Urinary creatinine excretion, measured glomerular filtration rate and CKD outcomes

Elena Tynkevich1,2, Martin Flamant3,4, Jean-Philippe Haymann5,6,7, Marie Metzger1,2, Eric Thervet8,9, Jean-Jacques Boffa6,7,10, François Vrtovsnik4,11, Pascal Houillier12,13, Marc Froissart1 and Bénédicte Stengel1,2 on behalf of the NephroTest Study Group

1Inserm Unit 1018, CESP, Research Centre in Epidemiology and Population Health, Villejuif, France, 2University Paris-Sud, UMRS 1018, Villejuif, France, 3Department of Physiology, AP-HP, Hôpital Bichat, Paris, France, 4University Paris Diderot, Paris, UMR 1149, Paris, France, 5Department of Physiology, AP-HP, Hôpital Tenon, Paris, France, 6Inserm UMR-S 1155, Paris, France, 7Paris and Sorbonne University, UPMC University-Paris 6, Paris, France, 8Department of Nephrology, AP-HP, Hôpital Européen Georges Pompidou, Paris, France, 9DHU Common and Rare Arterial Diseases, AP-HP, Hôpital Européen Georges Pompidou, Paris, France, 10Department of Nephrology, AP-HP, Hôpital Tenon, Paris, France, 11Department of Nephrology, AP-HP, Hôpital Bichat, Paris, France, 12University Paris Descartes-Paris 5, UMR 775, Paris, France and 13Department of Physiology, AP-HP, Hôpital Européen Georges Pompidou, Paris, France

Correspondence and offprint requests to: Bénédicte Stengel; E-mail: benedicte.stengel@inserm.fr

ABSTRACT

Background. Muscle wasting predicts mortality in patients with end-stage renal disease (ESRD), but its role in the progression of chronic kidney disease (CKD) is uncertain. We studied CKD outcomes associated with low muscle mass, assessed by urinary creatinine excretion (UCr).

Methods. The NephroTest cohort included 1429 patients with CKD stages 1–4 and both measured glomerular filtration rate (mGFR) (by 51Cr-EDTA) and estimated glomerular filtration rate (eGFR) (by CKD-Epidemiology Collaboration equation). We used cause-specific Cox models to estimate hazard ratios (HRs) for the competing risks of ESRD and death associated with gender-specific UCr quartiles.

Results. UCr was 13.6 ± 3.2 mmol/24 h (0.17 ± 0.05 mmol/kg/24 h) in men and 9.2 ± 2.1 (0.14 ± 0.05) in women. It was positively associated with mGFR, but not with eGFR. Over a median follow-up of 3.6 (2.1–5.8) years, 229 patients developed ESRD and 113 patients died before ESRD. Compared with patients in the highest UCr quartile, those in the lowest quartile had a higher crude HR (95% confidence interval) for pre-ESRD death: 4.3 (2.4–7.7), which was weakened, but remained statistically significant, independent of demographics, mGFR and several other factors: 2.1 (1.04–4.3). Their crude ESRD risk was not higher: HR: 0.95 (0.65–1.4), and even tended to be lower after adjusting for mGFR and log-proteinuria: HR: 0.70 (0.45–1.1). Adjustment for eGFR instead of mGFR reversed this relationship: HR: 1.7 (1.1–2.7).

Conclusions. In early stage CKD, low UCr is associated with higher risk for mortality, but not for ESRD. Using creatinine-based equation to adjust for GFR may bias the relationship of UCr with ESRD risk.

Keywords: chronic kidney disease, end-stage renal disease, glomerular filtration rate, mortality, muscle mass loss, urinary creatinine excretion

INTRODUCTION

While many studies have focused on the outcomes associated with obesity in chronic kidney disease (CKD), only a few have investigated those related to muscle wasting. The role of muscle mass loss, however, is increasingly recognized in the genesis of many chronic diseases [1]. It is common [2–4], known to predict mortality in patients on dialysis for end-stage renal disease (ESRD) [5–13], and has recently been shown to occur early in the course of CKD [14–18]. Whether it may also predict faster progression to ESRD is uncertain.

Daily urinary creatinine excretion (UCr), a specific biomarker of endogenous creatinine generation, has been shown to be reliable for assessing muscle mass in individuals at steady state [19–24]. It has been consistently associated with mortality in the general population [25], in transplant recipients [26] and in patients with cardiovascular disease (CVD) [27, 28] or type 2 diabetes [29]. Two recent studies reported that the greater the decline in UCr in patients with CKD stages 3–4, the higher the risk of both...
ESRD and death [14, 18]. In the first one, however, glomerular filtration rate (GFR) was estimated from creatinine-based equation [12]. Because muscle mass is a major determinant of serum creatinine independent of kidney function, eGFR tends to overestimate true GFR at low levels of UCr [17, 30]. Thus, an appropriate method to account for GFR in investigating outcomes associated with UCr should be independent of serum creatinine.

We therefore used data from the NephroTest cohort to study the relationships of UCr with the competing risks of pre-ESRD mortality and ESRD in CKD patients phenotyped with GFR measured by a reference method. To assess the extent to which these relationships may be biased, we analysed them while adjusting, in separate analyses, for measured GFR (mGFR) and for creatinine-based estimated GFR (eGFR), the latter to enable comparison with previous studies.

**Materials and methods**

**Study population and design**

The NephroTest cohort is a prospective multicentre study that enrolled 1827 adult patients with all stages of CKD and all nephropathy types referred by nephrologists to any of three departments of physiology for extensive workups [31]. Eligible patients were ≥18 years of age at inclusion and had neither started dialysis nor received a kidney transplant. Pregnant women were excluded. All patients gave written informed consent. The NephroTest study design was approved by an ethics committee (CCTIRS MG/CP09.503).

**Data collection and measurements**

During a 5-h in-person visit, a large set of clinical and laboratory indicators were collected, including blood pressure (BP), body mass index (BMI) and treatments received. At each visit, urinary clearance of $^{51}$Cr-EDTA (mGFR) and creatinine were determined as the average of 5–7 consecutive 30 min clearance periods (fractionated clearances), as previously described [32]. Patients also provided 24-h urine collection for measurement of daily UCr, and calculation of 24-h creatinine clearance. GFR was also estimated by the chronic kidney disease-epidemiology collaboration (CKD-EPI) equation [33]. Creatinine was measured with isotopic dilution mass spectrometry traceable creatinine in serum and with a modified kinetic Jaffe colorimetric method in urine. In addition to 99 patients with mGFR <15 mL/min/1.73 m$^2$ at baseline and 81 patients lost to follow-up, we excluded 84 patients with missing data for 24-h urine collection, 78 patients with missing data for both 24-h and fractional creatinine clearance and 56 patients with unreliable fractionated urinary clearances ($^{51}$Cr-EDTA and creatinine clearance) due to irregular or incomplete bladder voiding or to urine loss; 1429 patients remained for this analysis (Figure 1).

We then assessed the completeness of urine collection by calculating the ratio of 24-h creatinine clearance to fractionated creatinine clearance. When this ratio was higher or lower than 15%, reflecting excess or incomplete urine collection, we used 24-h UCr extrapolated from fractionated creatinine clearance, on the assumption that UCr is stable over the nychthemeron.

We used gender-specific quartiles to study UCr with cutoff points of 11.4, 13.3 and 15.5 mmol/24 h in men, and 7.6, 9.0 and 10.6 in women. Several nutritional markers were also measured including serum albumin, pre-albumin, HDL-cholesterol, triglycerides and urinary urea (mmol/24 h), reflecting protein intake. Patients were classified as having inflammation when at least one of the following markers was elevated: white blood cells (WBC) ≥7.5 × 10$^3$/mm$^3$, C-reactive protein (CRP) ≥10 mg/L and plasma fibrinogen of ≥5 g/L. Gender-specific thresholds were used to define low HDL-cholesterol: 1.0 mmol/L in men and 1.3 mmol/L in women. Urinary urea (mmol/24 h) and log-proteinuria were analysed as continuous variables.

Missing values accounted for <5% of most variables. Mean or mode imputation (by gender, where appropriate) was used for these variables in the multivariate analysis. For serum pre-albumin with 24% missing values, a missing category was created.

**Outcomes**

The primary end-points of this study were ESRD defined as initiation of renal replacement therapy (RRT) by dialysis or preemptive transplantation, and pre-ESRD mortality. Information about deaths and their causes and ESRD events was obtained either from patient medical records or linkage with the national death registry and the national ESRD REIN registry [34]. Survival time was defined as the period from the first visit to the date of the first event. Mortality and ESRD were recorded until 31 December 2010, and patients without any event were censored at this date.

We also studied mGFR decline as a secondary end-point. Over the study period, 942 patients (629 men and 313 women) had at least two mGFR measurements. As recently shown by the CKD Prognosis Consortium based on 35 cohorts including NephroTest, percentage change in GFR such as 20% reduction over 2 years may be used as an alternative end-point for CKD progression [35].

**Statistical analyses**

Patient characteristics were described according to gender-specific quartiles of UCr and variables expressed as percentages, means ± SD or medians (interquartile range). Differences between the four groups were tested with analysis of variance (ANOVA), the Kruskal–Wallis test or the Chi-square test, as appropriate. To understand how adjusting for eGFR instead of mGFR may potentially alter the relation of UCr with outcomes, we plotted the difference between eGFR and mGFR against UCr level.

We performed cause-specific Cox-regression analyses to estimate crude and adjusted hazard ratios (HRs) and 95% confidence intervals [HR (95% CI)] for the competing risks of pre-ESRD death and ESRD associated with gender-specific UCr quartiles, using the highest quartile as the reference category. The cause-specific approach has indeed been shown to be the most suitable to account for competing risks of concurrent events for aetiological studies [36]. When the relationship with outcomes was linear, adjustment variables were treated continuously. Otherwise, appropriate categorizations were used, e.g. BMI treated in quartiles. Assessment of the Schoenfeld residuals confirmed that none of the variables studied
violated the proportional-hazards assumption. Sequential models were developed for each outcome studied. Crude models, and models 1, adjusted for age, gender, ethnicity, height and centre, were similar for both outcomes. For pre-ESRD mortality, model 2 then included mGFR, model 3, log-proteinuria, model 4, traditional CVD risk factors and model 5, malnutrition-inflammation markers in addition to the variables in each of the previous models. For ESRD risk, model 2 included mGFR and an interaction term with time to account for non-proportionality of hazards for this variable. Then, model 3 included log-proteinuria, diabetes, systolic BP, anaemia, 24-h urinary urea and inflammation biomarkers. Models 2 and fully adjusted models 3 and 5 for both outcomes were also run with mGFR replaced by eGFR (models 2bis, model 3bis and model 5bis). In all models, we performed tests for linear trend across quartiles of UCr. Subsidiary analysis was performed with UCr treated as a continuous variable. We also investigated possible interactions between UCr, gender and proteinuria in the relationships with mortality and ESRD.

Finally, analysis of the association with percentage change in mGFR was performed in the sub-group of 942 patients with at least two measurements. We used a linear regression model to estimate mean annual mGFR decline over time and calculated percentage change per year as: (mean annual mGFR change)/(first mGFR) × 100%. We then used logistic regression models to estimate the odds ratios (ORs) and 95% CI for a fast progression, defined as a percentage change of mGFR >10% per year, associated with UCr quartiles, both before and after adjusting for the same variables as for the study of ESRD risk.

A two-sided P-value < 0.05 indicated statistical significance. Data analyses were performed with SAS software, version 9.3 (SAS institute, Cary, NC) and with R version 3.0.2 software (R Development Core Team, 2005).

## RESULTS

### Baseline patient characteristics and associations with UCr

Patients were mostly non-African men (Table 1). The distribution of mGFR classes was as follows: 17.6, 21.5, 31.6 and 29.3% for ≥60, 45–60, 30–45 and 15–30 mL/min/1.73 m², respectively. The most common CKDs were hypertensive and vascular nephropathy (34.1%), glomerulonephritis (15.3%) and diabetic nephropathy (9.5%). More than a third of the patients had at least one elevated inflammation biomarker.

Mean UCr was 0.17 ± 0.05 mmol/kg/24 h in men and 0.14 ± 0.05 in women. It was inversely associated with age. Both mGFR and 24-h proteinuria were positively associated with urinary creatinine, but eGFR was not. At higher UCr levels, eGFR tended to underestimate mGFR, while the latter was overestimated at lower levels of UCr (Figure 2). Patients with lower UCr also had significantly lower BMI, serum albumin, pre-albumin and urinary urea concentrations, and a higher prevalence of CVD history and anaemia. No association

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**Figure 1**: Study flow chart of patients with non-end-stage CKD.
was found with lipids, transferrin, inflammation, metabolic acidosis and potassium and phosphate levels.

**Association of UCr with pre-ESRD mortality risk**

Over a median (interquartile range) follow-up of 3.6 (2.1–5.8) years, 160 patients died (11.2%) of whom 113 (7.9%) before reaching ESRD. There were 48 cardiovascular deaths (37 before ESRD) and 85 non-cardiovascular deaths (60 before ESRD); 27 death causes were missing. Patients in the lowest UCr quartile had a crude excess risk of pre-ESRD death four times higher than those in the highest quartile (Table 2). The HR was strongly attenuated after adjusting for age, gender and ethnicity, but remained statistically significant in the fully adjusted model. In Models 2bis and 5bis, adjusting for eGFR instead of mGFR tended to strengthen this association. As expected, older age, history of CVD, diabetes, lower levels of BMI, mGFR and nutritional markers, and inflammation were all significantly and independently associated with increased mortality (Table 3). In contrast, systolic BP, serum lipids, 24-h urinary urea and log-proteinuria were not.

**Table 1. Baseline characteristics according to UCr quartiles in 1429 CKD patients**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>UCr (gender-specific quartiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First (lowest) (n = 357)</td>
<td>Second (n = 357)</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCr (mmol/24 h)</td>
<td>13.6 ± 3.2</td>
<td>9.8 ± 1.3</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td>462 (33.2)</td>
<td>32.5</td>
</tr>
<tr>
<td>UCr (mmol/24 h)</td>
<td>9.2 ± 2.1</td>
<td>6.6 ± 0.73</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59.0 ± 15.2</td>
<td>66.3 ± 12.2</td>
</tr>
<tr>
<td>African origin</td>
<td>185 (13.0)</td>
<td>5.0</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.4 ± 4.9</td>
<td>24.6 ± 4.4</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>406 (28.4)</td>
<td>29.4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>135.8 ± 21.1</td>
<td>136.8 ± 21.3</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75.8 ± 11.3</td>
<td>73.2 ± 11.4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1298 (90.8)</td>
<td>91.3</td>
</tr>
<tr>
<td>History of CVD</td>
<td>263 (18.4)</td>
<td>25.5</td>
</tr>
<tr>
<td><strong>Kidney function</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mGFR (mL/min per 1.73 m²)</td>
<td>42.6 ± 18.4</td>
<td>36.2 ± 13.9</td>
</tr>
<tr>
<td>eGFR (mL/min per 1.73 m²)</td>
<td>44.8 ± 21.2</td>
<td>43.9 ± 18.4</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>0.28 (0.13–0.92)</td>
<td>0.21 (0.10–0.65)</td>
</tr>
<tr>
<td><strong>Nutritional and inflammation markers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.3 (0.92–1.9)</td>
<td>1.3 (0.91–1.8)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>39.5 ± 4.3</td>
<td>39.1 ± 4.0</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>0.31 ± 0.07</td>
<td>0.30 ± 0.07</td>
</tr>
<tr>
<td>Pre-albumin (g/L)</td>
<td>2.3 ± 0.41</td>
<td>2.3 ± 0.39</td>
</tr>
<tr>
<td>Urinary urea (mmol/24 h)</td>
<td>376.5 ± 119.0</td>
<td>301.1 ± 92.0</td>
</tr>
<tr>
<td>CRP ≥10 mg/L</td>
<td>122 (8.5)</td>
<td>10.1</td>
</tr>
<tr>
<td>WBC count (10³/mm³)</td>
<td>6.5 ± 2.1</td>
<td>6.4 ± 1.8</td>
</tr>
<tr>
<td>Plasma fibrinogen (g/L)</td>
<td>3.8 ± 0.95</td>
<td>3.8 ± 0.99</td>
</tr>
<tr>
<td>Inflammationc</td>
<td>480 (33.6)</td>
<td>35.6</td>
</tr>
<tr>
<td><strong>Markers of metabolic complications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaemia</td>
<td>669 (46.8)</td>
<td>57.1</td>
</tr>
<tr>
<td>Metabolic acidosis</td>
<td>159 (11.1)</td>
<td>12.0</td>
</tr>
<tr>
<td>Serum potassium (mmol/L)</td>
<td>4.3 ± 0.48</td>
<td>4.3 ± 0.49</td>
</tr>
<tr>
<td>Serum phosphate (mmol/L)</td>
<td>1.1 ± 0.20</td>
<td>1.1 ± 0.19</td>
</tr>
</tbody>
</table>

BP, blood pressure; CVD, cardiovascular disease; mGFR, measured glomerular filtration rate; HDL, high-density lipoprotein; CRP, C-reactive protein; WBC, white blood cells.

*Fasting glucose ≥7 mmol/L or HbA1c ≥6.5 or antidiabetic treatment or reported diabetes.
*Any antihypertensive treatment or systolic BP ≥140 or diastolic BP ≥90 mmHg.
*At least one elevated inflammatory marker among WBC count, plasma fibrinogen and CRP.
*Haemoglobin <130 g/L in men and <120 g/L in women.
*Venous CO₂ <22 mmol/L or alkaline treatment.
significantly associated with mortality. In this fully adjusted model, the HRs for pre-ESRD death were similar for eGFR and mGFR (data not shown). Finally, the crude HR for pre-ESRD death associated with UCr treated continuously (per 1 mmol/24-h) was 0.90 (0.85–0.96) (P = 0.001) and 0.90 (0.83–0.99) (P = 0.03) in the fully adjusted model. Of note, the effect on mortality was mitigated by not censoring for ESRD with fully adjusted HRs of overall death for the 1st,
Table 4. Crude and adjusted ORs (95% CI) of a fast annual decline in mGFR according to UCr quartiles in 942 CKD patients

<table>
<thead>
<tr>
<th>Gender-specific quartiles of UCr</th>
<th>First (lowest)</th>
<th>Second</th>
<th>Third</th>
<th>Fourth (highest)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events/p.y.</td>
<td>44/1234.6</td>
<td>52/1430.8</td>
<td>62/1553.8</td>
<td>71/1616.3</td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>0.95 (0.65–1.4)</td>
<td>0.94 (0.66–1.3)</td>
<td>0.91 (0.65–1.3)</td>
<td>1 (0.79–1.23)</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.88 (0.58–1.3)</td>
<td>0.91 (0.62–1.3)</td>
<td>0.90 (0.63–1.3)</td>
<td>1 (0.56–2.3)</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>0.49 (0.33–0.74)</td>
<td>0.76 (0.53–1.1)</td>
<td>0.77 (0.54–1.1)</td>
<td>1 (0.001–2.4)</td>
<td></td>
</tr>
<tr>
<td>Model 2bis: Model 2 with mGFR replaced with eGFR</td>
<td>1.6 (1.04–2.4)</td>
<td>1.5 (1.03–2.2)</td>
<td>1.1 (0.77–1.6)</td>
<td>1 (0.01–3.7)</td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>0.68 (0.43–1.1)</td>
<td>0.93 (0.63–1.4)</td>
<td>0.87 (0.61–1.3)</td>
<td>1 (0.17–3.1)</td>
<td></td>
</tr>
<tr>
<td>Model 3bis: Model 3 with mGFR replaced with eGFR</td>
<td>1.7 (1.1–2.7)</td>
<td>1.6 (1.1–2.4)</td>
<td>1.2 (0.83–1.7)</td>
<td>1 (0.01–2.4)</td>
<td></td>
</tr>
</tbody>
</table>

Model 1: crude + age, gender, ethnicity, centre, height
Model 2: model 1 + mGFR
Model 2bis: Model 2 with mGFR replaced with eGFR
Model 3: model 2 + logarithm of 24-h proteinuria, diabetes, systolic blood pressure, anaemia, 24-h urinary urea, inflammation*
Model 3bis: Model 3 with mGFR replaced with eGFR

*At least one elevated inflammatory marker among WBC count, plasma fibrinogen and CRP.

Table 5. Crude and adjusted HRs (95% CI) of a fast annual decline in mGFR according to UCr quartiles in 942 CKD patients

<table>
<thead>
<tr>
<th>Gender-specific quartiles of UCr</th>
<th>First (lowest)</th>
<th>Second</th>
<th>Third</th>
<th>Fourth (highest)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events/at risk</td>
<td>51/220</td>
<td>56/238</td>
<td>57/238</td>
<td>71/246</td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>0.74 (0.49–1.1)</td>
<td>0.76 (0.51–1.1)</td>
<td>0.78 (0.52–1.2)</td>
<td>1 (0.16–3.7)</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.58 (0.36–0.91)</td>
<td>0.66 (0.43–1.0)</td>
<td>0.70 (0.46–1.1)</td>
<td>1 (0.02–3.7)</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>0.38 (0.23–0.63)</td>
<td>0.56 (0.35–0.89)</td>
<td>0.65 (0.41–1.0)</td>
<td>1 (0.0002–3.7)</td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>0.56 (0.31–1.0)</td>
<td>0.66 (0.39–1.1)</td>
<td>0.76 (0.46–1.2)</td>
<td>1 (0.06–3.7)</td>
<td></td>
</tr>
</tbody>
</table>

Model 1: crude + age, gender, ethnicity, centre, height
Model 2: model 1 + mean mGFR of overall visits
Model 3: model 2 + logarithm of 24-h proteinuria, diabetes, systolic blood pressure, anaemia, 24-h urinary urea, inflammation*

*At least one elevated inflammatory marker among WBC count, plasma fibrinogen and CRP.

Association of UCr with ESRD risk and mGFR decline

During follow-up, 229 (16.9%) patients initiated RRT for ESRD. No significant association was found between UCr and ESRD risk either before or after adjusting for demographic variables (Table 4). Model 2 showed a significant trend towards lower HRs with lower UCr after adjustment for mGFR. This trend was no longer significant after further adjustment for log-proteinuria, 24-h urinary urea and other risk factors for CKD progression, but the HR for the lowest quartile was still lower than one. Importantly, adjustment for eGFR instead of mGFR distorted this relationship and produced a significantly higher HR for ESRD in the lowest quartile. In the fully adjusted model, anaemia and a higher level of log-proteinuria were significantly associated with higher ESRD risk, while the relationship with systolic BP was on the borderline of statistical significance (Table 3). The higher the mGFR (or the eGFR, data not shown), the lower the risk for ESRD, with significant interaction with time. Using UCr as a continuous variable did not change these findings (data not shown). Of note, we found no significant interaction between UCr and either gender or proteinuria in their relationship with ESRD or mortality risks.

In the sub-group of 942 patients with repeated mGFR, median follow-up was 3.3 (2.0–5.5) years, mean mGFR decline was 1.7 ± 4.9 mL/min per year and 235 patients had an annual mGFR decline >10%. There was a trend towards lower ORs (95% CI) of a fast mGFR decline with lower UCr after adjustment for demographics and mGFR which was on the borderline of statistical significance after further adjustment for other confounders (Table 5).

DISCUSSION

This study showed that, in patients with CKD stages 1–4, low UCr was associated with a strong crude increased risk of pre-ESRD mortality, which was only partly explained by demographic variables and kidney function level. More importantly, in this large cohort of patients carefully phenotyped with mGFR, we found no crude association with ESRD risk or mGFR decline, and even a trend towards a lower risk for RRT initiation with lower UCr after adjusting for mGFR. Moreover, we highlight the potential risk for bias in the relationship of UCr with outcomes when adjustment is made for creatinine-based eGFR instead of mGFR. Overall, our findings provide
new insights into the relationships between muscle mass loss and clinical outcomes in early stage CKD.

Very few studies have investigated CKD outcomes associated with low muscle mass assessed by 24-h UCr. Both the Alberta Kidney Disease Network (AKDN) [14] and the Chronic Renal Insufficiency Cohort (CRIC) [18] studies reported excess deaths associated with lower UCr in patients with moderate CKD. In both studies, the HR for death was attenuated, but remained statistically significant after adjustment for confounders. Kidney function in these two studies, however, was estimated from creatinine-based equation [12] or serum cystatin (CRIC), and not measured. Our results are consistent with these findings, showing that the relationship of UCr with mortality was weakened, but remained statistically significant after adjusting for either mGFR or eGFR and other covariates. They are also in line with studies that point to an excess of deaths associated with altered muscle metabolism in the general population [25] or in transplant recipients [26], in patients with coronary heart disease [27, 37], chronic heart failure [28] and in type 2 diabetes [29]. An explanation for the relationship between low UCr and excess mortality may be that nutritional markers and/or low protein intake might be causes of low muscle mass. In the NephroTest study, as in the AKDN [14] and CRIC [18] studies, however, adjusting for protein intake and serum albumin did not significantly alter the observed association, nor was the association modified in our study by further adjustment for serum pre-albumin. These findings suggest that muscle mass loss in CKD mortality may play an independent role beyond that of patient nutritional status [38]. Although no mechanism is established for the wasting-death relationship, the role of several factors has been suggested including that of thrombocytosis and predisposition to thromboembolic events, arrhythmia-associated sudden death, suppression of the immune system and cachexia-related enhanced endotoxin bio-activity [11].

The relationship between UCr and CKD progression is debated. The AKDN and CRIC studies found that the decline in UCr was associated with increased ESRD risk, before and after adjusting for kidney function assessed by creatinine-based equation [14] or serum cystatin (CRIC) [18]. While similarly to these two studies, we observed a significant crude association between UCr and mortality, we found no crude association with ESRD risk despite lower mGFR at lower UCr level at baseline. This discrepancy mainly results from the lower number of person-years at risk in the lowest UCr quartile which is likely due to early mortality in patients with reduced muscle mass. Another possible explanation may be the lower level of 24-h proteinuria observed at lower UCr level in our study which was not present in the two studies above. Adjusting for proteinuria indeed slightly increased the HR for ESRD at low levels. Moreover, adjusting for mGFR tended to show an association opposite to that observed in these two studies. Adjusting for eGFR, however, produced a trend towards a significantly higher risk for ESRD similar to those found. We demonstrated that this reversal of ESRD risk resulted from the strong impact of UCr on the relationship between mGFR and eGFR. This finding suggests that decrease in UCr is not explained by demographic factors (age, gender and ethnicity) included in creatinine-based eGFR equations and makes eGFR inappropriate to adjust for kidney function in this analysis. In the CRIC study [18], however, adjustment was made for serum cystatin which is independent from serum creatinine, but may depend on factors other than only GFR [30]. Hence, although differences in study design or patient profile may explain discrepancies in findings, our results do not favour the hypothesis that low UCr would predict ESRD risk. Instead, the apparently lower risk for ESRD observed here suggests that dialysis may be started later in patients with reduced muscle mass due to overestimation of their true GFR by eGFR and possibly also because they have less metabolic acidosis, hyperkalaemia or hyperphosphataemia at low mGFR level when compared with their counterparts with normal muscle mass. It is worth noting that, unlike anaemia, these metabolic complications do not increase with decreasing UCr, while they strongly increase with decreasing mGFR [31] which argues for this hypothesis. Moreover, while a number of risk factors for mortality are also risk factors for ESRD and vice versa, it is interesting to underlie that markers of malnutrition including low BMI, hypoalbuminaemia, low pre-albumin and low UCr were all consistently associated with increased mortality in our study, but not with ESRD risk.

The major strengths of our study include its large sample size and the number of laboratory measurements, which enabled us to take major potential confounders into account in the associations under study. Another key strength is the use of a gold standard method to assess renal function, which, as shown above, made it possible to disentangle the impact of UCr and kidney function decline on mortality and ESRD. This study also has limitations, however. First, our findings are based on observational data, which do not allow for causal inference. Second, although no direct measure of muscle mass exists, it might be considered a limitation that we used UCr as a measure of muscle mass. However, reference methods such as computerized tomography (CT) or magnetic resonance imaging (MRI) measure total lean body mass and are therefore less specific than UCr [22]. Moreover, the time and money required for these methods make them impractical in large-scale clinical studies. The same is true for dual energy X-ray absorptiometry (DXA), which does not accurately differentiate between water and lean tissue, particularly in individuals with altered body water composition such as CKD patients [20, 39, 40]. In contrast, 24-h UCr is widely documented as a specific and reliable tool for assessing muscle mass [6, 7, 41], because it is directly proportional to total-body creatine content and skeletal muscle at steady state [23]. Its limitation is due to the frequency of inaccurate 24-h urine collections. However, in this study, we were able to assess the completeness of urine collection by calculating the ratio of fractionated creatinine clearance to 24-h creatinine clearance and used UCr extrapolated from fractionated creatinine clearance when this ratio showed evidence of more than 15% excess or incomplete collection.

**CONCLUSION**

In conclusion, muscle wasting, as reflected by low UCr, may predict mortality in patients with early stage CKD. In contrast, this study does not provide evidence for increased ESRD risk associated with low UCr in this population. Instead, the trend
towards a lower risk of ESRD observed here in patients with reduced muscle mass may reflect later dialysis start due to better tolerance of CKD complications and overestimation of true GFR by eGFR. Caution should thus be made in these patients in the assessment of kidney function using creatinine-based equation during the transition period to ESRD.

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APPENDIX


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