Fractional excretion of phosphorus and vascular calcification in stage 3 chronic kidney disease

Manuel Jiménez Villodres,[1] Guillermo García Gutiérrez,[2] Patricia García Frías,[1] José Rioja Villodres,[3] Mónica Martín Velázquez,[1] Miguel Ángel Sánchez Chaparro,[4,5] Carmen Pérez López,[4] Pedro Valdivielso[4,5]

[1]Nephrology Unit, Hospital Virgen de la Victoria, Málaga, Spain
[2]Radiology Unit, Hospital Virgen de la Victoria, Málaga, Spain
[3]Instituto de Investigación Biomédica de Málaga (IBIMA), University of Málaga, Málaga, Spain
[4]Internal Medicine Unit, Hospital Virgen de la Victoria, Málaga, Spain
[5]Department of Medicine and Dermatology, Instituto de Investigación Biomédica de Málaga (IBIMA), University of Málaga, Málaga, Spain

Correspondence to
Dr Miguel Ángel Sánchez Chaparro, Department of Internal Medicine and Dermatology, University of Málaga, Málaga 29016, Spain; masch@uma.es

Accepted 20 October 2018

To cite: Jiménez Villodres M, García Gutiérrez G, García Frías P, *et al*. *J Investig Med* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jim-2018-000852

ABSTRACT

The role of renal excretion of Pi in relation to vascular calcification (VC) in patients in the early stages of chronic kidney disease (CKD) is controversial. Thus, we determine the relation between fractional excretion of phosphorus (FEP) and VC, measured using two methods in a cross-sectional study of patients with stage 3 CKD. We recorded demographic data, anthropometry, comorbidities and active treatment. We measured 24-hour urine FEP and, in serum, measured fibroblast growth factor 23 (FGF23), α-Klotho, intact parathyroid hormone (iPTH), calcium and phosphorus. VC was measured by lateral abdominal radiography (Kauppila index (KI)) and CT of the abdominal aorta (measured in Agatston units). In 57% of subjects, abnormal VC was present when measured using CT, and in only 17% using lateral abdominal radiography. Factors associated with VC using CT were age, cardiovascular risk factors, vascular comorbidity, microalbuminuria and levels of FGF23, phosphorus and calcium x phosphorus product (CaxP); although only age (OR 1.25, 95% CI 1.11 to 1.41), smoking (OR 21.2, CI 4.4 to 100) and CaxP (OR 1.21, CI 1.06 to 1.37) maintained the association in a multivariate analysis. By contrast, only age (OR 1.35, 95% CI 1.07 to 1.74), CaxP (OR 1.14, CI 1.13 to 1.92) and FEP (OR 1.07,95% CI 1004 to 1.14) were associated with abnormal VC in the lateral abdominal radiography. In conclusion, in patients with stage 3 CKD, the detection of VC by abdominal CT is more sensitive than conventional X-rays. Moreover, CaxP is associated with cardiovascular risk factors and vascular comorbidity; quantification of FEPi in these patients provides additional clinical information in advanced VC detected by KI.

Significance of this study

What is already known about this subject?
- Vascular calcification is a common complication of advanced chronic kidney disease (CKD).
- Little is known in the early stages of CKD, but calcium x phosphorus product plays a major role.
- Renal excretion of phosphorus is regulated by PTH, fibroblast growth factor 23 and α-Klotho.

What are the new findings?
- Vascular calcification in stage 3 CKD is commonly detected by CT, but it is associated to arteriosclerosis disease and its risk factors.
- At this stage, vascular calcification in a lateral abdominal X-ray was uncommon but associated to the fractional excretion of phosphorus.
- CT is a more sensitive method to detect vascular calcification in the early stages of CKD.

How might these results change the focus of research or clinical practice?
- In patients with stage 3 CKD, quantification of fractional excretion of phosphorus i provides additional clinical information in advanced vascular calcification detected by Kauppila index.

INTRODUCTION

Cardiovascular morbidity and mortality in subjects with chronic kidney disease (CKD) has been widely described in the literature.[1] Not only has it been associated with a higher prevalence of traditional cardiovascular risk factors (CVRFs), but also with risk factors associated with CKD, such as albuminuria[2 3] or vascular calcification (VC).[4 5]

VC can be assessed in CKD by conventional radiology,[4 6] CT,[7 8] ultrasonography[9] or magnetic resonance,[10 11] all being complementary.

In the genesis of VC in CKD, traditional parameters such as calcium, phosphorus and parathormone[12] appear late; however, in the early stages, VC has been associated with changes in fibroblast growth factor 23 (FGF23), α-Klotho and phosphaturia.[13–15]

Clinical practice establishes that patients with stage 3–5 CKD are monitored for calcium, phosphorus and intact parathyroid hormone (iPTH) levels, and evaluated with a lateral abdominal radiography to monitor aortic calcification.[16] Also, serum phosphate levels should be maintained at normal levels (2.7–4.6 mg/dL) in CKD by means of diet and chelating agents.[16]

FEP is a simple, low-cost, easy-to-measure parameter, whose increase precedes the elevation of serum phosphate. Our objective is to

analyse the relationship of FEP in patients with stage 3 CKD, and the factors that regulate it (iPTH, FGF23 and α-Klotho), with the VC of the abdominal aorta using lateral abdominal radiography and ultrafast CT.

MATERIALS AND METHODS

We selected subjects with stage 3 CKD from the Department of Nephrology, University Hospital 'Virgen de la Victoria', Málaga, Spain. The criteria for inclusion were subjects with CKD, with a glomerular filtration rate (GFR) of between 30 and 60 mL/min/1.73 m^2 measured twice, male or female, between the ages of 18 and 70, and who signed a consent form. The exclusion criteria were pregnancy, diagnosis of primary or tertiary hyperparathyroidism, active malignant neoplasm, serum calcium levels >10 mg/dL and/or serum phosphate levels >4.6 mg/dL, steroid consumption for a period exceeding 6 months, treatment with calcium >500 mg of elemental calcium, calcimimetic or paricalcitol and non-calcium phosphate binders.

We recorded the presence of risk factors and cardiovascular comorbidities, medication, anthropometric data and blood pressure levels measured twice. Blood samples were obtained after fasting for 12 hours, and collection of a 24-hour urine specimen was made immediately prior to blood extraction, in order to limit the variability of phosphorus and other substances in the urine sample.[17]

The GFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[18] Creatinine, calcium, phosphate in serum and urine, albuminuria and proteinuria (mg/dL) were measured using the Siemens Dimension Vista System Flex analysis system. 25(OH)D (ng/mL) and the iPTH (pg/mL) were measured using electrochemiluminescence immunoassay. FGF23 (pg/mL) (Kainos) and α-Klotho (ng/mL) (Cusabio, Hong Kong, China) were measured using direct sandwich ELISA.

All participants underwent a lateral abdominal X-ray and a low-grade CT of the abdomen. VC was assessed by the Kauppila index (KI)[4 19] and by CT. The KI was categorised as normal (0 points), low risk (1–4), moderate risk (5–15) and high risk (16–24).[20] Abdominal Aortic Calcium Score (AACS) was shown as Agatston units.[7 19] Values >1025 22 HU were considered abnormal. The KI and AACS values are expressed as median and IQRs.

Statistical analysis was carried out using the SPSS V.22.0 (IBM). To compare between groups, we used the Student's t-test for independent samples (the Mann-Whitney test if the distribution was not normal) and the χ^2 test was used to calculate differences between categorical variables. To assess which factors were independently associated with VC, a forward stepwise (Wald) binary regression analysis was conducted, taking the AACS>1025 HU or a KI>4.0 as the dependent variable and the rest of the analysed factors as covariates. In a second instance, a lineal regression analyses taking AACS and KI as continuous variables was built using the same explanatory covariates. Moreover, $p<0.05$ was considered significant.

RESULTS

Characteristics of the sample

We selected 139 subjects through telephone interviews; 29 refused to participate in the study, 25 did not attend the tests and 5 patients were discarded because they did not meet the inclusion criteria. The final sample was made up of 80 subjects.

Tables 1 and 2 show the subjects included in our study, who were mainly patients with diabetic nephropathy and vascular/ischaemic and treated with antihypertensive ACE inhibitors/ARA II, either as monotherapy or in combination therapy. Three out of every four patients were taking statins, and 36% antiplatelet therapy. Regarding treatment related to phospho-calcium metabolism, 23 subjects were treated with calcifediol, 4 with oral calcium and 1 two with calcitriol. No individual presented with anaemia. Only 34 subjects presented with C-reactive protein (CRP) >3 mg/L. Of the 80 individuals, 46 had a GFR of <45 mL/min, while 34 had a GFR of >45 mL/min. 38% of subjects presented with iPTH >70 pg/mL.

VASCULAR CALCIFICATION

The KI value was 1 (0–3) with 13 patients (17%) showing a KI >4. The AACS value was 1265 (97–3827) AU; 45 patients (56%) had an AACS >1025 HU.

Tables 1 and 2 show the distribution of clinical and laboratory parameters separated according to the presence of normal or abnormal VC, determined by an abdominal CT and the KI. Patients with abnormal VC in the abdominal CT, were older, had a history of smoking, presented with hypertension, showed a higher prevalence of diabetic nephropathy and vascular/ischaemic, as well as greater cardiovascular comorbidities ($p<0.05$). Among the analytical parameters analysed, individuals with VC measured by abdominal CT presented significantly higher red blood cell distribution width (RDW), FGF23 serum and excretion of protein in the urine ($p<0.05$). Also, the levels of phosphorus and CaxP tended to be higher in this group with VC, although without significance ($p=0.06$). The FEP and α-Klotho was similar in both groups.

Only age (57±8 vs 63±5) and the levels of phosphorus, calcium, CaxP and FEP were significantly higher in subjects with VC and KI >4.

Table 3 shows that in the multivariate analysis only age, smoking and CaxP was independently associated with VC when measured by abdominal CT, while only age, CaxP product and FEP was associated with abnormal VC detected by the KI. In the lineal regression analyses (table 4), age, smoking, CaxP and α-Klotho were independently associated with VC.

To find out if the association between FGF23 and VC was associated with different levels of FEP, we decided to analyse patients according to whether they were above or below the median in both factors. Table 5 shows that there was no association between the ratios of FGF23/FEPi and the appearance of abnormal VC using the two methods discussed.

Figure 1 shows the correlation between AACS and the KI (Pearson's r^2 of 0.875 ($p<0.05$)). None of the patients with the KI>4 had a AACS <1025 HU.

DISCUSSION

Our study confirms that a high percentage of patients with stage 3 CKD suffer from VC at abdominal aorta level, 17% when we analyse it with the KI and 56% with CT. Besides

Table 1 Characteristics of the sample and its relationship with the pathological Abdominal Aortic Calcium Score (AACS) and Kauppila index (KI)*

Variable	Total sample	Normal AACS (n=35)	Pathological AACS (n=45)	Normal KI (n=65)	Abnormal KI (n=13)
Men†	58 (72)	22 (63)	36 (80)	47 (72)	9 (69)
Aged (years)†‡	58±8	54±9	61±6	57±8	63±5
Smoke†	55 (69)	17 (48.5)	38 (84)	44 (68)	9 (69)
Diabetes‡	30 (37.5)	10 (29)	20 (45)	22 (34)	8 (61)
HBP†	73 (91)	29 (83)	44 (98)	59 (91)	12 (93)
Refractory HBP†	17 (21.3)	1 (2.8)	16 (35.6)	12 (18.5)	5 (38)
Dyslipidaemia	63 (79)	25 (71)	38 (84)	52 (80)	10 (77)
CKD aetiology					
Vascular/ischaemic†	23 (29)	5 (14)	18 (40)	19 (29)	3 (23)
Diabetic†	15 (19)	5 (14)	10 (22)	11 (17)	4 (31)
Hereditary†‡	8 (10)	7 (20)	1 (2.2)	8 (12)	0 (0)
Cardiovascular comorbidity †‡§	18 (22.5)	4 (11.4)	14 (31)	17 (26)	0
Weight (kg)	83.2±14.8	83±14.7	83.4±15	84.5±15	76±10
Waist circumference (cm)	104±11	167±10	166±8	105±12	101±8
BMI (kg/m^2)	30±4.7	29.9±4.6	30.2±4.8	30.2±5	29.4±3.1
BMI (18–24.99)	10 (12.5)	6 (17)	4 (9)	9 (14)	1 (8)
BMI (25–30)	30 (37.5)	10 (29)	20 (44)	23 (35)	6 (46)
BMI (>30)	40 (50)	19 (54)	21 (47)	33 (51)	6 (46)
Systolic BP (mm Hg)	138.4±21.6	136.1±21.2	140.3±22	138±22	138±18
Diastolic BP (mm Hg)	84.5±13.5	85.4±13	83.8±14	85±13	79±13
Drug therapy N (%)					
Hypotensives	85(94)	31 (89)	44 (98)	61 (94)	12 (92)
Lipid-lowering drugs	63 (79)	25 (71)	38 (84)	52 (80)	10 (77)
Antiplatelets†	29 (36)	7 (20)	22 (49)	23 (35)	6 (46)
OHA	19 (63)	7 (70)	12 (60)	16 (73)	3 (37)
Insulin	14 (44)	6 (60)	8 (40)	9 (41)	7 (87)
Calcium carbonate	4 (5)	0	4 (9)	2 (3)	2 (15)

OHA and Insulin referred to subjects with diabetes.
*All the variables are expressed as N (%) and mean ±SD.
†p<0.05 for AACS.
‡p<0.05 for KI
§Includes cardiac pathology (aAcute coronary syndrome, coronary disease and stable angina), cerebrovascular (atherothrombotic stroke) and peripheral vascular.
BMI, body mass index; BP, blood pressure; CKD, chronic kidney disease; HBP, high blood pressure; OHA, oral hypoglycemic agents.

age and some VC risk factors, CaxP and FEP were associated independently with the presence of pathological VC.

Our stage 3 CKD series of patients has clinical features similar to those of other series of patients on a national scale with the same level of renal function deterioration[15 21 22] highlighting, in addition, a high ischaemic vascular and diabetic aetiology, and a very high prevalence of CVRF and their treatments, in particular antihypertensive treatment. Only 22% of patients included in the study had established cardiovascular disease (CVD).

It is well known that high serum phosphate (P) levels in the blood favour the development of VC in patients with CKD at any stage and raise cardiovascular mortality.[23 24] It is unsurprising, therefore, that the serum phosphorus and CaXP levels were higher in the VC group measured by CT than in the group without calcification (p<0.05, table 3), despite being considered acceptable levels.[16] Serum phosphorus levels are controlled by concentrations of iPTH and the FGF23-α-Klotho axis.[25] It has been shown in a meta-analysis that FGF23, besides exercising control over phosphorus and FEP, is an independent predictor of vascular mortality or mortality from whatever cause.[26]

In our study, patients with abnormal VC detected by CT had significantly higher levels of FGF23 compared with those who had no pathological VC (143±70 vs 115±42 pg/mL, p<0.05). Furthermore, we would expect to see a higher phosphaturic effect, but this did not happen with the FEP that was similar in both groups (tables 2 and 3). It has been noted that a greater 'resistance' to the phosphaturic effect of FGF23, translated into lower FEP than expected, is associated with a higher incidence of cardiovascular events[27] and VC in patients with stage 3–4 CKD.[15] More recent studies suggest that the association between FGF23 and the appearance of clinical events is independent of FEP.[28] Our results are in agreement with the latter since the prevalence of VC, as measured by both methods, was independent of the FGF23/FEP ratio.

Although α-Klotho in serum was not associated with abnormal VC, it was independently associated with VC, both as AACS and the KI when both parameters were considered

Table 2 Analytics parameters and its relationship with pathological Abdominal Aortic Calcium Score (AACS) and abnormal Kauppila index (KI)*

Parameter	Total sample	Normal AACS (n=35)	Pathological AACS (n=45)	Normal KI (n=65)	Abnormal KI (n=13)
Haemoglobin (g/dL)	13.8±1.7	13.8±1.7	13.7±1.7	13.8±1.8	13.5±0.8
Red blood cell distribution width (%)†	14.6±1.6	14±1.3	15.1±1.7	14.4±1.6	15.3±1.8
Glycaemia (mg/dL)‡	142±54	156±57	135±52	149±52	124±57
HbA1c (%)‡	7.1±1.2	7.4±1.3	7±1.2	7.2±1.3	6.9±1.0
Creatinin (mg/dL)	1.6±0.4	1.6±0.3	1.7±0.4	1.7±0.4	1.6±0.4
GFR (CKD-EPI) (mL/min/1.73 m^2)	43.8±9.7	44.6±8.1	43.3±10.8	43.7±9.5	45.7±10.3
Total cholesterol (mg/dL)	176±38	183±44	171±33	177±41	172±21
Cholesterol-HDL (mg/dL)	47±13	49±12	45±13	46±13	52±13
Cholesterol non-HDL (mg/dL)	129±38	134±44	126±32	132±40	120±21
Cholesterol-LDL (mg/dL)	95±33	101±39	91±28	96±36	93±19
Triglycerides (mg/dL)	190±115	179±109	199±120	203±120	135±71
CRP (mg/L)	7.9±3.5	8.1±3.8	7.7±3.2	7.4±3.2	11.1±3.9
Plasma calcium (mg/dL)§	8.8±0.4	8.8±0.4	8.8±0.5	8.7±0.4	9±0.5
Plasma phosphorus (mg/dL)†§	3.3±0.6	3.2±0.5	3.4±0.6	3.2±0.6	3.6±0.6
Calcium-phosphorus product (mg^2/dL^2)§	29.7±5.9	28.3±5.2	30.8±6.2	28.7±5.4	34±6.1
iPTH (pg/mL)	69.9±37.8	68.9±35.7	70.8±36.2	70.1±37	68.7±32.4
25(OH)vitamin D (ng/mL)	26.3±11.1	26.5±11.5	26.5±11.0	25.7±11.1	28.7±11.5
α–Klotho (ng/mL)	0.129±0.108	0.126±0.093	0.132±0.119	0.133±0.115	0.122±0.073
FGF23 (pg/mL)†	131.2±60.9	115.3±42.1	143.6±70.2	125.4±50.7	144±85
Albumin/creatinin rate (mg/g)†	102 (13–676)	27 (8–575)	113 (19–1022)	58 (10–657)	179 (19–608)
Proteinuria 24 hours (g/24 hours)†	0.19 (0.07–0.65)	0.18 (0.1–0.64)	0.21 (0.1–0.72)	0.17 (0.07–0.66)	0.19 (0.07–0.5)
FPE (%)	35.7±13.2	34±13	37±13	34.7±12.4	41.6±15.6

*All the variables are expressed as mean ±SD years median.
†p<0,05 for AACS.
‡Measures referred to the subgroup of diabetic patients (n=30).
§p<0,05 for KI.
CKD-EPI, chronic kidney disease epidemiology collaboration; CRP, C-reactive protein; FGF23, fibroblast growth factor 23; FPE, fractional phosphorus excretion; GFR, glomerular filtration rate; HDL, high-density lipoprotein; iPTH, intact parathyroid hormone; LDL, low-density lipoprotein.

as continuous variables (table 4). Serum α-Klotho predicts the progression of CKD,[29] besides it is associated to arterial stiffness[30] and plays a major role in protecting against VC.[31] Moreover, Klotho-hypomorphic mice suffer from VC.[32]

In terms of FEP, our population showed an average of 35.7%±13.2%, very similar to other series in stage 3 CKD.[17 33] The FEP was slightly, but not significantly, higher in those patients with VC as measured by the KI

Table 3 Multivariate analysis taken Abdominal Aortic Calcium Score (AACS)>1025 and Kauppila index (KI)>4 as dependent variables

Variable	Score * AACS	OR (95% CI) AACS	P values	Score † KI	OR (95% CI) KI	P values
Age	12.27	1.257 (1.115 to 1.417)	<0.05	5.12	1.356 (1.079 to 1.704)	<0.05
Smoke	11.79	21.276 (4.46 to 100)	<0.05			
Antihypertensive therapy	9.94					
HBP	5.49					
Comorbidity	4.37					
Antiplatelets	6.14					
RDW	9.15					
ACR	4.81					
FGF-23	4.29					
Calcium				5.97		
Phosphorus	3.55			4.43		
CaxP	3.75	1.210 (1.065 to 1.374)	<0.05	7.89	1.480 (1.138 to 1.925)	<0.05
FPE				2.44	1.073 (1.004 to 1.146)	<0.05

*Variables not included: gender, diabetes, α-Klotho, GFR, 25-OH-vitamin D, iPTH, FPE and statins.
†Variables not included in the equation: gender, diabetes, α-Klotho, FGF-23, 25-OH-vitamin D, iPTH, RDW, smoke, antihypertensive therapy and ACR.
CaxP, calcium x phosphorus product; FGF23, fibroblast growth factor 23; FPE, fractional phosphorus excretion; GFR, glomerular filtration rate; iPTH, intact parathyroid hormone; RDW, red blood cell distribution width.

Table 4 Lineal regression analyses for Abdominal Aortic Calcium Score (AACS) and Kauppila index (KI)

	AACS		KI	
	Standardised β coefficient	P values	Standardised β coefficient	P values
α-Klotho	0.461	0.001	0.265	0.012
CaxP	0.363	0.001	0.304	0.005
Age	0.300	0.002	0.327	0.003
Smoking	0.273	0.004		

CaxP, calcium x phosphorus product.

(34.7±12.4 vs 41.5%±15.6%, p=0.08), although in the multivariate analysis the FEP was associated independently with the presence of a KI>4 (OR 1.073, 95% CI 1.004 to 1.146, p<0.05). This association was maintained after adjusting in the logistic regression for factors such as iPTH, α-Klotho and, above all, FGF23. The fact that FEP was associated to the presence of an abnormal VC assessed by the KI but not to AACS (neither in the univariate nor in multivariate analyses) suggests that FEP is more related to medial calcification of the arterial wall than to the intima, detected by CT, linked to the arteriosclerotic process and associated to the common CVRFs. Nevertheless, it should be noted that we assessed the 24-hour FEP, which, much more than fasting FEP, reflects actual dietary exposure,[17 34] a factor we do not control.

In the univariate analysis, serum calcium levels are only associated with VC measured by the KI, although in the multivariate analysis, there was an independent association of the KI only with CaxP. In this context, it is important to register the use of supplements in the treatment of osteoporosis, a disease which frequently accompanies CKD.[35 36] In our study, only four subjects were taking an oral dose of calcium (500 mg of elemental calcium) for osteoporosis. These supplements might favour VC; therefore, clinical practice guidelines recommend limiting calcium <1 g/day due to the association between oral calcium supplements and calcification.[16]

Although there is an excellent correlation between VC as measured by the two methods (figure 1), described previously,[37 38] the diagnostic utility of both is not superimposable. As expected, more patients with abnormal VC were identified with CT than with lateral abdominal radiography, (56% vs 17%). In fact, all patients with a KI>4 had abnormal CT, but not the other way around. Our

Table 5 Relationship between fibroblast growth factor 23 (FGF23)/fractional phosphorus excretion (FPE) and vascular calcification (VC) grade

	Normal AACS (n=35)	Pathological AACS (n=45)	Normal KI (n=65)	Abnormal KI (n=13)
FGF-23 low FPE low	9 (26%)	8 (18%)	13 (20%)	4 (31%)
FGF-23 low FPE high	10 (28%)	13 (29%)	18 (28%)	5 (38%)
FGF-23 high FPE low	8 (23%)	15 (33%)	20 (31%)	2 (15%)
FGF-23 high FPE high	8 (23%)	9 (20%)	14 (21%)	2 (15%)

AACS, Abdominal Aortic Calcium Score; KI, Kauppila index.

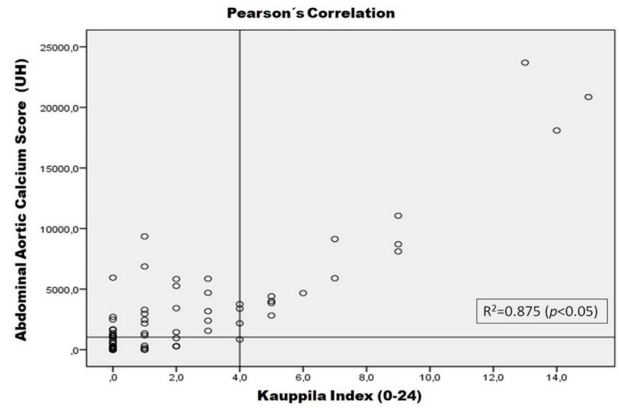

Figure 1 The correlation between Abdominal Aortic Calcium Score and Kauppila index.

results suggest that the higher sensitivity of the CT over lateral abdominal radiography lies in its ability to detect the calcification that accompanies the intimal atherosclerotic lesion, whereas the conventional X-ray seems only able to detect calcification of the middle layer of the artery, most associated with diabetes and CKD.[39 40] In fact, traditional risk factors for arteriosclerosis in patients with CKD and in general, such as age, smoking, high blood pressure, male gender, microalbuminuria, high RDW,[41] as well as vascular comorbidity and antiplatelet therapy,[42] were clearly associated with CV when identified by CT, but not with a conventional X-ray.

Most studies which support CV measured by the KI as a predictor of CVR are undertaken on elderly subjects[43 44] or those on dialysis.[20 45] Few studies measuring the KI focus on patients with stage 3 CKD, and all of them are carried out on patients with stage 3–4 CKD,[5 15 46 47] all having a lower GFR average than our population. Therefore, the finding in our study of a lower sensitivity to detect CVD of the KI in stage 3 may be relevant because there has been no study published with a population of equal magnitude for stage 3 (n=80). On the contrary, when VC is quantified in patients with stage 3 CKD by CT, there is seen to be a closer association with CVD.[48] In addition, a recent study indicates that in patients on haemodialysis, CT of the distal aorta and pelvic vessels gave a higher predictive value for vascular events and death.[49]

Our study is not without limitations, however, since it is cross-sectional, has no control group and the sample size is low. Nevertheless, it has the advantage that all patients have stage 3 CKD and that VC was analysed by two complementary methods, excluding patients with poor control of phosphorus plasma, according to current guidelines.

In conclusion, our study confirms that in patients with stage 3 CKD the detection of VC by abdominal CT is more sensitive than with a conventional X-ray. In addition, CaxP is associated with CVRF and vascular comorbidity, while the measurement of FEP in these patients only provides additional relevant clinical information for more advanced VC assessed by KI.

Acknowledgements Acknowledgement to the patients, for their generosity in collaborating with the study. To Sandra Meneaud (Fundación General de la

Universidad de Málaga, FGUMA) for translating the original manuscript into English.

Contributors MJV selected patients, review data, interpreted data, designed the project and gave final approval of the version to be published. GGG measured vascular calcification, interpreted data and gave final approval of the version to be published. PGF managed patients, interpreted data and gave final approval of the version to be published. JRV performed biochemical analyses, review data and gave final approval of the version to be published. MMV managed patients, interpreted data, review data and gave final approval of the version to be published. MÁSC performed statistical analyses, providing intellectual content of critical importance and gave final approval of the manuscript. CPL managed patients, interpreted data, review critically the manuscript and gave final approval of the version to be published. PV designed project, interpreted data and drafted the manuscript.

Funding This study has been financed with the help of the CTS-159 group of the Plan Andaluz de Innovación e Investigación (Andalusian Plan for Research and Innovation) (PAIDI).

Competing interests None declared.

Patient consent Not required.

Ethics approval Comité de Ética e Investigación de Málaga, Spain.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Schiffrin EL, Lipman ML, Mann JF. Chronic kidney disease: effects on the cardiovascular system. *Circulation* 2007;116:85–97.
2 Grimm RH, Svendsen KH, Kasiske B, *et al*. Proteinuria is a risk factor for mortality over 10 years of follow-up. MRFIT Research Group. Multiple risk factor intervention trial. *Kidney Int Suppl* 1997;63:S10–14.
3 Mann JF, Yi QL, Gerstein HC. Albuminuria as a predictor of cardiovascular and renal outcomes in people with known atherosclerotic cardiovascular disease. *Kidney Int Suppl* 2004;92:S59–62.
4 Kauppila LI, Polak JF, Cupples LA, *et al*. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. *Atherosclerosis* 1997;132:245–50.
5 Peeters MJ, van den Brand JA, van Zuilen AD, *et al*. Abdominal aortic calcification in patients with CKD. *J Nephrol* 2017;30:109–18.
6 Adragao T, Pires A, Lucas C, *et al*. A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. *Nephrol Dial Transplant* 2004;19:1480–8.
7 Agatston AS, Janowitz WR, Hildner FJ, *et al*. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990;15:827–32.
8 Ellison RC, Zhang Y, Wagenknecht LE, *et al*. Relation of the metabolic syndrome to calcified atherosclerotic plaque in the coronary arteries and aorta. *Am J Cardiol* 2005;95:1180–6.
9 Pignoli P, Tremoli E, Poli A, *et al*. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 1986;74:1399–406.
10 de Rotte AA, Koning W, Truijman MT, *et al*. Seven-tesla magnetic resonance imaging of atherosclerotic plaque in the significantly stenosed carotid artery: a feasibility study. *Invest Radiol* 2014;49:749–57.
11 Disthabanchong S, Boongird S. Role of different imaging modalities of vascular calcification in predicting outcomes in chronic kidney disease. *World J Nephrol* 2017;6:100–10.
12 Palmer SC, Hayen A, Macaskill P, *et al*. Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. *JAMA* 2011;305:1119–27.
13 Nitta K, Nagano N, Tsuchiya K. Fibroblast growth factor 23/klotho axis in chronic kidney disease. *Nephron Clin Pract* 2014;128(1-2):1–10.
14 Shimamura Y, Hamada K, Inoue K, *et al*. Serum levels of soluble secreted α-Klotho are decreased in the early stages of chronic kidney disease, making it a probable novel biomarker for early diagnosis. *Clin Exp Nephrol* 2012;16:722–9.
15 Craver L, Dusso A, Martinez-Alonso M, *et al*. A low fractional excretion of Phosphate/Fgf23 ratio is associated with severe abdominal Aortic calcification in stage 3 and 4 kidney disease patients. *BMC Nephrol* 2013;14:221.
16 Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Update Work Group. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). *Kidney International Supplements* 2017;7:1–59.
17 Evenepoel P, Meijers B, Viaene L, *et al*. Fibroblast growth factor-23 in early chronic kidney disease: additional support in favor of a phosphate-centric paradigm for the pathogenesis of secondary hyperparathyroidism. *Clin J Am Soc Nephrol* 2010;5:1268–76.
18 Levey AS, Stevens LA, Schmid CH, *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150:604–12.
19 An C, Lee HJ, Lee HS, *et al*. CT-based abdominal aortic calcification score as a surrogate marker for predicting the presence of asymptomatic coronary artery disease. *Eur Radiol* 2014;24:2491–8.
20 Verbeke F, Van Biesen W, Honkanen E, *et al*. Prognostic value of aortic stiffness and calcification for cardiovascular events and mortality in dialysis patients: outcome of the calcification outcome in renal disease (CORD) study. *Clin J Am Soc Nephrol* 2011;6:153–9.
21 Otero A, de Francisco A, Gayoso P, *et al*. Prevalence of chronic renal disease in Spain: results of the EPIRCE study. *Nefrologia* 2010;30:78–86.
22 Górriz JL, Molina P, Cerverón MJ, *et al*. Vascular calcification in patients with nondialysis CKD over 3 years. *Clin J Am Soc Nephrol* 2015;10:654–66.
23 McGovern AP, de Lusignan S, van Vlymen J, *et al*. Serum phosphate as a risk factor for cardiovascular events in people with and without chronic kidney disease: a large community based cohort study. *PLoS One* 2013;8:e74996.
24 Abajo M, Betriu À, Arroyo D, *et al*. Mineral metabolism factors predict accelerated progression of common carotid intima-media thickness in chronic kidney disease: the NEFRONA study. *Nephrol Dial Transplant* 2017;32:1882–91.
25 Lu X, Hu MC. Klotho/FGF23 axis in chronic kidney disease and cardiovascular disease. *Kidney Dis* 2017;3:15–23.
26 Qin Z, Liu X, Song M, *et al*. Fibroblast growth factor 23 as a predictor of cardiovascular and all-cause mortality in prospective studies. *Atherosclerosis* 2017;261:1–11.
27 Dominguez JR, Shlipak MG, Whooley MA, *et al*. Fractional excretion of phosphorus modifies the association between fibroblast growth factor-23 and outcomes. *J Am Soc Nephrol* 2013;24:647–54.
28 Bech AP, Bouma-de Krijger A, van Zuilen AD, *et al*. Impact of fractional phosphate excretion on the relation of FGF23 with outcome in CKD patients. *J Nephrol* 2015;28:477–84.
29 Liu QF, Ye JM, Yu LX, *et al*. Plasma s-Klotho is related to kidney function and predicts adverse renal outcomes in patients with advanced chronic kidney disease. *J Investig Med* 2018;66:669–75.
30 Kitagawa M, Sugiyama H, Morinaga H, *et al*. A decreased level of serum soluble Klotho is an independent biomarker associated with arterial stiffness in patients with chronic kidney disease. *PLoS One* 2013;8:e56695.
31 Yamada S, Giachelli CM. Vascular calcification in CKD-MBD: Roles for phosphate, FGF23, and Klotho. *Bone* 2017;100:87–93.
32 Lang F, Leibrock C, Pelzl L, *et al*. Therapeutic Interference With Vascular Calcification-Lessons From Klotho-Hypomorphic Mice and Beyond. *Front Endocrinol* 2018;9.
33 Isakova T, Wahl P, Vargas GS, *et al*. Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. *Kidney Int* 2011;79:1370–8.
34 Moe SM, Chen NX, Seifert MF, *et al*. A rat model of chronic kidney disease-mineral bone disorder. *Kidney Int* 2009;75:176–84.
35 Babayev R, Nickolas TL. Bone Disorders in Chronic Kidney Disease: An Update in Diagnosis and Management. *Semin Dial* 2015;28:645–53.
36 Lu KC, Wu CC, Yen JF, *et al*. Vascular calcification and renal bone disorders. *ScientificWorldJournal* 2014;2014:637065.
37 Bellasi A, Ferramosca E, Muntner P, *et al*. Correlation of simple imaging tests and coronary artery calcium measured by computed tomography in hemodialysis patients. *Kidney Int* 2006;70:1623–8.
38 Liabeuf S, Desjardins L, Diouf M, *et al*. The Addition of Vascular Calcification Scores to Traditional Risk Factors Improves Cardiovascular Risk Assessment in Patients with Chronic Kidney Disease. *PLoS One* 2015;10:e0131707.
39 Lanzer P, Boehm M, Sorribas V, *et al*. Medial vascular calcification revisited: review and perspectives. *Eur Heart J* 2014;35:1515–25.
40 Thompson B, Towler DA. Arterial calcification and bone physiology: role of the bone-vascular axis. *Nat Rev Endocrinol* 2012;8:529–43.
41 Oleksiak A, Kruk M, Lenarczyk E, *et al*. Biomarkers for risk stratification in secondary cardiovascular prevention. A role of red blood cell distribution width and calcium score. *Atherosclerosis* 2016;246:57–62.
42 Chen NX, Moe SM. Vascular calcification: pathophysiology and risk factors. *Curr Hypertens Rep* 2012;14:228–37.
43 Bolland MJ, Wang TK, van Pelt NC, *et al*. Abdominal aortic calcification on vertebral morphometry images predicts incident myocardial infarction. *J Bone Miner Res* 2010;25:505–12.

44 Rodondi N, Taylor BC, Bauer DC, *et al*. Association between aortic calcification and total and cardiovascular mortality in older women. *J Intern Med* 2007;261:238–44.
45 Okuno S, Ishimura E, Kitatani K, *et al*. Presence of abdominal aortic calcification is significantly associated with all-cause and cardiovascular mortality in maintenance hemodialysis patients. *Am J Kidney Dis* 2007;49:417–25.
46 Takayama Y, Yasuda Y, Suzuki S, *et al*. Relationship between abdominal aortic and coronary artery calcification as detected by computed tomography in chronic kidney disease patients. *Heart Vessels* 2016;31:1030–7.
47 Toussaint ND, Lau KK, Strauss BJ, *et al*. Using vertebral bone densitometry to determine aortic calcification in patients with chronic kidney disease. *Nephrology* 2010;15:575–83.
48 He J, Reilly M, Yang W, *et al*. Risk factors for coronary artery calcium among patients with chronic kidney disease (from the Chronic Renal Insufficiency Cohort Study). *Am J Cardiol* 2012;110:1735–41.
49 NasrAllah MM, Nassef A, Elshaboni TH, *et al*. Comparing different calcification scores to detect outcomes in chronic kidney disease patients with vascular calcification. *Int J Cardiol* 2016;220:884–9.