

MINERAL AND BONE DISORDERS AND VASCULAR PATHOLOGY IN ASYMPTOMATIC CKD NON DIALYSIS PATIENTS, NOT SUCH A CLEAR RELATIONSHIP

Jaume Almirall, Loreley Betancourt, Jose-Ramon Fortuño, Eugenio Berlanga, Juan Carlos Martinez-Ocaña, Esther Ponz
Parc Tauli Sabadell, Hospital Universitari. Serveis de Nefrologia, UDIAT Laboratori i Radiologia

Introduction

- Vascular pathology is highly prevalent from the early stages of CKD. The pathophysiology is complex and not fully understood
- In addition to the traditional risk factors, alterations of mineral and bone disorders seem to play a significant role
- Although there is little doubt of its relevance in advanced stages, its role in early-stage asymptomatic patients is less evident

Aim

- to evaluate the prevalence of vascular calcification, vascular dysfunction and left ventricular hypertrophy in asymptomatic patients with CKD stage 3-5ND and analyse its relationship with clinical and biological variables related with mineral and bone disorders

Material and methods

- Design: descriptive transversal, observational study
- Inclusion: 60 consecutive patients evaluated; a restrictive inclusion criteria will be applied so as not to "contaminate" the study sample:
 - Age: 40 - 65 years
 - CKD 3-5ND
 - asymptomatic, without previous CV disease
 - P < 5mg/dl without phosphorous binders
- Procedures:
 - Registry of the traditional CV risk factors (clinical and biological).
 - Biological measurements:
 - Biological variables analysed: Ca, P, PTH, 25(OH)D, 1-25(OH)₂, Fractional Phosphate Excretion (FPE), FGF-23, FPE/FGF-23 ratio and soluble Klotho; IL-6; T-troponin; NT pro BNP

Material and methods

Vascular function was assessed:

- with the SphygmoCor system, to obtain carotid-femoral pulse wave velocity, estimates of central aortic pressures and augmentation index
- vascular structural changes were analysed with measurement of intima-media thickness and evaluation of atheroma plaques using carotid ultrasound (ANTARES-Siemens). Image and measurements were carried out with the Syngo Art Health-US Workplace 3.0 software
- left ventricular hypertrophy and valve calcification were assessed by echocardiography study
- vascular calcification scores were obtained with Kauppila and Adragao indexes

Statistical procedure

- comparison of qualitative variables will be based on the chi-squared or Fisher exact test, while analysis of variance (ANOVA) will be used to compare the means of quantitative variables corresponding to independent samples. Nonparametric tests (Kruskal-Wallis) will also be used. Pearson or Spearman correlation coefficients will be used to analyse the correlation between variables according to their distribution.
- Multivariate linear regression models will be fitted for the quantitative variables, with logistic regression models for the dichotomised variables.
- Statistical significance will be considered as an alpha risk of 5% (P<0.05). The SPSS version 18.0 statistical package will be used for the analysis.

Results

- Patients: mean age: 54±8; 72% men
- Controls: 12 matched people with normal renal function
- Aetiology of CKD:
 - nephrosclerosis (biopsy proven): 28 (9)
 - diabetic nephropathy: 3
 - chronic glomerulonephritis: 8
 - polycystic kidney disease: 7
 - others: 12
 - unknown: 2
- Cr: 2.35±0.98 mg/dl
- eGFR(CKD-EPI): 32.8±11 mL/min/1.73m²
 - stage 3a: 18.3%
 - stage 3b: 38.3%
 - stage 4: 38.1%
 - stage 5: 1%

Results: General Descriptive analysis

Characteristics of the overall sample and control group

Variable	Control (n=12)	group (n=60)	Overall sample (n=80)	P value
Demographics				
Age		56 ± 5	54 ± 8	p NS
Female (%)		50 %	28 %	p NS
Cardiovascular Family history (%)		8 %	18 %	p NS
Dyslipidaemia (%)		33 %	75 %	p < 0.01
Diabetes mellitus (%)		0 %	18 %	p < 0.001
Active smokers (%)		0 %	20 %	p < 0.001
Body mass index		24.3 ± 2	29 ± 4.8	p < 0.000
Hypertension (%)		42 %	95 %	p < 0.004
Systolic blood pressure (SBP)		122.1 ± 17.1	141.7 ± 18.3	p < 0.003
Diastolic blood pressure		75.8 ± 8.5	85.3 ± 8.1	p < 0.003

Results: General Descriptive analysis: Laboratory data

Laboratory data	Control (n=12)	group (n=60)	Overall sample (n=80)	P value
Serum creatinine (mg/dl)	0.89 ± 0.22	2.35 ± 0.98		p < 0.000
eGFR (CKD-EPI)	84.4 ± 15	32.7 ± 11.8		p < 0.000
Glucose (mg/dl)	93.5 ± 11	98.2 ± 19		p < 0.005
Haemoglobin	13.9 ± 1.1	13.2 ± 2		p NS
pH	7.36 ± 0.03	7.30 ± 0.49		p < 0.000
Bicarbonate	26.7 ± 2.5	23.9 ± 4.1		p NS
Cholesterol (mg/dl)	205 ± 46	196 ± 43		p NS
LDL cholesterol	131 ± 41	120 ± 40		p NS
HDL cholesterol	55 ± 18	47 ± 12		p NS
Triglycerides	94 ± 41	169 ± 80		p < 0.000
Calcium	9.83 ± 0.35	9.41 ± 0.54		p NS
Phosphate	3.27 ± 0.59	3.59 ± 0.66		p NS
Ca x P	30.4 ± 6.0	32.7 ± 6.1		p NS
Albumin	44.6 ± 2.9	43.9 ± 2.9		p NS
PTH	41 ± 12	129 ± 83		p < 0.000
25 OH vitamin D (ng/ml)	25.2 ± 10	20.4 ± 17		p NS
1-25 (OH) ₂ vitamin D(pg/ml)	34.5 ± 10	25.3 ± 10.9		p = 0.02
po	0.13 ± 0.07	0.34 ± 0.32		p < 0.000
Proteinuria g/24h		1.02 ± 1.22		p < 0.000
FPE %	14.5 ± 6.8	40.1 ± 13.7		p < 0.000
FGF-23 (RU/ml)	89.8 ± 70	322.4 ± 350		p < 0.000
FPE/FGF-23	0.19 ± 0.08	0.23 ± 0.20		p NS
Soluble α Klotho (pg/ml)	614 ± 84	802 ± 208		p NS
T-troponin	6.38 ± 2.7	16.6 ± 18.9		p < 0.000
NT-proBNP	52.2 ± 38.2	154.5 ± 130		p < 0.000
IL-6	2.25 ± 1	5.17 ± 2.0		p < 0.000

Results: General Descriptive analysis: carotid ultrasound and arterial stiffness

	Control (n=12)	group (n=60)	Overall sample (n=80)	P value
Carotid ultrasound				
Intima Media Thickness	0.57±0.06	0.58±0.17		p NS
Carotid plaque		17%	83%	P=0.002
Calcified plaque		8%	25%	P=0.1
Arterial stiffness				
Pulse wave velocity	8.7 ± 2.5	9.82 ± 2.73		p NS
Augmentation index	23.4 ± 11.5	27 ± 8.6		p NS
Systolic Ao BP	113 ± 17	132 ± 18		p = 0.003
Diastolic Ao BP	76.7 ± 8.5	86.5 ± 8		p = 0.02

Univariate analysis: variables associated with the different functional and structural vascular results in the univariate analysis are the following:

Carotid ultrasound:

Intima Media Thickness	Pearson correlation	p
Age	0.40	0.002
Smoking habit	0.288	0.026
FPE/FGF-23	-0.321	0.012
Systolic blood pressure	0.237	0.068 (ns)

Carotid plaque (%)	Pearson correlation	p
Age	0.42	0.001
Sex (men=0, women=1)	-0.29	0.025
Diabetes mellitus	0.271	0.036
Smoking habit	0.294	0.023
FPE/FGF-23	-0.373	0.003
Triglycerides	0.272	0.036
FGF-23	0.260	0.045
Systolic blood pressure	0.253	0.052 (ns)

Calcified plaque (%)	Pearson correlation	p
Ca	0.254	0.051
FPE/FGF-23	-0.238	0.067 (ns)
Systolic blood pressure	0.252	0.052

Arterial stiffness:		
Pulse wave velocity	Pearson correlation	p
Age	0.573	0.000
Systolic blood pressure	0.443	0.000
FPE/FGF-23	-0.259	0.047
Diabetes mellitus	0.275	0.035
Dyslipidaemia	0.269	0.039
P	0.280	0.031
Troponin	0.299	0.021
25OH-VD	-0.247	0.059 (ns)

Augmentation index:		
Age	Pearson correlation	p
Age	0.377	0.003
Systolic blood pressure	0.292	0.024
FPE/FGF-23	-0.285	0.027
Sex (men=0, women=1)	0.455	0.000
NT-pro BNP	0.364	0.004
IL-6	0.312	0.015
FGF-23	0.295	0.022

Echocardiography:		
MVI/body surface	Pearson correlation	p
Systolic blood pressure	0.354	0.005
NT-pro BNP	0.286	0.027
FGF-23	0.335	0.009
PTH	0.371	0.004
FPE	0.284	0.028

Mitral Calcification (%)		
Age	Pearson correlation	p
Age	0.295	0.02
Systolic blood pressure	0.260	0.045
NT-pro BNP	0.390	0.002

RX Vascular Calcification		
Adragao score >2	Pearson correlation	p
Systolic blood pressure	0.455	0.000
FPE/FGF-23	-0.322	0.012
FGF23	0.250	0.054 (ns)

Kauppila score >6		
Age	Pearson correlation	p
Age	0.444	0.000
FPE/FGF-23	-0.272	0.036
Ca	0.238	0.067 (ns)
FGF23	0.176	0.178 (ns)

Summary of statistical associations in the univariate analysis

- IMT correlated with FPE/FGF ratio (p 0.01);
- carotid plaque with FGF-23 (p 0.045) and FPE/FGF ratio (p 0.003);
- calcified plaque correlates with Ca (p 0.05);
- PWV with serum P (p 0.03) and FPE/FGF ratio (p 0.047);
- AI with FGF-23 (p 0.02) and FPE/FGF ratio (p 0.02);
- LVM with PTH (p 0.004) and FGF-23 (p 0.009);
- Adragao with FGF-23 (p 0.05) and FPE/FGF ratio (p 0.01);
- Kauppila index with FPE/FGF ratio (p 0.03).

However, all these correlations lost their significance when adjusted in the multivariate analysis, being the observed vascular pathology best explained by variables related with traditional cardiovascular risk factors

Results of the multivariate analysis:

Multiple regression model						
Variable	non-standardised regression coefficient		standardised coefficient	Sig	95% CI for B	
	B	standard error			lower	upper
Intima Media Thickness:						
Age	0.007	0.003	0.353	0.007	0.002	0.012
Active smoking	0.123	0.049	0.291	0.015	0.025	0.222
SBP	0.001	0.001	0.121	0.341	-0.001	0.003
FPE/FGF-23	-0.025	0.008	-0.248	0.709	-0.181	0.110
Pulse wave velocity						
Age	0.165	0.036	0.523	0.000	0.080	0.236
Diabetes	1.256	0.735	0.174	0.069	-0.218	2.735
Phosphorus	1.073	0.521	0.266	0.044	0.028	2.118
Dyslipidaemia	0.875	0.655	0.108	0.208	-0.641	1.881
SBP	0.024	0.017	0.162	0.165	-0.010	0.059
25OH-VD	-0.027	0.017	-0.176	0.105	-0.061	0.006
FPE/FGF-23	1.809	1.081	0.218	0.100	-0.361	3.980
Augmentation index						
Age	0.213	0.099	0.213	0.034	0.015	0.412
Ca	1.118	0.260	0.374	0.000	0.584	1.677
Sex (1=men)	1.073	1.771	0.580	0.000	7.522	14.824
SBP	0.113	0.047	0.230	0.018	0.020	0.206
FPE/FGF-23	-3.200	2.669	-0.120	0.230	-8.843	2.142

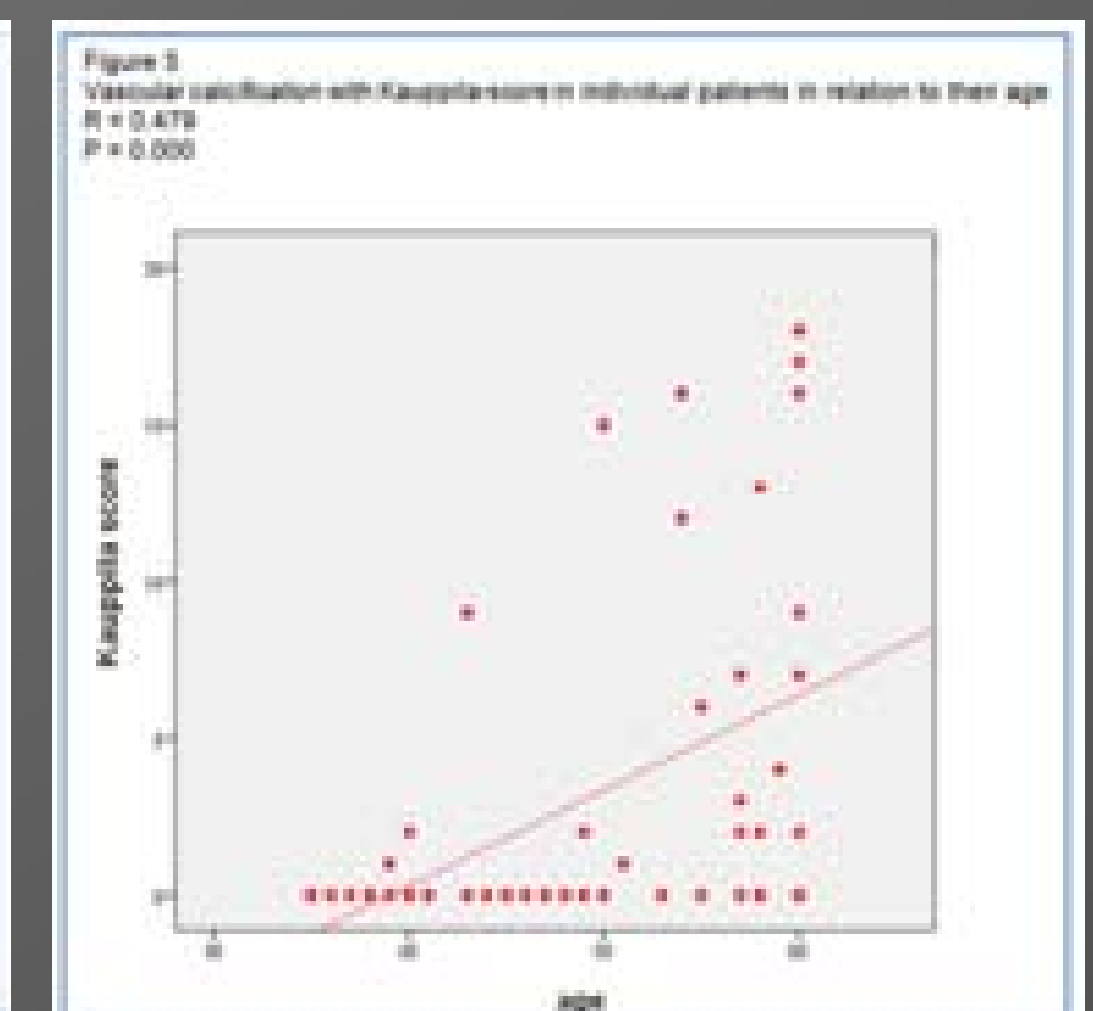
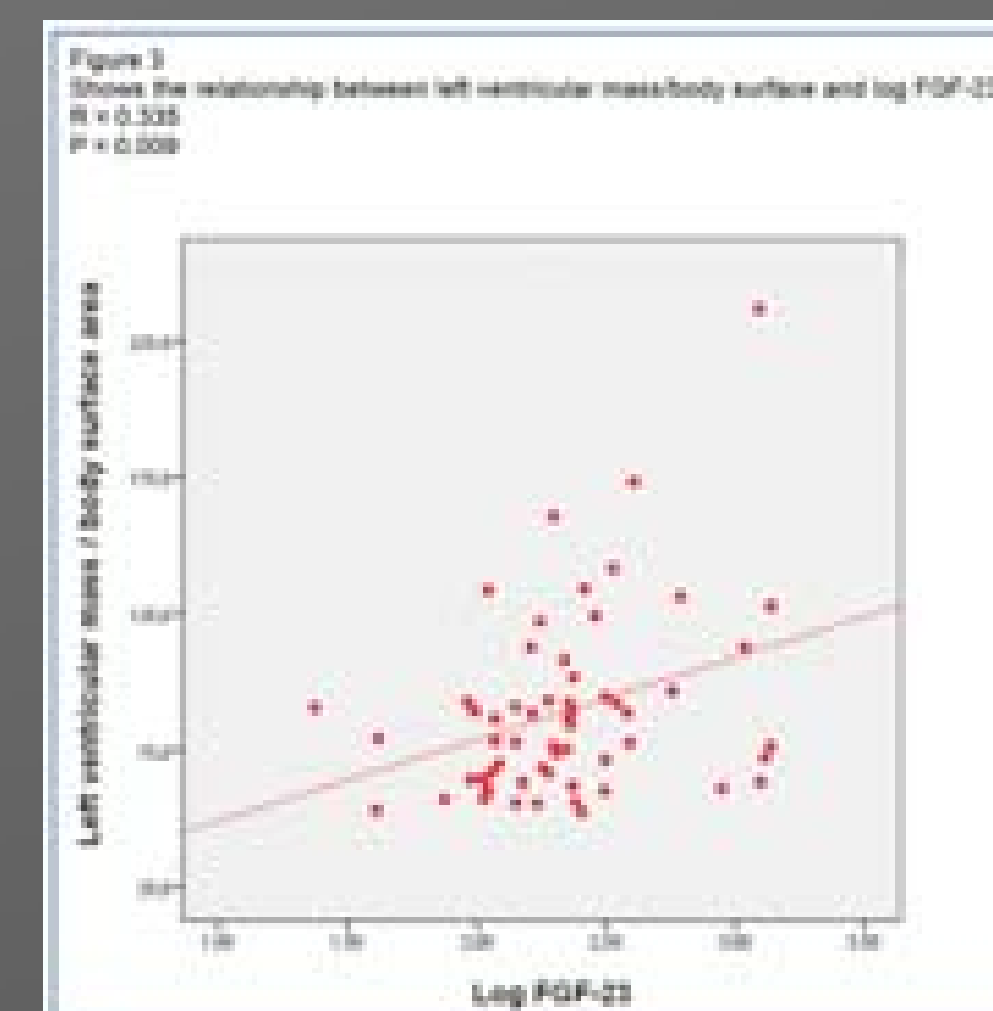
Echocardiography

Variable	non-standardised regression coefficient		standardised coefficient	Sig	95% CI for B	
	B	standard error			lower	upper
SBP	0.512	0.230	0.280	0.030	0.051	0.972
FGF-23	5.701	14.159	0.064	0.689	-22.663	34.064
PTH	0.112	0.061	0.278	0.072	-0.10	0.234

It is important to note the strong association observed between log FGF-23 and PTH with a Pearson correlation of 0.63 (p=0.000). This high co-linearity makes this multiple regression analysis difficult to interpret.

multivariate analysis -Logistic regression:

Carotid plaque %						
	B	SE	Wald	p	Odds Ratio	95% CI
Age	0.140	0.048	8.544	0.003	1.151	1.057 - 1.251
Smoking habit	2.271	1.156	3.668	0.058	9.690	1.054 - 88.666
Triglycerides	0.008	0.006	1.604	0.102	1.008	0.995 - 1.021
FPE/FGF-23	-0.809	1.209	0.460	0.495	0.424	0.084 - 2.138
Sex	-1.040	0.819	1.613	0.204	0.353	0.084 - 1.501
Diabetes Mellitus	1.425	1.205	1.390	0.236	4.190	0.584 - 30.317
Calcified plaque %						
Age	0.017	0.043	0.153	0.696	1.017	0.928 - 1.113
SBP	0.027	0.020	1.877	0.171	1.027	0.987 - 1.068
FPE/FGF-23	-0.608	1.364	0.208	0.625	0.514	0.084 - 3.117
P	0.312	0.046	6.930	0.007	1.369	1.254 - 1.494
Ca	1.100	0.609	3.060	0.080	3.017	0.884 - 10.422
Adragao score >2						
Age	-0.30	0.083	0.359	0.552	0.970	0.807 - 1.167
SBP	0.079	0.027	6.847	0.008	1.073	1.024 - 1.124
FPE/FGF-23	-2.743	1.466	3.366	0.067	0.064	0.010 - 0.442
Kauppila score >6						
Age	0.150	0.063	5.760	0.018	1.164	1.038 - 1.301
SBP	0.002	0.022	0.008	0.930	1.002	0.953 - 1.054
Ca	0.545	0.047	6.700	0.000	1.724	1.601 - 1.854
FPE/FGF-23	-1.516	1.300	1.380	0.244	0.200	0.040 - 0.960



Discussion, general aspects:

- The evidence that CRF carries an additional vascular risk
- The importance of traditional risk factors such as age, smoking habit, SBP, DM or dyslipidaemia.
- The association between the biological variables and the results of vascular studies is highly variable

Conclusions