

# Fibroblast Growth Factor 23 and Fetuin A are Independent Predictors for the Coronary Artery Disease Extent in Mild Chronic Kidney Disease

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**Background and objectives:** Cardiovascular disease in chronic kidney disease (CKD) is explained in part by traditional cardiovascular risk factors; by uremia-specific factors; and by abnormalities of mineral metabolism, factors involved in its regulation, and in the vascular calcification process.

**Design, setting, participants, & measurements:** In an unselected population of 177 patients with calculated GFR (eGFR) between 90 and 30 ml/min per 1.73 m<sup>2</sup>, the link between the mineral metabolism abnormalities (calcium, phosphorus, calcium-phosphorus product), regulatory factors (parathyroid hormone [PTH], intact PTH [iPTH], vitamin D, fibroblast growth factor 23 [FGF 23], and fetuin A), and the severity of coronary artery disease (CAD) assessed by coronary angiography were evaluated in three subgroups defined by tertiles of Gensini lesion severity score.

**Results:** The mean serum values for FGF 23 in the entire study population was 28.1 ± 17.3 RU/ml and for fetuin A was 473.1 ± 156.2 µg/ml. Patients with eGFR < 60 ml/min per 1.73 m<sup>2</sup> had significantly higher values of FGF 23 compared with patients with eGFR > 60 ml/min per 1.73 m<sup>2</sup>. The Gensini score values significantly correlated with gender; arterial hypertension; and HDL cholesterol, eGFR, iPTH, FGF 23, and fetuin A levels. After the adjustments for traditional and uremia-related cardiovascular risk factors, the FGF 23 and fetuin A remained significant predictors of the Gensini score.

**Conclusions:** This study suggests that in a relatively young population with mild-to-moderate alteration of kidney function and with less traditional cardiovascular risk factors, anomalies of the serum FGF 23 and fetuin A levels appear early in the course of disease and are independent major predictors for extent of CAD.

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Chronic kidney disease (CKD), defined as a calculated creatinine clearance of 15 to 60 ml/min per 1.73 m<sup>2</sup>, is associated with adverse outcomes, including ESRD and cardiovascular disease (CVD). The recent NEOERICA epidemiologic study established that approximately 25% of the population with CKD stages 3 to 5 (calculated GFR [eGFR] < 60 ml/min per 1.73 m<sup>2</sup>) had ischemic heart disease, more than double compared with patients without CKD (1). Similar data are available from the Framingham data analysis; once patients progress to eGFR < 45 ml/min per 1.73 m<sup>2</sup>, CVD burden is increased compared with individuals with more preserved renal function (2). Recent meta-analyses of multiple community-based data sets from different epidemiologic studies further

established the importance of CKD as a risk factor for all-cause mortality and CVD in the general population (3).

Deterioration of kidney function is accompanied by mineral metabolism disturbances that have been linked to the increased cardiovascular morbidity and mortality, possibly via cardiovascular calcifications (4–7). Vascular calcifications (VCs) appear as a consequence of a tightly regulated process, similar to bone osteogenesis. The (un)balance between promoters (hyperphosphatemia, inflammation) and inhibitors (fetuin A, others) is ultimately responsible for the high incidence and prevalence of VCs seen even in early stages of CKD.

Hyperphosphatemia is highly prevalent in CKD stage 4 and dialysis patients and induces phenotypic changes of the vascular smooth muscular cells into osteoblast-like cells, thus contributing decisively to calcification of the vessels (8–10). However, phosphate levels are maintained relatively normal until these late stages of CKD by the combined intervention of regulatory mechanisms: direct increase of parathyroid hormone (PTH) secretion, inhibition of the renal alpha-1-hydroxylase with subsequent decrease in calcitriol secretion, increase in

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fibroblast growth factor 23 (FGF 23) secretion (11). Shigematsu *et al.* (12), using 24-hour urine collection and cystatin C to estimate renal function, demonstrated that FGF 23 levels begins to rise early in CKD, as GFR falls under 80 ml/min per 1.73 m<sup>2</sup>, and correlates well with the other mineral metabolism disturbances—positively with PTH level and negatively with calcitriol levels. In a similar study, Gutierrez *et al.* (13) confirmed that FGF 23 levels increase as GFR falls below 60 ml/min per 1.73 m<sup>2</sup>, before the development of evident serum mineral abnormalities, and are independently associated with serum phosphate, fractional excretion of phosphate, and calcitriol deficiency. Emerging data are linking FGF 23 to cardiovascular calcification and mortality in ESRD patients on dialysis (14,15). However, in CKD, few data exist on the link between FGF 23 and CVD. A recent study by Gutierrez and collaborators (16) in CKD patients with GFR < 60 ml/min per 1.73 m<sup>2</sup> showed an association with increased risk of coronary artery calcifications in the highest tertile of FGF 23.

Fetuin A, a potent systemic inhibitor of soft tissue calcification, has been negatively related to VCs and cardiovascular mortality in dialysis populations (17). Again, there are only scarce and contradictory data on fetuin A levels in CKD stages 1 to 4 and their effect on vessel health. Mehrotra *et al.* (18) demonstrated an unexpected positive association between high levels of fetuin A and a higher calcification burden in diabetic patients in CKD stages 1 to 4.

The aim of the cross-sectional study presented here was to evaluate for the first time the possible relations between (1) mineral metabolism abnormalities calcium, phosphorus, calcium-phosphorus product (Ca×P); (2) factors that actively regulate calcium-phosphorus homeostasis and the VC process (PTH, vitamin D, FGF 23, and fetuin A); and (3) the severity of coronary artery disease (CAD) expressed as the extent of coronary artery stenosis in a group of unselected patients submitted to diagnostic coronary angiography and mild-to-moderate altered renal function (eGFR between 90 and 30 ml/min per 1.73 m<sup>2</sup>) without any specific treatment with calcium supplements, phosphate binders, or vitamin D.

## Materials and Methods

### Study Group

A total of 339 patients who underwent diagnostic coronary angiography in the Department of Cardiology at Fatih University Hospital during a period of 6 months (December 2008 and May 2009) were considered for screening at study entry. All patients had elective coronary angiography motivated by the presence of stable angina symptoms, with a high probability of coronary artery lesion(s) on the basis of noninvasive tests (positive dobutamine stress echocardiography and echocardiography abnormalities confirmed by exercise stress test).

Patients were eligible for inclusion if they had at the time of the angiographic examination a Cockcroft–Gault eGFR between 90 and 30 ml/min per 1.73 m<sup>2</sup>. Exclusion criteria were (1) eGFR > 90 ml/min per 1.73 m<sup>2</sup> or GFR < 30 ml/min per 1.73 m<sup>2</sup>, (2) presence of coronary artery bypass graft surgery history, (3) presence of nephrotic syndrome, (4) presence of primary hyperparathyroidism, (5) the use of calcium supplements or vitamin D treatment, and (6) patients with severe congestive heart failure (New York Heart Association class III to IV).

A total of 162 patients were excluded from the study: 83 patients had

GFR ≥ 90 ml/min per 1.73 m<sup>2</sup>, 40 patients had GFR ≤ 30 ml/min per 1.73 m<sup>2</sup> (34 patients were on dialysis), 11 patients had a positive history for coronary artery bypass graft surgery, 6 patients had nephrotic syndrome, 13 patients were using calcium- and vitamin-D-containing drugs, 2 patients were diagnosed with primary hyperparathyroidism, 9 patients had class IV congestive heart failure, and 4 patients declined participation in the study.

All 177 remaining patients were included in the study. The local hospital ethical committee approved the study protocol, and all patients signed an informed consent.

### Data Collection

Demographic and anthropometrical data (age, gender, comorbidities, actual treatment, smoking status, weight, height) were collected before the angiographic procedure from the individual charts and the electronic hospital database.

Blood pressure was measured on the nondominant arm on the morning of the procedure after a 5-minute resting interval. Hypertension was defined for the study purpose according to the 2007 European Society of Hypertension and of the European Society of Cardiology task force for the management of arterial hypertension guidelines (19).

In the morning of the procedure, after a 12-hour fasting period, blood samples were collected, stored, and analyzed by one single laboratory. Hemoglobin and serum levels of creatinine, calcium, phosphate, total cholesterol, HDL and LDL fractions, triglycerides, and 25-hydroxyvitamin D (25 OH D) were determined using standard measurement techniques. The GFR was calculated using the Cockcroft–Gault equation.

We defined the 25 OH D deficiency as values <10 ng/ml on the basis of the current Kidney Disease Improving Global Outcome recommendations (20).

Intact PTH (iPTH; normal range 10 to 69 pg/ml) was determined by the chemiluminescence method (Immulyte 2000; DPC, Los Angeles, CA).

In the study presented here, FGF 23 (C-terminal fragment) levels were measured using an ELISA kit according to the manufacturer's protocol (Immutopics, Inc., San Clemente, CA). The sensitivity of the second-generation human FGF 23 (C-terminal) ELISA as determined by the 95% confidence limit were calculated: the mean intra-assay precision and coefficient of variation were 33.7 (RU/ml) and 2.4%, respectively.

Serum fetuin A levels (μg/ml) were measured using a human fetuin A ELISA kit (BioVendor Laboratory Medicine, Inc., Brno, Czech Republic) in an ELISA plate reader (Biotek ELx808). Inter- and intra-assay coefficients of variation were 4.1% and 5.2%, respectively.

The urinary phosphate excretion and the urinary protein-to-creatinine ratio were determined from samples collected on the morning of the coronary angiography day.

### Imaging Procedure and Gensini Score Calculation

All patients underwent standard coronary angiography assessment performed by the same technician using a common technique. Two experienced physicians blinded to the study analyzed angiograms with a validated quantitative coronary angiographic system (Philips Allura Xper FD10). We estimated the extent of CAD using the Gensini score, which is a measure of the extent of myocardial ischemia and is computed by assigning a severity score to each coronary segment according to the degree of luminal narrowing and its geographic importance (21). Reduction in the diameter of the lumen, the roentgenographic appearance of concentric lesions, and eccentric plaques were evaluated (the corresponding Gensini scores for reductions of 25%, 50%, 75%, 90%, 99%, and complete occlusion were 1, 2, 4, 8, 16, and 32, respectively).

For each principal vascular segment, a multiplier was assigned according to the functional significance of the myocardial area supplied by this segment: left main coronary artery  $\times 5$ ; proximal segment of the left anterior descending coronary artery (LAD)  $\times 2.5$ ; proximal segment of the circumflex artery  $\times 2.5$ ; midsegment of the LAD  $\times 1.5$ ; right coronary artery distal segment of the LAD, posterolateral artery, and obtuse marginal artery  $\times 1$ ; and others  $\times 0.5$ .

### Statistical Analyses

All data are presented as mean  $\pm$  SD unless stated otherwise in the text. Continuous variables were checked for the normal distribution assumption using the Kolmogorov–Smirnov statistics, and those that did not satisfy the criteria were log-transformed to attain normal distribution. The study group was divided into three subgroups on the basis of the Gensini score tertiles. Significant differences between groups were assessed using the *t* test. The ANOVA test was used for multiple group comparisons of normally distributed variables. The  $\chi^2$  test was used to test differences in frequency distributions. All potential (physiologically meaningful) determinants of the Gensini score were investigated in a univariate screening procedure using the Pearson’s coefficient of correlation test. The nonparametric Spearman rho coefficient of correlation was used to assess correlations between variables without normal distribution. Significant determinants identified from this analysis were studied in a stepwise multiple regression model using the F statistic. All variables associated with these parameters with a level of significance  $<0.1$  were included in the tested model. Variables were forced in the model using a stepwise procedure. A *P* value  $<0.05$  for the final model was considered as statistically significant. Data were analyzed using the SPSS version 15.0 for Windows software (SPSS, Inc., Chicago IL).

### Results

A total of 177 patients that satisfied the selection criteria were included in the analysis. Demographic and biochemical data are presented in Table 1.

The mean values for parameters of mineral metabolism were largely situated in the normal range: serum calcium concentration was  $9.2 \pm 0.4$  mg/dl (17.5% of patients had hypocalcemia defined as a serum calcium  $<8.5$  mg/dl), the mean serum phosphate concentration was  $3.6 \pm 0.8$  mg/dl (11.9% had hyperphosphatemia defined as a serum phosphate  $>4.5$  mg/dl), and the  $\text{Ca}\times\text{P}$  was  $30.7 \pm 5.2$   $\text{mg}^2/\text{dl}^2$  (no patients had abnormal values). The mean iPTH value in the study population was  $69 \pm 38.6$  pg/ml and 37.9% of the patients had iPTH values  $>69$  pg/ml. In our group, the mean 25 OH D value was  $20.7 \pm 11.7$  ng/ml (31.6% of patients had 25 OH D deficiency).

The mean serum value for FGF 23 in the entire study population was  $28.1 \pm 17.3$  RU/ml (median 21.9 RU/ml; interquartile range 15.4 to 38.9 RU/ml) and for fetuin A was  $473.1 \pm 156.2$   $\mu\text{g}/\text{ml}$  (median 435.1  $\mu\text{g}/\text{ml}$ , interquartile range 358 to 557.9  $\mu\text{g}/\text{ml}$ ).

### Gensini Score Subgroup Analysis

The mean Gensini score in the study group was  $32.8 \pm 41.8$ . We further divided the patients in three subgroups according to tertiles of Gensini score. The demographic, biologic, and treatment characteristics of the three subgroups are shown in

Table 1. Demographic and biological data of the entire study group and in Gensini score tertiles

Data	Entire Group ( <i>n</i> = 177)	1st Tertile ( <i>n</i> = 57)	2nd Tertile ( <i>n</i> = 61)	3rd Tertile ( <i>n</i> = 59)	<i>P</i> for Trend
Age (years)	42.3 $\pm$ 13.7	44.7 $\pm$ 11.7	42.7 $\pm$ 12.4	40.2 $\pm$ 11.6	0.28
Gender (male, <i>n</i> ; %)	100; 56.5%	32; 56.7%	37; 60.7%	41; 69.5%	0.63
Hypertension ( <i>n</i> ; %)	104; 58.8	23; 40.3%	37; 60.7%	44; 74.5%	0.001
Diabetes mellitus ( <i>n</i> ; %)	37; 20.9%	9; 15.8%	12; 19.6%	16; 27.1%	0.31
Smoking ( <i>n</i> ; %)	75; 42.4%	22; 38.6%	24; 39.3%	29; 49.1%	0.42
eGFR (ml/min per 1.73 m <sup>2</sup> )	73.7 $\pm$ 9.5	76.7 $\pm$ 8.2	74.3 $\pm$ 6.9	70.0 $\pm$ 11.6	0.005
Serum creatinine (mg/dl)	1.1 $\pm$ 0.2	0.7 $\pm$ 0.1	0.8 $\pm$ 0.2	1.0 $\pm$ 0.3	0.001
Urinary protein-to-creatinine ratio (g/L)	0.16 $\pm$ 0.04	0.1 $\pm$ 0.07	0.15 $\pm$ 0.1	0.27 $\pm$ 0.7	0.042
Total cholesterol (mg/dl)	195.3 $\pm$ 47.2	198.2 $\pm$ 46.7	197.2 $\pm$ 42.4	190.4 $\pm$ 52.8	0.33
LDL cholesterol (mg/dl)	120.6 $\pm$ 38.4	126.3 $\pm$ 35.9	120.5 $\pm$ 36.8	115.5 $\pm$ 42.5	0.38
HDL cholesterol (mg/dl)	42.6 $\pm$ 12.1	44.7 $\pm$ 11.7	42.7 $\pm$ 12.4	40.2 $\pm$ 11.6	0.29
Triglycerides (mg/dl)	169.5 $\pm$ 102.7	151.8 $\pm$ 86.0	174.4 $\pm$ 88.9	179.0 $\pm$ 127.8	0.35
Calcium (mg/dl)	9.2 $\pm$ 0.4	9.2 $\pm$ 0.41	9.3 $\pm$ 0.44	8.9 $\pm$ 0.7	0.76
Phosphorus (mg/dl)	3.34 $\pm$ 0.6	3.3 $\pm$ 0.6	3.5 $\pm$ 0.7	3.6 $\pm$ 0.8	0.62
Ca $\times$ P (mg <sup>2</sup> /dl <sup>2</sup> )	30.7 $\pm$ 5.2	30.1 $\pm$ 5.7	32.4 $\pm$ 6.1	30.7 $\pm$ 5.6	0.08
iPTH (pg/ml)	66.1 $\pm$ 36.3	60.8 $\pm$ 32.1	71.5 $\pm$ 44	74.3 $\pm$ 37.4	0.08
25 OH D (ng/ml)	20.7 $\pm$ 11.4	21 $\pm$ 9.7	19.6 $\pm$ 7.3	19.8 $\pm$ 7.5	0.97
FGF 23 (ru/ml)	28.1 $\pm$ 17.3	15.1 $\pm$ 6.1	20.1 $\pm$ 5.3	48.6 $\pm$ 16.0	0.0001
Fetuin A ( $\mu\text{g}/\text{ml}$ )	473.1 $\pm$ 156.2	563.5 $\pm$ 166.5	464.7 $\pm$ 125.3	394.4 $\pm$ 129.0	0.0001
ACE inhibitor ( <i>n</i> ; %)	44; 24.9%	8; 14.1%	16; 26.2%	20; 33.9%	0.07
ARB ( <i>n</i> ; %)	36; 20.6%	11; 19.3%	10; 16.4%	15; 25.4%	0.5
Statins ( <i>n</i> ; %)	46; 25.9%	5; 8.7%	14; 22.9%	27; 45.7%	0.002

ACE, angiotensin conversion enzyme; ARB, angiotensin receptor blockers.

Table 1. Patients in the highest Gensini score tertile had statistically significant lower GFR values ( $70 \pm 11.6$  ml/min per  $1.73 \text{ m}^2$  versus  $76.7 \pm 8.2$  ml/min per  $1.73 \text{ m}^2$  in the first tertile versus  $74.3 \pm 6.9$  ml/min per  $1.73 \text{ m}^2$  in the second tertile;  $P$  for trend = 0.005). There was a statistically significantly higher prevalence of hypertension in the third tertile compared with the first and second tertiles of Gensini score ( $P$  for trend = 0.001). The use of  $\beta$ -blockers and statins was also statistically significantly higher in the third tertile compared with the first two tertiles of Gensini score ( $P$  for trend were 0.02 and 0.002, respectively) (see Table 1). Mean values of total, LDL, and HDL cholesterol did not differ significantly between subgroups, but the percent of patients with abnormal levels of the above-mentioned parameters decreased significantly in the third tertile, most probably reflecting a more aggressive statin treatment. The mean values of triglycerides were abnormal in all subgroups; a significant increase in triglyceride values was recorded in the third tertile compared with the first tertile (see Table 1).

The mean values of the mineral metabolism markers calcium, phosphorus,  $\text{Ca} \times \text{P}$ , iPTH, and 25 OH D did not vary across the three Gensini score tertiles and remained in the normal range with very low percentages of abnormalities for the calcium and phosphorus values.

Across the Gensini tertiles FGF 23 values increased significantly in the third tertile compared with the first tertile ( $P$  for trend = 0.001), whereas fetuin A values decreased significantly ( $P$  for trend = 0.001).

#### Univariate and Multivariate Analysis for the Gensini Score

The Gensini score values significantly correlated in univariate analysis with gender ( $R = -0.181$ ,  $P = 0.016$ ), presence of hypertension ( $R = 0.203$ ,  $P = 0.007$ ), HDL cholesterol level ( $R = -0.158$ ,  $P = 0.047$ ), eGFR ( $R = -0.315$ ,  $P = 0.001$ ), iPTH ( $R = 0.152$ ;  $P = 0.044$ ), FGF 23 ( $R = 0.868$ ;  $P = 0.001$ ), and fetuin A levels ( $R = -0.491$ ;  $P = 0.001$ ) but not with the vitamin D values.

All of the parameters that significantly correlated with the Gensini score and other factors considered physiologically as potentially relevant for the atherosclerotic process were introduced in standard multivariate regression analysis in a two-step procedure using the enter method. In the first step we wanted to see the independent influence of the mineral metabolism parameters and of FGF 23 and fetuin A on the Gensini lesion score as a measure of the atherosclerotic process in our population. In this first step, FGF 23 ( $\beta = 0.819$ ;  $P = 0.001$ ) and

fetuin A ( $\beta = -0.096$ ;  $P = 0.001$ ) were the only statistically significant independent predictors that correlated with the Gensini score (Table 2). The model explains 76.4% of the variation in the Gensini score ( $P = 0.001$  for the model), the most important part of the variation being explained by the FGF 23.

In the second step, we adjusted for the presence of traditional risk factors (gender, hypertension, smoking, diabetes, lipid profile) and GFR. Even after these adjustments, the FGF 23 ( $\beta = 0.616$ ;  $P = 0.000$ ) and fetuin A ( $\beta = -0.186$ ;  $P = 0.002$ ) remained statistically significant predictors of the Gensini score (Table 2b). The model explains 76.5% of the variation in the Gensini score, with  $P = 0.002$  for the model.

#### Subgroup Analysis for the Patients with an eGFR < 60 ml/min per $1.73 \text{ m}^2$

To better understand the influence of the FGF 23 and fetuin A on the coronary artery calcification process, we further analyzed patients with a eGFR < 60 ml/min per  $1.73 \text{ m}^2$  ( $n = 21$  patients) and compared them with subjects with a GFR between 60 and 90 ml/min.

Patients with eGFR < 60 ml/min per  $1.73 \text{ m}^2$  had significantly higher values of FGF 23 compared with patients with eGFR > 60 ml/min per  $1.73 \text{ m}^2$  ( $43.3 \pm 21.6$  RU/ml versus  $25.9 \pm 15.7$  RU/ml;  $P = 0.001$ ) but similar mean values for fetuin A ( $433.1 \pm 175.3$   $\mu\text{g/ml}$  versus  $478.5 \pm 153.3$   $\mu\text{g/ml}$ ;  $P = 0.2$ ). As expected in the group of patients with eGFR < 60 ml/min per  $1.73 \text{ m}^2$ , there were significant abnormalities of the mineral metabolism parameters: higher iPTH ( $90.7 \pm 47.6$  pg/ml versus  $66.1 \pm 36.6$  pg/ml;  $P = 0.006$ ), lower serum calcium values ( $8.5 \pm 0.9$  mg/dl versus  $9.1 \pm 0.5$  mg/dl;  $P = 0.001$ ), and higher serum phosphorus ( $4.2 \pm 1.1$  mg/dl versus  $3.5 \pm 0.7$  mg/dl;  $P = 0.001$ ).

The mean Gensini score value in this group was significantly higher compared with patients with eGFR > 60 ml/min per  $1.73 \text{ m}^2$  ( $73.5 \pm 52.3$  versus  $27.5 \pm 37.2$ ,  $P = 0.001$ ), suggesting a higher burden of coronary lesions in this group.

In univariate analysis (using the Spearman rho coefficient of correlation), only FGF 23 ( $R = 0.687$ ,  $P = 0.001$ ) and fetuin A ( $R = -0.641$ ,  $P = 0.002$ ) were significantly correlated with the Gensini score. In multivariate regression analysis, the FGF 23 remained the only significant determinant of the Gensini score ( $\beta = 0.74$ ;  $P = 0.001$ ) in patients with GFR < 60 ml/min, even after adjustment for traditional risk factors, whereas fetuin A lost its independent predictive value in the model. The model explains 63.8% of the variation in the Gensini score, with  $P = 0.007$  for the model.

Table 2a. Multivariate regression analysis for the Gensini score: step 1

	Unstandardized $\beta$	Standardized $\beta$	$P$	95% Confidence Interval for $\beta$
Constant	4.23		0.4	$-310.5 \pm 123.3$
Calcium (mg/dl)	-1.1	0.140	0.4	$-14.1 \pm 34$
Phosphorus (mg/dl)	-1.4	0.414	0.4	$-28.3 \pm 74.1$
25 OH D (ng/ml)	-0.09	-0.019	0.6	$-0.5 \pm 0.3$
iPTH (pg/ml)	0.04	0.034	0.4	$-0.4 \pm 0.1$
FGF 23 (ru/ml)	1.9	0.819	0.001	$1.8 \pm 2.2$
Fetuin A ( $\mu\text{g/ml}$ )	-0.03	-0.096	0.023	$-0.5 \pm -0.004$



Table 2b. Multivariate regression analysis for the Gensini score: step 2

	Unstandardized $\beta$	Standardized $\beta$	P	95% Confidence Interval for $\beta$
Constant	1.1		0.978	$-80.9 \pm 83.2$
Hypertension	3.2	0.038	0.384	$-4.1 \pm 10.4$
Smoking	-0.9	-0.011	0.788	$-7.9 \pm 6$
Diabetes	-1.2	-0.011	0.796	$-10.3 \pm 7.7$
Gender	-0.6	-0.007	0.880	$-8.4 \pm 7.1$
Total cholesterol (mg/dl)	-0.019	-0.021	0.642	$-0.1 \pm 0.1$
Triglycerides (mg/dl)	0.01	0.018	0.684	$-0.03 \pm 0.04$
eGFR (ml/min per 1.73 m <sup>2</sup> )	-0.1	-0.022	0.657	$-0.5 \pm 0.3$
Calcium (mg/dl)	0.06	0.001	0.987	$-7.1 \pm 7.2$
Phosphorus (mg/dl)	-1.1	-0.018	0.694	$-6.2 \pm 4.2$
iPTH (pg/ml)	0.05	0.038	0.376	$-0.05 \pm 0.1$
25 OH D (ng/ml)	-0.1	-0.017	0.693	$-0.5 \pm 0.4$
FGF 23 (ru/ml)	1.9	0.812	0.000	$1.7 \pm 2.2$
Fetuin A ( $\mu$ g/ml)	-0.03	-0.093	0.044	$-0.05 \pm -0.001$

## Discussion

In this cross-sectional study in a group of unselected patients with CAD and mild-to-moderate altered renal function as estimated from the Cockcroft–Gault equation, we report for the first time a significant association between small increases in FGF 23 levels, decreased levels of fetuin A, and increased coronary artery lesions objectively assessed by coronary angiography. These two factors were shown to be independently associated in multivariate analysis with the Gensini score and explain 76.4% of the variance in CAD scores. Most importantly, this association remained statistically significant after adjustment for established cardiovascular risk factors such as gender, age, lipid profile, hypertension, smoking, diabetes, and GFR. Moreover, the mineral metabolism parameters (serum calcium, phosphorus, iPTH, or 25 OH D levels) were not found to be statistically significant predictors of the Gensini score of coronary lesions in multivariate analysis in our patients.

It is well recognized that CAD is more prevalent and severe in CKD stages 3 to 5 and ESRD patients on maintenance dialysis (1–5). It is still unsolved if in patients with kidney disease the basic biology underlying CVD is similar to that acknowledged for patients without kidney disease; nevertheless many more risk factors are present as a consequence of the renal dysfunction and thus involved in the accelerated atherosclerotic process. Similar to the general population (22), we found in our group a significant relationship between the Gensini score and classical cardiovascular risk factors: Gensini score correlated with the presence of hypertension and HDL cholesterol serum values. In multivariate analysis, male gender and hypertension remained statistically significant predictors of a more severe Gensini score.

Available data in the literature demonstrate that patients with a similar GFR range to our study group had an increased risk of overt atherosclerotic CAD (1). In previously reported studies, after adjusting for the traditional risk factors, GFR still emerged as an important predictor for the excess cardiovascular mortality found in CKD patients (1,3). Our study patients had elective coronary angiography motivated by the presence

of angina symptoms, with a high probability of coronary artery lesion on noninvasive tests and a range of eGFR from 30 to 90 ml/min per 1.73 m<sup>2</sup>. In our univariate analysis, the eGFR correlated with the Gensini score, supporting the presumption that CKD is a major risk factor for CAD. However this association does not answer the very important question of which CKD-related pathophysiologically relevant pathway might be involved and thus therapeutically targeted in CAD. In the adjusted multivariate analysis that includes FGF 23 and fetuin A as predictors of the Gensini score, GFR lost its statistical significance as an independent predictor of CAD. Our data suggest the hypothesis that GFR might only be a surrogate measure, including the intimate hormonal disturbances of the mineral metabolism that may ultimately lead (together with other aggressive stimuli such as inflammation and endothelial dysfunction) to accelerated VCs. Moreover, the recent Mild-to-Moderate Kidney Disease study indicated FGF 23 to be a significant CKD progression predictor; one can speculate that introduction of FGF 23 in risk prediction analyses could complement/replace GFR as a major outcome predictor (23).

Clinical studies have shown that mineral metabolism markers (calcium, phosphorus, PTH, and vitamin D) are associated with cardiovascular mortality and VCs in ESRD patients on dialysis (9). At the same time, there is increasing recognition of a link between atherosclerosis and phosphate metabolism in CKD patients (24). Traditionally, the disturbed phosphate homeostasis was seen from the vitamin D-PTH axis perspective, but new insights and clarifications were provided by the discovery of FGF 23. Similar to PTH, FGF 23 inhibits the Na/P<sub>i</sub>-dependent phosphate reabsorption in the proximal tubule (leading to phosphaturia) and inhibits alpha-1-hydroxylase, thus lowering 25 OH D levels and vitamin-D-dependent reabsorption of phosphate in the gut (11). FGF 23 rises early in CKD to maintain normal phosphorus levels; therefore, even small increases, in the normal range, in phosphorus may trigger significantly greater increases in FGF 23 levels (11–13).

The association of FGF 23 with the Gensini coronary lesion score

was present in our study in univariate analysis and maintained in the multivariate analysis after adjustment for traditional risk factors and GFR, suggesting a possible independent role in CAD pathogenesis. Furthermore, in the subgroup analysis restricted to the patients with  $eGFR < 60$  ml/min per  $1.73\text{ m}^2$ , FGF 23 remained significantly associated with the Gensini score even if the strength of the association diminished compared with the entire study group.

To our knowledge, the study of Gutierrez *et al.* (16) is the only one in the literature addressing the relationship between FGF 23 and CAD in a CKD population. Their study enrolled 162 patients with various degrees of CKD from  $GFR > 60$  ml/min per  $1.73\text{ m}^2$  to  $< 30$  ml/min per  $1.73\text{ m}^2$ , and the CAD severity was assessed by means of computed tomography and Agatston score. In their analysis, the FGF 23 was not associated with the log-transformed coronary artery calcification score, but the highest tertile of FGF 23 was associated with a 2.4-fold increased risk of calcifications. Our study included patients with mild-to-moderate alterations in  $eGFR$ , with similar cardiovascular risk factors, but without treatment with vitamin D or phosphorus binders. Most importantly we used a more direct target-organ end point derived from coronary angiography and not a surrogate marker for coronary lesions.

Fetuin A is a protein synthesized by the liver that it is downregulated by inflammation; it is an important mediator of insulin resistance and inhibits the *de novo* formation and precipitation of basic calcium phosphate (17). In CKD patients, fetuin A values decrease as renal function declines (25). Serum concentrations of fetuin A are further depressed in patients with ESRD on dialysis, and lower serum concentrations were independently associated with risk of cardiovascular and all-cause mortality in this population (26,27).

In our study, the fetuin A serum level were significantly lower in the third (more severe CAD) subgroup, in accordance with (higher) GFR values, confirming previous observations in the literature. The fetuin A level correlated with the Gensini score in univariate and multivariate regression analysis; after adjustment for the classical cardiovascular risk factors and GFR values fetuin A remained an independent prognostic factor of CAD severity. To our knowledge, only one other study investigated the link between fetuin A and cardiovascular mortality and morbidity in CKD patients. In a cross-sectional study including 88 patients with CKD stages 3 and 4, type 2 diabetes mellitus, and significant proteinuria (7 g/d), Mehrotra *et al.* (18) described a significant correlation between high serum fetuin A levels and CAD, again indirectly assessed by the Agatston coronary artery calcium (CAC) score. Their findings contradict the current knowledge of low fetuin A associated with CAC in CKD population. They explained the high fetuin A values seen in their patients with advanced diabetic nephropathy by the increased hepatic synthesis seen in proteinuric renal diseases and by a possible link with the increased insulin resistance seen in CKD. In this context, they were not able to make the difference between direct effects of fetuin A on CAC and an indirect effect mediated through higher proatherogenic insulin resistance present in this type of patient. Our study investigated a larger population, not limited to diabetic patients. Furthermore, a significant effect of lipid profile abnormalities was not found, thus we consider as more plausible the role of fetuin A as a systemic inhibitor of coronary calcifications.

There are several limitations of our study. First, this is a cross-

sectional analysis that precludes causality and direct relationships between VCs and mineral metabolism. Second, we used an unselected population and estimated the renal function using the Cockcroft–Gault formula, which is known to be a less precise estimation of the renal function. Third, we had no data about our patients' inflammatory status, which is a well known leading factor for atherosclerosis progression in CKD. The relative strength of our study is the design. We assessed a population in which the interference of traditional cardiovascular risk factors was minimized and biases in mineral metabolism markers related to treatment with phosphate binders and vitamin D supplements were excluded. Most importantly, in addition to calcium and phosphorus levels, all hormones currently implicated in maintaining calcium-phosphorus homeostasis were investigated together with direct assessment of end-point organ damage. Finally, a plausible pathophysiological mechanism is unveiled: Alterations in GFR that associate with small alterations in FGF 23 and fetuin A and with high-normal phosphorus determine a decreased inhibition of VCs, thus leading to increased coronary atherosclerotic lesions.

In conclusion, our study suggests that in a relatively young population with mild-to-moderate alterations in renal function and with less traditional cardiovascular risk factors, small modifications in serum levels of FGF 23 and fetuin A appear early in the course of disease evolution and are independent major predictors for CAD extent.

## Disclosures

None.

## References

1. de Lusignan S, Chan T, Stevens P, O'Donoghue D, Hague N, Dzregah B, Van Vlymen J, Walker M, Hilton S: Identifying patients with chronic kidney disease from general practice computer records. *Fam Pract* 22: 234–241, 2005
2. Parikh NI, Hwang SJ, Larson MG, Levy D, Fox CS: Chronic kidney disease as a predictor of cardiovascular disease (from the Framingham Heart Study). *Am J Cardiol* 102: 47–53, 2008
3. Weiner DE, Tighiouart H, Amin MG, Stark PC, Macleod B, Griffith JL, Salem DN, Levey AS, Sarnak MJ: Chronic kidney disease as a risk factor for cardiovascular disease and all cause mortality: A pooled analysis of community-based studies. *J Am Soc Nephrol* 15: 1307–1315, 2004
4. Kramer H, Toto R, Peshock R, Cooper R, Victor R: Association between chronic kidney disease and coronary artery calcification: The Dallas Heart Study. *J Am Soc Nephrol* 16: 507–513, 2005
5. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, Salusky IB: Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 342: 1478–1483, 2000
6. Haydar AA, Hujairi NM, Covic AA, Pereira D, Rubens M, Goldsmith DJ: Coronary artery calcification is related to coronary atherosclerosis in chronic renal disease patients: A study comparing EBCT-generated coronary artery calcium scores and coronary angiography. *Nephrol Dial Transplant* 19: 2307–2312, 2004
7. Noordzij M, Korevaar JC, Dekker FW, Boeschoten EW, Bos

- WJ, Krediet RT, Bossuyt PM, Geskus RB; NECOSAD study group: Mineral metabolism and mortality in dialysis patients: A reassessment of the K/DOQI guideline. *Blood Purif* 26: 231–237, 2008
8. Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, Sherrard DJ, Andress DL: Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol* 16: 520–528, 2005
  9. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM: Mineral metabolism, mortality and morbidity in maintenance hemodialysis. *J Am Soc Nephrol* 15: 2208–2218, 2004
  10. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM: Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 87: E10–E17, 2000
  11. Negri AL: FGF 23 in chronic kidney disease and kidney post-transplant patients. *Nefrología* 29: 196–202, 2009
  12. Shigematsu T, Kazama JJ, Yamashita T, Fukumoto S, Hosoya T, Gejyo F, Fukagawa M: Possible involvement of circulating fibroblast growth factor 23 in the development of secondary hyperparathyroidism associated with renal insufficiency. *Am J Kidney Dis* 44: 250–256, 2004
  13. Gutierrez O, Isakova T, Rhee E, Shah A, Holmes J, Collerone G, Jüppner H, Wolf M: Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. *J Am Soc Nephrol* 16: 2205–2215, 2005
  14. Jean G, Terrat JC, Vanel T, Hurot JM, Lorriaux C, Mayor B, Chazot C: High levels of serum fibroblast growth factor (FGF)-23 are associated with increased mortality in long haemodialysis patients. *Nephrol Dial Transplant* 24: 2792–2796, 2009
  15. Gutierrez O, Mannstadt M, Isakova T, Rauh-Hain J, Tamez H, Shah A, Smith K, Lee H, Thadhani R, Jueppner H, Wolf M: Fibroblast Growth Factor 23 and mortality among hemodialysis patients. *N Engl J Med* 359: 584–592, 2008
  16. Gutierrez OM, Januzzi JL, Isakova T, Laliberte K, Smith K, Collerone G, Sarwar A, Hoffmann U, Coglianese E, Christenson R, Wang TJ, deFilippi C, Wolf M: Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. *Circulation* 119: 2545–2552, 2009
  17. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Böhm R, Metzger T, Wanner C, Jahnke-Dechent W, Floege J: Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: A cross-sectional study. *Lancet* 361: 827–833, 2003
  18. Mehrotra R, Westenfeld R, Christenson P, Budoff M, Ipp E, Takasu J, Gupta A, Norris K, Ketteler M, Adler S: Serum fetuin-A in nondialyzed patients with diabetic nephropathy: Relationship with coronary artery calcification. *Kidney Int* 67: 1070–1077, 2005
  19. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Erdine S, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Lindholm LH, Viigimaa M, Adamopoulos S, Agabiti-Rosei E, Ambrosioni E, Bertomeu V, Clement D, Erdine S, Farsang C, Gaita D, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Waeber B, Williams B: Management of Arterial Hypertension of the European Society of Hypertension; European Society of Cardiology: 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 25: 1105–1187, 2007
  20. KDIGO Clinical Practice Guideline for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease—Mineral and Bone Disorder (CKD-MBD). *Kidney Int* 76[Suppl 113]: S1–S130, 2009
  21. Gensini GG: A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol* 51: 606, 1983
  22. Neaton JD, Wentworth D: Serum cholesterol, blood pressure, cigarette smoking, and death from coronary heart disease. Overall findings and differences by age for 316,099 white men. Multiple Risk Factor Intervention Trial Research Group. *Arch Intern Med* 152: 56–64, 1992
  23. Fliser D, Kollerits B, Neyer U, Ankerst DP, Lhotta K, Lingenhel A, Ritz E, Kronenberg F; for the MMKD Study Group: Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: The Mild to Moderate Kidney Disease (MMKD) study. *J Am Soc Nephrol* 18: 2600–2608, 2007
  24. Kanbay M, Goldsmith DJ, Akcay A, Covic C: Phosphate—The silent stealthy cardiorenal culprit in all stages of chronic kidney disease. *Blood Purif* 27: 220–230, 2009
  25. Ketteler M: Fetuin A and extraosseous calcifications in uremia. *Curr Opin Nephrol Hypertens* 14: 337–342, 2005
  26. Stenvinkel P, Wang K, Qureshi AR, Axelsson J, Pecoits-Filho R, Gao P, Barany P, Lindholm B, Jogestrand T, Heimbürger O, Holmes C, Schalling M, Nordfors L: Low fetuin-A levels are associated with cardiovascular death: Impact of variations in the gene encoding fetuin. *Kidney Int* 67: 2383–2392, 2005
  27. Wang AY, Woo J, Lam CW, Wang M, Chan IH, Gao P, Lui SF, Li PK, Sanderson JE: Associations of serum fetuin-A with malnutrition, inflammation, atherosclerosis and valvular calcification syndrome and outcome in peritoneal dialysis patients. *Nephrol Dial Transplant* 20: 1676–1685, 2005

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