urine albumin-to-creatinine ratio categories, serum albumin, hemoglobin, low-density lipoprotein

Model B: Model A plus use of use of aspirin, beta blockers, statins, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, active vitamin D, nutritional vitamin D, phosphate binders, and steroids.

Model C: Model B plus serum calcium, phosphate and parathyroid hormone.

* For quartile analyses, p values correspond to tests for linear trend

IL-6, interleukin-6; CRP, high-sensitivity C-reactive protein; SD, standard deviation

Table 3

Dual effects of inflammatory markers and FGF23 on all-cause mortality

	Hazard Ratio (95% Confidence Interval)							
	Unadjusted	**P**	**Model A**	**P**	**Model B**	**P**	**Model C**	**P**
IL-6 and FGF23								
Separate Cox proportional hazards models of each individual exposure (from Table 2)								
per 1 SD of lnIL-6	1.52 (1.43–1.61)	< .0001	1.38 (1.28–1.49)	< .0001	1.38 (1.28–1.48)	< .0001	1.35 (1.25–1.46)	< .0001
per 1 SD of lnFGF23	1.64 (1.53–1.76)	< .0001	1.53 (1.40–1.67)	< .0001	1.51 (1.38–1.65)	< .0001	1.45 (1.32–1.60)	< .0001
Single Cox proportional hazards model that includes both IL-6 and FGF23								
per 1 SD of lnIL-6	1.39 (1.30–1.48)	< .0001	1.30 (1.20–1.41)	< .0001	1.30 (1.20–1.41)	< .0001	1.29 (1.18–1.40)	< .0001
per 1 SD of lnFGF23	1.47 (1.36–1.58)	< .0001	1.42 (1.30–1.55)	< .0001	1.40 (1.28–1.54)	< .0001	1.36 (1.23–1.50)	< .0001
CRP and FGF23								
Separate Cox proportional hazards models of each individual exposure (from Table 2)								
per 1 SD of lnCRP	1.39 (1.27–1.51)	< .0001	1.31 (1.19–1.43)	< .0001	1.28 (1.17–1.40)	< .0001	1.28 (1.16–1.40)	< .0001
per 1 SD of lnFGF23	1.64 (1.53–1.76)	< .0001	1.53 (1.40–1.67)	< .0001	1.51 (1.38–1.65)	< .0001	1.45 (1.32–1.60)	< .0001
Single Cox proportional hazards model that includes both CRP and FGF23								
per 1 SD of lnCRP	1.26 (1.15–1.37)	< .0001	1.23 (1.13–1.35)	< .0001	1.22 (1.11–1.33)	< .0001	1.22 (1.11–1.33)	< .0001
per 1 SD of lnFGF23	1.58 (1.46–1.70)	< .0001	1.48 (1.35–1.62)	< .0001	1.46 (1.34–1.60)	< .0001	1.41 (1.28–1.55)	< .0001

Model A: Stratified by site and adjusted for age, sex, race, ethnicity, body mass index, diabetes, smoking status, history of cardiovascular disease, systolic blood pressure, estimated glomerular filtration rate, urine albumin-to-creatinine ratio categories, serum albumin, hemoglobin, low-density lipoprotein

Model B: Model A plus use of use of aspirin, beta blockers, statins, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, active vitamin D, nutritional vitamin D, phosphate binders, and steroids.

Model C: Model B plus serum calcium, phosphate and parathyroid hormone.

IL-6, interleukin-6; CRP, high-sensitivity C-reactive protein; SD, standard deviation

Table 4

Multivariable-adjusted hazard ratios of mortality according to combined quartiles of IL-6 and FGF23.

		Total N	Deaths, N	HR (95%CI)
FGF23 Quartile 1	IL6 Quartile 1	420	24	Reference
	IL6 Quartile 2	256	24	1.15 (0.63–2.11)
	IL6 Quartile 3	171	15	1.12 (0.58–2.17)
	IL6 Quartile 4	122	20	1.83 (0.98–3.44)
FGF23 Quartile 2	IL6 Quartile 1	293	18	0.93 (0.49–1.74)
	IL6 Quartile 2	251	25	1.08 (0.59–1.97)
	IL6 Quartile 3	252	34	1.64 (0.95–2.83)
	IL6 Quartile 4	173	30	2.15 (1.22–3.78)
FGF23 Quartile 3	IL6 Quartile 1	172	11	0.89 (0.43–1.86)
	IL6 Quartile 2	270	38	1.94 (1.13–3.35)
	IL6 Quartile 3	296	47	1.99 (1.17–3.38)
	IL6 Quartile 4	231	50	2.65 (1.55–4.51)
FGF23 Quartile 4	IL6 Quartile 1	85	11	1.94 (0.90–4.15)
	IL6 Quartile 2	191	26	1.91 (1.03–3.53)
	IL6 Quartile 3	250	49	2.75 (1.60–4.74)
	IL6 Quartile 4	442	128	4.38 (2.65–7.23)

Stratified by site and adjusted for age, sex, race, ethnicity, body mass index, diabetes, smoking status, history of CVD, systolic blood pressure, estimated glomerular filtration rate, urine albumin-to-creatinine ratio categories, serum albumin, hemoglobin, low-density lipoprotein; use of aspirin, beta blockers, statins and angiotensin-converting enzyme inhibitor or angiotensin II receptor blockers, active vitamin D, nutritional vitamin D, phosphate binders, and steroids; serum calcium, phosphate and parathyroid hormone.

Table 5

Multivariable-adjusted hazard ratios of mortality according to combined quartiles of CRP and FGF23.

		Total N	Deaths, N	HR (95%CI)
FGF23 Quartile 1	CRP Quartile 1	299	15	Reference
	CRP Quartile 2	274	23	1.51 (0.77–2.99)
	CRP Quartile 3	208	26	2.19 (1.13–4.26)
	CRP Quartile 4	188	19	1.72 (0.84–3.53)
FGF23 Quartile 2	CRP Quartile 1	259	20	1.33 (0.67–2.66)
	CRP Quartile 2	269	30	1.70 (0.88–3.26)
	CRP Quartile 3	257	30	1.95 (1.02–3.73)
	CRP Quartile 4	183	27	2.32 (1.19–4.51)
FGF23 Quartile 3	CRP Quartile 1	231	30	2.02 (1.05–3.87)
	CRP Quartile 2	245	30	1.98 (1.03–3.82)
	CRP Quartile 3	243	41	2.85 (1.52–5.33)
	CRP Quartile 4	250	45	2.87 (1.54–5.36)
FGF23 Quartile 4	CRP Quartile 1	185	26	2.86 (1.45–5.65)
	CRP Quartile 2	177	32	3.32 (1.71–6.43)
	CRP Quartile 3	261	57	3.36 (1.80–6.25)
	CRP Quartile 4	344	99	5.54 (3.04–10.09)

Stratified by site and adjusted for age, sex, race, ethnicity, body mass index, diabetes, smoking status, history of CVD, systolic blood pressure, estimated glomerular filtration rate, urine albumin-to-creatinine ratio categories, serum albumin, hemoglobin, low-density lipoprotein; use of aspirin, beta blockers, statins and angiotensin-converting enzyme inhibitor or angiotensin II receptor blockers, active vitamin D, nutritional vitamin D, phosphate binders, and steroids; serum calcium, phosphate and parathyroid hormone.