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Original Article

ORIGINAL ARTICLE

Fibroblast growth factor 23 but not copeptin is independently associated with kidney failure and mortality in patients with chronic kidney disease

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ABSTRACT

Background. Copeptin and intact fibroblast growth factor 23 (iFGF23) increase early during chronic kidney disease (CKD) and may be predictive of unfavourable outcomes. The aim of this study was to evaluate their respective associations with renal and vital outcomes in CKD patients.

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Methods. We included CKD patients from the NephroTest cohort with concomitant measurements of plasma copeptin and iFGF23 concentrations and isotopic glomerular filtration rate measurement (mGFR). The primary endpoint was a composite outcome including kidney failure (KF) (dialysis initiation, pre-emptive transplantation or a 57% decrease of mGFR, corresponding to doubling of serum creatinine) or death before KF. Hazard ratios (HRs) of the primary endpoint associated with log-transformed copeptin and iFGF23 concentrations were estimated by Cox models. The slope of mGFR over time was analysed using a linear mixed model.

Results. A total of 329 CKD patients (243 men, mean age 60.3 ± 14.6 years) were included. Among them, 301 with an mGFR >15 ml/min/1.73 m^2 were included in survival and mGFR slope analyses. During a median follow-up of 4.61 years (quartile 1–quartile 3: 3.72–6.07), 61 KFs and 32 deaths occurred. Baseline iFGF23 concentrations were associated with the composite outcome after multiple adjustments {HR 2.72 [95% confidence interval (CI) 1.85–3.99]}, whereas copeptin concentrations were not [HR 1.01 (95% CI 0.74–1.39)]. Neither copeptin nor iFGF23 were associated with mGFR slope over time.

Conclusion. Our study shows for the first time in population of CKD patients an independent association between iFGF23 and unfavourable renal and vital outcomes and shows no such association regarding copeptin, encouraging the integration of iFGF23 measurement into the follow-up of CKD.

LAY SUMMARY

Copeptin and intact fibroblast growth factor 23 (iFGF23) are circulating proteins that increase early during chronic kidney disease (CKD) and may be predictive of unfavourable outcomes. We included 301 CKD patients before end-stage renal disease from the NephroTest cohort with concomitant measurements of plasma copeptin and iFGF23 concentrations and direct measurement of glomerular filtration rate (GFR). Baseline iFGF23 concentrations but not those of copeptin were associated after multiple adjustments with a composite outcome reflecting an unfavourable renal or vital evolution: kidney failure (KF) (dialysis initiation, pre-emptive transplantation or 57% decrease of mGFR) or death before KF. Neither copeptin nor iFGF23 were associated with measured GFR slope over time in additional analyses. It is the first time that these two potential biomarkers have been tested in a population of well-phenotyped CKD patients. Our study encourages the integration of iFGF23 measurement into the follow-up of CKD patients, whatever the CKD cause.

Keywords: biomarkers, chronic kidney disease, copeptin, fibroblast growth factor 23, glomerular filtration rate

INTRODUCTION

Chronic kidney disease (CKD) is associated with early hormonal modifications. Some of these could be predictive of CKD progression, kidney failure (KF) and/or mortality and may therefore improve the management of CKD patients, provided they can be easily measured in routine care. Among these potential hormonal biomarkers, fibroblast growth factor 23 (FGF23) and vasopressin [1] have been shown to increase early during CKD, although this is less clearly established for vasopressin.

FGF23 is secreted by osteocytes and osteoblasts and circulates as an intact peptide (iFGF23), which can be cleaved in N-terminal and C-terminal fragments (cFGF23). α-Klotho, a protein expressed at the cell surface of some organs, forms complexes with FGF receptors, thus increasing their affinity for FGF23 [2]. Both iFGF23 and cFGF23 can be measured in plasma using enzyme-linked immunosorbent assays (ELISAs). FGF23 controls plasma phosphate concentrations by decreasing renal phosphate reabsorption, decreasing calcitriol synthesis and stimulating calcitriol catabolism in the proximal tubule [3]. Plasma phosphate and calcitriol concentrations exert a feedback on FGF23 production. FGF23 concentration increases when glomerular filtration rate (GFR) declines, preventing an excessive increase in plasma phosphate concentration [4]. Elevated iFGF23 concentration has been independently associated with GFR decline [5] and mortality in CKD patients [6]. High iFGF23 concentrations have also been reported to be positively associated with urinary albumin excretion (UAE) [7].

Vasopressin, a peptide that induces water reabsorption, is stimulated by an increase in plasma osmolality, but also by severe hypovolaemia. Copeptin, a peptide co-secreted in equimolar amounts, is a surrogate marker of vasopressin [8].

Plasma copeptin concentration has been shown to predict albuminuria occurrence [9] and to be positively associated with UAE [10] and GFR decline in patients with autosomal dominant polycystic kidney disease (ADPKD) [11], diabetic nephropathy [12], immunoglobulin A (IgA) nephropathy [13] and kidney transplantation [14]. Plasma copeptin concentration could be associated with the development of CKD since vasopressin is known to increase renal energy expenditure [15]. Several publications also suggest that high fluid intake, by inhibiting vasopressin, could have beneficial effects on renal function, whatever the cause of CKD [16].

In renal transplant recipients, copeptin and cFGF23 were positively associated, independently from GFR [17], suggesting a potential common pathophysiological pathway between these two hormonal systems. This is supported by experimental studies linking vasopressin and α-Klotho expression [18] or showing inhibition of FGF23 by vasopressin [19].

The aim of our study was to determine the potential independent associations of iFGF23 and copeptin with the rate of decline of measured GFR (mGFR), KF or overall mortality in CKD patients.

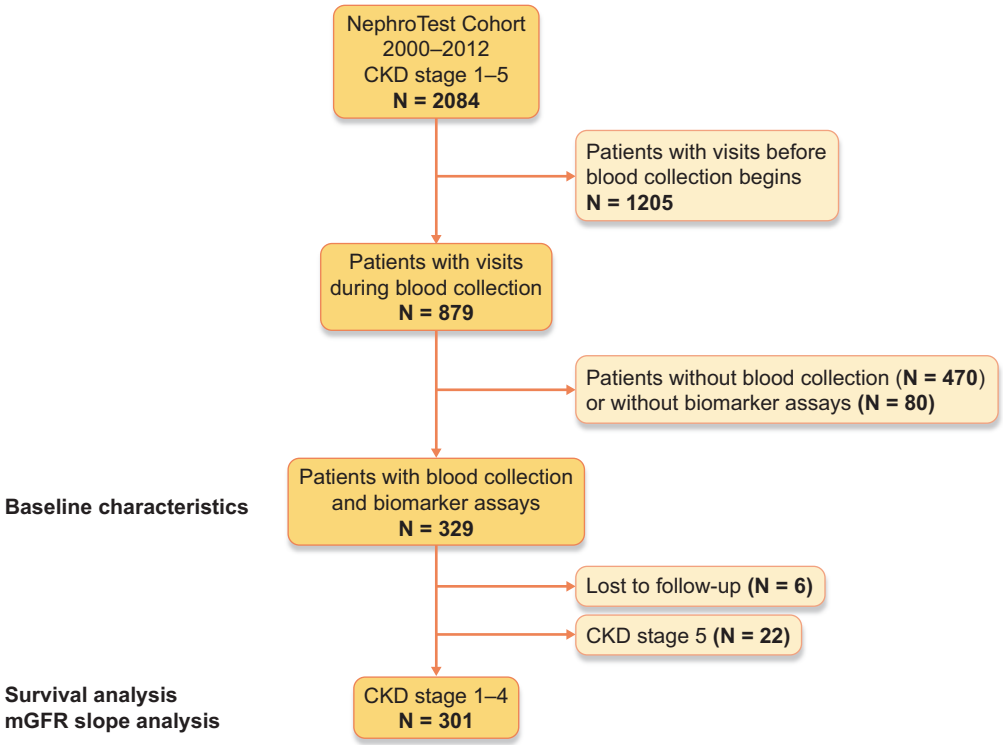

Figure 1: Flow chart of the study.

MATERIALS AND METHODS

Study population

The NephroTest study is a prospective hospital-based cohort initiated in 2000, enrolling adult patients with CKD stages 1–5 [20], not on dialysis nor living with a kidney transplant, not pregnant and referred to any of three French nephrology departments for yearly extensive work-up. Between January 2000 and December 2012, 2084 patients were enrolled in this study. In a subgroup of 329 patients from two of the three centres, copeptin and iFGF23 concentrations were assessed from plasma collection, which began in 2009. Of these 329 patients, 301 with an mGFR >15 ml/min/1.73 m^2 were included in the survival analyses and in the mGFR slope analysis (Fig. 1). All patients signed an informed consent before inclusion in the cohort. The NephroTest study was approved by an ethics committee (DGRI CCTIRS MG/CP09.503) and adheres to the Declaration of Helsinki.

Data collection and biological assessment

As previously reported, 5-hour in-person visits for an extensive nephrological work-up provided demographic, clinical and laboratory data [21]. Medical history was collected at the inclusion visit. Diabetes was defined by a diagnosis of diabetes mellitus, use of an antidiabetic treatment or fasting plasma glucose ≥7 mmol/L [22]. Cardiovascular history was defined as the history of myocardial infarction, angioplasty/coronary artery bypass graft, stroke and heart failure.

The following measurements were performed after an overnight fast: creatinine, sodium, glucose, albumin, total cholesterol, ionized calcium, phosphate, 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], serum parathyroid hormone (PTH), osmolality in plasma and osmolality on a urinary spot. A 24-hour urine collection was performed to determine urinary excretion of creatinine, albumin, phosphate, calcium and urea. Urinary albumin:creatinine ratio (ACR) was calculated from 24-hour urine concentrations. Protein intake was estimated with the Maroni formula using 24-hour urinary urea nitrogen [23]. Urinary osmolarity was calculated from 24-hour urine concentrations of urea, sodium and potassium [24]. Plasma samples were aliquoted and kept frozen at −80°C until centralized measurements of copeptin (Brahms human Ct-proAVP LIA kit, B·R·A·H·M·S, Germany (as a subsidiary of Thermo Fisher Scientific, USA)) and iFGF23 (human intact-FGF23 ELISA kit; Kainos, Tokyo, Japan).

GFR was measured by chromium-51-labeled ethylenediaminetetraacetic acid (^{51}Cr-EDTA) renal clearance at enrolment and at each following visit, as previously described [25]. Briefly, 1.8–3.7 MBq of ^{51}Cr-EDTA (GE Healthcare, Velizy, France) was injected intravenously as a single bolus. One hour was allowed for distribution of the tracer into the extracellular fluid, then renal ^{51}Cr-EDTA clearance was determined by averaging five to six consecutive 30-min clearance periods. In case of extracellular volume expansion, a continuous infusion protocol was used [26].

Outcomes/endpoint

Our primary endpoint was a composite outcome including KF, defined by dialysis initiation or pre-emptive kidney transplantation or by a 57% decrease of mGFR (corresponding to doubling of serum creatinine [27, 28]) or death during follow-up before the occurrence of KF. Renal replacement therapy and vital status were ascertained by linkage with the French Renal Epidemiology and Information Network registry of dialysis and transplantation and the national death registry. All survival data were right-censored on 31 December 2013 or the date of the last visit

for the few patients not identified in registries. A secondary endpoint was the slope of the mGFR decline during follow-up.

Statistical analysis

First, baseline characteristics in the overall sample of patients were described using median [interquartile range quartile 1 (Q1)–quartile 3 (Q3)] or mean [± standard deviation (SD)], according to the distribution of quantitative variables and percentage and number for qualitative variables.

Variables with a right-skewed distribution were log-transformed. Associations between log-transformed biomarkers (copeptin and iFGF23) and patients' characteristics were assessed before and after adjustment for mGFR using Pearson correlation coefficients and partial correlation coefficients.

In the patients with CKD stages 1–4 at baseline, we used cause-specific Cox models to estimate crude and adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) of our composite outcome according to log-transformed copeptin and iFGF23 concentrations. Sex, age, mGFR and log-ACR were forced in the final model while the other covariates were selected using a stepwise procedure (entry if $P < .3$, exit if $P > .2$). Variables tested were elevated blood pressure (>140/90 mmHg, using the average of three measurements), protein intake (g/kg/day), sub-Saharan African origin, body mass index (<19, 20–24, 25–29, ≥30 kg/m^2), cardiovascular history, smoking status, renin–angiotensin system inhibitor treatment, albuminemia (<35 g/L), diabetes, hypercholesterolaemia (treatment or total cholesterol >5 or >6 mmol/L depending on cardiovascular history), plasma phosphate, log(PTH), 25(OH)D and 1,25$(OH)_2$D. The Cox model assumption of proportional hazards was met for all covariates. Finally, penalized splines were used in fully adjusted Cox models to represent the functional relationship between log-transformed biomarker concentrations and the composite outcome risk. A sensitivity analysis was also carried out using KF as the outcome in the survival analysis. We also performed a multivariate analysis of the determinants of copeptin and iFGF23, by multiple linear regression, incorporating variables known to be associated with either FGF23 or copeptin [7, 29, 30]. Then we used a linear mixed model [31] with random intercepts and slopes to study the association between baseline log-transformed copeptin and iFGF23 concentrations and mGFR slope (in ml/min/year). We estimated β and the SDs adjusted for baseline mGFR (<30, 30–44, ≥45 ml/min/1.73 m^2), as well as for the above covariates and the number of mGFR measurements. The covariance matrix for the random effects was estimated for each group of baseline mGFRs separately and robust sandwich variance estimators were used to estimate variances of regression coefficients. Interactions with time were tested for all covariates. Only those that were statistically significant according to the Wald test and improved the model according to the Akaike information criterion were included in the final model.

All variables have <3% missing data (except protein intake, at 8%), which led us to impute by the median for continuous variables and by the most frequent class for categorical variables. Analyses were performed with SAS version 9.4 software (SAS Institute, Cary, NC, USA) and R 3.1.3 (R Foundation for Statistical Computing, Vienna, Austria) [32].

RESULTS

Baseline characteristics of the study group

The baseline characteristics of the patients are reported in Table 1. The patients' mean age was 60.3 ± 14.6 years and almost three-quarters of them were men. The mean mGFR was 36.9 ± 17.2 ml/min/1.73 m^2 and the median ACR was 9.4 mg/mmol creatinine (IQR 1.7–60.5). Supplementary Table 1 shows data according to the mGFR classes. Twenty-three percent of the patients (76/329) were diabetic, of which 37% (28/76) had diabetic nephropathy. Fifteen patients had polycystic kidney disease. A flowchart is shown in Fig. 1.

Associations between copeptin, FGF23 and patients' characteristics

Copeptin and iFGF23 concentrations exhibited markedly higher values with decreasing baseline mGFR, with $r = -0.55$ ($P < .0001$) and $r = -0.59$ ($P < .0001$), respectively. Copeptin was significantly higher in men than women [23.1 pmol/L (IQR 10.4–39.8) versus 13.5 (6.0–33.6), respectively; $P < .0027$], as already reported in many studies, whereas iFGF23 showed no gender difference [67 pg/ml (IQR 45–120) versus 62 (43–110), $P = .55$]. Neither copeptin nor iFGF23 differ according to ethnic origin, diabetes, smoking status and type of nephropathy (data not shown). Copeptin and iFGF23 were significantly higher with increasing baseline ACR, protein intake and plasma osmolality, independent of mGFR. After adjustment for mGFR, copeptin concentration remained positively correlated with plasma sodium concentration and urinary osmolarity, whereas iFGF23 remained positively correlated with plasma phosphate concentration and negatively correlated with 1,25$(OH)_2$D concentration (Table 2). iFGF23 and copeptin concentrations were positively associated ($r = 0.43$, $P < .0001$) and this association remained significant but became weaker after adjustment for mGFR ($r = 0.16$, $P = .005$). Finally, iFGF23 and copeptin concentrations were not significantly associated in the multivariate regression models taking into account their respective determinants (Supplementary Table 2a and 2b).

Survival analysis of the composite outcome

Among the 301 patients with an mGFR >15 ml/min/1.73 m^2 who were included in the survival analysis during a median follow-up of 4.61 years (IQR 3.72–6.07), 57 were dialysed, 1 had a preemptive kidney transplantation, 3 had a 57% mGFR decrease and 32 died before reaching KF. Higher concentrations of copeptin and iFGF23 increased the risk of a first event of the composite outcome in unadjusted analysis (Table 3, Model 0). However, after adjustment for mGFR (Table 3, Model 1) and adjustment for other covariates (Table 3, Model 2), the HR for the composite outcome according to copeptin concentration was not statistically different from 1. In contrast, the HR for the composite outcome according to iFGF23 concentration remained >1. When adding simultaneously iFGF23 and copeptin in the models (Table 3, Model 3), estimated HR did not change. Similar results were found for KF outcome analysed separately (Table 3). The linear no-threshold relationship between iFGF23 concentration and the risk of occurrence of a first event of the composite outcome was confirmed using penalized spline regression (Fig. 2a), whereas there was no relationship between copeptin concentration and the composite outcome (Fig. 2b).

Similar associations were found after excluding the 88 patients with diabetes mellitus ($n = 76$) and/or polycystic kidney disease ($n = 15$) (Supplementary Table 3).

It should be noted that plasma phosphate and 1,25$(OH)_2$D concentrations were not associated with the composite outcome. Adjusting for these variables did not modify the HR for the composite outcome according to the iFGF23 concentration (data not shown).

Table 1: Patients' characteristics ($N = 329$).

Characteristics	Values
Age (years), mean ± SD	60.3 ± 14.6
Men, % (*n*)	73.9 (243)
Sub-Saharan Africa origin, % (*n*)	11.6 (38)
BMI (kg/m^2), mean ± SD	26.6 ± 5.4
Diabetes mellitus, % (*n*)	23.1 (76)
Hypercholesterolemia, % (*n*)	9.3 (30)
Lipid-lowering treatment, % (*n*)	62.5 (205)
Renin–angiotensin system inhibitor treatment, % (*n*)	88.1 (289)
History of cardiovascular disease[a], % (*n*)	19.6 (64)
Never/current/past smoker, % (*n*)	46.8 (154)/13.4 (44)/39.8 (131)
Nephropathy, % (*n*)	
Diabetic	8.5 (28)
Glomerular	16.7 (55)
Vascular	34.0 (112)
Polycystic kidney disease	4.6 (15)
Interstitial chronic disease	14.6 (48)
Other	21.6 (71)
Copeptin (pmol/L), median (Q1–Q3)	21 (9–38)
Intact FGF23 (pg/ml), median (Q1–Q3)	66 (44–118)
mGFR (ml/min/1.73 m^2), mean ± SD	36.9 ± 17.2
ACR (mg/mmol), median (Q1–Q3)	9.4 (1.7–60.5)
Systolic blood pressure (mmHg), mean ± SD	135 ± 21
Diastolic blood pressure (mmHg), mean ± SD	74 ± 12
Plasma osmolality (mosmol/kg H_2O), mean ± SD	301 ± 9
Fasting urinary osmolality (mosmol/kg H_2O), mean ± SD	472 ± 139
Urinary osmolarity (mosmol/L),[b] mean ± SD	365 ± 115
Plasma Na^+ (mmol/L), mean ± SD	139.1 ± 2.8
Albuminemia (g/L), mean ± SD	38.1 ± 3.8
Urinary urea excretion (mmol/24 h), mean ± SD	351 ± 133
Estimated protein intake (g/kg/24 h)[c], mean ± SD	1.01 ± 0.25
Ionized Ca^{2+} (mmol/L), mean ± SD	1.23 ± 0.07
Urinary calcium excretion (mmol/24 h), median (Q1–Q3)	1.03 (0.52–1.88)
25(OH)D (ng/ml), median (Q1–Q3)	21.4 (14.4–28.8)
1,25$(OH)_2$D (pg/ml), median (Q1–Q3)	28.0 (19.0–39.0)
PTH (pg/ml), median (Q1–Q3)	64 (41–90)
Serum phosphate (mmol/L), mean ± SD	1.09 ± 0.23
Urinary phosphate excretion (mmol/24 h), mean ± SD	21.2 ± 8.6

BMI: body mass index.
[a]Defined as a history of myocardial infarction, angioplasty/coronary artery bypass graft, stroke or heart failure.
[b]Urinary osmolarity was calculated from the 24-hour urine collection.
[c]By the Maroni formula.

mGFR slope analysis

Among the 301 patients with mGFR >15 ml/min/1.73 m^2 included in the mGFR slope analysis (median number of GFR measurements: 3; minimum 1, maximum 7), 269 had two or more sequential mGFR measurements. Of note, patients with a single GFR measurement contributed to the intercept estimation but not to the GFR slope. The median follow-up time from the first plasma collection was 2.29 years (IQR 1.34–3.92). Using a linear mixed model, the estimated mean decrease in mGFR was −1.37 ± 0.22 ml/min/year. We found a crude relationship between copeptin concentration and mGFR slope: −0.69 ± 0.22 ml/min/year/1 unit of log copeptin ($P < .01$). The association between baseline iFGF23 and mGFR slope was nearly significantly different from zero: −0.77 ± 0.44 ml/min/year/1 unit of log iFGF23 ($P = .08$). However, when adjusting for other factors associated with baseline mGFR and mGFR slope, including ACR and protein intake, neither copeptin nor iFGF23 remained associated with mGFR slope (Table 4).

DISCUSSION

Using a well-phenotyped population, we found that iFGF23 was associated with our composite outcome (death and KF), whereas copeptin was not. Neither copeptin nor iFGF23 were associated with the mGFR slope.

After kidney transplantation, copeptin and FGF23 were reported to be positively associated, independent of GFR [17]. We show a positive association between iFGF23 and copeptin in univariate analysis after adjustment for mGFR that disappeared in the multivariate model taking account of their respective determinants, suggesting that the two biomarkers may have common determinants other than GFR (e.g. UAE or protein intake).

After multiple adjustments, iFGF23 concentrations remained positively associated with an increased risk of both our composite outcome and KF alone. A positive association has already been shown between high FGF23 concentrations and the risk of CKD [33], renal failure [5, 34] and mortality in CKD patients [34]. All these studies used eGFR based on plasma creatinine, not mGFR as in our study.

Table 2: Correlation between FGF23 or copeptin plasma concentrations and biological parameters (N = 329 patients).

Parameters	FGF23 (log)				Copeptin (Log)			
	r^a	P-value	Partial r^b	P-value	R^a	P-value	Partial r^b	P-value
Age	0.04	.50	−0.1	.08	0.00	.96	**−0.13**	**.02**
BMI	0.12	.03	0.07	.20	0.07	.18	0.02	.71
Systolic blood pressure	0.07	.18	−0.02	.65	0.1	.08	0.01	.82
Diastolic blood pressure	0.04	.46	−0.02	.78	0.17	.002	**0.14**	**.01**
Na^+	0.06	.24	0.02	.73	0.19	.0004	**0.18**	**.0012**
Ca^{2+}	0.00	.96	−0.05	.33	0.09	.11	0.06	.28
Phosphate	0.59	<.0001	**0.48**	**<.0001**	0.23	<.0001	0.02	.69
PTH (log)	0.34	<.0001	0.06	.27	0.43	<.0001	**0.21**	**<.0001**
25(OH)D (log)	0.07	.20	0.1	.08	−0.06	.30	−0.06	.28
$1,25(OH)_2D$ (log)	−0.41	<.0001	**−0.26**	**<.0001**	−0.28	<.0001	−0.1	.08
Plasma osmolality	0.56	<.0001	**0.33**	**<.0001**	0.51	<.0001	**0.27**	**<.0001**
ACR (log)	0.32	<.0001	**0.17**	**.002**	0.39	<.0001	**0.27**	**<.0001**
Fasting urinary osmolality	−0.36	<.0001	−0.08	.15	−0.27	<.0001	0.02	.66
24-hour urinary urea excretion	−0.08	.13	0.07	.22	−0.03	.56	**0.12**	**.03**
24-hour estimated protein intake	−0.08	.18	**0.19**	**.001**	0.01	.82	**0.29**	**<.0001**
24-hour urinary calcium excretion (log)	−0.27	<.0001	−0.04	.48	−0.25	<.0001	−0.03	.54
24-hour urinary phosphate excretion	0.01	.90	**0.16**	**.003**	−0.04	.42	0.09	.10
Urinary osmolarity	−0.17	.002	−0.03	.57	0.11	.06	**0.31**	**<.0001**

BMI: body mass index.
Variables with right-skewed distribution were log-transformed.
Statistically significant P-values are in bold.
[a]Pearson correlation coefficient.
[b]Pearson partial correlation coefficients after adjustment for mGFR.

Table 3: HR (95% CI) of KF or death before KF (composite outcome) and of KF according to log-transformed FGF23 and copeptin concentrations (N = 301).

Outcome	Model 0	Model 1	Model 2	Model 3
Composite outcome (KF or death)				
FGF23 (log)	**3.27 (2.52, 4.25)**	**2.04 (1.45, 2.86)**	**2.72 (1.85, 3.99)**	**2.72 (1.85, 3.99)**
Copeptin (log)	**2.01 (1.55, 2.60)**	1.26 (0.94, 1.68)	1.01 (0.73, 1.41)	1.01 (0.74, 1.39)
KF				
FGF23 (log)	**4.20 (3.06, 5.77)**	**2.21 (1.46, 3.33)**	**3.06 (1.84, 5.09)**	**3.03 (1.82, 5.05)**
Copeptin (log)	**2.63 (1.86, 3.71)**	**1.47 (1.01, 2.13)**	1.17 (0.78, 1.77)	1.12 (0.75, 1.68)

Model 0: FGF23 or copeptin (crude HR).
Model 1: Model 0 + mGFR.
Model 2: Model 1 + age, gender, race, body mass index, history of cardiovascular disease, renin–angiotensin system inhibitor treatment, urinary ACR (log), protein intake and 25(OH)D.
Model 3: Model 2 combining FGF23 and copeptin.
Kidney failure is defined by dialysis initiation or pre-emptive kidney transplantation or 57% decrease of mGFR, corresponding to doubling of serum creatinine.
HRs statistically different from 1 are highlighted in bold.

The pathophysiological link between FGF23 increase and poor renal prognosis remains to be determined. FGF23 may induce chronic inflammation, which is known to promote CKD progression [35]. Indeed, Dai *et al.* [36] identified FGF23-responsive transcripts and activation of networks associated with renal damage and chronic inflammation in kidneys of CKD mouse models with elevated FGF23. Also, chronic inflammation can stimulate FGF23 synthesis [37], which could further promote inflammation.

The association of plasma FGF23 concentration with our composite endpoint may also reflect its extrarenal effects. Elevated FGF23 concentrations are associated with a higher mortality risk, mainly from cardiovascular diseases [38]. High FGF23 concentration is indeed associated with left ventricular hypertrophy [39], with a proven pathophysiological link [40]. FGF23 may also inhibit erythropoiesis in CKD patients [41] and thus promote anaemia, which is a risk factor for mortality in CKD.

It is also known that proteinuria leads to tubular resistance to the action of FGF23 and higher phosphate levels [7]. Therefore, in patients with overt albuminuria and CKD, the important elevation of FGF23 concentrations [42] could by itself [38–41] and/or via hyperphosphatemia [43] impair the prognosis.

Our study shows no association between plasma copeptin concentration and the composite outcome. This result is discrepant with several studies [11–15]. It probably results from the very strict adjustments used in our analyses or may result from the specificity of previously studied CKD populations. Indeed, the association between copeptin and renal prognosis has been demonstrated in some specific aetiologies of CKD: ADPKD [11], diabetic nephropathy [12], IgA nephropathy [13] and after kidney transplantation [14]. The link between vasopressin or copeptin

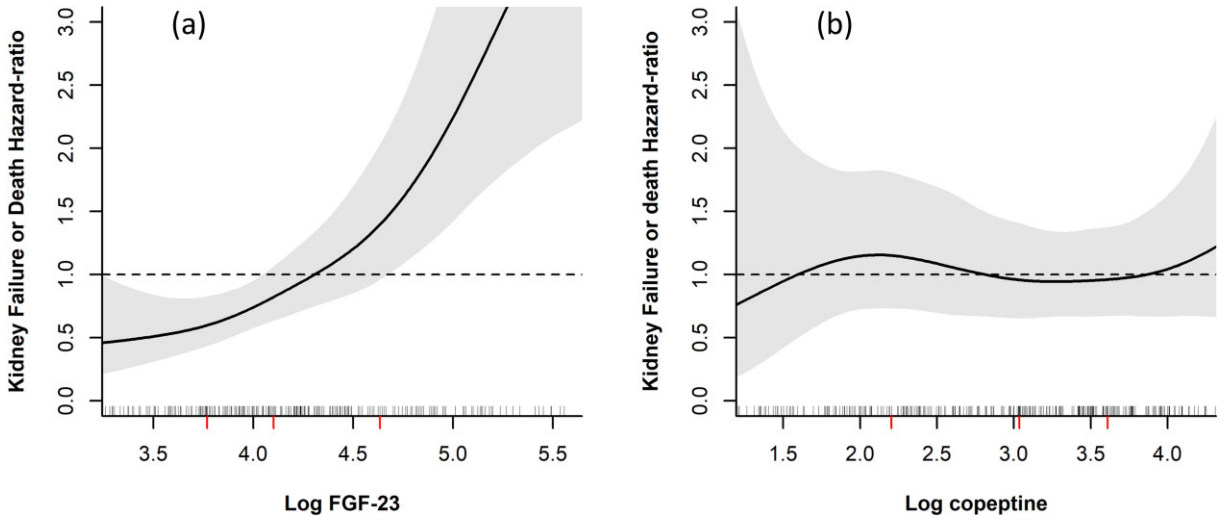

Figure 2: Estimated adjusted HR with 95% CIs for the association of log **(a)** FGF23 and **(b)** copeptin with a composite outcome using a penalized splines estimator. N = 301. Ticks represent distribution of values. HR was plotted only for values between the 5th and 95th percentiles. Composite outcome: kidney failure defined by dialysis initiation or pre-emptive kidney transplantation or 57% decrease of mGFR (corresponding to doubling of serum creatinine) or death before kidney failure during follow-up. Variables tested were centre, elevated blood pressure (>140/90 mmHg), protein intake (g/kg/day), sub-Saharan African origin, body mass index (<19, 20–24, 25–29, >30 kg/m^2), cardiovascular history, smoking status (never, former, current), renin–angiotensin system inhibitor treatment, albuminemia (<35 g/L), diabetes, hypercholesterolaemia (treatment or total cholesterol >5 or >6 mmol/L depending on the presence of cardiovascular history), plasma phosphate, log(PTH), 25(OH)D and 1,25$(OH)_2$D.

Table 4: Linear mixed model analysis testing the association between copeptin or FGF23 with mean differences at baseline (in ml/min) and change over time of mGFR (in ml/min/year). N = 301.

Variables	Model using Copeptin concentration**		Model using FGF23 concentration**	
	$\beta \pm$ SD	P-value	$\beta \pm$ SD	P-value
Intercept[a] (ml/min)	**51.25 ± 1.64**	**<.001**	**48.53 ± 1.80**	**<.001**
Factors associated with baseline mGFR				
Age, per year	**−0.23 ± 0.06**	**<.001**	**−0.22 ± 0.05**	**<.001**
Women *vs* men	**−11.65 ± 1.66**	**<.001**	**−8.4 ± 1.64**	**<.001**
BMI (kg/m^2)				
<19	**−9.74 ± 2.57**	**<.001**	**−11.12 ± 3.95**	**.005**
20–25	0 (ref)		0 (ref)	
25–30	2.64 ± 1.84	.15	**4.02 ± 1.77**	**.02**
≥30	**4.55 ± 1.87**	**.02**	**6.15 ± 1.78**	**<.001**
Log (ACR)	−0.81 ± 0.43	.06	**−1.4 ± 0.4**	**<.001**
History of cardiovascular disease	**−4.16 ± 1.66**	**.01**	**−4.34 ± 1.6**	**.007**
Protein intake, per 0.1 g/kg/24 hours	**2.15 ± 0.47**	**<.001**	**1.79 ± 0.48**	**<.001**
Log (biomarker)	**−9.65 ± 0.86**	**<.001**	**−12.6 ± 1.08**	**<.001**
mGFR slope (ml/min/year)[b]	**−0.73 ± 0.31**	**.02**	**−0.82 ± 0.27**	**.003**
Factors associated with mGFR slope				
Log (ACR)	**−0.48 ± 0.11**	**<.0001**	**−0.51 ± 0.11**	**<.0001**
Protein intake, per 0.1 g/kg/24 hours	**−0.23 ± 0.10**	**.02**	**−0.23 ± 0.10**	**.02**
Log (biomarker)	−0.30 ± 0.24	.22	−0.38 ± 0.44	.38

[a]Baseline mean mGFR and mean mGFR slope estimated for a 60-year-old man with protein intake of 1 g/kg/24 hours, ACR of 8.4 mg/mmol, without history of cardiovascular disease, a BMI of 20–25 and a copeptin concentration of 7.63 pmol/L or an FGF23 of 39 pg/ml (median value of first tertile in men).
[b]Models were always adjusted for centre (interaction with time).
Variables with right-skewed distribution were log-transformed.
Statistically significant *P*-values are highlighted in bold.
BMI: body mass index.

and cyst development in ADPKD is known [44]. This likely explains the predictive value of copeptin in this particular population. Vasopressin is elevated in diabetes mellitus, and its role in diabetic nephropathy [15] is supported by experimental and epidemiological studies [12]. There is currently no pathophysiological hypothesis providing a link between vasopressin (measured as copeptin) and IgA nephropathy or renal transplantation. However, the NephroTest cohort did not include transplanted patients. An association between copeptin and mortality was described [45] in a population of dialysed patients hospitalized for

coronary angiography or with type 2 diabetes mellitus. However, these specific populations are not representative of all CKD patients.

Finally, no association between iFGF23 and mGFR slope was found despite the association of baseline iFGF23 concentrations with the composite outcome. This may be explained by an insufficient power of our study to reveal modest differences in mGFR slope. The association between FGF23 and the risk of mortality [6] may otherwise be stronger than that between FGF23 and the rate of mGFR decline. Moreover, the initiation of renal replacement therapy depends not only on the decrease in GFR, but also on the clinical tolerance of this low GFR, the vascular access and the biological complications of CKD. FGF23 may thus be associated with poor tolerance to a low GFR, e.g. due to left ventricular hypertrophy [40] and/or anaemia [41].

Our study has several limitations. The number of patients included in our analyses is relatively low, but this is a counterpart of using very robust data with direct GFR measurement and a well-described cohort. Another limitation of our study is that circulating α-Klotho was not measured. Of note, the choice to measure iFGF23 (not cFGF23) relies on the fact that C-terminal fragments can bind, but not transactivate, the FGFR/Klotho complex, acting as a competitive inhibitor for iFGF23 [46]. Because of this limited sample size, a composite outcome was used, which did not allow the study of associations of each biomarker with mortality per se. The follow-up time may have been too short to obtain a sufficiently precise mGFR slope evolution. Finally, the cause of death of the patients in our cohort was not available.

Besides these limitations, our study has several significant strengths. Its main strength is the use of mGFR, based on a standardized method that was identical for all participants. To our knowledge, this is the first time that these two biomarkers of interest, which are both elevated in CKD, have been studied simultaneously in the same population, allowing them to be reliably compared with each other. Another strength is the use of prospectively collected data with very few missing data. Finally, we were able to adjust our results for many confounding factors, given the accuracy and large size of the database of the NephroTest study.

In conclusion, our study brings new information regarding the independent association between plasma iFGF23 concentration and unfavourable renal and vital outcomes in CKD patients and shows no such association with copeptin. It is the first time that these two potential biomarkers have been tested in a population of well-phenotyped CKD patients. Our study encourages the integration of iFGF23 measurement into the follow-up of CKD patients, whatever the cause of CKD.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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AUTHORS' CONTRIBUTIONS

A.M.C. contributed to the writing of the article. M.M. contributed to statistical analysis and the writing of the article. L.B. contributed to the design of the study and the writing of the article. C.G. supervised copeptin measurements. M.B. contributed to the writing of the article. S.B. supervised copeptin measurements. C.P.B. contributed to the writing of the article. B.S. supervised statistical analysis. P.H. contributed to the design of the study and the writing of the article. D.P. supervised FGF23 measurements. M.C. contributed to the design of the study and the writing of the article.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study may be shared by the NephroTest study group upon reasonable request.

CONFLICT OF INTEREST STATEMENT

M.C. received consulting fees from Withings, Home Biosciences, Usense, Alnylam Pharmaceuticals, Advicenne and Viatris and a grant from BioHealth Laboratory over the 3 years prior to submission. The other authors have no conflicts of interest. The results presented in this article have not been published previously in whole or part.

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