Fibroblast Growth Factor 23 and the Risk of Infection-Related Hospitalization in Older Adults

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ABSTRACT

Within monocytes, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] is important for production of cathelicidins, which in turn, are critical for antibacterial action. Fibroblast growth factor 23 (FGF23) decreases 1,25$(OH)_2$D production and thus, could increase infection risk. We examined this possibility in 3141 community-dwelling adults ages ≥65 years old at baseline in the Cardiovascular Health Study using Cox proportional hazards models to examine the association between FGF23 concentrations and first infection-related hospitalizations and determine whether associations differed by the presence of CKD (eGFR<60 ml/min per 1.73 m^2 [*n*=832] or urine albumin-to-creatinine ratio >30 mg/g [*n*=577]). Mean±SD age of participants was 78±5 years old, 60% of participants were women, and the median plasma FGF23 concentration was 70 (interquartile range, 53–99) relative units per milliliter. In fully adjusted models, higher FGF23 concentrations associated with higher risk of first infection-related hospitalization (hazard ratio [HR], 1.11; 95% confidence interval [95% CI], 1.03 to 1.20 per doubling of FGF23) during a median follow-up of 8.6 years. In participants with or without CKD (defined by eGFR), FGF23 concentration associated with first infection-related hospitalization with HRs of 1.24 (95% CI, 1.08 to 1.42) and 1.06 (95% CI, 0.97 to 1.17) per doubling of FGF23, respectively (*P*=0.13 for interaction). Associations did not differ between groups when stratified by urine albumin-to-creatinine ratio. In sensitivity analyses, the addition of serum calcium, phosphorus, 25-hydroxyvitamin D, intact parathyroid hormone, and 24,25-dihydroxyvitamin D did not meaningfully change the estimates. In conclusion, in community-dwelling older adults, higher plasma FGF23 concentrations independently associated with the risk of first infection-related hospitalization.

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Fibroblast growth factor 23 (FGF23) is a protein synthesized by osteocytes and osteoblasts that acts as a phosphaturic factor. It also suppresses renal synthesis of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] by inhibiting expression of the enzyme 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1).[1,2] Numerous epidemiologic studies have shown high circulating FGF23 concentrations to be independently associated with adverse outcomes, including mortality and cardiovascular events, particularly among individuals with CKD, who have very high FGF23 circulating concentrations.[3–6]

Older adults account for a disproportionate amount of infection-related hospitalizations in the United States, and this number continues to

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rise.[7,8] Additionally, even moderate degrees of decreased kidney function are associated with a clinically significant increase in risk of infection-related hospitalization.[9] Higher FGF23 concentrations are associated with increased risk of infection in patients on chronic hemodialysis[10]; however, to our knowledge, similar associations have not been previously evaluated in older adults without ESRD.

FGF23 has recently been identified as a regulator of innate immunity and has been shown to suppress CYP27B1 in PBMC monocytes from healthy humans similar to its inhibition of CYP27B1 in the kidney, decreasing intracrine production of $1,25(OH)_2D$, concomitant with suppressed production of antibacterial cathelicidins.[11] Similarly, FGF23 treatment in peritoneal dialysate monocyte effluents from uremic patients decreases mRNA expression of CYP27B1.[11] Additionally, recent evidence showed that elevated FGF23 levels in the setting of CKD limit leukocyte recruitment and impair host defense.[12]

The purpose of this study was to determine the association of plasma FGF23 concentrations with first infection-related hospitalization in older community-dwelling adults. Additionally, we sought to determine whether this association was modified by the presence of CKD. We hypothesized that higher circulating FGF23 concentrations would be independently associated with first infection-related hospitalization and that the association would be stronger in individuals with CKD.

RESULTS

Participant Characteristics at Baseline

Among the 3141 study participants, the mean±SD age was 78±5 years old, 60% (n=1885) were women, and 16% (n=507) were black. The mean±SD eGFR was 70.8±19.4 ml/min per 1.73 m^2, and the median urine albumin-to-creatinine ratio (ACR) was 8.7 (interquartile range [IQR], 4.69–20.37) mg/g. Thirty-six percent had CKD (eGFR<60 ml/min per 1.73 m^2 [n=832] and/or urine ACR >30 mg/g [n=577]). The median plasma FGF23 concentration was 70 (IQR, 53–99) relative units per milliliter.

Compared with participants in the lowest FGF23 quartile, those with higher concentrations were older, more frequently women, less likely to be black, and had higher prevalence of chronic diseases, lower eGFR, higher urine ACR, and higher systemic inflammatory markers. Individuals with higher FGF23 concentrations also had higher serum calcium, phosphorus, and intact parathyroid hormone (iPTH) concentrations, whereas 25-hydroxyvitamin D [25(OH)D], 24,25-dihydroxyvitamin D [$24,25(OH)_2D$], and the vitamin D metabolic ratio [VMR; $24,25(OH)_2D$-to-25(OH)D ratio] concentrations were similar across FGF23 quartiles (Table 1).

First Infection-Related Hospitalization

During a median follow-up of 8.6 years (maximum of 17.1 years), 1164 participants (37%) had an infection-related hospitalization. The incidence rate for first infection-related hospitalization was the greatest for the highest quartile of FGF23 concentrations (5.8 infection-related hospitalizations per 100 person-years) (Table 2). Across all quartiles of FGF23 concentrations, the incidence rates for first infection-related hospitalization were greater for participants with eGFR<60 ml/min per 1.73 m^2 (quartile 4 incidence rate: 7.4 infection-related hospitalizations per 100 person-years) than those with eGFR≥60 ml/min per 1.73 m^2 (quartile 4 incidence rate: 4.8 infection-related hospitalizations per 100 person-years).

Relation between FGF23 and First Infection-Related Hospitalization

In analysis adjusted for age, race, and sex (model 1), higher plasma FGF23 concentrations were associated with a higher risk of first infection-related hospitalization (Table 3). This association was attenuated but remained significant after additional adjustment for smoking status, body mass index (BMI), diabetes, congestive heart failure (CHF), coronary heart disease (CHD), eGFR, and urine ACR (model 2). After additional adjustment for albumin, c-reactive protein (CRP), and IL-6 (model 3), the plasma FGF23 concentrations in the highest quartile (compared with the lowest quartile) were associated with a 26% greater risk of first infection-related hospitalization (95% confidence interval [95% CI], 5% to 53%). Similar results were obtained when FGF23 was modeled continuously (hazard ratio [HR] per doubling of FGF23, 1.11; 95% CI, 1.03 to 1.20).

To explore any differential associations of FGF23 with first infection-related hospitalization in patients with and without CKD, we performed the same analysis after stratification by CKD status using two different stratification variables (eGFR<60 and ≥60 ml/min per 1.73 m^2 and urine ACR ≤30 and >30 mg/g) (Table 4). The P values for the FGF23 × eGFR and FGF23 × urine ACR interaction terms were 0.13 and 0.58, respectively. In the final model, the point estimates for first infection-related hospitalization were qualitatively stronger in the subset with eGFR<60 ml/min per 1.73 m^2 than in those with higher eGFR. Each doubling in FGF23 was associated with 24% higher risk of infection-related hospitalization in individuals with eGFR<60 ml/min per 1.73 m^2 (95% CI, 8% to 42%) compared with a nonsignificant 6% higher risk in those with higher eGFR (95% CI, 3% lower to 17% higher). In contrast, associations of FGF23 with infection hospitalization were similar irrespective of albuminuria status. Additional *post hoc* analyses showed no group differences when stratified by the presence of diabetes, CHF, and CHD (Supplemental Table 1) (P values for interaction terms are P=0.52, P=0.82, and P=0.60, respectively).

In secondary analysis using a negative binomial regression, there was no significant association of FGF23 with number of infection-related hospitalizations (entire cohort [model 3]: HR per doubling of FGF23, 1.06; 95% CI, 0.98 to 1.15). When the Cox proportional hazards analysis was limited to only first hospitalization due to bacterial infections or infections considered likely be bacterial in origin, the estimate of association

Table 1. Demographic characteristics and laboratory measurements by quartile of FGF23

Variable	Quartile 1 <53.34 RU/ml, *n*=786	Quartile 2 =53.34–70.34 RU/ml, *n*=785	Quartile 3 =70.35–99.33 RU/ml, *n*=786	Quartile 4 >99.33 RU/ml, *n*=784	*P* Value (for Trend)
Age, yr	77±5	77±5	78±4	79±5	<0.001
Men, *n* (%)	369 (47%)	343 (44%)	281 (36%)	263 (34%)	<0.001
Black race, *n* (%)	181 (23%)	121 (15%)	98 (13%)	107 (14%)	<0.001
Smoking status, *n* (%)					0.002
Never	335 (43%)	324 (41%)	308 (39%)	303 (39%)	
Former	411 (52%)	411 (52%)	399 (51%)	404 (52%)	
Current	40 (5%)	50 (6%)	79 (10%)	77 (10%)	
Diabetes, *n* (%)	104 (13%)	129 (16%)	138 (18%)	199 (25%)	<0.001
CHF, *n* (%)	23 (3%)	44 (6%)	57 (7%)	156 (20%)	<0.001
CHD, *n* (%)	144 (18%)	161 (21%)	188 (24%)	261 (33%)	<0.001
BMI, kg/m^2	26.3±4.3	26.6±4.1	27.3±4.8	27.6±5.3	<0.001
eGFR, ml/min per 1.73 m^2	80.8±18.1	74.4±16.7	69.3±16.2	58.6±19.4	<0.001
ACR, mg/g	6.9 [4.3–14.3]	8.4 [4.5–17.3]	8.3 [4.7–19.0]	13.5 [6.1–54.0]	<0.001
Albumin, g/dl	3.80±0.30	3.81±0.30	3.78±0.29	3.76±0.30	<0.01
CRP, mg/L	4.08±9.38	4.19±7.25	4.97±6.60	5.64±8.24	<0.001
IL-6, pg/ml	3.1±2.1	3.4±2.3	3.9±2.5	4.6±2.7	<0.001
Calcium, mg/dl	9.70±0.53	9.79±0.58	9.78±0.55	9.81±0.64	0.02
Phosphorus, mg/dl	3.70±0.53	3.72±0.51	3.79±0.57	3.95±0.56	<0.001
25(OH)D, ng/ml	27.8±10.3	29.0±11.2	29.4±12.6	27.6±11.4	>0.99
iPTH, pg/ml	43.7±18.2	45.8±21.2	47.1±21.8	62.9±51.9	<0.001
24,25(OH)$_2$D, ng/ml	3.4±1.8	3.6±1.8	3.5±1.9	3.2±1.8	0.22
VMR	0.121±0.040	0.124±0.040	0.120±0.042	0.116±0.042	0.08

Data are mean±SD, *n* (%), or median [IQR]. eGFR was calculated by the cystatin C equation. Data are from a subgroup with a random sample of *n*=881 participants for the following variables: calcium, phosphorus, 25(OH)D, iPTH, 24,25(OH)$_2$D, and VMR. RU, relative unit.

with FGF23 remained similar (model 3: HR per doubling of FGF23, 1.13; 95% CI, 1.05 to 1.22).

Last, we conducted sensitivity analyses in 881 participants randomly selected for other mineral metabolism measurements. Results were similar after additional adjustment for circulating 25(OH)D, iPTH, calcium, phosphorus, and 24,24(OH)$_2$D or VMR (Table 5).

DISCUSSION

In ambulatory, community-dwelling older adults, higher plasma FGF23 concentrations were independently associated with the risk of first infection-related hospitalization. To our knowledge, this is the first study examining the association of FGF23 with infectious outcomes in older adults without ESRD, and it is of potential clinical relevance. Infection-related hospitalizations are associated with significant mortality and have a major economic effect on health care spending.[8] In older adults, hospitalization attributable to infectious disease is substantial and continues to rise over time[7,8]; persons with CKD have both increased complications and longer lengths of stay.[13] Reduced kidney function, even moderately, is associated with a linear increase in risk of first infection-related hospitalization.[9] Importantly, the mechanisms contributing to this risk are incompletely understood.[14] Measurement of FGF23 may provide new insights into risk of infection in community-living older adults and patients with kidney function decline.

To the best of our knowledge, only one other study has examined the association between FGF23 and infectious outcomes, and that study was in a population of patients on chronic hemodialysis.[10] In that study, higher circulating FGF23 concentrations were independently associated with higher risk of infection as defined by time to first infectious hospitalization or infectious death. Finding a similar association in a population of community-dwelling older adults is a novel observation, may provide new insights to mechanisms contributing to infection risk in older persons, and may ultimately have treatment implications for individuals at high risk of infection.

The mechanisms that may link increased circulating FGF23 with infection are incompletely understood. As was also the case in the recent study in patients on chronic hemodialysis,[10] inflammation did not seem to be an important mechanism, because adjustment for CRP and IL-6 did not significantly attenuate this association; however, these measurements were temporally separated from the outcomes and may have increased at the time of infection-related hospitalization. In sensitivity analyses further adjusted for other markers of mineral metabolism, the association was also unchanged. Although we did not assess the independent association of 25(OH)D with first infection-related hospitalization, it has been previously shown that higher circulating 25(OH)D

Table 2. Incidence rates of first infection-related hospitalization by FGF23 quartile for the overall cohort and stratified by eGFR

FGF23	No. of Events	Person-yr	Rate per 100 person-yr
Overall Cohort			
Quartile 1: <53.34 RU/ml (*n*=786)	266	79.6	3.3
Quartile 2: 53.34–70.34 RU/ml (*n*=785)	293	75.1	3.9
Quartile 3: 70.34–99.33 RU/ml (*n*=786)	297	71.2	4.2
Quartile 4: >99.33 RU/ml (*n*=784)	308	52.7	5.8
eGFR≥60 ml/min per 1.73 m^2			
Quartile 1: <53.34 RU/ml (*n*=720)	244	74.1	3.3
Quartile 2: 53.34–70.34 RU/ml (*n*=639)	233	62.9	3.7
Quartile 3: 70.34–99.33 RU/ml (*n*=570)	209	55.7	3.8
Quartile 4: >99.33 RU/ml (*n*=380)	153	31.7	4.8
eGFR<60 ml/min per 1.73 m^2			
Quartile 1: <53.34 RU/ml (*n*=66)	22	5.5	4.0
Quartile 2: 53.34–70.34 RU/ml (*n*=146)	60	12.3	4.9
Quartile 3: 70.34–99.33 RU/ml (*n*=216)	80	15.5	5.7
Quartile 4: >99.33 RU/ml (*n*=404)	155	21.0	7.4

RU, relative unit.

concentrations are associated with decreased risk of infection in patients on chronic hemodialysis[10] and community-dwelling adults.[15] However, it is possible that circulating markers of mineral metabolism do not reflect local $1,25(OH)_2D$ production, and these pathways may still mediate the association of FGF23 with first infection-related hospitalization, consistent with evidence that FGF23 reduces $1,25(OH)_2D$ production locally in monocytes.[11] Because FGF23 inhibits conversion of 25(OH)D to $1,25(OH)_2D$ by CYP27B1, elevated FGF23 may still predispose older adults to infection risk, even in the presence of adequate 25(OH)D. Thus, administering active vitamin D in older adults experiencing infection may have beneficial effects and is a compelling hypothesis for future research.

CYP27B1 and the vitamin D receptor are expressed in immune cells, and $1,25(OH)_2D$ may have autocrine or paracrine roles.[16] In response to binding of bacterial wall LPS, CYP27B1 and the vitamin D receptor expression are upregulated, increasing conversion of 25(OH)D to $1,25(OH)_2D$. This subsequently increases transcription of cathelicidins, bactericidal proteins that are particularly dependent on sufficient circulating 25(OH)D.[17,18] FGF23 has been shown to decrease CYP27B1 expression in monocytic cells, decreasing production of $1,25(OH)_2D$, concomitant with suppression of antibacterial cathelicidins.[11] The association with FGF23 was similar when a subanalysis was performed including only bacterial infections or infections considered likely to be bacterial in origin, consistent with a potential role of FGF23 in the transcription of cathelicidins. Recent evidence indicates that FGF23 may also directly impair neutrophil recruitment and host defense in the setting of CKD by interfering with leukocyte integrin activation and recruitment.[12]

Although the test for interaction was not statistically significant, the association of FGF23 with first infection-related hospitalization was qualitatively stronger by comparison of point estimates in participants with CKD as defined by eGFR<60 ml/min per 1.73 m^2. Other studies have also shown a stronger association of FGF23 with adverse outcomes in patients with CKD.[3,19] FGF23 may be particularly harmful in CKD by limiting conversion of 25(OH)D to $1,25(OH)_2D$.[20] Alternatively, it has been proposed that high FGF23 may mark a novel axis of kidney dysfunction, providing information about dimensions beyond those obtained by eGFR or urine ACR.[3] It is unclear why a moderate effect modification was seen on the basis of eGFR but not by urine ACR categories, but this may be due to the fact that albuminuria is measuring an earlier stage of kidney disease or reflects the ability of the kidney to maintain mineral metabolism homeostasis less. Thus, these results for first infection-related hospitalization differ from the association between FGF23 and both all-cause mortality and cardiovascular events, in which results were found to be stronger in individuals with CKD defined by either eGFR<60 ml/min per 1.73 m^2 or a urine ACR ≥30 mg/g.[3] Of note, little is known regarding the association of proteinuria with risk of infection as well as the risk of infection in general in nondialysis-dependent CKD.[14]

There was a significant association of FGF23 with first infection-related hospitalization using Cox proportional hazards analysis but not with number of infection-related

Table 3. Associations (HRs and 95% CIs) of FGF23 with first infection-related hospitalization

Model	Quartile 1 <53.34 RU/ml, *n*=786	Quartile 2 =53.34–70.34 RU/ml, *n*=785	Quartile 3 =70.35–99.33 RU/ml, *n*=786	Quartile 4 >99.33 RU/ml, *n*=784	Per Doubling of FGF23
Model 1	Reference	1.20 (1.01 to 1.41)	1.28 (1.08 to 1.51)	1.88 (1.58 to 2.22)	1.30 (1.22 to 1.39)
Model 2	Reference	1.08 (0.92 to 1.28)	1.07 (0.90 to 1.28)	1.31 (1.09 to 1.58)	1.14 (1.06 to 1.22)
Model 3	Reference	1.08 (0.91 to 1.28)	1.05 (0.88 to 1.25)	1.26 (1.05 to 1.53)	1.11 (1.03 to 1.20)

Model 1: age, race, and sex. Model 2: model 1, smoking status, BMI, diabetes, CHD, CHF, eGFR (cystatin C equation), and urine ACR. Model 3: model 2, albumin, CRP, and IL-6. RU, relative unit.

Table 4. Associations (HRs and 95% CIs) of FGF23 with first infection-related hospitalization stratified by kidney disease status (eGFR and urine ACR)

Model	Quartile 1 <53.34 RU/ml, *n*=786	Quartile 2 =53.34–70.34 RU/ml, *n*=785	Quartile 3 =70.35–99.33 RU/ml, *n*=786	Quartile 4 >99.33 RU/ml, *n*=784	Per Doubling of FGF23
eGFR≥60 ml/min per 1.73 m^2					
Model 1	Reference	1.15 (0.96 to 1.38)	1.18 (0.98 to 1.42)	1.58 (1.29 to 1.94)	1.19 (1.09 to 1.29)
Model 2	Reference	1.05 (0.87 to 1.26)	1.03 (0.85 to 1.25)	1.25 (1.01 to 1.55)	1.09 (0.99 to 1.19)
Model 3	Reference	1.04 (0.87 to 1.25)	0.99 (0.82 to 1.21)	1.19 (0.96 to 1.49)	1.06 (0.97 to 1.17)
eGFR<60 ml/min per 1.73 m^2					
Model 1	Reference	1.34 (0.82 to 2.19)	1.54 (0.96 to 2.46)	2.14 (1.35 to 3.40)	1.42 (1.26 to 1.59)
Model 2	Reference	1.31 (0.80 to 2.16)	1.31 (0.81 to 2.11)	1.59 (0.98 to 2.58)	1.26 (1.10 to 1.44)
Model 3	Reference	1.35 (0.82 to 2.22)	1.33 (0.82 to 2.16)	1.62 (1.00 to 2.64)	1.24 (1.08 to 1.42)
ACR≤30 mg/g					
Model 1	Reference	1.13 (0.94 to 1.36)	1.26 (1.05 to 1.50)	1.61 (1.32 to 1.95)	1.23 (1.13 to 1.33)
Model 2	Reference	1.06 (0.88 to 1.27)	1.12 (0.93 to 1.35)	1.28 (1.04 to 1.57)	1.12 (1.03 to 1.23)
Model 3	Reference	1.05 (0.88 to 1.26)	1.09 (0.91 to 1.32)	1.24 (1.00 to 1.53)	1.11 (1.02 to 1.21)
ACR>30 mg/g					
Model 1	Reference	1.38 (0.88 to 2.16)	1.24 (0.78 to 1.96)	2.16 (1.42 to 3.30)	1.37 (1.21 to 1.54)
Model 2	Reference	1.22 (0.77 to 1.92)	0.86 (0.53 to 1.40)	1.33 (0.82 to 2.15)	1.17 (1.00 to 1.36)
Model 3	Reference	1.19 (0.75 to 1.88)	0.82 (0.50 to 1.35)	1.24 (0.76 to 2.02)	1.13 (0.96 to 1.32)

Model 1: age, race, and sex. Model 2: model 1, smoking status, BMI, diabetes, CHD, CHF, eGFR (cystatin C equation), and urine ACR. Model 3: model 2, albumin, CRP, and IL-6. RU, relative unit.

hospitalizations using a negative binomial regression; the latter method measures the rate of infection (*i.e.,* number of infections per person-time), whereas Cox regression assesses the hazard rate of first infection. Thus, because there was longer person-time follow-up when using the negative binomial regression model, there was more attenuation of the association due to length of time between the baseline measurement of FGF23 and the outcome of interest. Additionally, the difference may be explained by the fact that FGF23 levels may be important for first infectious event but less so for later events, perhaps because prior infection may be a more important risk factor for subsequent infections.

There are several important limitations to this study. Because of the nature of the analysis, the results are observational only. Future studies should confirm these findings and test whether supplementation with $1,25(OH)_2D$ may decrease infection rates in older adults with elevated FGF23 concentrations. We adjusted for several important covariates, but the results may be subject to residual confounding. Whether residual confounding by severity of kidney function may influence this association requires future study and confirmation in other settings. We hypothesize that FGF23 may be related to infection-related hospitalizations by influencing $1,25(OH)_2D$ production within monocytes. With available data within the Cardiovascular Health Study (CHS), we were not able to test monocyte $1,25(OH)_2D$ production on the basis of FGF23 concentrations, and we did not have systemic $1,25(OH)_2D$ or cathelicidin concentrations available due to limited sample volume. However, $24,25(OH)_2D$ levels and VMR were measured as novel markers of vitamin D activity and adequacy and indirect markers of $1,25(OH)_2D$; $24,25(OH)_2D$ is the most abundant product of vitamin D catabolism. Because 24-hydroxylase is directly induced by $1,25(OH)_2D$, lower serum levels of $24,25(OH)_2D$ indicate diminished vitamin D activity,[21,22] and VMR reflects vitamin D adequacy.[23] Additionally, the findings may not apply to populations of younger adults, and International Classification of Diseases,

Table 5. Associations (HRs and 95% CIs) of FGF23 with first infection-related hospitalization for the 881 participants in a random subgroup with measurements of markers of mineral metabolism

Model	Quartile 1 <53.34 RU/ml, *n*=223	Quartile 2 =53.34–70.34 RU/ml, *n*=228	Quartile 3 =70.35–99.33 RU/ml, *n*=220	Quartile 4 >99.33 RU/ml, *n*=210	Per Doubling of FGF23
Model 1	Reference	1.25 (0.90 to 1.73)	1.50 (1.08 to 2.08)	2.96 (2.15 to 4.09)	1.61 (1.43 to 1.81)
Model 2	Reference	1.10 (0.79 to 1.53)	1.34 (0.96 to 1.88)	2.19 (1.54 to 3.13)	1.42 (1.24 to 1.63)
Model 3	Reference	1.07 (0.77 to 1.49)	1.27 (0.91 to 1.79)	2.07 (1.44 to 2.97)	1.37 (1.19 to 1.57)
Model 4a	Reference	1.04 (0.75 to 1.45)	1.24 (0.88 to 1.75)	2.02 (1.41 to 2.91)	1.35 (1.17 to 1.55)
Model 4b	Reference	1.04 (0.74 to 1.45)	1.25 (0.89 to 1.75)	1.99 (1.38 to 2.86)	1.34 (1.17 to 1.55)

Model 1: age, race, and sex. Model 2: model 1, smoking status, BMI, diabetes, CHD, CHF, eGFR (cystatin C equation), and urine ACR. Model 3: model 2, albumin, CRP, and IL-6. Model 4a: model 3, calcium, phosphorus, 25(OH)D, iPTH, and $24,25(OH)_2D$. Model 4b: model 3, calcium, phosphorus, iPTH, and the VMR [$24,25(OH)_2D$-to-25(OH)D ratio]. RU, relative unit.

9th Revision, Clinical Modification (ICD-9-CM) codes were used to ascertain the outcome of interest.

However, there are also several important strengths, including a large number of participants from a community-based population, long-term follow-up, and a large number of infectious hospitalizations. Because of the nature and data collected in the CHS, it was possible to perform comprehensive adjustment for covariates. A large subgroup was also available to study the role of CKD in the association of FGF23 with infectious outcomes. Last, markers of mineral metabolism were measured in a subgroup of participants to provide additional mechanistic insight.

In conclusion, in community-dwelling older adults, higher plasma FGF23 concentrations were independently associated with risk of first infection-related hospitalization. Future studies are needed to confirm postulated mechanisms underlying the associations and determine whether administering $1,25(OH)_2D$ or reducing FGF23 may reduce the risk of infectious outcomes.

CONCISE METHODS

Study Design

The study methods of the CHS, a prospective, longitudinal study of older community-dwelling adults, have been described previously.[24] Briefly, participants were recruited from Medicare eligibility lists within four counties: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania. Eligible participants were 65 years of age or older, were expected to remain in the area for 3 years after recruitment, were not receiving active treatment for cancer, and were able to provide informed consent without a proxy. The original cohort was recruited in 1989–1990 and a second cohort of 687 black participants was recruited in 1992–1993 for a total of 5888 participants. We conducted FGF23 measurements at the 1996–1997 study visit, which we considered the baseline time point for this analysis. This visit was selected as the baseline visit, because it is the first CHS study visit at which urine collections were obtained for ACR measurement. Among 4413 individuals who participated in this visit, 3406 had eGFR measurements and never previously thawed plasma samples available for FGF23 measurement. Of those, we excluded individuals with missing or insufficient blood specimens for FGF23 measurement (n=69) or missing covariate data (n=196), resulting in a final analytic sample of 3141 participants for this analysis. All participants provided written informed consent, and the study was approved by the investigational review boards at the participating centers.

Study Variables

Plasma FGF23 was measured using a commercially available ELISA kit (Immunotopics, San Clemente, CA) that recognizes two epitopes on the C-terminal side of FGF23.[25] Fasting (8 hours) EDTA plasma specimens collected at the 1996–1997 visit, stored at −70°C, and not previously thawed were thawed in 2010 for measurement. Our estimates of intra-assay and interassay coefficients of variation ranged from 7.4% to 10.6%.

The time to first infection-related hospitalization was determined as any hospitalization with a principal discharge diagnosis of bacteremia, septicemia, endocarditis, pulmonary, genitourinary, gastrointestinal, soft tissue, bone, or joint infection through June 30, 2013. All discharge diagnoses were on the basis of the ICD-9-CM codes (Supplemental Material).

Confounders related to FGF23 and infection-related hospitalization were selected *a priori* as potential covariates, and all were measured during the same study visit as FGF23. Race was determined by self-report and for this analysis, categorized as black or nonblack. Smoking was defined as current, former, or never. Diabetes was defined as the use of insulin or oral hypoglycemic agents, fasting glucose level ≥126 mg/dl, or nonfasting glucose level >200 mg/dl. CHF and CHD were determined on the basis of a combination of self-reported disease at baseline or adjudicated events occurring between baseline and the 1996–1997 study visit. BMI was calculated using body weight measured using a calibrated balance beam scale and height measured with a wall-mounted stadiometer. Cystatin C was measured using a BNII Nephelometer (Dade Behring, Deerfield, IL) and chosen as the primary measure of kidney function.[26] eGFR was calculated for cystatin C using an equation derived from a pooling of cohorts that used iothalamate clearance as the criterion standard ($eGFR=76.7\times \text{cystatin C}^{-1.19}$).[27] Urine ACR (milligrams per gram) was determined from random morning urine samples. Urine albumin was measured by rate nephelometry using the Array 360 CE PROTEIN ANALYZER (Beckman Instruments, Fullerton, CA), and urine creatinine was measured on a Kodak Ektachem 700 Analyzer (Eastman Kodak Company, Rochester, NY). Urine ACR was log transformed for analysis.

CRP was measured using an ELISA developed by the CHS central blood laboratory (CHS Blood Laboratory, Colchester, VT), and IL-6 was measured by ultrasensitive ELISA (R&D Systems, Minneapolis, MN).[28] We took a random sample of 881 individuals at the year 9 examination for additional measurements of mineral markers. Individuals were eligible if they were not missing serum creatinine, cystatin C, or urine ACR. Within this random sample, total 25(OH)D [sum of $25(OH)D_2$ and $25(OH)D_3$] and $24,25(OH)_2D$ were measured using immunoaffinity purification and liquid chromatography-tandem mass spectrometry, and iPTH (Beckman-Coulter DxI Automated TwoSite Immunoassay; Beckman-Coulter, Inc., Brea, CA),[21,29] calcium, and phosphorus were measured using a standard clinical chemistry analyzer. VMR was calculated as the $24,25(OH)_2D$-to-25(OH)D ratio as a reflection of vitamin D adequacy.[23] The subgroup was very similar to the entire cohort in terms of demographics and clinical characteristics.

Statistical Analyses

Covariates were summarized by FGF23 quartile and presented as mean (SD) or median (IQR) for continuous variables and n (%) for categorical variables. Incidence rates were examined by quartile of FGF23 and stratified by eGFR. The longitudinal association between FG23 and first infection-related hospitalization was analyzed using Cox proportional hazards. Participants were censored at death or loss for follow-up. All analyses evaluated FGF23 using

the log-transformed continuous variable and quartiles, with the lowest quartile serving as the reference category. The initial model was adjusted for age, race, and sex. Model 2 was further adjusted for smoking status, BMI, diabetes, CHD, CHF, eGFR, and urine ACR. Model 3 was additionally adjusted for CRP, albumin, and IL-6. Because we were interested in understanding the proposed relationship in participants with and without CKD, we also stratified the analyses by two biomarkers of CKD status (eGFR$<$60 and $\geq$60 ml/min per 1.73 m^2 and urine ACR $\leq$30 and $>$30 mg/g)[30] and tested for multiplicative interactions by CKD status. However, irrespective of *P* values, we planned *a priori* to explore these associations in separate strata according to CKD status, because previous epidemiologic studies have reported stronger association between FGF23 and outcomes in subjects with CKD.[3,19] Additional *post hoc* analyses were performed with stratification by the presence of diabetes, CHF, and CHD.

As a secondary analysis, we repeated the above analyses using a negative binomial regression to examine the association of FGF23 with number of infection-related hospitalizations. We also restricted infection type to bacterial infections or infections considered likely to be bacterial in origin (Supplemental Material). All analyses were also repeated in the subgroup analyses adding serum calcium, phosphorus, iPTH, 25(OH)D, and 24,25$(OH)_2$D (model 4a) or VMR (model 4b) to the final model for 881 participants who were randomly selected for these measurements. Two-tailed values of $P<0.05$ were considered statistically significant for all analyses including interaction terms. All statistical analyses were performed with STATA 12.1.

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A full list of principal Cardiovascular Health Study investigators and institutions can be found at CHS-NHLBI.org.

DISCLOSURES

None.

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