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ORIGINAL ARTICLE

Reclassification of chronic kidney disease patients for end-stage renal disease risk by proteinuria indexed to estimated glomerular filtration rate: multicentre prospective study in nephrology clinics

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ABSTRACT

Background. In non-dialysis chronic kidney disease (CKD), absolute proteinuria (Uprot) depends on the extent of kidney damage and residual glomerular filtration rate (GFR). We therefore evaluated, as compared with Uprot, the strength of association of proteinuria indexed to estimated GFR (eGFR) with end-stage renal disease (ESRD) risk.

Methods. In a multi-cohort prospective study in 3957 CKD patients of Stages G3–G5 referred to nephrology clinics, we tested two multivariable Cox models for ESRD risk, with either

Uprot (g/24 h) or filtration-adjusted proteinuria (F-Uprot) calculated as Uprot/eGFR $\times 100$.

Results. Mean $\pm$ SD age was 67 ± 14 years, males 60%, diabetics 29%, cardiovascular disease (CVD) 34%, eGFR 32 ± 13 mL/min/1.73 m^2, median (interquartile range) Uprot 0.41 (0.12–1.29) g/24 h and F-Uprot 1.41 (0.36–4.93) g/24 h per 100 mL/min/1.73 m^2 eGFR. Over a median follow-up of 44 months, 862 patients reached ESRD. At competing risk analysis, ESRD risk progressively increased when F-Uprot was 1.0–4.9 and ≥ 5.0 versus <1.0 g/24 h per 100 mL/min/1.73 m^2 eGFR in Stages G3a–G4 (P < 0.001) and Stage G5 (P $= 0.002$).

Multivariable Cox analysis showed that Uprot predicts ESRD in Stages G3a–G4 while in G5 the effect was not significant; conversely, F-Uprot significantly predicted ESRD at all stages. The F-Uprot model allowed a significantly better prediction versus the Uprot model according to Akaike information criterion. Net reclassification improvement was 12.2% (95% confidence interval 4.2–21.1), with higher reclassification in elderly, diabetes and CVD, as well as in diabetic nephropathy and glomerulonephritis, and in CKD Stages G4 and G5.

Conclusions. In patients referred to nephrology clinics, F-Uprot predicts ESRD at all stages of overt CKD and improves, as compared with Uprot, reclassification of patients for renal risk, especially in more advanced and complicated disease.

Keywords: ESRD, estimated GFR, net reclassification index, proteinuria, risk stratification

ADDITIONAL CONTENT

An author video to accompany this article is available at: https://academic.oup.com/ndt/pages/author_videos.

INTRODUCTION

Non-dialysis chronic kidney disease (ND-CKD) patients referred to a nephrologist have peculiar clinical features, as compared with unreferred patients, including more advanced kidney and cardiovascular disease (CVD), greater incidence of end-stage renal disease (ESRD) versus mortality, and specific modifiable determinants of either outcome [1–7]. In these patients, improving risk stratification is essential to select those requiring a more intensive management, in terms of monitoring and treatment, and therefore to optimize clinical practice of the limited workforce of nephrologists [8].

In adjusted survival analyses, 24-h proteinuria (Uprot) predicts ESRD [3–7, 9–13]. However, Uprot has an intrinsic pathophysiological limitation because it depends not only on the extent of kidney damage but also on the number and function of residual nephrons. Consequently, a low Uprot level can herald a better prognosis in untreated patients, as well as in responders to antiproteinuric therapy or, alternatively, be merely a consequence of low glomerular filtration rate (GFR). In the latter case, proteinuria alone may lose its prognostic significance likely because metabolic and haemodynamic factors associated with low GFR play a major role in renal risk stratification. Therefore, moderate proteinuria, which is nowadays prevalent in nephrology clinics, may not associate with a more favourable prognosis [14].

Indexing 24-h proteinuria to estimated GFR (eGFR) value [filtration-adjusted proteinuria (F-Uprot)] may overcome this problem by offering to the clinical nephrologist a cost-effective and easily computable biomarker that includes the information on proteinuria and GFR in a single parameter [15]. A similar approach has been used for improving risk stratification by means of body mass index (BMI), which is a mathematical function containing weight and height.

Although being potentially relevant for nephrology practice and research, the role of GFR-indexed proteinuria in identifying high-risk patients remains unexplored so far. We therefore tested the strength of association of F-Uprot with ESRD risk, as compared with Uprot, in a large population of ND-CKD Stages G3–G5 patients under nephrology care.

MATERIALS AND METHODS

Study design

This is a multicentre prospective study examining 3957 patients from six established cohorts that enrolled Caucasian ND-CKD patients, with Stages G3–G5 in 94% cases, under nephrology care in 128 Italian outpatients CKD clinics [6, 7, 16–19] (Figure 1). All studies were approved by Institutional Review Boards and patients gave written consent to use their clinical data.

Cohorts were originally built to collect prospective information of patients referred to CKD clinics from ≥6 months prior to the basal study visit. They shared the same procedures (see below), endpoints (ESRD and all-cause death) and exclusion criteria (renal replacement therapy, acute kidney injury in the 6 months prior baseline, active malignancy, life expectancy <6 months, advanced liver or heart disease). For this study, additional exclusion criteria were duplicated patients, eGFR >60 mL/min/1.73 m^2, missing information on Uprot and eGFR, or no follow-up.

Patients were followed from basal visit up to ESRD, that is, start of chronic dialysis therapy or kidney transplantation, all-cause death or 31 December 2015, and censored on the date of the last nephrology clinic visit.

Procedures

Nephrologists collected medical history including CKD cause and CVD (stroke, coronary heart disease, heart failure, peripheral vascular disease), performed physical examination and registered laboratory results, therapy and events in anonymous electronic case reports that were periodically sent to the coordinating centre for quality analysis and storage.

Laboratory protocols were standardized with in-house analyses. We quantified proteinuria by 24-h urine collections; collection was considered inaccurate, and repeated, if creatinine excretion was outside the expected range [7]. eGFR was calculated by the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI); since creatinine was not standardized to isotope-dilution mass spectrometry values, levels were reduced by 5% according to Skali et al. [20]. In each patient, F-Uprot was calculated as (Uprot/eGFR) ×100 and expressed as g/24 h per 100 mL/min/1.73 m^2 [15].

Statistical analysis

Continuous variables are reported according to their distribution as either mean ± SD or median and interquartile range (IQR) and compared by one-way analysis of variance or Kruskal–Wallis test. Categorical variables are reported as percentage and compared by Chi-squared test. Since patients with one or more missing variable among CVD, BMI, blood pressure
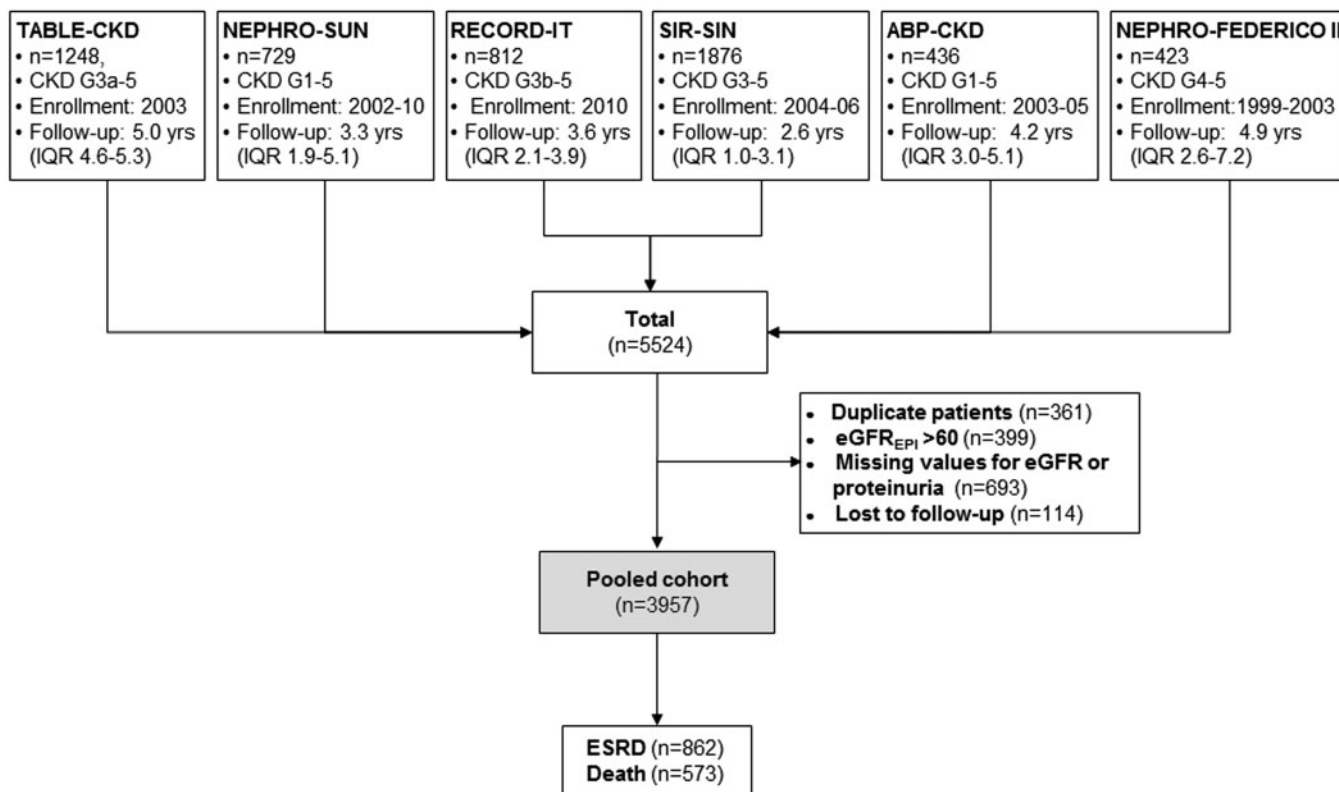
FIGURE 1: Study flow chart. TABLE, Target Blood pressure LEvels in CKD Study; NEPHRO-SUN, Nephrology of Second University of Naples Study; RECORD-IT, REport of COmorbidities in non-Dialysis Renal Disease Population in Italy; SIR-SIN, Studio Italiano indicatori di Risultato multipli – epidemiologia dell'insufficienza renale cronica in Italia della Società Italiana di Nefrologia; ABP-CKD, Ambulatory Blood Pressure Study in Chronic Kidney Disease; NEPHRO-FEDERICO II, Nephrology of Federico II University of Naples Study.

(BP), phosphorus and haemoglobin did not differ from those with a complete data set in terms of demographic, clinical characteristics and outcomes, we imputed missing data to include all patients in survival models by implementing a multiple imputation method [21]. Median follow-up was estimated by inverse Kaplan–Meier approach. Incidence rates of ESRD were reported as number of events/person-time and 95% confidence interval (CI) was calculated assuming a Poisson distribution.

Study hypothesis was tested according to Pencina *et al.* [22, 23]. We built two multivariable Cox proportional hazards models, the first with Uprot and the second where Uprot was replaced by F-Uprot. According to previous studies, Uprot and F-Uprot were evaluated as having a specific CKD stage effect (from G3a to G5) [7, 14, 24]. Nonlinear association between proteinuria and ESRD was tested by modelling predictor as restricted cubic spline (RCS) with four knots defined *a priori* at 0, 25th, 50th and 75th percentile and retained in the final model if a significant nonlinear effect was found.

We used Cox analyses to estimate hazard ratio (HR) and 95% CI because the cause-specific relative hazards are more appropriate for studying the cause of diseases in the case of competing event [25]. Indeed, ESRD and death before ESRD are competitive events, that is, occurrence of death prevents dialysis initiation or kidney transplantation. Either model was adjusted for the same basal covariates (age, gender, BMI, diabetes, history of CVD, current smoking, haemoglobin, systolic BP, use of anti-renin–angiotensin system, phosphorus, eGFR) that were

identified *a priori* as risk factors on the basis of previous similar studies [1–7].

Cox models were stratified by (i) cohort to take into account potential differences in the basal ESRD risk across the six cohorts and (ii) CKD stage because baseline risk of ESRD highly differs across stages, and, moreover, the prognostic role of Uprot on ESRD remarkably changes by stage [7, 10].

Risk thresholds of F-Uprot were derived from splines. Threshold locations corresponded to a break point in the linearity that indicate a change in slope for the log-HR function and, from a clinical point of view, to a change on the risk function [26]; this allowed identification of three F-Uprot categories (<1.0, 1.0–4.9 and ≥ 5.0 g/24 h per 100 mL/min/1.73 m^2 of eGFR). The association between F-Uprot categories (low, intermediate and high) and ESRD was estimated by cumulative incidence, which accounts for competing risk of death, and compared by Gray's test [27]. Calibration of the two models was tested by the Greenwood–Nam–D'Agostino (GND) test [28], and graphically displayed for either model by plotting observed and predicted ESRD risk across deciles of risk.

To compare the performance of the two models (F-Uprot versus Uprot), we used a series of metrics. First, goodness-of-fit analysis for competing models was compared by using Akaike information criterion (AIC) [29]. Secondly, we tested discrimination ability of each model by calculating the *c*-index for stratified Cox proportional hazard model as a weighted average of the stratum-specific *c*'s, with weights equal to the number of

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Table 1. Basal characteristics of patients overall and by CKD stage

	Overall ($n = 3957$)	G3a ($n = 786$)	G3b ($n = 1411$)	G4 ($n = 1341$)	G5 ($n = 419$)	P
Age, years	67 ± 14	64 ± 13	67 ± 14	68 ± 13	67 ± 13	<0.001
Gender (male), %	59.7	69.7	59.7	56.3	51.6	<0.001
Diabetes, %	29.3	26.7	28.5	31.9	28.4	0.06
CVD, %	34.4	33.3	34.3	36.2	30.5	0.16
BMI, kg/m^2	27.6 ± 4.9	27.7 ± 4.3	27.7 ± 4.8	27.6 ± 5.2	27.0 ± 4.8	0.07
Smokers, %	12.6	16.2	12.0	12.0	10.3	0.007
Primary kidney disease, %						<0.001
HTN	32.0	37.8	34.0	29.1	23.6	
DN	15.6	13.8	14.0	18.7	14.8	
GN	13.6	12.1	15.0	12.8	14.6	
TIN	8.2	9.3	7.3	8.4	8.3	
PKD	4.4	3.6	3.6	4.7	7.5	
Other/unknown	26.2	23.4	26.2	26.4	31.2	
BP, mmHg	140 ± 19/80 ± 11	140 ± 20/81 ± 11	139 ± 19/80 ± 10	141 ± 20/80 ± 11	143 ± 19/81 ± 11	<0.001
eGFR, mL/min/1.73 m^2	32 ± 13	51 ± 4	37 ± 4	23 ± 4	12 ± 3	–
Calcium, mg/dL	9.3 ± 0.7	9.4 ± 0.6	9.4 ± 0.6	9.2 ± 0.7	9.1 ± 0.7	<0.001
Phosphorus, mg/dL	3.8 ± 0.8	3.4 ± 0.7	3.6 ± 0.7	3.9 ± 0.8	4.4 ± 0.9	<0.001
PTH (pg/mL)	90 (56–157)	58 (38–84)	78 (51–120)	121 (73–194)	199 (105–349)	<0.001
Serum albumin, g/dL	4.0 ± 0.5	4.0 ± 0.5	4.0 ± 0.5	3.9 ± 0.5	4.0 ± 0.5	<0.001
Haemoglobin, g/dL	12.6 ± 1.8	13.6 ± 1.7	12.9 ± 1.7	12.0 ± 1.7	11.4 ± 1.5	<0.001
Total cholesterol, mg/dL	196 ± 44	196 ± 43	197 ± 43	196 ± 47	193 ± 45	0.41
Uprot, g/24 h	0.41 (0.12–1.29)	0.19 (0.08–0.68)	0.29 (0.10–0.92)	0.66 (0.20–1.54)	1.10 (0.52–2.14)	<0.001
F-Uprot, g/24 h per100 mL/min eGFR	1.41 (0.36–4.93)	0.36 (0.14–1.32)	0.73 (0.28–2.54)	2.94 (0.89–7.13)	9.95 (4.69–19.69)	<0.001
Urinary Na, mmol/24 h	148 ± 64	161 ± 65	145 ± 64	143 ± 64	144 ± 66	<0.001
Low sodium intake, %	24.3	16.4	25.4	27.2	26.5	<0.001
BP lowering drugs, no./pt	2.3 ± 1.2	2.0 ± 1.2	2.2 ± 1.2	2.4 ± 1.3	2.4 ± 1.3	<0.001
Diuretics, % pts	50.1	38.9	50.1	56.2	51.8	<0.001
Anti-RAS, % pts	72.2	73.5	76.2	72.2	56.6	<0.001
Epoetin, % pts	13.7	2.1	7.2	20.0	35.7	<0.001
Statin, % pts	31.4	30.9	33.2	31.4	26.0	0.05

Data are mean ± SD or median (IQR).

HTN, hypertensive nephropathy; DN, diabetic nephropathy; GN, glomerulonephritis; TIN, tubule-interstitial nephropathies; PKD, polycystic kidney disease; eGFR, GFR estimated by the CKD-EPI equation; PTH, parathyroid hormone; Uprot, 24 h proteinuria; Low sodium intake, urinary Na ≤100 mmol/24 h; RAS, renin–angiotensin system; pt, patient.

informative pairs. CI of difference between *c*-indexes was calculated by the bootstrap method; bootstrap CI was calculated with 1000 replicates and using the percentile method [30, 31]. Thirdly, reclassification capacity was tested by evaluating continuous net reclassification improvement (NRI), considered as the most objective measure to test improvement of risk prediction for outcomes with no established category of risk, as in the case of ESRD [22, 23, 32, 33]. NRI was also computed by main clinical subgroups to assess generalizability. To confirm the extent of reclassification, we also computed the integrated discrimination improvement (IDI), which is the difference in discrimination slope between the two models, where the discrimination slope is the mean difference in predicted probabilities between patients with and without event [34].

We also assessed in our data the prediction accuracy of the widely used 4-variable kidney failure risk equation (KFRE) developed by Tangri *et al.* [35]. Protein-to-creatinine ratio was transformed to albumin-to-creatinine ratio (ACR) by dividing by 2.655 for men and 1.7566 for women, and ACR was log-transformed. These conversion factors, also used elsewhere [36], were obtained by Dr N. Tangri (personal communication).

A two-tailed P-value <0.05 was considered significant. Data were analysed using STATA version 14.0 (College Station, TX, USA), Hmisc, Nricens and Cmprsk packages of R software

3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) and SAS version 9.4 (SAS Inc., Cary, NC, USA).

RESULTS

The whole population was characterized by a high risk profile for ESRD, as evidenced by the high prevalence of diabetes, CVD and severity of kidney disease. Conversely, nutritional status was adequate in most patients, as testified by the normal levels of serum albumin and the trivial percentage (<2%) of patients with low BMI (<18.7 and <20.0 kg/m^2 in females and males, respectively). From Stages G3a to G5, Uprot, F-Uprot, BP, intact parathyroid hormone and serum phosphorus increased while haemoglobin, albumin and serum calcium decreased (Table 1).

Low to moderate proteinuria (Uprot ≤0.5 g/24 h) was observed in 54% overall, with high rates in age >65 years (61%), diabetes (48%) and CVD (58%); in these three subgroups, eGFR was similarly low (35 ± 12, 36 ± 12 and 36 ± 12 mL/min/1.73 m^2, respectively).

Survival analysis

Follow-up for survival lasted a median 44 months (IQR 34–64 months); 862 ESRD events and 573 all-cause deaths were observed with incidence rate of 5.99 (95% CI 5.60–6.41) and 3.98 per 100 patient/years (95% CI 3.66–4.32), respectively.

Table 2. Multivariable Cox models estimating risk of ESRD with proteinuria expressed as either Uprot (g/24 h) or F-Uprot (g/24 h per 100 mL/min/1.73 m^2)

		Model with Uprot				Model with F-Uprot		
		HR	95% CI	P		HR	95% CI	P
Age (1 year)		0.98	0.97–0.98	<0.001		0.98	0.97–0.98	<0.001
Male gender		1.41	1.22–1.64	<0.001		1.41	1.22–1.64	<0.001
BMI (kg/m^2)		0.97	0.96–0.99	<0.001		0.97	0.96–0.99	<0.001
Diabetes		0.99	0.84–1.17	0.921		0.97	0.82–1.14	0.678
CVD		1.29	1.10–1.52	0.002		1.30	1.11–1.53	0.002
Current smoking		1.32	0.93–1.38	0.220		1.14	0.93–1.39	0.206
Haemoglobin (g/dL)		0.94	0.90–0.98	0.009		0.94	0.89–0.98	0.009
Systolic BP (1 mmHg)		1.004	1.001–1.007	0.029		1.004	1.001–1.009	0.034
Anti-RAS		0.99	0.85–1.16	0.915		0.98	0.84–1.15	0.840
Phosphorus (mg/dL)		1.20	1.09–1.31	<0.001		1.20	1.10–1.31	<0.001
eGFR (mL/min/1.73 m^2)		0.90	0.88–0.92	<0.001		0.91	0.89–0.93	<0.001
Uprot or F-Uprot[a]								
Stage G3a		1.20	1.09–1.33	<0.001		1.10	1.04–1.15	<0.001
Stage G3b	0.5 versus 0.15	2.24	0.90–5.57	<0.001	5 versus 1	1.75	0.71–4.33	<0.001
	1.0 versus 0.15	2.79	1.15–6.80		10 versus 1	2.51	1.01–6.23	
Stage G4	0.5 versus 0.15	1.64	1.07–2.51	<0.001	5 versus 1	2.24	1.59–3.14	<0.001
	1.0 versus 0.15	2.27	1.61–3.21		10 versus 1	2.57	1.84–3.60	
Stage G5		1.01	0.96–1.06	0.753	5 versus 1	1.34	0.82–2.17	0.048
					10 versus 1	1.57	1.01–2.48	

Models are stratified for cohort and CKD stage. We report in bold statistically significant hazards.

[a]HRs of Uprot and F-Uprot with ESRD are reported in the plots (Figure 2) where association with ESRD outcome is not linear. Nonlinear HRs for ESRD with Uprot (Stages G3b and G4) and F-Uprot (Stages G3b, G4 and G5) reported in the table are computed for selected values. Linear association of HRs for ESRD with Uprot (Stages G3a and G5) and F-Uprot (Stage G3a) are reported in the table only.

RAS, renin–angiotensin system.

Table 2 shows the two Cox models with either Uprot or F-Uprot. Each model resulted well calibrated, as testified by the results of the GND tests (P = 0.997 for Uprot model and P = 0.960 for F-Uprot model). Supplementary Appendix, Figure S1 depicts the close relationship between observed and predicted ESRD across risk deciles for either Uprot or F-Uprot model. Younger age, male gender, lower BMI, CVD, higher phosphate and systolic BP, and lower eGFR and haemoglobin were associated with ESRD in either model.

Uprot showed a significant association with ESRD across Stages G3a–G4 while the effect was not significant in Stage G5. Conversely, higher F-Uprot heralded ESRD at all stages (Table 2 and Figure 2); specifically, ESRD risk increased sharply up to F-Uprot 1.0 g/24 h per 100 mL/min/1.73 m^2 and progressively increased from 1.0 to 4.9, while risk excess became less pronounced for values ≥5.0 particularly in stages G4 and G5. HRs for selected values are reported in Table 2. When using F-Uprot thresholds to evaluate the role of F-Uprot in competing risk analysis (Figure 3), risk of ESRD significantly increased with higher F-Uprot at all stages.

When including in the Kidney Disease Improving Global Outcomes (KDIGO) risk table the F-Uprot thresholds identified in survival analyses (Table 3), we found that these values modified the prevalence of patients in risk categories including patients with significant proteinuria (A2 and A3); in particular, patients with similar Uprot were classified to different risk category in the presence of moderate to severe eGFR impairment (G3b, G4).

The model with F-Uprot performed better than that with Uprot in terms of AIC (7262.8 versus 7304.8, respectively), while c-index did not differ (Uprot: 0.736, 95% CI 0.715–0.760; F-Uprot: 0.736, 95% CI 0.714–0.758). Figure 4 depicts

reclassification of patients by F-Uprot. Overall, the F-Uprot model outperformed the Uprot model with a total NRI of 12.2% (95% CI 4.2–21.1). In particular, reclassification increased in elderly, diabetes and CVD, as well as in diabetic nephropathy and glomerulonephritis. Improved reclassification was also observed in advanced disease (CKD Stages G4 and G5). NRI distinct for events and non-events is reported in Supplementary Appendix, Table S1. The overall IDI was 0.04 (95% CI 0.01–0.07).

The stratified c-index of KFRE was 0.724 (95% CI 0.703–0.747), that is, significantly lower by 0.012 (95% CI 0.002–0.020) with respect to the F-Uprot model. Calibration of KFRE in our population was also suboptimal (P = 0.013, Supplementary Appendix, Figure S1).

DISCUSSION

The major finding of the current study is that F-Uprot predicts ESRD across all stages of overt disease. Survival analyses tested the effect of F-Uprot independent of eGFR within each of the four CKD stages examined (Table 2 and Figure 2). This approach of gaining a stage-specific effect is considered superior to standard analyses obtained in the whole population [37]. In this regard, the incremental contribution of F-Uprot resulted significant also in stage G5. This is a major difference from Uprot, which did not show any predictive role at G5; a similar attenuation or lack of any association of Uprot with ESRD in lower GFR strata has been previously reported [2, 3, 7, 10]. Superiority of F-Uprot versus Uprot in predicting ESRD in advanced CKD is not a trivial finding because nowadays the rate of progression to

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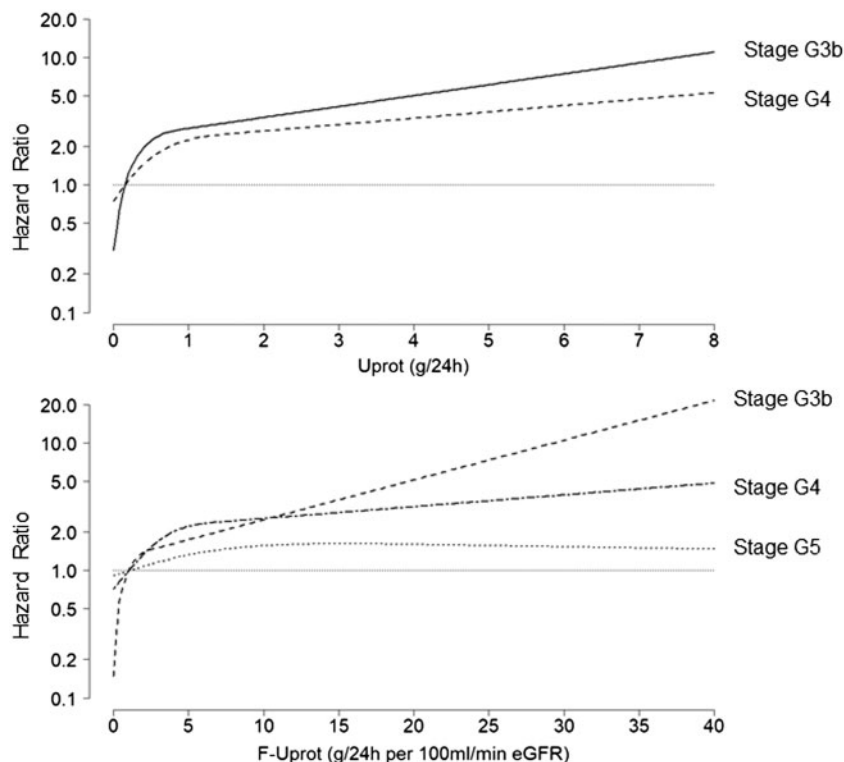
FIGURE 2: RCS plots of the relationship between Uprot (top) and F-Uprot (bottom) and ESRD by CKD stage where effect was nonlinear. Lines indicate the HR estimates. Uprot reference level is 0.15 g/24 h. F-Uprot reference level is 1 g/24 h per 100 mL/min/1.73 m^2 eGFR. HRs are stratified by cohort and CKD stage and adjusted for all variables in Table 2. P-values for Uprot: <0.001 for Stage G3b, <0.001 for Stage G4. P-values for F-Uprot: <0.001 for Stage G3b, <0.001 for Stage G4 and 0.048 for Stage G5.
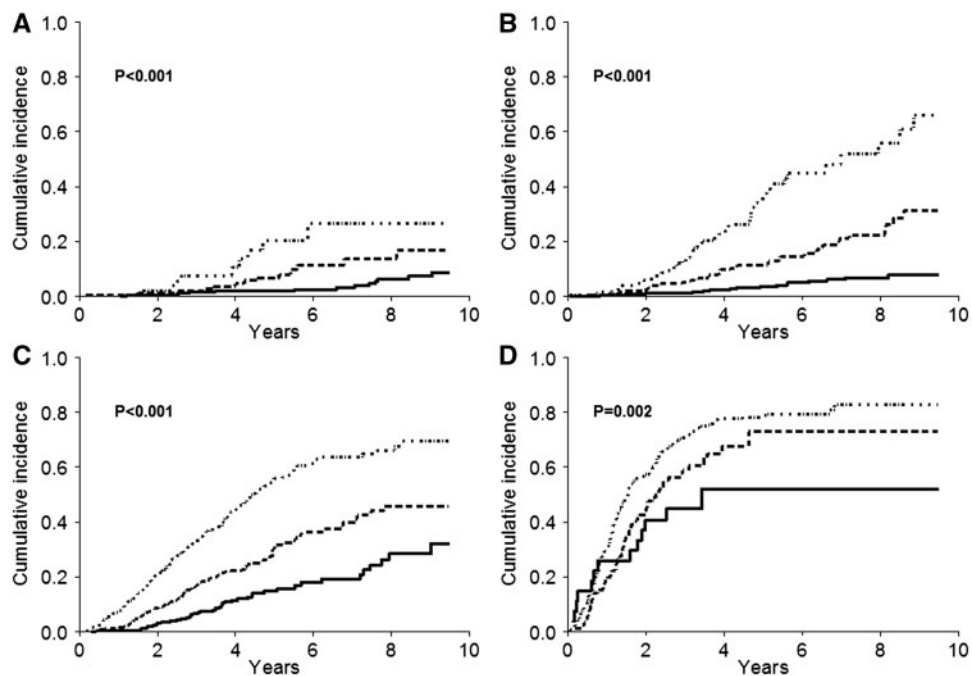
FIGURE 3: Cumulative incidence probability of ESRD, by competing risk analysis accounting for death before ESRD, for the three main F-Uprot categories identified by spline analyses in Figure 2. F-Uprot values in the categories were: low-solid line (<1.0 g/24 h per 100 mL/min/ 1.73 m^2 eGFR), intermediate-dashed line (1.0–4.9 g/24 h per 100 mL/min/1.73 m^2 eGFR) and high-dotted line (≥5.0 g/24 h per 100 mL/min/ 1.73 m^2 eGFR). (**A**) Stage G3a, (**B**) Stage G3b, (**C**) Stage G4, (**D**) Stage G5. P-values are derived from Gray's test.
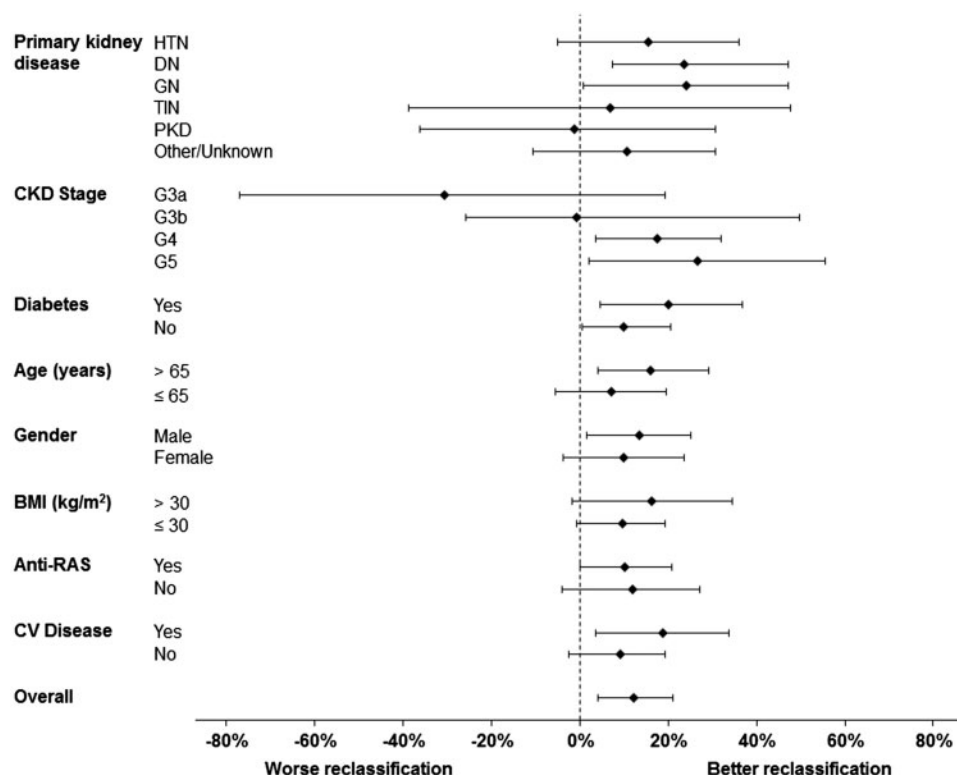
FIGURE 4: NRI (*x* axis) with 95% CIs comparing the model with Uprot and the model with F-Uprot, overall and by clinical subgroups (*y*-axis). HTN, hypertensive nephropathy; DN, diabetic nephropathy; GN, glomerulonephritis; TIN, tubulointerstitial nephropathies; PKD, polycystic kidney disease; CV, cardiovascular; Anti-RAS, inhibitors of renin–angiotensin system.

Table 3. Classification of patients in the KDIGO risk categories according to F-Uprot cut-offs

	A1 (Uprot <150 mg/24 h)	A2 (Uprot 150–500 mg/24 h)	A3 (Uprot >500 mg/24 h)
Stage G3a (eGFR 60–45)			
Patients/events (*N*)	351/9	194/7	241/26
Patients with F-Uprot			
Low, %	100	99	3
Intermediate, %	0	0.5	72
High, %	0	0	24
Stage G3b (eGFR 44–30)			
Patients/events (*N*)	460/9	395/24	556/95
Patients with F-Uprot			
Low, %	100	81	0
Intermediate, %	0	18	67
High, %	0	0	33
Stage G4 (eGFR 30–15)			
Patients/events (*N*)	257/25	319/72	765/295
Patients with F-Uprot			
Low, %	100	31	0
Intermediate, %	0	69	38
High, %	0	0	62
Stage G5 (eGFR <15)			
Patients/events (N)	30/16	68/39	321/245
Patients with F-Uprot			
Low, %	90	0	0
Intermediate, %	10	96	6
High, %	0	4	94

F-Uprot values in the categories were: low (<1.0 g/24 h per 100 mL/min/1.73 m^2 eGFR), intermediate (1.0–4.9 g/24 h per 100 mL/min/1.73 m^2 eGFR) and high (≥ 5.0 g/24 h per 100 mL/min/1.73 m^2 eGFR).

dialysis in referred patients has become heterogeneous even at Stages G4–G5 [38, 39].

The 'intended use' of F-Uprot is to help clinicians to improve management of CKD patients. High F-Uprot in fact is useful in identifying and triaging CKD patients who require a higher level of care in terms of frequency of visits and intensity of treatment, as well as in timely planning of dialysis. On the other hand, lower F-Uprot allows definition of low-risk patients thus avoiding time-consuming visits and overzealous (and potentially risky) treatments. In this regard, the finding that the F-Uprot model was well calibrated is essential to correctly implement this new biomarker in clinical practice [22, 23, 28]. To this aim, we established two risk thresholds of F-Uprot (1.0 and 5.0 g/24 h per 100 mL/min/1.73 m^2 eGFR), derived from RCS analysis, and tested the association with ESRD taking into account the competing risk of death (Figure 3); analysis disclosed an incidence of ESRD almost double when F-Uprot values increased above each threshold at all CKD stages.

Accurate tests that complement current approaches to prediction of CKD progression are eagerly sought to facilitate clinical decision-making [40]. Therefore, new biomarkers are currently being tested. Use of cystatin C-based eGFR in meta-analyses that mostly include studies in the general population, allowed a 10% reclassification for ESRD risk in comparison with the traditional creatinine-based eGFR [41]. More recently, the CKD273 classifier, based on 273 peptides in the urinary proteome, has been found to provide NRI of 30% for the risk of eGFR decline in early CKD [42]. In our study, the prognostic

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role of F-Uprot on ESRD appears to be stronger as compared with Uprot as it was associated with significant improvement of NRI. We found that 12.2% of patients were correctly reclassified for ESRD risk by F-Uprot using NRI. Reclassification was confirmed by IDI. This result is hardly comparable to the findings of the two previous studies due mainly to differences of study design; indeed we enrolled patients referred to CKD clinics with more advanced CKD and we used the hard endpoint of ESRD.

We also assessed in our data the prediction performance of the KFRE, which has shown excellent performances in North America and across other countries and was implemented in electronic medical records and laboratory information systems [35]. KFRE showed a lower discrimination in comparison with the F-Uprot model and may further support the importance of this new biomarker and the need for future studies. We also found a suboptimal calibration (Supplementary Appendix, Figure S1), which is however expected considering the different basal risk of our population of referred patients.

Of note, at variance with NRI results, and goodness-of-fit (AIC) as well, c-index did not change. Indeed, good traditional models, that is, with high baseline c-index (as in our model with Uprot), hardly improve after adding a new biomarker [33]. On the other hand, it has been proposed to consider NRI as the main statistical tool to validate new markers beyond the area under the curve (C statistic) [22, 23, 33, 36]. The paradigm of this new approach is the Framingham Heart Study, where NRI indicated that high-density lipoprotein cholesterol offers statistically significant improvement in the performance of a coronary heart disease model even though no improvement of c-index was observed [22].

Theoretically, it is possible that the improved performance of F-Uprot versus Uprot is merely due to that fact that it implicitly contains also information on eGFR. This possibility is likely reduced because all survival analyses were adjusted for basal eGFR besides being stratified by CKD stage; however, a potential residual confounding from eGFR due to the different parameterization in the F-Uprot formula with respect to the linear eGFR used for adjustment may still remain.

On the other hand, indexing proteinuria to eGFR may allow a more accurate assessment of filtration barrier disease [15]. Uprot, in fact, does not take into account the functioning nephron mass through which protein leak occurs. Therefore, the damage on the single nephron induced by the same amount of proteinuria through the interstitial protein trafficking may be more pronounced when functioning nephron mass is reduced (low eGFR) than in the presence of a higher number of functioning nephrons (high eGFR). Such information cannot be provided by Uprot while it can be captured by F-Uprot, which, by incorporating eGFR, allows a more precise estimate of proteinuria-induced damage on residual nephrons.

The impact of F-Uprot on stratification of patients for ESRD risk is more evident for specific subgroups. This already emerges when including F-Uprot into the KDIGO classification of basal risk profile (Table 3); the effect of F-Uprot on patients reclassification becomes manifest in those with significant proteinuria (A2–A3) and eGFR ranging from 45 to 15 mL/min/1.73 m^2 (G3b–G4). F-Uprot estimate may therefore improve the KDIGO concept of the two-dimensional matrix of eGFR and albuminuria in patients that, being referred to tertiary nephrology care, likely have a more advanced disease. More important, when taking into account the renal outcome (Figure 4), higher NRI was confirmed in kidney diseases with higher proteinuria, that is, diabetic nephropathy and glomerulonephritis where Uprot was 0.8 (0.2–2.1) and 1.2 (0.5–2.3) g/24 h, respectively, while Uprot in other diagnoses was 0.3 (0.1–0.8) g/24 h. Higher reclassification was also observed in patients with CVD, diabetics and older patients (Figure 4); in these subgroups, Uprot was moderate but associated with low eGFR. This suggests that F-Uprot allows better estimates of ESRD risk in the emerging low-proteinuric phenotype occurring in the context of a chronic ischaemic damage of the kidney [14, 24, 43–45]. Higher NRI was similarly observed in lower GFR strata.

This study has two main limitations. First, our results derive from a Caucasian referred CKD population and, therefore, findings may not hold true in other ethnic groups or in unreferred CKD patients. Second, analysis of risk factors was based on a single data collection; studies are therefore needed to verify whether changes over time of F-Uprot allows additional improvement of ESRD risk stratification, as recently proposed for Uprot [46]. In addition, the F-Uprot formula should be used with caution in the context of nephrotic syndrome; in these patients, in fact, both Modification of Diet in Renal Disease 4-variable formula and CKD-EPI overestimate the eGFR due to a higher tubular creatinine excretion [47]. In our cohort, we could not address this issue because only 28 patients (0.7%) had nephrotic syndrome.

Strengths of the study are the sample size, which was relatively large when considering the study setting (tertiary nephrology care), the persistence of result after stratification for the six cohorts, which indicates generalizability of findings to referred patients, and the adjustment of analyses for several variables including basal eGFR.

In conclusion, in CKD patients under nephrology care, proteinuria indexed to eGFR acts as an independent predictor of ESRD across the whole spectrum of overt disease. This association is stronger than that observed with Uprot. Consequently, F-Uprot improves reclassification of CKD patients for ESRD risk, with the effect being more evident in patients with more advanced and complicated disease.

F-Uprot is easily assessable at no additional cost and summarizes in one single parameter Uprot and eGFR. These advantages suggest the primary use in nephrology clinical practice. In addition, F-Uprot may facilitate identification and enrollment of 'progressors' in randomized trials, with consequent reduction of sample size and length of follow-up. Results also call for more studies estimating the effect of F-Uprot as surrogate endpoint in trials on nephroprotective interventions.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

All authors approved the final version and significantly contributed to the work as they conceived the study (M.P., P.C., R.M. and L.D.N.), and/or acquired data (R.M., C.Z., V.B., F.L., G.T., F.M., L.D.M. and D.R.), and/or played an important role in interpreting the results (C.Z., V.B., G.C., F.L., G.T., L.D.V., F.M. and H.J.L.H.) and/or drafted the manuscript (M.P. and L.D.N.) and/or revised it (P.C., R.M., C.Z., V.B., G.C., F.L., G.T., L.D.V., F.M., L.D.M., D.R. and H.J.L.H.).

CONFLICT OF INTEREST STATEMENT

All the authors declared no conflict of interest. The results presented in this paper have not been published previously in whole or part.

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Hepcidin, iron indices and bone mineral metabolism in non-dialysis chronic kidney disease

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ABSTRACT

Background. Few studies have examined the association between hepcidin, iron indices and bone mineral metabolism in non-dialysis chronic kidney disease (CKD) patients.

Methods. We reviewed the data of 2238 patients from a large-scale multicenter prospective Korean study (2011–16) and excluded 214 patients with missing data on markers and related medications of iron and bone mineral metabolism, hemoglobin, blood pressure and causes of CKD. Multivariate linear regression analysis was used to identify the association between iron and bone mineral metabolism.

Results. The proportion of CKD Stages 1–5 were 16.2, 18.7, 37.1, 21.6 and 6.4%, respectively. Per each 10% increase in transferrin saturation (TSAT), there was a 0.013 mmol/L decrease in phosphorus [95% confidence interval (CI) −0.021 to −0.004; P = 0.003] and a 0.022 nmol/L increase in logarithmic

25-hydroxyvitamin D (Ln-25OHD) levels (95% CI 0.005–0.040; P = 0.019). A 1 pmol/L increase in Ln-ferritin was associated with a 0.080 ng/L decrease in Ln-intact parathyroid hormone (Ln-iPTH; 95% CI −0.122 to −0.039; P < 0.001). Meanwhile, beta (95% CI) per 1 unit increase in phosphorus, Ln-25OHD and Ln-iPTH for the square root of the serum hepcidin were 0.594 (0.257–0.932; P = 0.001), −0.270 (−0.431 to −0.108; P = 0.001) and 0.115 (0.004–0.226; P = 0.042), respectively. In subgroup analysis, the relationship between phosphorus, 25OHD and hepcidin was strongest in the positive-inflammation group.

Conclusions. Markers of bone mineral metabolism and iron status, including hepcidin, were closely correlated to each other. Potential mechanisms of the relationship warrant further studies.

Keywords: bone mineral metabolism, chronic kidney disease, hepcidin, iron