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**Extracellular fluid volume is associated with incident end-stage kidney disease and mortality in patients with chronic kidney disease.**

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**Abbreviations:**

BP: blood pressure

BSA: body surface area

CKD: chronic kidney disease

ECF: extracellular fluid

ESKD: end-stage kidney disease

GFR: glomerular filtration rate

eGFR: estimated glomerular filtration rate

mGFR: measured glomerular filtration rate

**ABSTRACT**

Volume overload has been shown to be an independent risk factor for mortality in patients receiving chronic dialysis, but data in non-dialysis patients are scarce. Therefore we evaluated the prognostic value of extracellular fluid (ECF) volume for chronic kidney disease (CKD) progression and mortality in a prospective hospital-based cohort with CKD stage 1-4 (NephroTest Study). ECF (scaled to body surface area) and the measured glomerular filtration rate (mGFR) were determined using the distribution volume and clearance of  $^{51}\text{Cr}$ -EDTA, respectively. Cause-specific Cox and linear mixed-effect regression models were used to analyze the association of ECF with end-stage kidney disease (ESKD) and mortality, and with mGFR decline, respectively. The 1593 patients were mean age 58.8 years, 67% were men, mean mGFR of 43.6 mL/min/1.73m<sup>2</sup> and mean ECF 15.1 L/1.73m<sup>2</sup>. After a median follow-up of 5.3 years, ESKD occurred in 324 patients and 185 patients died before ESKD. In multivariable analysis, ECF was significantly associated with the risk of ESKD (hazard ratio per 1L/1.73m<sup>2</sup> increase: 1.14; 95% confidence interval [1.07; 1.21]) and with a faster GFR decline (adjusted mean difference in mGFR slope per 1L/1.73m<sup>2</sup> increase -0.14 [-0.23; -0.05] mL/min/year). The relationship of ECF with mortality was non-linear and not significant (per 1L/1.73m<sup>2</sup> increase 0.92, [0.73; 1.16]), below 15L/1.73m<sup>2</sup>, but significant (1.28; [1.14-1.45]) above 15L/1.73m<sup>2</sup>. Thus, in this large cohort of carefully phenotyped patients with CKD, ECF was an independent risk factor of CKD progression and mortality. Hence, close monitoring and treatment of fluid overload are important for the clinical management of patients with non-dialysis CKD.

**Key words:** chronic kidney disease, extracellular fluid volume, volume overload, sodium.

## INTRODUCTION

Impaired renal salt and water excretion, in combination with other factors such as hypoalbuminemia, often result in chronic extracellular fluid (ECF) overload during chronic kidney disease (CKD).<sup>1</sup> In hemodialysis patients, several large-scale studies have shown that fluid overload is a strong and independent risk factor for mortality.<sup>2,3</sup> In contrast, studies evaluating the role of fluid overload on renal function and mortality in patients with non-dialysis CKD yielded conflicting results.<sup>4-6</sup> In addition, ECF was estimated using single or multifrequency bioelectrical impedance analysis, and not isotope dilution, which is the most direct and accurate method, although not routinely available.<sup>7-10</sup> Similarly, glomerular filtration rate (GFR) was estimated and not measured by a reference method. Therefore, the relationship between ECF and renal outcome and mortality during CKD remains uncertain.

The aim of this prospective observational study was to evaluate the association of ECF with progression of CKD, with end-stage kidney disease (ESKD), and with mortality occurring before ESKD, in non-dialysis CKD patients, using gold standard measurements of ECF and GFR.

## RESULTS

### Baseline characteristics

1593 patients with available ECF and CKD stage 1 to 4 from the prospective observational Nephrotest cohort were included in the study. Characteristics of the patients are reported in [Table 1](#). Mean age was  $58.8 \pm 15.1$  years, 66.7% were men, 87.8% had a history of hypertension, and 27.0% had diabetes. Mean mGFR was  $43.6 \pm 18.6$  mL/min/1.73m<sup>2</sup>. Mean ECF was  $15.1 \pm 2.2$  L/1.73m<sup>2</sup>. Distribution of ECF, crude or scaled to body surface area (BSA) according to gender is shown in [Supplementary Figure S1](#). ECF, anthropometric parameters, and diuretic prescription, in function of GFR subgroups, are reported in [Supplementary Figure S2](#). BSA and crude ECF decreased as GFR decreased, so that the relationship between ECF and mGFR was attenuated when ECF was scaled to BSA: ECF slightly decreased from 15.7 to 14.5 L/1.73m<sup>2</sup> when GFR decreased from > 60ml/min to 15-30 ml/min. Loop diuretics increased from 16% in patients with GFR>60ml/min to 43% in patients with GFR from 15 to 30 mL/min.

### Determinants of ECF

Compared with patients in the first tertile of ECF, patients in the third tertile were older, more often men, more likely to have a history of hypertension and diabetes, had a higher systolic and diastolic blood pressure (BP), body mass index (BMI), mGFR and urinary protein to creatinine ratio (uPCR), and a lower plasma albumin concentration. 24-hour urine sodium excretion did not differ between the three tertiles of ECF ([Table 1](#)). Associations were similar in multivariable analysis ([Supplementary Table S1](#)).

### **A higher ECF is associated with ESKD and mortality**

After a median follow-up of 5.3 [IQR: 3.0;7.4] years, 324 (20.3%) patients reached ESKD and 185 (11.6%) of those who did not reach ESKD died (67 [4.2%] from cardiovascular cause). Cumulative incidence of death at 5 years was significantly higher in the third tertile of ECF (3.8; 95%CI [2.3;5.9], 5.4 [3.5;7.8] and 13.2 [10.2;16.6] %, for the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> tertile, respectively,  $P < 0.001$ ), with a similar pattern for cardiovascular mortality ([Supplementary Figure S3](#)) while no difference was observed across tertiles of ECF on ESKD occurrence ([Figure 1](#)). Penalized splines representing the relationship between ECF and adjusted Hazard Ratios (HR) of death and ESKD are shown in [Figure 2](#). The relationship between ECF and ESKD was linear ([Figure 2, left panel](#)). In contrast, the relationship between ECF and mortality before ESKD showed an inflection point, with an increasing risk observed for ECF values above 15 L/1.73m<sup>2</sup> ([Figure 2, right panel](#)). Cause-specific Cox regression models showed that ECF was significantly associated with ESKD after adjustment for confounders (adjusted HR per 1 L/1.73m<sup>2</sup> increase of ECF: 1.14, 95%CI[1.07;1.21],  $P < 0.001$ ), and with mortality before ESKD above a threshold of 15L/1.73m<sup>2</sup> (adjusted HR per 1L/1.73m<sup>2</sup> increase of ECF below 15L/1.73m<sup>2</sup>, 0.92 [0.73 ; 1.16],  $P = 0.49$ , and above 15L/1.73m<sup>2</sup>, 1.28 [1.14;1.45],  $P < 0.001$ ) ([Table 2](#)). When the model was adjusted for systolic blood pressure as a time-varying covariate, the association between ECF and ESKD was slightly weaker (HR per 1 L/1.73m<sup>2</sup> increase of ECF: 1.12, 95%CI [1.05;1.19],  $P < 0.001$ ) ([Table 2](#)). E-values were 1.42 for the association between ECF and ESKD, and 1.88 for the association between ECF and mortality before ESKD. Associations between ECF and the risks of ESKD or mortality did not depend on uPCR, blood pressure, diabetes, age, gender or mGFR (p-values for interaction tests non-significant). Similar results were observed using ECF categorized in tertiles ([Supplementary Figure S4](#)), stratification for baseline eGFR instead of baseline mGFR ([Supplementary Table](#)



S2), and using multiple imputations for missing data (Supplementary Table S2). Similar associations were also observed when ECF was expressed as percentage of body weight (Supplementary Table S3).

### **A higher ECF is associated with a faster mGFR decline**

Analyses of longitudinal data (median number of visits: 2 [1 - 4] per patient, median duration between two consecutive visits 1.1 [1.0 ; 1.5] years) showed that the mean mGFR slope was -1.64, 95%CI [-1.82 ; -1.45] mL/min/year in the total population and -1.31[-1.60 ; -1.01], -1.49 [-1.81 ; -1.17] and -2.28 [-2.63 ; -1.92]mL/min/year for the first, second and third tertiles of ECF, respectively. In the fully adjusted linear mixed-effect model, ECF was significantly associated with a faster mGFR decline (mean difference in mGFR slope per 1L/1.73m<sup>2</sup> increase in ECF: -0.14, 95%CI [-0.23; -0.05] mL/min/year, P=0.002) (Table 3, Figure 3). Similar results were observed when ECF was analyzed in tertiles (Table 3) and in the subgroup of patients with at least 2 visits (Supplementary Table S4). Analyses yielded similar trends when GFR was estimated using the deindexed CKD-EPI formula (Supplementary Table S5).

## DISCUSSION

In this multicenter study conducted in 1593 patients with CKD stage 1 to 4, with a median follow-up of 5.3 years, a higher ECF was independently associated with ESKD (linear association) and death (non-linear association, with an increasing risk as ECF increased above a threshold of 15 L/1.73m<sup>2</sup>). These findings are robust because they rely on gold-standard methods for ECF and GFR measurement<sup>9-15</sup> in a large cohort of nearly 1600 carefully phenotyped patients, and analyses were adjusted for multiple potential confounders. In addition, we showed an association of a higher ECF with mGFR decline, strengthening our results.

Studies evaluating the role of fluid overload on renal function and mortality in patients with non-dialysis CKD have yielded conflicting results. In the largest study so far, recently published, Bansal *et al* showed in 3751 patients from the CRIC cohort that a shorter vector length – a bioelectrical impedance analysis-derived marker of overhydration – was significantly associated with the risk for heart failure, with adjusted HR for first (highest hydration state) *versus* third and fourth quartiles of 1.28, 95% CI [1.01 ; 1.61], but not with all-cause mortality or CKD progression.<sup>4</sup> In contrast, all previous pioneer works evaluating the prognostic role of ECF in patients with non-dialysis CKD had shown that fluid overload was associated with mortality and/or CKD progression, but they suffered a number of limitations as they were conducted in smaller cohorts, with composite endpoints, and bioelectrical impedance analysis (and not a gold standard such as isotope dilution) was used to assess fluid volume.<sup>7,8,10</sup> Thus, in a single-center cohort of 472 non-dialysis patients with CKD stage 4-5, Tsai *et al.* reported that fluid overload was associated with an increased risk

of renal replacement therapy (adjusted HR for third *versus* first tertile = 3.16, 95%CI [1.33;7.50]) and with a faster GFR decline (-1.10 [-2.06;-0.13] ml/min/1.73m<sup>2</sup> per year in the third *versus* first tertile), after a median follow-up of 17.3-months.<sup>6</sup> In the same cohort of patients, fluid overload was later reported to be associated with the combined endpoint of all-cause mortality and cardiovascular morbidity.<sup>5</sup> In a single-center prospective cohort of 338 patients with CKD stage 3 to 5 followed for a median of 2.1 years, Hung *et al.* showed that patients with volume overload were at a higher risk for the composite endpoint of ESKD or decline in estimated GFR  $\geq$  50%, and for cardiovascular morbidity and mortality.<sup>16</sup> Finally, in a retrospective cohort study of 149 patients with CKD followed for nearly 5 years, Tai *et al.* showed that the ratio of ECF over expected total body water was independently associated with the combined endpoint of ESKD or GFR decline  $\geq$  50%.<sup>17</sup> Our study, based on a large cohort and gold-standard GFR and ECF measurements, provides evidence in favor of a deleterious effect of fluid overload on renal function and on mortality in patients with CKD. The decrease in mGFR attributable to 1L/1.73m<sup>2</sup> increase in ECF was 0.14mL/min/year. A 10% ECF increase in a patient with an ECF value of 15.1 L/1.73m<sup>2</sup> (mean value in the cohort) would thus correspond to a yearly decrease in mGFR of 0.21 mL/min/1.73m<sup>2</sup>. Mean mGFR slope in our population was -1.64mL/min/year. The yearly mGFR loss solely attributable to age is estimated to be approximately 0.35 to 0.75 ml/min/1.73m<sup>2</sup> in healthy subjects<sup>18</sup> and in our cohort, GFR decrease due to uncontrolled hypertension was 0.61 mL/min/1.73m<sup>2</sup> and that due to a higher (one log-unit) uPCR was 0.43 mL/min/1.73m<sup>2</sup>. Therefore, the independent effect of ECF on GFR decrease is quantitatively small. However, ECF may be increased by several liters in some patients with marked fluid overload, leading to a potentially clinically relevant contribution to GFR decline, independent of its effects on blood pressure and proteinuria. In addition, ECF is also an independent determinant of

uncontrolled hypertension<sup>19</sup> and is associated with increased proteinuria, both of which also contributing to its deleterious effect on renal function.

A number of underlying mechanisms may explain the association between volume overload and adverse events. Volume overload is a prominent feature in conditions associated with a grim prognosis such as cirrhosis, nephrotic syndrome and heart failure, in which sodium and water retention are combined with arterial underfilling.<sup>20</sup> In addition, uPCR and low albumin concentration, both well-described prognostic factors for adverse outcome in patients with CKD,<sup>21,22</sup> are associated with increased ECF, as confirmed in the present study, possibly through ENaC-mediated sodium retention.<sup>22</sup> Although the associations we observed were similar after adjustment for pre-existing cardiovascular disease (including heart failure), plasma albumin concentration and uPCR, suggesting that these are not the only factors explaining the observed associations, we cannot exclude the possibility that the results might reflect the increased retention of salt and water in such comorbid conditions and be explained by residual confounding. However, 24-hour urinary sodium excretion increased as ECF increased in this cohort, whereas in patients with heart failure and liver cirrhosis, urinary sodium excretion is expected to decrease when fluid overload worsens. Furthermore, even though volume-dependent increase in blood pressure may in part explain the link between fluid overload and adverse outcomes, the associations of ECF with ESKD and mortality were independent of blood pressure. In the same vein, the steeper decline of GFR observed for a higher ECF persisted after adjustment for blood pressure and uPCR, even though both factors were themselves associated with GFR decline.

Increased salt intake tends to increase ECF in patients with CKD, who have a higher salt-sensitivity.<sup>23-26</sup> Therefore, salt intake may play a crucial role in the link between fluid

overload and adverse outcomes. However, in our study, 24-hour urine sodium excretion, a surrogate for salt intake, was not associated with outcomes, and our results persisted after adjustment for this covariate.

Finally, there is a pathophysiological plausibility for the observed link between ECF and mortality and ESKD to be, at least in part, causal and independent of all above-mentioned factors. Indeed, fluid overload-induced increased venous pressure increases interstitial pressure, and alters renal microcirculation, leading to impaired renal function.<sup>27</sup> In addition, increased mean circulatory filling pressure associated with fluid overload may induce pressure-independent alterations of structure and function of large arteries.<sup>6, 28, 29</sup> Finally, recent studies have suggested that fluid overload may also induce inflammatory processes by translocation of endotoxin fragments through congested bowel wall, and/or by inducing splanchnic ischemia, thereby increasing mortality, both in non-dialysis and in hemodialysis patients.<sup>29-31</sup> However, our study was observational and by definition prone to confounding, as illustrated by the fairly low calculated E-values (see Methods)<sup>32</sup>: only randomized interventional studies targeting different levels of ECF control using diuretics or low sodium intake can provide evidence for the prognostic value of fluid overload during CKD. Of note, our results suggest that close attention to fluid status should be paid in future blood pressure target randomized trials. Indeed, in the Systolic Blood Pressure Intervention Trial (SPRINT),<sup>33</sup> one cannot rule out that part of the beneficial effect observed in the intensive treatment arm may be related to the higher rate of diuretic prescription and therefore potentially to ECF reduction, as suggested by the markedly lower rate of heart failure but not of stroke.

Several large observational studies conducted in patients with CKD showed that a higher salt intake is associated with mortality and cardiovascular events.<sup>34, 35</sup> The association between salt intake and CKD progression is more controversial.<sup>34, 36</sup> Interventional studies of low sodium intake<sup>23, 26, 37-40</sup> and/or diuretics<sup>40, 41</sup> have shown a reduction of proteinuria and blood pressure. However randomized controlled trials did not demonstrate that reduction of sodium intake, or diuretics – independently of blood pressure reduction – reduces cardiovascular disease and mortality or slows CKD progression.<sup>36-38, 42-44</sup>

Surprisingly, measured ECF did not increase, and even slightly decreased, as mGFR decreased. This was largely explained by the fact that patients with a lower mGFR had a lower body weight, BMI, BSA, and muscle mass as assessed by 24-hour urinary creatinine excretion, as previously reported, including in the NephroTest cohort.<sup>45, 46</sup> However, the positive association between ECF and GFR, although markedly attenuated, remained after adjustment for BSA, at least in CKD stage 2 to 4. The cross-sectional analysis of ECF in this hospital-based observational cohort therefore does not reflect the natural history of ECF during CKD.<sup>6, 16, 17, 29, 41, 47</sup> Interpretation of ECF values in this population receiving optimized care is complex as all patients are carefully followed-up by nephrologists and largely prescribed diuretics, with a marked increase in diuretic prescription when GFR decreased ([Supplementary Figure S2](#)).

Our study underlines that ECF is an important parameter in the management of patients with CKD. Nevertheless, physical examination and search for peripheral edema, blood pressure measurement, diagnosis of orthostatic hypotension, or weight variations are not sufficient for detection of small variations in ECF, such as those expected during non-dialysis

CKD.<sup>47</sup> Even if isotopic measurement provides information on both ECF and GFR and is recommended in difficult situations, this gold standard exploration is not routinely available. The use of different complementary tools (such as bioelectrical impedance spectroscopy, echocardiography, and biomarkers) is thus necessary to monitor ECF as accurately as possible and to adjust treatment in these patients. ECF can be controlled with dietary advice<sup>26</sup> and diuretic use<sup>41</sup> which are the cornerstone of the treatment of fluid retention in CKD patients. Even if diuretic doses are not available in the NephroTest database, results of our study suggest that therapeutic inertia (both for pharmacological treatment and dietary sodium restriction) partly explains the increase in ECF. Only 56% of the patients in the third tertile of ECF, and 55% of the patients with mGFR below 30mL/min, were prescribed diuretics. Likewise, sodium intake, estimated from 24-hour urinary sodium excretion, was on average 155mmol (3.6g) per day, which is above the recommended intake, especially in patients with CKD.<sup>48</sup> In addition, 24-hour urinary sodium excretion increased with ECF, from 145 mmol/day in the first tertile to 168 mmol/day in the third tertile. Tight control and monitoring of sodium balance should be considered early in the course of CKD, in combination with other reno-protective treatments such as renin-angiotensin-system blockers.

To our knowledge, this is the largest study evaluating the prognostic value of ECF on renal function and mortality in non-dialysis CKD patients, using gold standard methods of ECF and GFR measurements. Our study has some limitations. First, it was an observational study, by definition prone to confounding despite multiple adjustments. In addition, GFR and ECF were derived from the same <sup>51</sup>Cr-EDTA clearance samples, which may generate a spurious relationship between the two variables. However, measurement errors (mainly due to urine

loss) would be expected to generate an opposite association (underestimation of GFR when ECF is overestimated), and similar results were observed when eGFR was used instead of mGFR, making this hypothesis unlikely. Another limitation is that GFR slope analyses were based on a median of only two measurements. Moreover, although ECF was measured using a reference method, independent measurement of ECF using bioelectrical impedance spectroscopy, or biological markers of plasma volume (such as vasopressin or natriuretic peptides), would have strengthened our results. Finally, patients from the NephroTest study are closely followed up and receive optimized care, so that this cohort is not an appropriate setting to analyze the independent effect of GFR on ECF; in addition, due the tight control of ECF in this population, this observational study may underestimated the strength of the association between ECF and adverse outcomes.

In conclusion, our results show that ECF is an independent risk factor for ESKD and mortality in patients with non-dialysis CKD, suggesting that it is important to carefully monitor and maintain ECF in these patients, in addition to other hydro-electrolytic disturbance and cardiovascular risk factors. Nevertheless, future prospective interventional studies are needed to confirm the benefit of ECF control in CKD.



## **PATIENTS AND METHODS**

### **Study design and participants**

The NephroTest study is a prospective hospital-based tricentric cohort (Physiology departments of Tenon, Bichat and Georges Pompidou Hospitals, Paris, France), which enrolled 2084 adult patients with CKD of all stages and of various aetiologies, from January 2000 to December 2012.<sup>49</sup> Pregnancy, a past history of renal transplantation, and dialysis were exclusion criteria. Patients with no available ECF measurement at baseline (n=288), with CKD stage 5 at inclusion (n=103), or lost to follow-up (n=100) were excluded from the study, leaving 1593 patients for this analysis.

### **Data collection**

Patients were referred by their nephrologist to one of the three renal physiology units for extensive work-up during a 5-hour in-person visit, including GFR and ECF measurements. Patients were asked to collect 24-hour urine the day before admission, with indications given by a trained nurse and detailed in a written information document. Past medical history including underlying renal disease (as reported by the nephrologist), treatment, anthropometric data and a large set of clinical and laboratory variables were collected.

### **GFR and ECF measurements**

Measured GFR (mGFR) was determined by renal clearance of <sup>51</sup>Cr-EDTA (GE Healthcare, Vélizy, France), as previously described.<sup>50</sup> Briefly, a single dose of 1.8-3.5 MBq of <sup>51</sup>Cr-EDTA was injected intravenously. After allowing 1.5 hours for equilibration of the tracer in the ECF, urine was collected and discarded. Average renal <sup>51</sup>Cr-EDTA clearance was then determined

from the average of six consecutive 30-minute clearance periods. Blood was drawn at the midpoint of each clearance period. ECF was determined as the average of the six consecutive measurements, each of them being calculated as the remaining quantity of the tracer (injected quantity of  $^{51}\text{Cr-EDTA}$ , “ $Q_{\text{injected}}$ ”, minus excreted quantity of the tracer, “ $Q_{\text{excreted}}$ ”), divided by the extrapolated serum concentration of the tracer at the corresponding time point. To take into account morphological differences between patients, ECF was scaled to body surface area (BSA); the latter being calculated using Dubois’ formula.<sup>51</sup>

$$ECF(L/1.73m^2) = \frac{Q_{\text{injected}} - Q_{\text{excreted}}}{\text{plasma EDTA concentration}} \times \frac{1.73}{BSA}$$

ECF was analyzed both as continuous variable and in tertiles (the first tertile being used as the reference category). Sensibility analyses were conducted with ECF expressed as a ratio of body weight, expressed in percentage, instead of scaled to BSA.

## Outcomes

Progression to ESKD was defined by initiation of chronic dialysis or pre-emptive renal transplantation. ESKD and vital status were obtained by record linkage with the French REIN Registry (*Réseau Epidémiologique et Information en Néphrologie*, Paris, France) and the national death registry (*Répertoire National d’Identification des Personnes Physiques*, RNIPP, INSEE, France), respectively. Causes of death were obtained by the national registry CapiDC (*Centre d’épidémiologie sur les causes médicales de Décès*) using the CIM-10 classification. For each death in the Nephrotest cohort, the main cause of death adjudicated was by three independent physicians. Survival data were censored on December 31, 2013.

## Statistical analysis

Categorical variables are reported as frequencies and percentages and continuous variables are reported as mean $\pm$ SD or median (interquartile range, IQR), as appropriate. Patients' characteristics were compared across tertiles of ECF using Chi-2 test or one-way analysis of variance for qualitative and quantitative variables, respectively.

Determinants of ECF treated quantitatively were analyzed using a multivariable linear regression model (see methodological details in the [Supplementary Material](#)). Cumulative incidence curves were estimated with Aalen-Johansen method ("cuminc" function of the R package "cmprsk") to take competing risks into account.<sup>52</sup> Crude and adjusted hazard ratios (HRs) of ESKD and of mortality before ESKD associated with ECF were estimated using cause-specific Cox regression models. Penalized splines were used in the fully adjusted Cox model to represent the functional relationship between ECF treated quantitatively and the risk of ESKD (or death before ESKD). In order to test linear relationships between ECF and outcomes (ESKD and mortality), we compared fully adjusted Cox models including ECF expressed as continuous variable *versus* pspline function of ECF, using the likelihood ratio test.

Adjustment covariates, selected *a priori* as potential confounders, included age, gender, ethnicity (African origin *versus* others), site of recruitment, diabetic status (no diabetes, diabetes without diabetic nephropathy, diabetes with diabetic nephropathy), body mass index (BMI), elevated blood pressure (in two classes: < or  $\geq$  140/90 mmHg), urinary protein-to-creatinine ratio (uPCR, log-transformed), 24-hour urinary sodium excretion (corrected by potential collection bias using the ratio of creatinine clearance in the collection over fractionated creatinine clearance, as previously described<sup>53</sup>), diuretics and renin-angiotensin

system inhibitors. For mortality, previous cardiovascular event (myocardial infarction or angioplasty or stroke or heart failure) and plasma albumin concentration were also entered in the model. Proportional hazard assumption was checked for each covariate using  $\log(-\log(S))$  and Schoenfeld residuals against time. Because this assumption was not verified for mGFR, stratification for baseline mGFR level was performed using six classes of mGFR: >60, 50-60, 40-50, 30-40, 20-30, 15-20 mL/min/1.73m<sup>2</sup>. Stratification for baseline eGFR level was also performed in sensitivity analyses. In order to adjust for GFR as a continuous variable, while taking into account its time-dependence, an additional model including a mGFR time-dependent coefficient was performed. Finally, sensitivity analyses incorporating systolic blood pressure as a continuous time-varying covariate were also performed. Interactions between ECF and age, gender, uPCR, blood pressure, and mGFR were tested.

Missing data were less than 5% ([Supplementary Table S5](#)) and assumed to be missing at random. Single and multiple imputations were performed using the chained equation method (R package “mice”, 100 imputed datasets, 20 iterations).<sup>54</sup> Primary analyses were performed using single imputations for missing data. Sensitivity analyses, using multiple imputations and complete cases were also performed. For each model, the E-value – reflecting the minimal strength of association that potential unmeasured confounders would need to have with both ECF and outcomes to explain away the observed association – was calculated.<sup>32</sup>

Finally, the association between baseline ECF and mGFR decline (expressed in mL/min/year) was analyzed using a multivariable linear mixed-effect regression model with random intercept and slope (“lmer” function of the R package “lme4”).<sup>55-57</sup> The main analysis was

conducted in the total population (n=1593), as mixed-effect regression model allows accounting for patients with only one visit at baseline to reduce selection bias under the missing at random assumption. A sensitivity analysis was conducted in patients with at least two mGFR measurements (n=1009). Detailed methods and covariates are provided in the [Supplementary Material](#). Single imputations were performed for missing data. A 2-tailed p-value <0.05 was considered statistically significant. All statistical analyses were conducted using R 3.4 software.

### **Ethics statements**

All patients signed informed consent before inclusion in the cohort. The NephroTest study was approved by an ethic committee (*Direction Générale pour la Recherche et l'Innovation* (DGRI) ; Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé (CCTIRS). Ref: DGRI CCTIRS MG/CP09.503, July 9<sup>th</sup>, 2009).

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## Disclosures

The authors declared no relevant competing of interests.

## Authorship

ALF, EVP, GG conceived and designed the study, analyzed and interpreted the data. ALF, GG, MM performed statistical analyses and designed tables and figures. MF, FV, BS, MM, JPH, JJB, PH, ET conceived and coordinated the Nephrotest study. ALF and EVP drafted the manuscript. All authors made critical revision of the manuscript for important intellectual content.

## Supplementary material

**Supplementary methods.** Determinants of ECF and Linear mixed-effect regression models.

**Table S1.** Multivariable linear regression model to identify independent determinants of extracellular fluid volume, crude (L), or scaled to body surface area (L/1.73m<sup>2</sup>).

**Table S2.** Cause-specific Cox regression models for the association of extracellular fluid volume (L/1.73m<sup>2</sup>) with end-stage kidney disease and with mortality, using single or multiple imputations for missing data, or complete cases.

**Table S3.** Cause-specific Cox regression models for the association of extracellular fluid volume (% body weight) with end-stage kidney disease and for mortality.

**Table S4.** Decline of measured glomerular filtration rate according to extracellular fluid volume in patients with at least 2 visits (n=1009).

**Table S5.** Decline of estimated glomerular filtration rate according to extracellular fluid volume (n=1593).

**Table S6.** Number of missing data for baseline covariates.

**Figure S1.** Distribution of extracellular fluid volume according to gender.

**Figure S2.** Anthropometric characteristics of the patients according to level of baseline measured glomerular filtration rate.

**Figure S3.** Cumulative incidence rates of cardiovascular mortality according to tertiles of extracellular fluid volume.

**Figure S4.** Adjusted hazard ratios of end-stage kidney disease and mortality (total and cardiovascular) associated with tertiles of extracellular fluid volume.

Supplementary information is available at *Kidney International's* website

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**Table 1. Baseline clinical and biological characteristics in total population and according to tertiles of extracellular fluid volume.**

	Total n = 1593	ECF (L/1.73m <sup>2</sup> )			p
		1 <sup>st</sup> tertile n = 531 [8.88 - 14.04[	2 <sup>nd</sup> tertile n = 531 [14.04 - 15.83[	3 <sup>rd</sup> tertile n = 531 [15.83- 23.29]	
<b>Demographics and clinical characteristics</b>					
Age (years)	58.8 ± 15.1	53.2 ± 15.2	58.8 ± 14.3	64.2 ± 13.6	<0.001
Gender (men)	1063 (66.7)	249 (46.9)	365 (68.7)	449 (84.6)	<0.001
Ethnicity (Sub-Saharan African origin)	217 (14.3)	80 (15.8)	80 (15.8)	57 (11.2)	0.053
Height (cm)	167.1 (9.4)	164.6 (9.6)	167.7 (9.4)	169.1 (8.6)	<0.001
Weight (kg)	74.6 (16.3)	67.1 (13.7)	74.4 (13.9)	82.2 (17.3)	<0.001
Body surface area (m <sup>2</sup> )	1.83 (0.22)	1.73 (0.20)	1.83 (0.20)	1.92 (0.21)	<0.001
BMI (kg/m <sup>2</sup> )	26.6 ± 5.1	24.7 ± 4.3	26.4 ± 4.4	28.7 ± 5.8	<0.001
Tobacco consumption					<0.001
Non smoker	873 (54.8)	345 (65.0)	284 (53.5)	244 (46.0)	
Former smoker	506 (31.8)	113 (21.3)	168 (31.6)	225 (42.4)	
Current smoker	214 (13.4)	73 (13.7)	79 (14.9)	62 (11.7)	
Systolic blood pressure (mmHg)	135 ± 20.2	130 ± 18.6	135 ± 19.7	141 ± 20.8	<0.001
Diastolic blood pressure (mmHg)	75 ± 11.5	74 ± 11.8	75 ± 11.9	76 ± 10.8	0.024
Elevated blood pressure (≥140 and/or 90 mmHg)	573 (37.3)	144 (28.3)	187 (36.7)	242 (46.8)	<0.001
<b>Medical history</b>					
Hypertension	1399 (87.8)	441 (83.1)	459 (86.4)	499 (94.0)	<0.001
Diabetes mellitus	430 (27.0)	77 (14.5)	120 (22.6)	233 (43.9)	<0.001
Dyslipidemia	277 (18.0)	110 (21.4)	84 (16.3)	83 (16.3)	0.047
Previous cardiovascular event	288 (18.4)	61 (11.7)	81 (15.5)	146 (28.1)	<0.001
Underlying renal disease					<0.001
Diabetic nephropathy	154 (9.7)	18 (3.4)	34 (6.4)	102 (19.2)	
Glomerular	224 (14.1)	102 (19.2)	69 (13.0)	53 (10.0)	
Vascular	410 (25.7)	110 (20.7)	146 (27.5)	154 (29.0)	
Polycystic kidney disease	100 (6.3)	35 (6.6)	39 (7.3)	26 (4.9)	
Interstitial	150 (9.4)	69 (13.0)	51 (9.6)	30 (5.6)	
Other or unknown conditions	555 (34.8)	197 (37.1)	192 (36.2)	166 (31.3)	
<b>Treatment</b>					
Number of antihypertensivedrugs	2.3 ± 1.6	2.0 ± 1.4	2.3 ± 1.6	2.7 ± 1.6	<0.001
ACEi and/or ARB (%)	1186 (74.5)	378 (71.2)	386 (72.7)	422 (79.5)	0.004
Diuretics	759 (47.7)	209 (39.4)	253 (47.6)	297 (56.0)	<0.001
Loop diuretic	454 (28.5)	114 (21.5)	137 (25.8)	203 (38.3)	<0.001
Thiazide diuretic	326 (20.5)	96 (18.1)	121 (22.8)	109 (20.6)	0.169
Amiloride	17 (1.1)	5 (0.9)	6 (1.1)	6 (1.1)	0.943
Aldosterone antagonist	43 (2.7)	13 (2.5)	19 (3.6)	11 (2.1)	0.291
Statin	693 (43.6)	196 (37.0)	217 (40.9)	280 (52.8)	<0.001
<b>Biological parameters</b>					
eGFR CKD-Epi (mL/min/1.73m <sup>2</sup> )	45.9 ± 21.8	45.2 ± 23.2	46.1 ± 21.7	46.3 ± 20.4	0.668
mGFR (mL/min/1.73m <sup>2</sup> )	43.6 ± 18.6	40.6 ± 17.9	44.5 ± 18.5	45.6 ± 19.0	<0.001
mGFR (mL/min/1.73m <sup>2</sup> )					<0.001
≥ 60	299 (18.8)	77 (14.5)	105 (19.8)	117 (22.0)	
[45-60[	366 (23.0)	108 (20.3)	129 (24.3)	129 (24.3)	
[30-45[	503 (31.6)	168 (31.6)	171 (32.2)	164 (30.9)	
[15-30[	425 (26.7)	178 (33.5)	126 (23.7)	121 (22.8)	
Measured ECF (L)	16.1 ± 3.6	12.9 ± 2.0	15.8 ± 1.8	19.6 ± 3.1	<0.001
Measured ECF (L/1.73m <sup>2</sup> )	15.1 ± 2.2	12.8 ± 1.0	14.9 ± 0.5	17.6 ± 1.6	<0.001
Hemoglobin (g/dL)	12.74 (1.60)	12.68 (1.63)	12.83 (1.59)	12.70 (1.60)	0.267
Protein (g/L)	70.2 ± 6.0)	70.5 ± 6.3	70.5 ± 5.7	69.6 ± 6.0	0.026
Albumin (g/L)	39.5 ± 4.4	39.9 ± 4.1	40.0 ± 4.1	38.8 ± 4.8	<0.001

24-h urinary sodium excretion (mmol/24h)	155 ± 73.0	145 ± 71.7	153 ± 68.4	168 ± 77.3	<0.001
24-h urinary potassium excretion (mmol/24h)	65 ± 26	59 ± 22.9	65 ± 24.7	72 ± 29.1	<0.001
24-h urinary sodium / potassium ratio	2.6± 1.5	2.7 ± 1.7	2.6± 1.5	2.6 ± 1.3	0.210
Protein to creatinine ratio (mg/mmol)	80.8 ± 144.1	72.3 ± 114.7	67.0 ± 113.9	103.2 ± 188.8	<0.001

Continuous data are expressed in mean ± SD and categorical data are expressed in n (%). Diabetes was either self-reported or defined as fasting glycemia ≥ 7 mmol/L or antidiabetic drug treatment. Previous cardiovascular event was defined as a history of stroke, ischemic heart disease (angioplasty, surgical coronary bypass, or myocardial infarction), or heart failure. Dyslipidemia was defined as a total cholesterol > 6 mmol/L or > 5mmol/L in case of a previous cardiovascular event. ACEi: angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blockers; ECF: extracellular fluid volume; mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate, calculated using the CKD-EPI formula.

**Table 2. Cause-specific Cox regression models: effects of 1L/1.73m<sup>2</sup> increase in extracellular fluid volume on end-stage kidney disease and mortality.**

Outcome	Models	HR [95% CI]	p-value
<b>ESKD</b>			
n=1593	Events (n)	324	
	Crude model	1.03 [0.98 ; 1.08]	0.29
	Adjusted model (1)*	1.14 [1.07 ; 1.21]	<0.001
	Adjusted model (2)*	1.10 [1.04 ; 1.17]	0.001
	Adjusted model (3)	1.12 [1.05 ; 1.19]	<0.001
<b>Mortality</b>			
≤ 15 L/1.73m <sup>2</sup> (n=827)	Events (n)	63	
	Crude	1.00 [0.81 ; 1.24]	0.99
	Adjusted model	0.92 [0.73 ; 1.16]	0.49
> 15 L/1.73m <sup>2</sup> (n=766)	Events (n)	122	
	Crude model	1.27 [1.16 ; 1.39]	<0.001
	Adjusted model (1)	1.28 [1.14 ; 1.45]	<0.001
	Adjusted model (2)	1.28 [1.14 ; 1.44]	<0.001
	Adjusted model (3)	1.29 [1.15 ; 1.46]	<0.001

Crude and adjusted hazard ratios are indicated for 1L/1.73m<sup>2</sup> increase in extracellular fluid volume. Analyses were adjusted for the following covariates: age, gender, site of inclusion, ethnicity, body mass index, diabetic status (no diabetes, diabetes without diabetic nephropathy, diabetes with diabetic nephropathy), elevated blood pressure, urinary protein-to-creatinine ratio (log-transformed), 24-hour urinary sodium excretion, diuretics and renin-angiotensin system inhibitors. For mortality, models were also adjusted for previous cardiovascular events (myocardial infarction or angioplasty or stroke or heart failure) and plasma albumin concentration. Single imputations were used for missing data. ESKD: end-stage kidney disease.

(1) Model stratified for baseline measured glomerular filtration rate

(2) Model adjusted for mGFR (expressed as a continuous variable, as a time-dependent coefficient).

(3) Model adjusted for systolic blood pressure as a continuous time-varying covariate

\* When the model was fully adjusted, but neither stratified nor adjusted for mGFR, HR for ESKD was 0.98 [0.92 ; 1.04],  $p=0.44$ , showing that GFR is the covariate explaining that the crude analysis reveals no significant association between ECF and ESKD.

**Table 3. Decline of measured glomerular filtration rate according to extracellular fluid volume (n=1593).**

	Mean difference in mGFR slopes (mL/min/year)			
	Model 0	Model 1	Model 2	Model 3
<b>ECF analyzed as a continuous variable</b>				
<b>ECF (per 1L/1.73m<sup>2</sup>)</b>	-0.19 [-0.28 ; -0.10]	-0.20 [-0.29 ; -0.1]	-0.15 [-0.25 ; -0.06]	-0.14 [-0.23 ; -0.05]
<b>Elevated blood pressure</b>			-0.78 [-1.19 ; -0.37]	-0.61 [-1.01 ; -0.21]
<b>Protein-to-creatinine ratio</b>				-0.43 [-0.55 ; -0.32]
<b>ECF analyzed in tertiles</b>				
<b>ECF</b>	Ref	Ref	Ref	Ref
<b>1<sup>st</sup>tertile [8.88 - 14.04[</b>				
<b>2<sup>nd</sup>tertile [14.04 - 15.83[</b>	-0.09 [-0.54 ; 0.35]	-0.10 [-0.55 ; 0.35]	0.02 [-0.43 ; 0.47]	-0.02 [-0.46 ; 0.41]
<b>3<sup>rd</sup>tertile [15.83 - 23.29]</b>	-0.86 [-1.34 ; -0.39]	-0.90 [-1.37 ; -0.42]	-0.72 [-1.20 ; -0.24]	-0.55 [-1.02 ; -0.08]
<b>Elevated blood pressure</b>			-0.85 [-1.25 ; -0.44]	-0.68 [-1.08 ; -0.29]
<b>Protein-to-creatinine ratio</b>				-0.42 [-0.54 ; -0.31]

ECF was analyzed as a continuous variable (results are indicated per 1L/73m<sup>2</sup> increase in ECF), or in tertiles.

Model 0: time, baseline values of ECF and mGFR levels (>60, 45-60, 30-44, 15-29 mL/min/1.73m<sup>2</sup>), and interaction terms between time and GFR and time and ECF.

Model 1: Model 0 + all baseline covariates (age, gender, ethnicity, recruitment site, body mass index, diabetic status (no diabetes, diabetes without diabetic nephropathy, diabetes with diabetic nephropathy), elevated blood pressure (< or ≥ 140/90 mmHg), urinary protein-to-creatinine ratio (log-transformed, per 1-log unit increase\*), 24-h urinary sodium excretion, diuretics, and renin-angiotensin system inhibitors).

Model 2: Model1 + interaction term between time and elevated blood pressure.

Model 3: Model 2 + interaction terms between time and urinary protein-to-creatinine ratio and between time and site of inclusion.



mGFR decline was modeled using a linear mixed-effect regression model. Mean differences in mGFR slopes are expressed in mL/min/year. ECF: extracellular fluid volume; mGFR measured glomerular filtration rate.

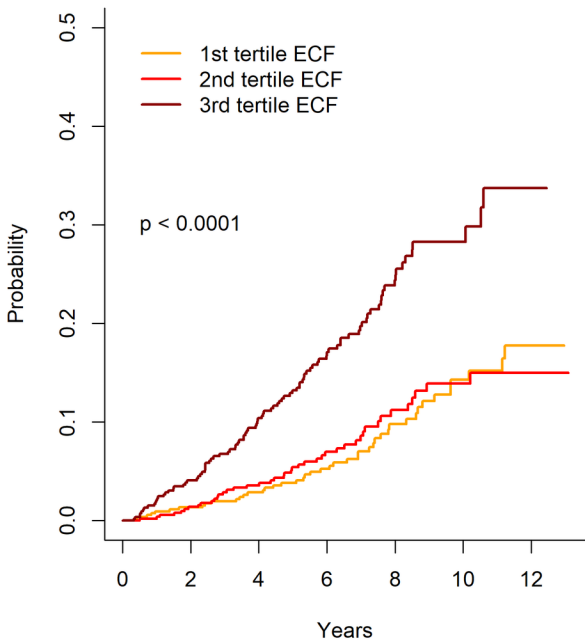
\*The indicated coefficient corresponds to a 2.72-fold increase in protein-to-creatinine ratio.

**Figure 1. Cumulative incidence rates of mortality and end-stage kidney disease according to tertiles of extracellular fluid volume.** ECF: extracellular fluid volume; ESKD: end-stage kidney disease.

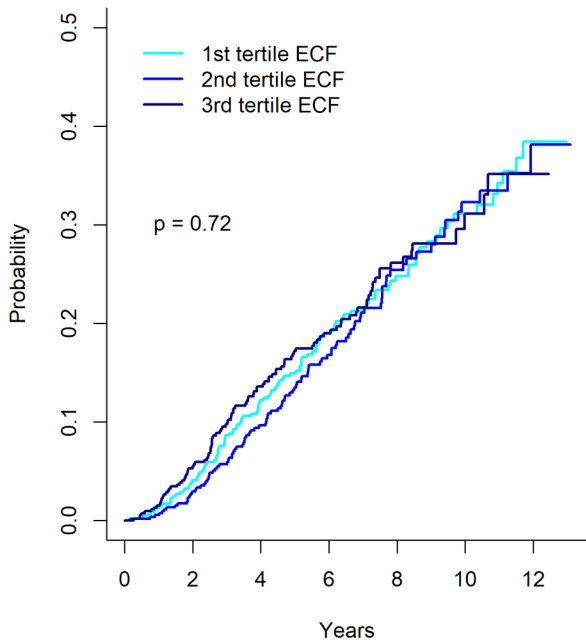
**Figure 2. Estimated adjusted hazard ratios and 95% confidence intervals for the association of extracellular fluid volume with end-stage kidney disease and with mortality, using penalized-splines estimators.** Cause-specific Cox regression models were adjusted for the following covariates: age, gender, site of inclusion, ethnicity, body mass index, diabetic status (no diabetes, diabetes without diabetic nephropathy, diabetes with diabetic nephropathy), elevated blood pressure, urinary protein-to-creatinine ratio (log-transformed), 24-hour urinary sodium excretion, diuretics and renin-angiotensin system inhibitors. For mortality, models were also adjusted for previous cardiovascular events (myocardial infarction or angioplasty or stroke or heart failure) and plasma albumin concentration. Models were stratified for baseline measured glomerular filtration rate. Single imputations were used for missing data. ECF: extracellular fluid volume, scaled to body surface area ( $L/1.73m^2$ ). ESKD: end-stage kidney disease.

**Figure 3. Three-dimensional modeling of the decline of glomerular filtration rate according to baseline extracellular fluid volume.** Arrows indicate the expected GFR slopes for the highest (red arrow) and the lowest (blue arrow) ECF values. The X-axis indicates time (expressed in years), the Y-axis indicates GFR (expressed in mL/min) and the Z-axis indicates ECF (expressed in  $L/1.73m^2$ ).

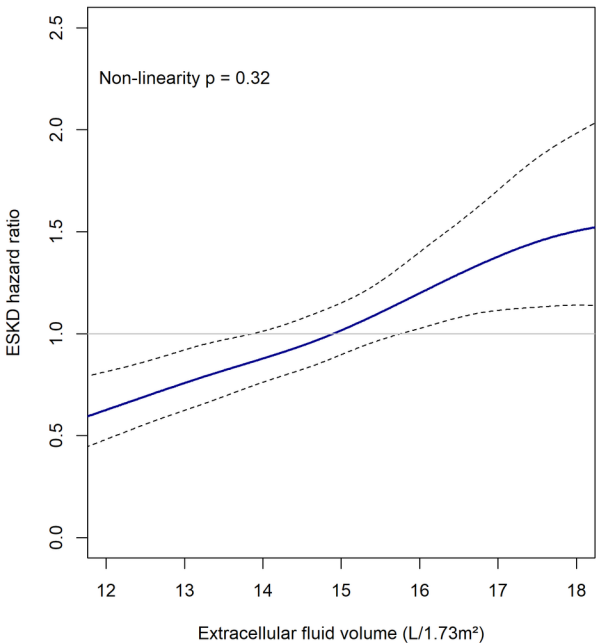
**Cumulative incidence of mortality  
(tertiles of ECF)**



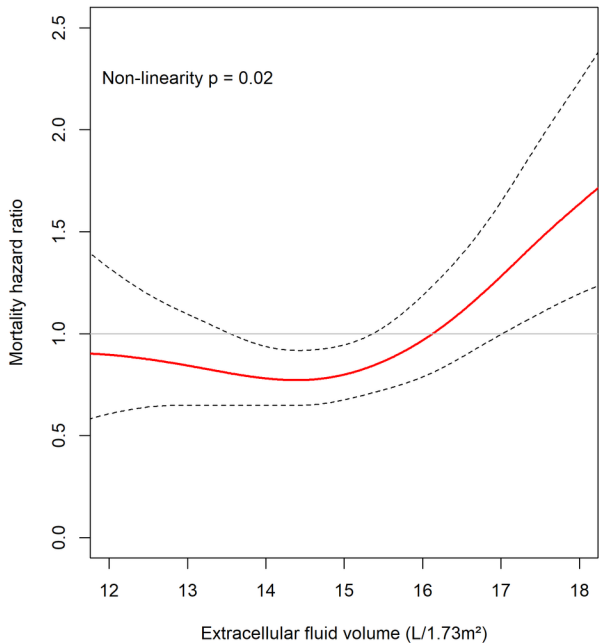
**Cumulative incidence of ESKD  
(tertiles of ECF)**

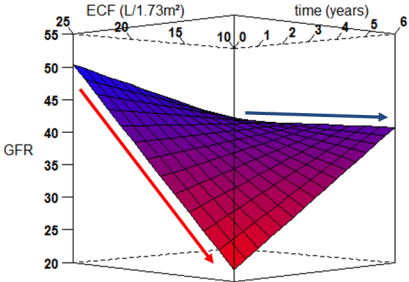


**Estimated adjusted hazard ratio for the association of ECF with ESKD**



**Estimated adjusted hazard ratio for the association of ECF with mortality**





# Extracellular fluid volume is associated with incident end-stage kidney disease and mortality in patients with chronic kidney disease.

## NephroTest cohort

1593 patients



- Prospective cohort
- CKD stage 1-4
- All etiologies
- Measured extracellular fluid volume (ECF) and GFR: distribution volume and clearance of  $^{51}\text{Cr}$ -EDTA.

## Statistical analyses

-ECF in L/1.73m<sup>2</sup>.

Association of ECF with:

- **ESKD and mortality:** Cox regression models
- **mGFR decline:** linear mixed-effect regression models
- Median follow-up: 5.3 [IQR: 3.0 ; 7.4] years

## Results

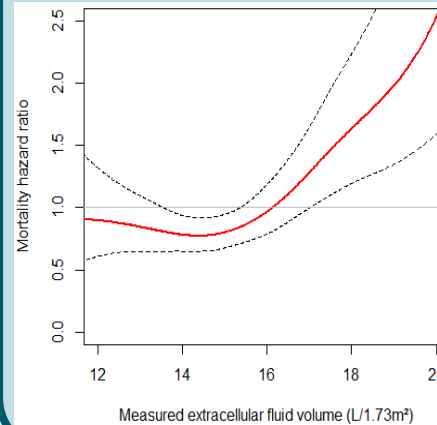
**Mortality**  
(11.6%)

**ESKD**  
(20.3%)

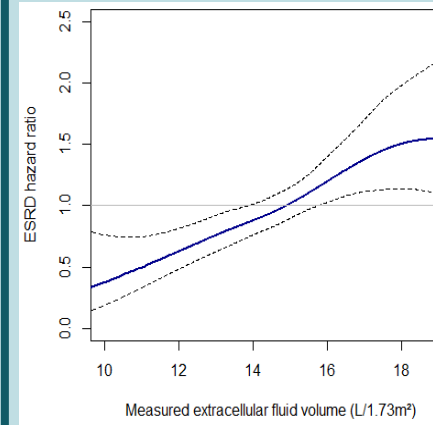
**mGFR decline**  
-1.64 [-1.82 ; -1.45] mL/min/y

For 1L/1.73m<sup>2</sup> increase in ECF:

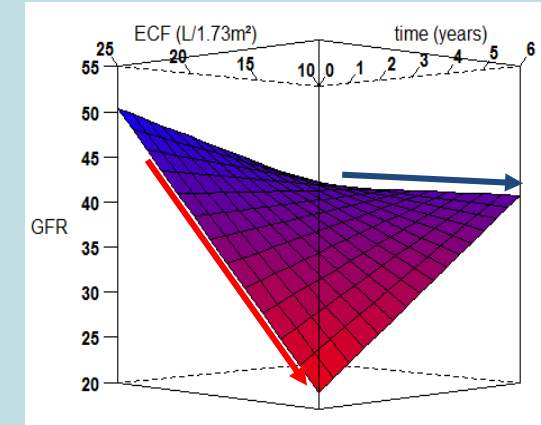
**HR = 1.28 [1.14 ; 1.45]**  
above 15L/1.73m<sup>2</sup>



**HR = 1.14 [1.07 ; 1.21]**



Mean difference in mGFR slope  
**-0.14 [-0.23 ; -0.05] mL/min/y**



## CONCLUSION:

ECF is independently associated with CKD progression and mortality. Close monitoring and treatment of fluid overload are important for the clinical management of patients with non-dialysis CKD.