ORIGINAL RESEARCH

Extracellular Fluid Volume Is an Independent Determinant of Uncontrolled and Resistant Hypertension in Chronic Kidney Disease: A NephroTest Cohort Study

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***Background*—**Hypertension is highly prevalent during chronic kidney disease (CKD) and, in turn, worsens CKD prognosis. We aimed to describe the determinants of uncontrolled and resistant hypertension during CKD.

***Methods and Results*—**We analyzed baseline data from patients with CKD stage 1 to 5 (NephroTest cohort) who underwent thorough renal explorations, including measurements of glomerular filtration rate (clearance of ^{51}Cr-EDTA) and of extracellular water (volume of distribution of the tracer). Hypertension was defined as blood pressure (BP; average of 3 office measurements) ≥140/90 mm Hg or the use of antihypertensive drugs. In 2015 patients (mean age, 58.7±15.3 years; 67% men; mean glomerular filtration rate, 42±15 mL/min per 1.73 m^2), prevalence of hypertension was 88%. Among hypertensive patients, 44% and 32% had uncontrolled (≥140/90 mm Hg) and resistant (uncontrolled BP despite 3 drugs, including a diuretic, or ≥4 drugs, including a diuretic, regardless of BP level) hypertension, respectively. In multivariable analysis, extracellular water, older age, higher albuminuria, diabetic nephropathy, and the absence of aldosterone blockers were independently associated with uncontrolled BP. Extracellular water, older age, lower glomerular filtration rate, higher albuminuria and body mass index, male sex, African origin, diabetes mellitus, and diabetic and glomerular nephropathies were associated with resistant hypertension.

***Conclusions*—**In this large population of patients with CKD, a lower glomerular filtration rate, a higher body mass index, diabetic status, and African origin were associated with hypertension severity but not with BP control. Higher extracellular water, older age, and higher albuminuria were independent determinants of both resistant and uncontrolled hypertension during CKD. Our results advocate for the large use of diuretics in this population. **(*J Am Heart Assoc*. 2018;7:e010278. DOI: 10.1161/JAHA.118.010278.)**

Key Words: chronic kidney disease • extracellular water • hypertension • resistant hypertension • uncontrolled hypertension

High rates of uncontrolled hypertension and resistant hypertension, both associated with a poor cardiovascular and renal prognosis,[1–5] have been reported in patients with chronic kidney disease (CKD).[6–8] Most epidemiological studies on treatment and control of hypertension were conducted in cohorts meant to be representative of the general population, such as the National Health and Nutrition Examination Surveys (NHANESs).[9,10] Few data on the factors associated with hypertension control and resistance were obtained specifically in patients with CKD.[7] Several

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Accompanying Data S1 and Tables S1 through S5 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.010278

*A complete list of the NephroTest Study Group investigators can be found in the Appendix at the end of the article.

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Received July 10, 2018; accepted August 16, 2018.

Clinical Perspective

What Is New?

- In this large cohort of patients with chronic kidney disease, a lower glomerular filtration rate was a risk factor for resistant hypertension, but was not independently associated with uncontrolled hypertension, whereas a higher extracellular water rate appeared to be independently associated with both uncontrolled hypertension and resistant hypertension.

What Are the Clinical Implications?

- Our results suggest that chronic kidney disease does not prevent blood pressure control, provided adequate treatment, including a tight control of fluid overload, is administered.

small-scaled studies have suggested that volume overload plays a key role for hypertension control during CKD,[11,12] but extracellular water (ECW) was estimated, using multifrequency bioimpedance, as the most direct and accurate method to measure extracellular fluid volume and isotope dilution; however, this measurement is cumbersome and not routinely available.

The aim of the study was to define the rates and the determinants of hypertension, uncontrolled hypertension, and apparent treatment-resistant hypertension in a population of patients with CKD who underwent thorough renal explorations, including gold standard measurement of glomerular filtration rate (GFR) and ECW.

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 various causes, stages 1 to 5, from January 2000 to December 2012. Pregnancy, a history of renal transplantation, and dialysis were exclusion criteria. Data from the baseline visit were used in this cross-sectional study. Drug treatment and blood pressure (BP) values were missing for 2 and 67 patients, respectively, so that 2015 patients were included in this study (Figure 1). All patients signed informed consent before inclusion in the cohort. The NephroTest study was approved by an ethics committee (Direction Générale de la Recherche et de l'Innovation; Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé; reference, DGRI Comité Consultatif sur le Traitement de l'Information en Matière de Recherche dans le Domaine de la Santé MG/CP09.503; July 9, 2009). The database, analytic methods, and study materials will not be made available to other researchers for purposes of replicating the procedure, because of restrictions on data sharing for the NephroTest study from the National Commission for Data Protection and Liberties.

Procedures

Patients were referred by their nephrologist to 1 of the 3 renal physiology units for extensive workup during a 5-hour in-person visit, including GFR measurement. 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. Medical history, treatment, anthropometric data, and a large set of clinical and laboratory variables were collected.

GFR and ECW Measurements

Measured GFR (mGFR) was determined by renal clearance of ^{51}Cr-EDTA (GE Healthcare, Vélizy, France), as previously described.[13] Briefly, a single dose of 1.8 to 3.5 MBq of ^{51}Cr-EDTA was injected intravenously. After allowing 1.5 hours for equilibration of the tracer in the extracellular fluid, urine was collected and discarded. Average renal ^{51}Cr-EDTA clearance was then determined from the average of 6 consecutive 30-minute clearance periods. Blood was drawn at the midpoint of each clearance period. ECW was calculated after the equilibrium period, as the remaining quantity of the tracer divided by the serum concentration of the tracer, and expressed in liters. To take into account the expected ECW for a given sex and weight, ECW was expressed as a ratio of measured over theoretical ECW; the latter was calculated as follows: theoretical ECW=a+b×body weight (a=7.35, b=0.135 in men and a=5.27, b=0.134 in women).[14] ECW was treated in ratio over theoretical ECW in the main analysis and in liters in a secondary analysis.

To consider potentially excessive or incomplete 24-hour urine collections, 24-hour urinary parameters were corrected by dividing the measured value by the ratio of creatinine clearance in the collection versus the fractionated urinary clearance of creatinine in the 6 timed periods of GFR measurement, as previously described.[15]

BP Measurement and Definitions

BP was calculated as the average of 3 measurements taken with an automated device by a trained observer, after 5 minutes of rest in a seated patient. Hypertension was defined as a systolic BP ≥140 mm Hg and/or a diastolic BP

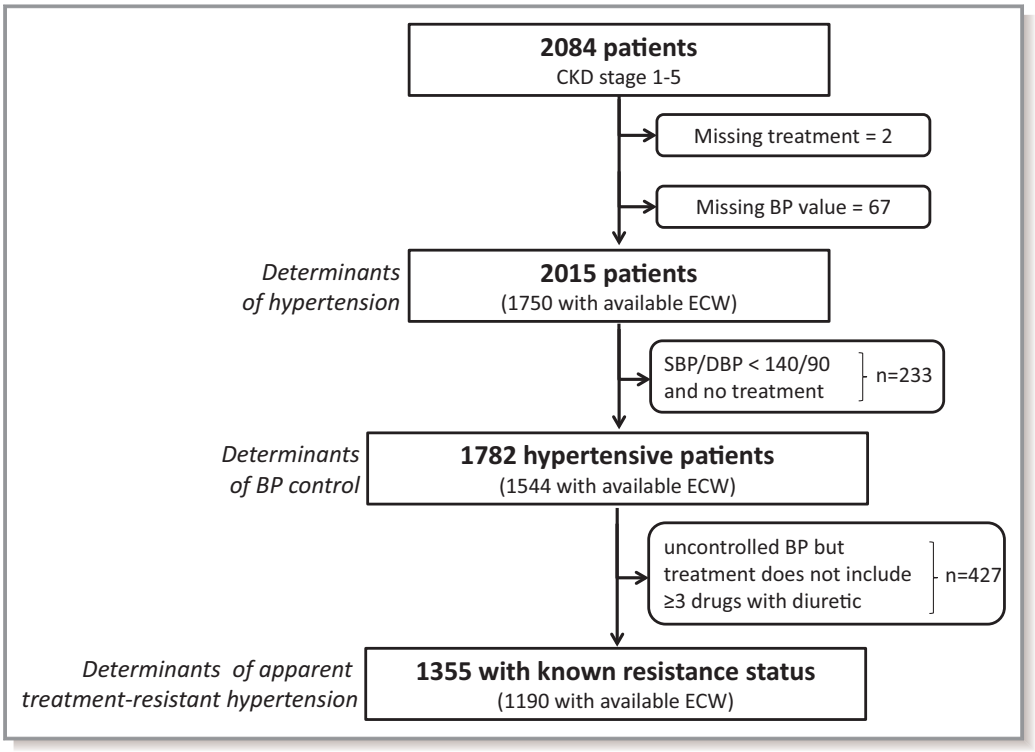


Figure 1. Flow diagram of study population. BP indicates blood pressure; CKD, chronic kidney disease; DBP, diastolic BP; ECW, extracellular water; SBP, systolic BP.

≥90 mm Hg, and/or the current use of antihypertensive drugs. β Blockers, diuretics, and blockers of the renin-angiotensin system prescribed for cardiovascular reasons or proteinuria in an otherwise normotensive patient with no history of hypertension (n=64 patients) were not considered as antihypertensive drugs so as to avoid an upwardly biased hypertension prevalence rate. BP was controlled if systolic BP was <140 mm Hg and diastolic BP was <90 mm Hg. Apparent treatment-resistant hypertension was defined as uncontrolled BP despite at least 3 drugs, including a diuretic, or controlled BP under ≥4 drugs, including a diuretic.

Statistical Analysis

Prevalence of hypertension was described in 2015 patients, and prevalences of uncontrolled and apparent treatment-resistant hypertension were described in 1782 hypertensive patients. For each condition, prevalence was calculated in the whole population, as well as according to mGFR level (≥60, 45–59, 30–44, 15–29, and <15 mL/min per 1.73 m^2). Characteristics of the patients were analyzed in the whole population as well as by hypertension, hypertension control, and hypertension resistance status. Groups were compared using Kruskal-Wallis tests for continuous variables and χ^2 tests for categorical variables. Number and types of antihypertensive drugs were analyzed in the whole population and by GFR subgroups. Cochran-Armitage tests for trend by GFR level were performed for each drug type.

Crude and fully-adjusted odds ratios (ORs) with 95% confidence intervals (95% CIs) were estimated from logistic regression models for hypertension, uncontrolled hypertension, and apparent treatment-resistant hypertension, according to ECW (in L or in ratio over theoretical ECW) and other patient characteristics (details about the choice of covariates for each dependent variable are given in Data S1). Because of technical issues or irregular urine voiding, ECW measurement was missing at random in 265 of the 2015 patients (Figure 1). Logistic regression models for hypertension, uncontrolled BP, and apparent treatment-resistant hypertension were first treated by complete case analysis for ECW, and missing values for other covariates were replaced by median for continuous variables and by the most frequent classes for categorical variables. Accordingly, determinants of hypertension were analyzed in 1750 patients with available ECW measurement, and determinants of uncontrolled hypertension were analyzed in 1544 hypertensive patients among them (Figure 1). Determinants of apparent treatment-resistant hypertension among hypertensive patients were analyzed in 1355 patients who also had a known resistance status (ie, after exclusion of patients with uncontrolled hypertension and <3 drugs or at least 3 drugs without a diuretic, because these could not

be classified as resistant or not). A secondary analysis of the determinants of resistant hypertension was performed in the total population of hypertensive patients. Finally, in sensitivity analyses, we performed multiple imputations of our data set (n=5 imputed data set; fully conditional specification using all covariates, including outcomes; maximum, 100 iterations) using all covariates in Table 1 and dependent variables, performed final models on each complete data set, and finally combined the estimated ORs using Rubin's rules.[16] All analyses were conducted using SAS 9.4 or R 3.3 (https://www.R-project.org/).

Results

Demographic data and baseline characteristics of the patients are given in Table 1 for the total population and in Table 2 by hypertension, hypertension control, and hypertension resistance status. Mean age was 58.7±15.3 years, 67% were men, 14% were of African origin, and 27% had diabetes mellitus. Mean systolic BP was 136±20 mm Hg, and mean diastolic BP was 75±12 mm Hg. Mean mGFR was 42.0±20.0 mL/min per 1.73 m^2, and mean ECW was 16.2±3.8 L. Type of nephropathies were diabetic, glomerular, vascular, polycystic, and interstitial nephropathies in 10%, 14%, 27%, 6%, and 9% of the patients, respectively. Median sodium intake, estimated from sodium excretion in the 24-hour urine collection, was 3.4 g/d, corresponding to an 8.5-g salt intake (Table 1). Prevalence of hypertension was 88% in the total population, but increased from 75% to 96% for an mGFR ≥60 to an mGFR <15 mL/min per 1.73 m^2 (Figure 2A and 2C).

Antihypertensive drugs in the population of hypertensive patients (n=1782), and by GFR subgroup, are indicated in Table 3. A diuretic was part of the treatment in 54% of hypertensive patients. Prevalence of uncontrolled hypertension was 44% (34% in patients with mGFR ≥60 mL/min per 1.73 m^2, with a progressive increase, up to 52% in patients with mGFR <15 mL/min per 1.73 m^2, as illustrated in Figure 2A and 2C). Among patients with uncontrolled BP, 46% were taking at least 3 drugs, including a diuretic, and 44% were taking ≤2 antihypertensive drugs. Most patients (73.6%) with uncontrolled hypertension had isolated systolic hypertension, 23.6% had systolodiastolic hypertension, and 2.7% had isolated diastolic hypertension. Apparent treatment-resistant hypertension (uncontrolled BP despite at least 3 drugs, including a diuretic, or controlled BP with ≥4 drugs, including a diuretic) was found in 32% of all hypertensive patients, with a progressive increase from 23% for an mGFR ≥60 mL/min per 1.73 m^2 to 49% in patients with an mGFR <15 mL/min per 1.73 m^2 (Figure 2B and 2C).

In multivariable analysis, a higher ECW was an independent determinant of hypertension, with an OR of 1.19 (95% CI, 1.05–1.35) per 10% increase when expressed as a ratio of theoretical ECW, and an OR of 1.10 (95% CI, 1.03–1.18) per 1-L increase of absolute ECW (Table 4, Table S1). Other independent determinants of hypertension included older age, higher body mass index (BMI), African origin, diabetes mellitus, previous cardiovascular event, lower mGFR, and higher albuminuria (Table 4). The association between BMI and hypertension

Table 1. Characteristics of the Patients (n=2015)

Characteristic	Value	Missing, N
Age, y	58.7±15.3	0
Men	67	0
Sub-Saharan African origin	14	108
BMI, kg/m^2	26.6±5.2	0
Previous cardiovascular event	18	39
Smoking status (current/former/never)	14/31/55	0
Diabetes mellitus	27	0
SBP, mm Hg	136±20	0
DBP, mm Hg	75±12	0
mGFR, mL/min per 1.73 m^2	42.0±20.0	0
eGFR (CKD-EPI), mL/min per 1.73 m^2	44.4±22.9	0
Extracellular water, L	16.2±3.8	265
ECW ratio over theoretical ECW	0.97±0.15	265
Type of nephropathy		0
Diabetic	10	
Glomerular	14	
Vascular	27	
Polycystic	6	
Interstitial	9	
Other or unknown	34	
Natriuresis, mmol/24 h*	146 (107–192)	258
Kaliuresis, mmol/24 h*	61.5 (45.9–78.5)	258
24-h Urinary Na/K ratio	2.37 (1.71–3.25)	120
Albuminuria, mg/mmol creatinine	8.9 (1.6–51.0)	64
[Na], mmol/L	140±3	1
[K], mmol/L	4.3±0.5	3
Plasma uric acid, μmol/L	422±110	7
[HCO_3–], mmol/L	25.8±3.2	12

Data are given as mean±SD, percentage, or median (interquartile range). BMI indicates body mass index; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DBP, diastolic blood pressure; ECW, extracellular water; mGFR, measured glomerular filtration rate; SBP, systolic blood pressure.
*Values corrected for inaccurate 24-hour urine collection using the ratio of 24-hour creatinine clearance over fractionated creatinine clearance, as detailed in the Methods section.

Table 2. Characteristics of the Patients by Hypertension, Hypertension Control, and Hypertension Resistance Status

	Total Population (N=2015)			Hypertensive Patients (N=1782)					
	Hypertension			Uncontrolled Hypertension			Apparent Treatment-Resistant Hypertension		
Characteristic	No (N=233)	Yes (N=1782)	*P* Value	No (N=996)	Yes (N=786)	*P* Value	No (N=1204)	Yes (N=578)	*P* Value
Age, y	47.6±16.3	60.2±14.5	<0.0001	57.2±15.1	64.1±12.6	<0.0001	58.9±15.1	62.9±12.9	<0.0001
Men	55.4 (129)	68.2 (1215)	<0.0001	65.8 (655)	71.2 (560)	0.014	65.1 (784)	74.6 (431)	<0.0001
Sub-Saharan African origin	10.7 (24)	14.4 (243)	0.13	15.4 (144)	13.2 (99)	0.20	11.9 (135)	19.7 (108)	<0.0001
BMI, kg/m^2	24.0±4.5	27.0±5.2	<0.0001	26.6±5.2	27.4±5.1	0.0001	26.1±4.9	28.8±5.3	<0.0001
Previous cardiovascular event	3.0 (7)	19.9 (347)	<0.0001	18.2 (176)	22.0 (171)	0.044	15.6 (183)	28.7 (164)	<0.0001
Smoking status									
Former	17.2 (40)	33.0 (588)	<0.0001	29.4 (293)	37.5 (295)	0.001	31.3 (377)	36.5 (211)	0.028
Current	15.5 (36)	13.5 (241)		14.7 (146)	12.1 (95)		14.7 (177)	11.1 (64)	
Diabetes mellitus	7.7 (18)	30.0 (535)	<0.0001	24.4 (243)	37.2 (292)	<0.0001	21.8 (262)	47.2 (273)	<0.0001
mGFR, mL/min per 1.73 m^2	53.3 (38.9–70.1)	37.4 (26.5–51.6)	<0.0001	38.2 (27.3–53.5)	36.2 (24.5–49.8)	0.0008	39.1 (28.1–53.7)	33.8 (22.4–46.6)	<0.0001
Extracellular water, L	14.4±3.4	16.4±3.8	<0.0001	15.9±3.7	17.0±3.8	<0.0001	15.9±3.5	17.5±4.0	<0.0001
ECW ratio over theoretical ECW	0.93±0.14	0.97±0.15	0.0015	0.95±0.14	0.99±0.16	<0.0001	0.96±0.15	0.99±0.16	0.0065
Type of nephropathy									
Diabetic	1.7 (4)	11.5 (205)	<0.0001	7.3 (73)	16.8 (132)	<0.0001	6.4 (77)	22.1 (128)	<0.0001
Glomerular	18.0 (42)	13.9 (247)		17.1 (170)	9.8 (77)		15.5 (187)	10.4 (60)	
Vascular	1.3 (3)	29.9 (532)		27.1 (270)	33.3 (262)		26.2 (316)	37.4 (216)	
Polycystic	3.0 (7)	5.9 (106)		7.2 (72)	4.3 (34)		7.6 (91)	2.6 (15)	
Interstitial	21.5 (50)	7.5 (133)		8.4 (84)	6.2 (49)		10.1 (122)	1.9 (11)	
Other or unknown	54.5 (127)	31.4 (559)		32.8 (327)	29.5 (232)		34.1 (411)	25.6 (148)	
Natriuresis, mmol/24 h	132 (103–183)	147 (108–193)	0.028	145 (106–191)	151 (109–195)	0.033	143 (107–188)	156 (114–202)	0.001
Kaliuresis, mmol/24 h	59 (44–75)	62 (46–79)	0.14	61 (46–78)	63 (47–80)	0.12	61 (46–79)	63 (45–78)	0.56
24-h Urinary Na/K ratio	2.32 (1.70–3.11)	2.37 (1.72–3.26)	0.37	2.36 (1.72–3.21)	2.38 (1.71–3.33)	0.56	2.33 (1.68–3.20)	2.48 (1.84–3.36)	0.007
ACR, mg/mmol creatinine	4.98 (0.91–25.2)	9.64 (1.78–56.4)	<0.0001	6.47 (1.52–35.0)	18.46 (2.42–87.5)	<0.0001	7.25 (1.53–41.7)	20.0 (2.90–86.3)	<0.0001
[Na], mmol/L	140±2	140±3	0.76	140±3	140±3	0.17	140±3	140±3	0.82
[K], mmol/L	4.09±0.38	4.29±0.51	<0.0001	4.30±0.51	4.28±0.51	0.49	4.30±0.50	4.26±0.55	0.22
Plasma uric acid, μmoL/L	369±100	429±109	<0.0001	432±111	426±107	0.20	419±102	450±120	<0.0001
[HCO_{3-}], mmol/L	26.4 (24.4–28.0)	26.0 (23.8–28.0)	0.11	26.0 (23.7–27.8)	26.0 (24.0–28.0)	0.44	26.0 (23.8–27.8)	26.2 (23.9–28.1)	0.25

Continuous data are expressed as mean±SD or median (interquartile range), and groups were compared using Kruskal-Wallis test. Categorical data are expressed as percentage (number), and groups were compared using χ^2 test. ACR indicates albumin/creatinine ratio; BMI, body mass index; ECW, extracellular water; mGFR, measured glomerular filtration rate.

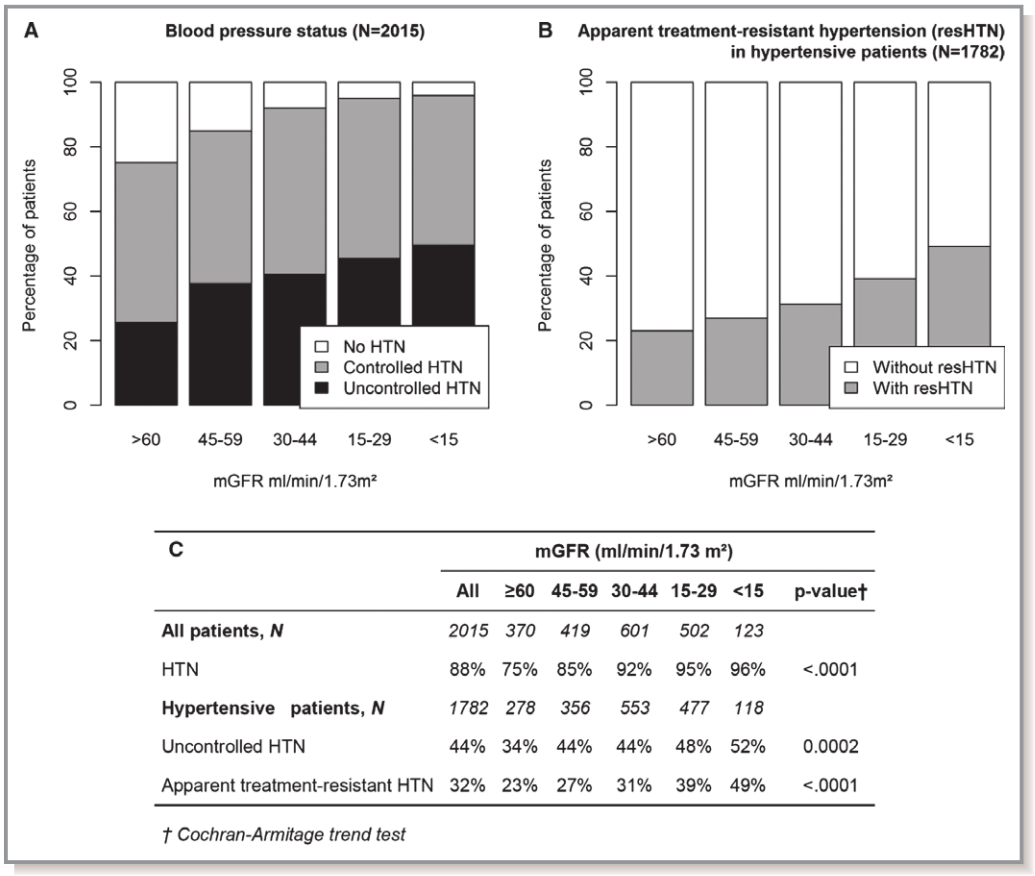
DOI: 10.1161/JAHA.118.010278

disappeared when absolute ECW value (in liters) was entered in the model, instead of its ratio over theoretical ECW (Table S1).

In the population of hypertensive patients, multivariable analysis for the determinants of uncontrolled hypertension showed that older age, higher albuminuria, diabetic nephropathy, and higher ECW (OR per 10% as a ratio over theoretical ECW, 1.11 [95% CI, 1.02–1.20]; and OR per 1 L, 1.07 [95% CI, 1.02–1.11]) were significantly associated with an increased risk of uncontrolled hypertension, whereas the use of aldosterone blockers was significantly associated with a decreased risk of uncontrolled hypertension (Table 5, Table S2). mGFR was not independently associated with hypertension control (OR per −10 mL/min per 1.73 m^2, 1.00 [95% CI, 0.99–1.00]; P=0.4).

Multivariable analysis for the determinants of apparent treatment-resistant hypertension was conducted in the population of hypertensive patients, with the exclusion of patients with uncontrolled hypertension despite no treatment (n=50) and 1 (n=116), 2 (n=182), or ≥3 drugs with no diuretics (n=79) because these patients may or may not be resistant would they be properly treated (Table 6, Table S3). Thus, resistant hypertension status defined a more severe status than nonresistant hypertension in this analysis. Older age, higher BMI, albuminuria, ECW (OR per 10% as a ratio over theoretical ECW, 1.12 [95% CI, 1.01–1.23]; and OR per 1 L, 1.08 [95% CI, 1.03–1.14]), lower mGFR, male sex, African origin, and diabetes mellitus were significantly associated with an increased risk of apparent treatment-resistant hypertension (Table 6). Compared with interstitial nephropathy, the type of nephropathy with the strongest association with apparent treatment-resistant hypertension was diabetic nephropathy (OR, 9.03; 95% CI, 3.84–21.21). A secondary analysis performed in the total population of 1782 hypertensive patients yielded similar results (Table S4).

In all analyses, similar results were obtained when 24-hour sodium and potassium excretions (instead of their ratio) were entered in the model separately (Table S5).



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	mGFR (ml/min/1.73 m²)						
	All	**≥60**	**45-59**	**30-44**	**15-29**	**<15**	**p-value†**
All patients, *N*	*2015*	*370*	*419*	*601*	*502*	*123*	
HTN	88%	75%	85%	92%	95%	96%	<.0001
Hypertensive patients, *N*	*1782*	*278*	*356*	*553*	*477*	*118*	
Uncontrolled HTN	44%	34%	44%	44%	48%	52%	0.0002
Apparent treatment-resistant HTN	32%	23%	27%	31%	39%	49%	<.0001

† Cochran-Armitage trend test

Figure 2. Prevalence of hypertension, uncontrolled hypertension, and apparent treatment-resistant hypertension by glomerular filtration rate (GFR) subgroups. A, Blood pressure status in the total population (n=2015). B, Apparent treatment-resistant hypertension in hypertensive patients (n=1782). C, Hypertension in all participants and uncontrolled hypertension and apparent treatment-resistant hypertension in hypertensive patients (n=1782). mGFR indicates measured GFR.

Table 3. Antihypertensive Treatments in NephroTest Hypertensive Patients (n=1782)

Variable	All	mGFR, mL/min per 1.73 m^2					*P* Value
		≥60 (N=278)	45–59 (N=356)	30–44 (N=553)	15–29 (N=477)	<15 (N=118)	
No. of antihypertensive drugs							<0.0001*
0	2.8 (50)	4.3 (12)	3.7 (13)	2.2 (12)	2.5 (12)	0.8 (1)	
1	19.3 (344)	26.6 (74)	26.1 (93)	19.0 (105)	13.0 (62)	8.5 (10)	
2	26.2 (467)	30.2 (84)	25.6 (91)	24.8 (137)	27.0 (129)	22.0 (26)	
3	24.6 (439)	20.9 (58)	24.4 (87)	26.8 (148)	23.5 (112)	28.8 (34)	
≥4	27.0 (482)	18.0 (50)	20.2 (72)	27.3 (151)	34.0 (162)	39.8 (47)	
Any diuretic	54.3 (967)	48.2 (134)	47.5 (169)	55.0 (304)	58.1 (277)	70.3 (83)	<0.0001†
Loop diuretic	33.6 (599)	16.5 (46)	22.5 (80)	32.9 (182)	45.5 (217)	62.7 (74)	<0.0001†
Thiazide diuretic	22.3 (398)	29.9 (83)	27.0 (96)	24.8 (137)	14.7 (70)	10.2 (12)	<0.0001†
Aldosterone blocker	2.8 (50)	4.3 (12)	2.8 (10)	2.5 (14)	2.7 (13)	0.8 (1)	0.096†
Converting enzyme inhibitor	51.6 (919)	46.8 (130)	45.8 (163)	53.3 (295)	55.1 (263)	57.6 (68)	0.001†
Angiotensin II receptor antagonist	43.9 (782)	44.2 (123)	43.0 (153)	44.3 (245)	42.3 (202)	50.0 (59)	0.73†
Calcium channel blocker	49.8 (887)	41.0 (114)	44.4 (158)	50.1 (277)	55.8 (266)	61.0 (72)	<0.0001†

Data are given as percentage (number). mGFR indicates measured glomerular filtration rate.
*χ^2 Test.
†Cochran-Armitage test for trend.

Results from sensitivity analyses showed that complete case analysis for ECW and multiple imputations give similar ORs of hypertension, uncontrolled BP, and apparent treatment-resistant hypertension analysis, according to ECW and their other determinants (Tables S1 through S4).

Table 4. Determinants of Hypertension in the Population With ECW Measurement (n=1750)

Variable	Crude OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
ECW, L	1.18 (1.13–1.24)	<0.0001	…	…
ECW ratio over theoretical ECW	1.22 (1.09–1.36)	0.0005	1.19 (1.05–1.35)	0.008
Age, y	1.06 (1.05–1.07)	<0.0001	1.04 (1.03–1.06)	<0.0001
Sex (women vs men)	0.54 (0.41–0.73)	<0.0001	0.82 (0.57–1.17)	0.2710
BMI 25–30 vs <25 kg/m^2	2.42 (1.74–3.37)	<0.0001	1.58 (1.07–2.32)	0.021
BMI ≥30 vs <25 kg/m^2	4.54 (2.73–7.56)	<0.0001	2.15 (1.20–3.83)	0.010
Ethnicity (African origin vs other)	1.41 (0.90–2.23)	0.14	2.28 (1.33–3.89)	0.003
Diabetes mellitus	6.40 (3.61–11.3)	<0.0001	2.16 (1.16–4.03)	0.015
Previous cardiovascular event	10.1 (4.11–24.6)	<0.0001	3.96 (1.56–10.0)	0.004
Smoking status (past vs none)	2.69 (1.80–4.01)	<0.0001	1.43 (0.91–2.24)	0.12
Smoking status (active vs none)	1.06 (0.70–1.60)	0.78	1.40 (0.86–2.28)	0.18
mGFR, per −10 mL/min per 1.73 m^2	1.40 (1.30–1.50)	<0.0001	1.22 (1.10–1.35)	0.0002
Log albuminuria, mg/mmol creatinine	1.17 (1.09–1.27)	<0.0001	1.19 (1.08–1.31)	0.0006
[Na], /mmol/L	0.99 (0.94–1.04)	0.73	0.98 (0.92–1.05)	0.60
[K], /mmol/L	2.29 (1.66–3.16)	<0.0001	1.77 (1.16–2.71)	0.008
[HCO_3−], /mmol/L	0.98 (0.93–1.02)	0.34	1.11 (1.04–1.18)	0.003
Plasma uric acid, /10 μmol/L	1.06 (1.05–1.08)	<0.0001	1.03 (1.01–1.05)	0.0008
Ratio Na/K 24-h urine	1.01 (1.00–1.02)	0.28	1.00 (0.99–1.01)	0.83

Crude and adjusted ORs (95% CIs) of hypertension are indicated, as well as *P* values. ORs were adjusted for all covariates and recruitment site. Fully adjusted ORs for ECW expressed in L are shown in Table S2. BMI indicates body mass index; CI, confidence interval; ECW, extracellular water; mGFR, measured glomerular filtration rate; OR, odds ratio.

Table 5. Determinants of Uncontrolled Hypertension in the Patients With Hypertension and ECW Measurement (n=1544)

Variable	Crude OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
ECW, L	1.08 (1.05–1.11)	<0.0001	…	…
ECW ratio over theoretical ECW	1.20 (1.12–1.29)	<0.0001	1.11 (1.02–1.20)	0.013
Age, y	1.03 (1.03–1.04)	<0.0001	1.03 (1.02–1.04)	<0.0001
Sex (women vs men)	0.76 (0.61–0.95)	0.014	0.81 (0.63–1.06)	0.12
BMI 25–30 vs <25 kg/m^2	1.24 (0.99–1.57)	0.064	1.23 (0.88–1.72)	0.22
BMI ≥30 vs <25 kg/m^2	1.39 (1.07–1.81)	0.015	1.07 (0.83–1.39)	0.60
Ethnicity (African origin vs other)	0.89 (0.67–1.18)	0.43	1.13 (0.83–1.55)	0.44
Diabetes mellitus	1.74 (1.40–2.17)	<0.0001	1.02 (0.76–1.38)	0.90
Previous cardiovascular event	1.19 (0.93–1.53)	0.17	0.82 (0.62–1.09)	0.18
Smoking status (past vs none)	1.39 (1.11–1.73)	0.004	1.16 (0.90–1.50)	0.25
Smoking status (active vs none)	0.89 (0.65–1.22)	0.46	0.94 (0.66–1.33)	0.73
mGFR, per −10 mL/min per 1.73 m^2	1.08 (1.03–1.14)	0.0042	1.00 (0.99–1.00)	0.39
Log albuminuria, mg/mmol creatinine	1.19 (1.13–1.26)	<0.0001	1.27 (1.19–1.36)	<0.0001
Type of nephropathy				
Diabetic	2.58 (1.81–3.69)	<0.0001	2.13 (1.19–3.83)	0.011
Glomerular	0.66 (0.46–0.93)	0.018	0.77 (0.45–1.31)	0.33
Vascular	1.41 (1.09–1.82)	0.009	1.40 (0.88–2.23)	0.15
Polycystic	0.76 (0.48–1.20)	0.25	1.11 (0.61–2.03)	0.73
Interstitial	1 (Reference)	…	1 (Reference)	…
Other or unknown	0.87 (0.58–1.31)	0.51	0.99 (0.62–1.57)	0.96
No. of antihypertensive treatments	1.08 (1.00–1.16)	0.039	0.93 (0.84–1.04)	0.19
Diuretic	0.90 (0.74–1.11)	0.33	1.01 (0.75–1.35)	0.97
Aldosterone blocker	2.64 (1.30–5.39)	0.008	0.45 (0.21–0.98)	0.046
[Na], /mmol/L	1.02 (0.98–1.06)	0.27	1.32 (0.88–1.99)	0.18
[K], /mmol/L	0.92 (0.75–1.12)	0.41	0.78 (0.60–1.00)	0.049
[HCO_3−], /mmol/L	1.02 (0.98–1.05)	0.36	1.03 (0.99–1.07)	0.20
Plasma uric acid, /10 μmol/L	0.99 (0.99–1.00)	0.26	0.99 (0.98–1.00)	0.26
Ratio Na/K 24-h urine	1.00 (1.00–1.01)	0.627	1.00 (0.99–1.01)	0.77

Crude and adjusted ORs (95% CIs) of uncontrolled hypertension are indicated, as well as *P* values. ORs were adjusted for all covariates and recruitment site. Fully adjusted ORs for ECW expressed in L are shown in Table S3. BMI indicates body mass index; CI, confidence interval; ECW, extracellular water; mGFR, measured glomerular filtration rate; OR, odds ratio.

Discussion

In this analysis conducted in 2015 patients with CKD, stage 1 to 5, who underwent gold standard GFR and ECW measurements, we showed that ECW was an independent determinant of hypertension, uncontrolled hypertension, and apparent treatment-resistant hypertension. In addition, we identified that mGFR, BMI, ethnicity, male sex, and diabetes mellitus were significantly associated with apparent treatment-resistant hypertension but not uncontrolled hypertension, whereas age, albuminuria, and diabetic nephropathy were associated with both uncontrolled and resistant hypertension.

The prevalences of hypertension, uncontrolled hypertension, and apparent treatment-resistant hypertension are in the same range orders as in previous studies conducted in patients with CKD. In the CRIC (Chronic Renal Insufficiency Cohort) study conducted in 3612 outpatients recruited between 2003 and 2007, with an estimated GFR between 20 and 70 mL/min per 1.73 m^2,[6] prevalence of hypertension was 86% (versus 88% in our study); and in hypertensive patients, BP was controlled in 67% (versus 56% in our study). Likewise, in a primary care cohort of 10 040 patients with CKD, stage 3 to 5, conducted in Kent (UK) between 2004 and 2008, prevalence of hypertension was 84%, half of which were controlled[17]; and in

Table 6. Determinants of Apparent Treatment-Resistant Hypertension in the Patients With Hypertension and ECW Measurement (n=1190)

Variable	Crude OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
ECW, L	1.16 (1.12–1.20)	<0.0001	…	…
ECW ratio over theoretical ECW	1.19 (1.09–1.29)	<0.0001	1.12 (1.01–1.23)	0.026
Age, y	1.03 (1.02–1.04)	<0.0001	1.02 (1.01–1.03)	0.003
Sex (women vs men)	0.59 (0.46–0.75)	<0.0001	0.68 (0.50–0.93)	0.017
Ethnicity (African origin vs other)	1.79 (1.31–2.45)	0.0003	2.56 (1.74–3.76)	<0.0001
BMI 25–30 vs <25 kg/m^2	2.31 (1.74–3.06)	<0.0001	1.70 (1.23–2.35)	0.001
BMI ≥30 vs <25 kg/m^2	4.02 (2.93–5.51)	<0.0001	2.64 (1.83–3.81)	<0.0001
Diabetes mellitus	3.39 (2.63–4.38)	<0.0001	1.52 (1.07–2.16)	0.018
Previous cardiovascular event	2.14 (1.61–2.83)	<0.0001	1.29 (0.93–1.80)	0.12
Smoking status (past vs none)	1.31 (1.02–1.69)	0.038	0.97 (0.71–1.33)	0.86
Smoking status (active vs none)	0.83 (0.58–1.19)	0.31	0.74 (0.48–1.15)	0.18
mGFR, per −10 mL/min per 1.73 m^2	1.22 (1.14–1.30)	<0.0001	1.19 (1.10–1.29)	<0.0001
Log albuminuria, mg/mmol creatinine	1.24 (1.16–1.31)	<0.0001	1.19 (1.10–1.28)	<0.0001
Type of nephropathy		<0.0001		
Diabetic	23.8 (11.0–51.2)	<0.0001	9.03 (3.84–21.21)	<0.0001
Glomerular	3.10 (1.49–6.47)	0.003	3.01 (1.37–6.64)	0.006
Vascular	9.06 (4.52–18.1)	<0.0001	6.09 (2.90–12.77)	<0.0001
Polycystic	1.68 (0.70–4.05)	0.25	2.14 (0.84–5.46)	0.11
Interstitial	1 (Reference)	…	1 (Reference)	…
Other or unknown	3.97 (1.98–7.95)	0.0001	2.74 (1.30–5.81)	0.008
Ratio Na/K 24-h urine	1.01 (1.00–1.02)	0.025	1.00 (1.00–1.01)	0.40

Patients with unknown resistance status (uncontrolled hypertension and <3 drugs or at least 3 drugs without a diuretic) were excluded from this analysis. Crude and adjusted ORs (95% CIs) of apparent treatment-resistant hypertension are indicated, as well as *P* values. ORs were adjusted for all covariates and recruitment site. Fully adjusted ORs for ECW expressed in L are shown in Table S4. The secondary analysis conducted in all hypertensive patients is shown in Table S5. BMI indicates body mass index; CI, confidence interval; ECW, extracellular water; mGFR, measured glomerular filtration rate; OR, odds ratio.

participants with CKD from NHANES IV, hypertension was controlled (<140/90 mm Hg) in 56% of the subjects.[18]

Two definitions are encountered for resistant hypertension.[7] One definition is uncontrolled BP despite the use of at least 3 drugs, including a diuretic. Because we aimed for resistant hypertension to be a marker of severity, and not of hypertension control, we did the following: (1) chose the second definition of resistant hypertension (uncontrolled BP despite 3 drugs, including a diuretic, or the use of ≥4 drugs, including a diuretic, regardless of BP level); and (2) excluded patients with uncontrolled BP but inappropriate treatment from the main analysis. Among US adults from NHANES, 8.9% of hypertensive participants (12.8% of treated hypertensive participants) had resistant hypertension (defined as uncontrolled BP despite 3 different drug classes or the use of at least 4 antihypertensive drug classes regardless of BP, with no requirement for the use of a diuretic, although 86% of patients with resistant hypertension used a diuretic).[9] In 470 386 hypertensive individuals in the Kaiser Permanente Southern California health system, 12.8% (15.3% of those receiving medication) have resistant hypertension. The prevalence of resistant hypertension was much higher in our study (32% of hypertensive patients), as expected in patients with CKD. Indeed, studies conducted in patients with CKD found prevalences of resistant hypertension ranging from 11%[19] to 40%,[20] with an increasing prevalence as GFR decreases.[21] In the CRIC study, factors associated with resistant hypertension were age, male sex, black race, diabetes mellitus, higher BMI, lower GFR, and higher proteinuria, all also identified to be independent predictors of resistant hypertension in our study.

Comparison of the determinants associated with uncontrolled and resistant hypertension allowed us to define factors independently associated with the severity of hypertension (as assessed by the apparent treatment-resistant hypertension status), but not uncontrolled hypertension. Indeed, determinants of a more severe hypertension do not necessarily predict a poorer control, provided appropriate treatment is prescribed. This was the case for a more advanced kidney disease (lower mGFR), a higher BMI, African origin, male sex, and diabetes mellitus, all independently associated with

resistant hypertension, but not uncontrolled hypertension. Noteworthy, the lack of an association between GFR and BP control had previously been shown in the CRIC study[6] of patients with CKD as well as in NHANES.[18] As previously shown in the CRIC study cohort,[6] this likely reflects a more aggressive treatment in patients with a lower GFR, because 58% of the patients with mGFR between 15 and 30 mL/min per 1.73 m^2 received at least 3 antihypertensive drugs versus 39% of the patients with a GFR >60 mL/min per 1.73 m^2.

Therapeutic inertia (both for nutritional and pharmacological treatment) might be a cause of poorly controlled BP. Sodium intake, estimated from 24-hour urinary sodium excretion, was 3.4 g/d, hence above the recommended intake of 1.5 to 2 g/d,[22,23] despite the well-described salt sensitivity of BP in patients with CKD.[24–26] In addition, 44% of the patients with uncontrolled BP received <3 drugs, suggesting that therapeutic inertia might be a more common cause of poorly controlled BP than resistant hypertension, as previously highlighted in NHANES.[9]

Increased sympathetic and renin-angiotensin system activities, endothelial dysfunction, and increased arterial stiffness are among the multiple mechanisms that contribute to the pathogenesis of hypertension during CKD.[27] Another key pathophysiological factor is altered renal sodium excretion, leading to fluid retention.[27] ECW has been shown to increase during CKD, even in the early stage of the disease,[11,28,29] and is thought to play a crucial role in the development of hypertension in this population.[30–32] However, no large study on the factors associated with hypertension in CKD ever relied on gold standard measurement of ECW, based on isotope dilution, because this technique is not routinely available. In our large cohort of patients with CKD, ECW, measured as the volume of distribution of ^{51}Cr-EDTA, was independently associated with hypertension, uncontrolled hypertension, and apparent treatment-resistant hypertension, after adjustment for multiple potentially confounding variables, including BMI, albuminuria, urinary sodium excretion, and plasma sodium concentration. Interestingly, BMI was not independently associated with hypertension when absolute ECW, instead of its ratio over theoretical ECW, was entered in the model. Similar findings were reported in 40 patients with CKD who underwent 24-hour ambulatory BP measurement and total body water assessment with bioelectrical impedance, suggesting that BMI was less involved in BP control when body water imbalance was entered in the model.[12] Likewise, male sex was no longer associated with resistant hypertension when absolute ECW was entered in the model, suggesting that increased ECW in men may contribute to the severity of hypertension. The ratio of ECW over theoretical ECW was chosen for the main analysis because the absolute value of ECW is strongly correlated with anthropometric parameters. In addition, although one ought to be careful when interpreting these observational data, it is of interest to note the aldosterone blockers were significantly associated with hypertension control, although the rate of antialdosterone treatment was low because of a cohort recruited since 2000. Previous reports have shown the beneficial effect of aldosterone antagonists in patients with CKD.[33–35] Likewise, a randomized trial conducted in patients with resistant hypertension[36] showed that an approach based on combined diuretics was more efficient in controlling BP than an approach based on sequential blockade of the renin-angiotensin system, and the recent randomized studies, PATHWAY-2 (Prevention and Treatment of Hypertension With Algorithm based Therapy-2) and ReHOT (Resistant Hypertension Optimal Treatment), demonstrated that spironolactone was the most efficient fourth-line treatment in resistant hypertension.[37,38] The key role of ECW reduction through sodium restriction[25,39] or diuretic treatment[31,40] for hypertension control in CKD has been shown by previous studies. Altogether, these data suggest the need for a larger use of diuretics, including aldosterone antagonists, in hypertensive patients with CKD.

Strengths of our study include the quality of GFR and ECW assessment, measured with renal clearance of ^{51}Cr-EDTA and determination of the volume of distribution of the tracer, respectively; hence, these are gold standard methods rarely available in large cohorts. In addition, analyses were adjusted for multiple confounding factors, including plasma sodium and potassium, which are often overlooked, although they are highly linked with ECW and should be considered when studying the association between ECW and BP.[41]

Our study has several limitations. First, it is an observational study with no predefined guidelines about patient care and antihypertensive treatment. On the other hand, information obtained in real-life conditions is complementary to data obtained in the controlled and standardized conditions of a randomized trial. Furthermore, our analysis was based on office BP measurement during a single visit. Repeated office measurements or, ideally, out-of- office measurements, such as ambulatory BP measurements, would have provided a higher diagnosis accuracy, and in particular would have helped identifying patients with pseudoresistant hypertension. Finally, because of the initial recruitment of this cohort (ie, patients with CKD referred by their nephrologist for an extensive workup), we can only study factors associated with prevalence, not incidence, of hypertension, uncontrolled BP, and resistant hypertension in patients with CKD.

Appendix

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Sources of Funding

The NephroTest chronic kidney disease cohort study is supported by the following grants: INSERM GIS-IReSP AO 8113LS TGIR, French Ministry of Health AOM 09114, INSERM AO 8022LS, Agence de la Biomédecine R0 8156LL, AURA and Roche 2009-152-447G. Hôpitaux Paris Nord Val de Seine provided financial support for publication fees.

Disclosures

None.

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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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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 51Cr-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^2 and mean ECF 15.1 L/1.73m^2. 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^2 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^2 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^2 increase 0.92, [0.73; 1.16]), below 15L/1.73m^2, but significant (1.28; [1.14-1.45]) above 15L/1.73m^2. 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.

Kidney International (2019) **96,** 1020–1029; https://doi.org/10.1016/j.kint.2019.06.017

KEYWORDS: chronic kidney disease; extracellular fluid volume; sodium; volume overload

Impaired renal salt and water excretion, in combination with other factors such as hypo-albuminemia, often result in chronic ECF overload with CKD.[1] In hemodialysis patients, several large-scale studies have shown that fluid overload is a strong and independent risk factor for mortality.[2,3] In contrast, studies evaluating the role of fluid overload on renal function and mortality in patients with non-dialysis CKD yielded conflicting results.[4–6] In addition, ECF was estimated using single- or multi-frequency bioelectrical impedance analysis, and not isotope dilution, which is the most direct and accurate method, although not routinely available.[7–10] Similarly, 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 ESKD, and with mortality occurring before ESKD, in non-dialysis CKD patients, using the "gold-standard" measurements of ECF and GFR.

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Received 22 February 2019; revised 14 May 2019; accepted 7 June 2019; published online 16 July 2019

RESULTS

Baseline characteristics

A total of 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 ± 15.1 years; 66.7% were men; 87.8% had a history of hypertension; and 27.0% had diabetes. Mean mGFR was 43.6 ± 18.6 ml/min per 1.73 m^2. Mean ECF was 15.1 ± 2.2 L/1.73 m^2. Distribution of ECF, crude or scaled to body surface area (BSA), according to sex, is shown in Supplementary Figure S1. ECF, anthropometric parameters, and diuretic prescription, for GFR subgroups, are reported in Supplementary Figure S2. BSA and crude ECF decreased as GFR decreased, so the relationship between ECF and mGFR was attenuated when ECF was scaled to BSA: ECF decreased slightly from 15.7 to 14.5 L/1.73 m^2 when GFR decreased from >60 ml/min to 15–30 ml/min. Loop diuretics increased from 16% in patients with GFR >60 ml/min to 43% in patients with GFR of 15–30 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 higher systolic and diastolic blood pressure (BP), body mass index, mGFR, and urinary protein-to-creatinine ratio (uPCR), a higher 24-hour urine sodium excretion, and a lower plasma albumin concentration (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 (interquartile range: 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 causes). Cumulative incidence of death at 5 years was significantly higher in the third tertile of ECF (3.8%, 95% confidence interval [CI] [2.3, 5.9]; 5.4%, 95% CI [3.5, 7.8], and 13.2%, 95% CI [10.2, 16.6], for the 1st, 2nd, and 3rd tertile, respectively, $P < 0.001$), with a similar pattern for cardiovascular mortality (Supplementary Figure S3), whereas no difference was observed across tertiles of ECF for ESKD occurrence (Figure 1). Penalized splines representing the relationship between ECF and adjusted hazard ratios (HRs) 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/1.73 m^2 (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/1.73 m^2 increase of ECF: 1.14, 95% CI [1.07, 1.21], $P < 0.001$), and with mortality before ESKD above a threshold of 15 L/1.73 m^2 (adjusted HR per 1 L/1.73 m^2 increase of ECF below 15 L/1.73 m^2, 0.92, 95% CI [0.73, 1.16], $P = 0.49$, and above 15 L/1.73 m^2, 1.28, 95% CI [1.14, 1.45], $P < 0.001$) (Table 2). When the model was adjusted for systolic BP as a time-varying covariate, the association between ECF and ESKD was slightly weaker (HR per 1 L/1.73 m^2 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, BP, diabetes, age, sex, or mGFR (P values for interaction tests nonsignificant). Similar results were observed using ECF categorized in tertiles (Supplementary Figure S4), stratification for baseline estimated glomerular filtration rate instead of baseline mGFR (Supplementary Table S2), and multiple imputations for missing data (Supplementary Table S2). Similar associations were also observed when ECF was expressed as a 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 2 consecutive visits 1.1 [IQR: 1.0–1.5 years]) showed that the mean mGFR slope was –1.64, 95% CI (–1.82, –1.45) ml/min per year in the total population, and –1.31, 95% CI (–1.60, –1.01) ml/min per year; –1.49, 95% CI (–1.81, –1.17) ml/min per year, and –2.28, 95% CI (–2.63, –1.92) ml/min per 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 1 L/1.73 m^2 increase in ECF: –0.14, 95% CI [–0.23, –0.05] ml/min per 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 Chronic Kidney Disease–Epidemiology Collaboration (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 (nonlinear association, with an increasing risk as ECF increased above a threshold of 15 L/1.73 m^2). These findings are robust because they rely on gold-standard methods for ECF and GFR measurement[9–15] in a large cohort of nearly 1600 carefully phenotyped patients, and analyses were adjusted for multiple potential confounders. In addition, we found 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 to date, recently published, Bansal *et al.*[4] showed in 3751 patients from the chronic renal insufficiency cohort (CRIC) that a shorter vector length—a bioelectrical impedance

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

		ECF (L/1.73 m^2)			
	Total n = 1593	1st tertile n = 531 [8.88–14.03]	2nd tertile n = 531 [14.04–15.82]	3rd tertile n = 531 [15.83–23.29]	*P*
Demographics and clinical characteristics					
Age (yr)	58.8 ± 15.1	53.2 ± 15.2	58.8 ± 14.3	64.2 ± 13.6	<0.001
Sex (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^2)	1.83 (0.22)	1.73 (0.20)	1.83 (0.20)	1.92 (0.21)	<0.001
Body mass index (kg/m^2)	26.6 ± 5.1	24.7 ± 4.3	26.4 ± 4.4	28.7 ± 5.8	<0.001
Tobacco consumption					<0.001
Nonsmoker	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 (mm Hg)	135 ± 20.2	130 ± 18.6	135 ± 19.7	141 ± 20.8	<0.001
Diastolic blood pressure (mm Hg)	75 ± 11.5	74 ± 11.8	75 ± 11.9	76 ± 10.8	0.024
Elevated blood pressure (≥140 and/or 90 mm Hg)	573 (37.3)	144 (28.3)	187 (36.7)	242 (46.8)	<0.001
Medical history					
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)	
Treatment					
Number of antihypertensive drugs	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
Biological parameters					
eGFR CKD-EPI (ml/min per 1.73 m^2)[a]	45.9 ± 21.8	45.2 ± 23.2	46.1 ± 21.7	46.3 ± 20.4	0.668
mGFR (ml/min per 1.73 m^2)	43.6 ± 18.6	40.6 ± 17.9	44.5 ± 18.5	45.6 ± 19.0	<0.001
mGFR (ml/min per 1.73 m^2)					<0.001
mGFR ≥60	299 (18.8)	77 (14.5)	105 (19.8)	117 (22.0)	
45 ≤ mGFR < 60	366 (23.0)	108 (20.3)	129 (24.3)	129 (24.3)	
30 ≤ mGFR < 45	503 (31.6)	168 (31.6)	171 (32.2)	164 (30.9)	
15 ≤ mGFR < 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/1.73 m^2)	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/24 h)	155 ± 73.0	145 ± 71.7	153 ± 68.4	168 ± 77.3	<0.001
24-h urinary potassium excretion (mmol/24 h)	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

ACEi, angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blocker; CKD-EPI, Chronic Kidney Disease–Epidemiology Collaboration; ECF, extracellular fluid volume; eGFR: estimated glomerular filtration rate; mGFR: measured glomerular filtration rate;
[a]Calculated using the CKD-EPI formula.
Continuous data are expressed as mean ± SD; categorical data are expressed as 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 total cholesterol >6 mmol/L or >5 mmol/L in case of a previous cardiovascular event.

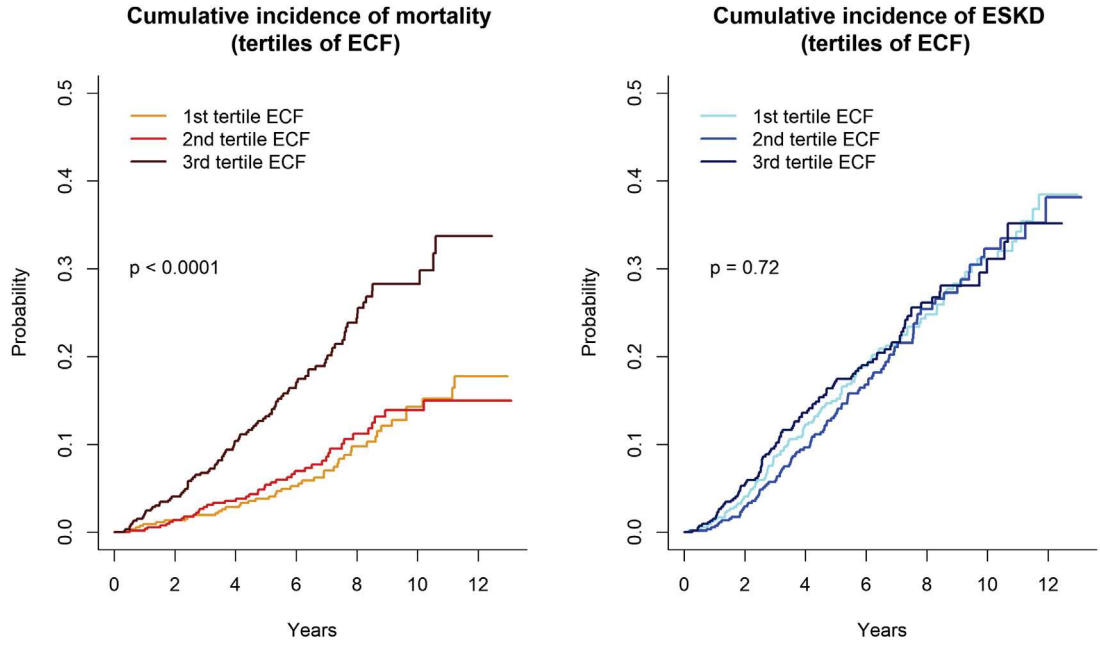

Figure 1 | Cumulative incidence rates of mortality and end-stage kidney disease according to tertiles of extracellular fluid volume (ECF). ESKD, end-stage kidney disease.

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. In contrast, all previous pioneer works evaluating the prognostic role of ECF in patients with non-dialysis CKD showed that fluid overload was associated with mortality and/or CKD progression. However, they

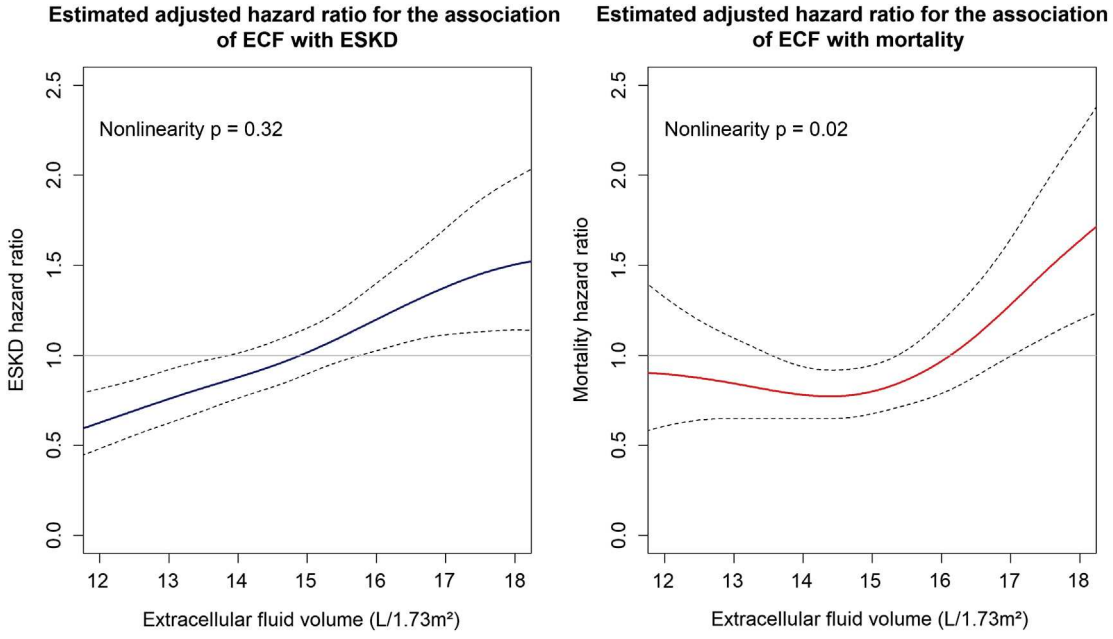

Figure 2 | Estimated adjusted hazard ratios and 95% confidence intervals for the association of ECF with end-stage kidney disease (ESKD) and with mortality, using penalized-splines estimators. Cause-specific Cox regression models were adjusted for the following covariates: age, sex, site of inclusion, 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/1.73 m^2).

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

Outcome	Models	HR [95% CI]	*P* value
ESKD			
n = 1593	Events (n)	324	
	Crude model	1.03 [0.98, 1.08]	0.29
	Adjusted model (1)[a]	1.14 [1.07, 1.21]	<0.001
	Adjusted model (2)[a]	1.10 [1.04, 1.17]	0.001
	Adjusted model (3)	1.12 [1.05, 1.19]	<0.001
Mortality			
≤15 L/1.73 m^2	Events (n)	63	
(n = 827)	Crude	1.00 [0.81, 1.24]	0.99
	Adjusted model	0.92 [0.73, 1.16]	0.49
>15 L/1.73 m^2	Events (n)	122	
(n = 766)	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

CI, confidence interval; ESKD, end-stage kidney disease; HR, hazard ratio.
[a]When the model was fully adjusted, but neither stratified nor adjusted for measured glomerular filtration rate, HR for ESKD was 0.98 (95% CI [0.92, 1.04], $P = 0.44$, showing that glomerular filtration rate is the covariate explaining that the crude analysis reveals no significant association between extracellular fluid volume and ESKD.
Crude and adjusted HRs are indicated for 1 L/1.73 m^2 increase in extracellular fluid volume. Analyses were adjusted for the following covariates: age, sex, 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. Model (1) was stratified for baseline measured glomerular filtration rate. Model (2) was adjusted for measured glomerular filtration rate (expressed as a continuous variable, as a time-dependent coefficient). Model (3) was adjusted for systolic blood pressure as a continuous time-varying covariate.

suffered from a number of limitations: they were conducted in smaller cohorts; they had composite endpoints; and bioelectrical impedance analysis (and not a gold standard such as isotope dilution) was used to assess fluid volume.[7,8,10] Thus, in a single-center cohort of 472 non-dialysis patients with CKD stage 4–5, Tsai *et al.*[6] 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 95% CI [–2.06, –0.13] ml/min per 1.73 m^2 per year in the third vs. first tertile), after a median follow-up of 17.3 months. 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.[5] 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.*[16] showed that patients with volume overload were at a higher risk for the composite endpoint of ESKD or decline in estimated GFR ≥50%, and for cardiovascular morbidity and mortality. Finally, in a retrospective cohort study of 149 patients with CKD followed for nearly 5 years, Tai *et al.*[17] showed that the ratio of ECF over expected total body water was independently associated with the combined endpoint of ESKD or GFR decline ≥50%. 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 1 L/1.73 m^2 increase in ECF was 0.14 ml/min per year. A 10% ECF increase in a patient with an ECF value of 15.1 L/1.73 m^2 (mean value in the cohort) would thus correspond to a yearly decrease in mGFR of 0.21 ml/min per 1.73 m^2. Mean mGFR slope in our population was –1.64 ml/min per year. The yearly mGFR loss attributable to solely age is estimated to be approximately 0.35 to 0.75 ml/min/1.73 m^2 in healthy subjects,[18] and in our cohort, the GFR decrease due to uncontrolled hypertension was 0.61 ml/min per 1.73 m^2, and that due to a higher (one log-unit) uPCR was 0.43 ml/min per 1.73 m^2. Therefore, the independent effect of ECF on

Table 3 | Decline of measured glomerular filtration rate according to ECF (n = 1593)

	Mean difference in mGFR slopes (ml/min per yr)			
	Model 0	Model 1	Model 2	Model 3
ECF analyzed as a continuous variable				
ECF (per 1 L/1.73 m^2)	–0.19 [–0.28, –0.10]	–0.20 [–0.29, –0.1]	–0.15 [–0.25, –0.06]	–0.14 [–0.23, –0.05]
Elevated blood pressure			–0.78 [–1.19, –0.37]	–0.61 [–1.01, –0.21]
Protein-to-creatinine ratio[a]				–0.43 [–0.55, –0.32]
ECF analyzed in tertiles				
ECF				
1st tertile [8.88–14.03]	Ref	Ref	Ref	Ref
2nd tertile [14.04–15.82]	–0.09 [–0.54, 0.35]	–0.10 [–0.55, 0.35]	0.02 [–0.43, 0.47]	–0.02 [–0.46, 0.41]
3rd tertile [15.83–23.29]	–0.86 [–1.34, –0.39]	–0.90 [–1.37, –0.42]	–0.72 [–1.20, –0.24]	–0.55 [–1.02, –0.08]
Elevated blood pressure			–0.85 [–1.25, –0.44]	–0.68 [–1.08, –0.29]
Protein-to-creatinine ratio[a]				–0.42 [–0.54, –0.31]

ECF, extracellular fluid volume; mGFR, measured glomerular filtration rate; Ref, referent.
[a]The indicated coefficient corresponds to a 2.72-fold increase in protein-to-creatinine ratio.
ECF was analyzed as a continuous variable (results are indicated per 1 L/1.73 m^2 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 per 1.73 m^2), and interaction terms between time and GFR and time and ECF. Model 1: Model 0 + all baseline covariates (age, sex, ethnicity, recruitment site, body mass index, diabetic status (no diabetes, diabetes without diabetic nephropathy, diabetes with diabetic nephropathy), elevated blood pressure (< or ≥140/90 mm Hg), 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: Model 1 + 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 per year.

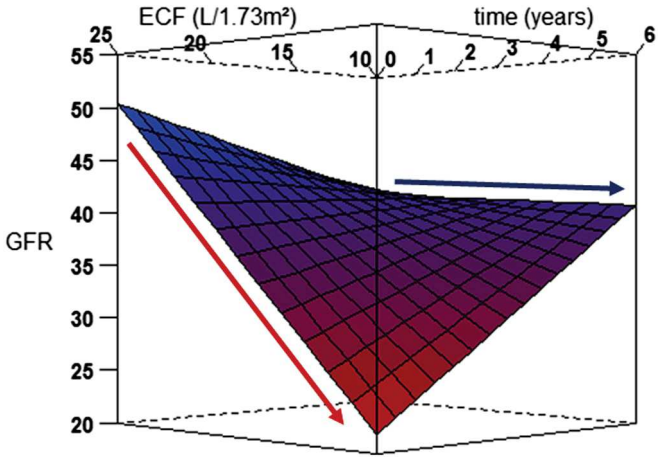

Figure 3 | Three-dimensional modeling of the decline of glomerular filtration rate (GFR) according to baseline extracellular fluid volume (ECF). Arrows indicate the expected GFR slopes for the highest (red arrow) and the lowest (blue arrow) ECF values. The *x*-axis indicates time (expressed in years);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

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 BP and proteinuria. In addition, ECF is also an independent determinant of uncontrolled hypertension[19] and is associated with increased proteinuria, both of which also contribute 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.[20] In addition, uPCR and low albumin concentration, both well-described prognostic factors for adverse outcome in patients with CKD,[21,22] are associated with increased ECF, as confirmed in the present study, possibly through epithelial sodium channel–mediated sodium retention.[22] Although the associations we observed were similar after adjustment for preexisting 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 a volume-dependent increase in BP 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 keeping with this possibility, 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.[23–26] 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 likelihood that the observed link between ECF and mortality and ESKD is, 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.[27] In addition, increased mean circulatory filling pressure associated with fluid overload may induce pressure-independent alterations of structure and function of large arteries.[6,28,29] 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, in both non-dialysis and hemodialysis patients.[29–31] However, our study was observational and by definition prone to confounding, as illustrated by the fairly low calculated E-values (see Methods)[32]; 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 should be paid to fluid status in future blood pressure targeted randomized trials. Indeed, for the Systolic Blood Pressure Intervention Trial (SPRINT),[33] one cannot rule out the possibility 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 level of salt intake is associated with mortality and cardiovascular events.[34,35] The association between salt intake and CKD progression is more controversial.[34,36] Interventional studies of low sodium intake[23,26,37–40] and/or diuretics[40,41] have shown a reduction of proteinuria and blood pressure. However, randomized controlled trials did not demonstrate that reduction of sodium intake, or diuretics—independent of blood pressure reduction—reduces cardiovascular disease and mortality or slows CKD progression.[36–38,42–44]

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, body mass index, BSA, and muscle mass, as assessed by 24-hour urinary creatinine excretion, as previously reported, including in the NephroTest cohort.[45,46] However, the positive association between ECF and GFR,

although markedly attenuated, remained after adjustment for BSA, at least in CKD stages 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.[6,16,17,29,41,47] Interpretation of ECF values in this population receiving optimized care is complex, as all patients are carefully followed up by nephrologists and are usually prescribed diuretics, with a marked increase in diuretic prescription when GFR decreased (Supplementary Figure S2).

Our study highlights ECF as 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, and weight variations are not sufficient for detection of small variations in ECF, such as those expected during non-dialysis CKD.[47] 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[26] and diuretic use,[41] which are the cornerstones of the treatment of fluid retention in CKD patients. Even though diuretic doses are not available in the NephroTest database, results of our study suggest that therapeutic inertia (for both pharmacologic 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 30 ml/min, were prescribed diuretics. Likewise, sodium intake, estimated from 24-hour urinary sodium excretion, was on average 155 mmol (3.6 g) per day, which is above the recommended intake, especially in patients with CKD.[48] In addition, 24-hour urinary sodium excretion increased with ECF, from 145 mmol/d in the first tertile to 168 mmol/d in the third tertile. Tight control and monitoring of sodium balance should be considered early in the course of CKD, in combination with other renoprotective 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 the gold-standard ECF and GFR measurements. Our study has some limitations. First, it was an observational study, which by definition is prone to confounding despite multiple adjustments. In addition, GFR and ECF were derived from the same 51Cr-EDTA clearance samples, which may generate a spurious relationship between the 2 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 estimated glomerular filtration rate was used instead of mGFR, making this hypothesis unlikely. Another limitation is that GFR slope analyses were based on a median of only 2 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 appropriate for analyzing the independent effect of GFR on ECF; in addition, due to the tight control of ECF in this population, this observational study may have 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 careful monitoring and maintenance of ECF in these patients is important, along with other hydro-electrolytic disturbance and cardiovascular risk factors. Nevertheless, future prospective interventional studies are needed to confirm the benefit of ECF control in CKD.

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 etiologies, from January 2000 to December 2012.[49] 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 who were 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 1 of the 3 renal physiology units for extensive workup 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 was determined by renal clearance of 51Cr-EDTA (GE Healthcare, Vélizy, France), as previously described.[50] Briefly, a single dose of 1.8–3.5 MBq of 51Cr-EDTA was injected intravenously. After allowing 1.5 hours for equilibration of the tracer in the ECF, urine was collected and discarded. Average renal 51Cr-EDTA clearance was then determined from the average of 6 consecutive 30-minute clearance periods. Blood was drawn at the midpoint of each clearance period. ECF was determined as the average of the 6 consecutive measurements, each of them being calculated as the remaining quantity of the tracer (injected quantity of 51Cr-EDTA [$Q_{injected}$] minus the excreted quantity of the tracer [$Q_{excreted}$]), divided by the extrapolated serum concentration of the

tracer at the corresponding time point. To take into account morphologic differences among patients, ECF was scaled to BSA, the latter being calculated using Dubois' formula.[51] ECF was calculated as follows:

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

ECF was analyzed both as a 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 preemptive renal transplantation. ESKD and vital status were obtained by record linkage with the French REIN Registry (Renal Epidemiology and Information Network, Paris, France) and the national RNIPP (Répertoire National d'Identification des Personnes Physiques) registry for identification of persons, respectively. Causes of death were obtained from the national CépiDc registry (Centre d'Épidémiologie sur les Causes Médicales de Décès—Epidemiology Center for Medical Causes of Death) using the CIM-10 (classification internationale des maladies) classification. For each death in the Nephrotest cohort, the main cause of death was adjudicated by 3 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 ± SD or median (interquartile range), as appropriate. Patients' characteristics were compared across tertiles of ECF using the χ^2 test or a 1-way analysis of variance, for qualitative and quantitative variables, respectively.

Determinants of ECF treated quantitatively were analyzed using a multivariable linear regression model (see methodologic details in the Supplementary Methods). Cumulative incidence curves were estimated with the Aalen-Johansen method ("cuminc" function of the R package "cmprsk") to take competing risks into account.[52] Crude and adjusted 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 a continuous variable versus spline function of ECF, using the likelihood ratio test. Adjustment covariates, selected *a priori* as potential confounders, included age, sex, ethnicity (African origin vs. others), site of recruitment, diabetic status (no diabetes, diabetes without diabetic nephropathy, diabetes with diabetic nephropathy), body mass index, elevated blood pressure (in 2 classes: $<$ or $\geq$140/90 mm Hg), 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[53]), 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. The 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 6 classes of mGFR: >60, 50–60, 40–50, 30–40, 20–30, and 15–20 ml/min per 1.73 m^2. Stratification for baseline estimated glomerular filtration rate 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 an mGFR time-dependent coefficient was used. Finally, sensitivity analyses incorporating systolic blood pressure as a continuous time-varying covariate were also performed. Interactions between ECF and age, sex, uPCR, blood pressure, and mGFR were tested.

Missing data were less than 5% (Supplementary Table S6) and were 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).[54] 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.[32]

Finally, the association between baseline ECF and mGFR decline (expressed in ml/min per year) was analyzed using a multivariable linear mixed-effect regression model with random intercept and slope ("lmer" function of the R package "lme4").[55–57] The main analysis was conducted in the total population (n = 1593), as a 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 2 mGFR measurements (n = 1009). Detailed methods and covariates are provided in the Supplementary Methods. 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.

Consent and ethics

All patients signed informed consent before inclusion in the cohort. The NephroTest study was approved by an ethics committee (Direction Generale 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; reference: DGRI CCTIRS MG/CP09.503, July 9, 2009).

APPENDIX

The NephroTest Study Group list of all investigators

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DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

The NephroTest CKD cohort study is supported by the following grants: INSERM GIS-IReSP AO 8113LS TGIR, French Ministry of Health

AOM 09114, Inserm AO 8022LS, Agence de la Biomédecine R0 8156LL, AURA, and Roche 2009-152-447G.

AUTHOR CONTRIBUTIONS

ALF, EVP, and GG conceived and designed the study, and analyzed and interpreted the data. ALF, GG, and MM performed statistical analyses and designed tables and figures. MF, FV, BS, MM, JPH, JJB, PH, and ET conceived and coordinated the Nephrotest study. ALF and EVP drafted the article. All the authors made critical revisions of the article 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/1.73 m^2).
Table S2. Cause-specific Cox regression models for the association of extracellular fluid volume (L/1.73 m^2) 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 sex.
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 material is linked to the online version of the paper at www.kidney-international.org.

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JIM Original Article

doi: 10.1111/joim.13151

Trajectory of extracellular fluid volume over time and subsequent risks of end-stage kidney disease and mortality in chronic kidney disease: a prospective cohort study

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Abstract. Faucon A-L, Leffondré K, Flamant M, Metzger M, Boffa J-J, Haymann J-P, Houillier P, Thervet E, Vrtovsnik F, Proust-Lima C, Stengel B, Vidal-Petiot E, Geri G (Université Paris-Saclay, Paris; Univ. Bordeaux, ISPED, Bordeaux; Hôpital Bichat and INSERM U1149, Paris; Université de Paris, Paris; Hôpital Tenon; Université Pierre et Marie Curie; Hôpital Tenon; Hôpital Européen Georges Pompidou and Centre de Recherche des Cordeliers; Hôpital Européen Georges Pompidou; Hôpital Bichat, Paris; Hôpital Ambroise Paré, Boulogne-Billancourt; Université Paris-Saclay, Université Versailles Saint Quentin en Yvelines, Versailles, France). Trajectory of extracellular fluid volume over time and subsequent risks of end-stage kidney disease and mortality in chronic kidney disease: a prospective cohort study (Original Article). *J Intern Med*, 2020;https://doiorg/10.1111/joim.13151

Background. Extracellular fluid volume (ECF) is independently associated with chronic kidney disease (CKD) progression and mortality in patients with CKD, but the prognostic value of the trajectory of ECF over time beyond that of baseline value is unknown.

Objectives. To characterize ECF trajectory and evaluate its association with the risks of end-stage kidney disease (ESKD) and mortality.

Methods. From the prospective tricentric NephroTest cohort, we included 1588 patients with baseline measured glomerular filtration rate (mGFR) $\geq$15 mL min^{-1}/1.73 m^2 and ECF measurement. ECF and GFR were measured repeatedly using the distribution volume and clearance of ^{51}Cr-EDTA, respectively. ESKD and mortality were traced through record linkage with the national registries. Adjusted shared random-effect joint models were used to analyse the association between the trajectory of ECF over time and the two competing outcomes.

Results. Patients were mean age 58.7 years, 66.7% men, mean mGFR of 43.6 $\pm$ 18.6 mL min^{-1}/1.73 m^2 and mean ECF of 16.1 $\pm$ 3.6 L. Over a median follow-up of 5.3 [IQR: 3.0;7.4] years, ECF increased by 136 [95%CI 106;167] mL per year on average, whilst diuretic prescription and 24-hour urinary sodium excretion remained stable. ESKD occurred in 324 (20.4%) patients, and 185 (11.6%) patients died before ESKD. A higher current value of ECF was associated with increased hazards of ESKD (adjusted hazard ratio [aHR]: 1.12 [95%CI 1.06;1.18]; $P < 0.001$ per 1 L increase in ECF), and death before ESKD (aHR: 1.10 [95%CI 1.04;1.17]; $P = 0.002$).

Conclusions. The current value of ECF was associated with the risks of ESKD and mortality, independent of multiple potential confounders, including kidney function decline. This highlights the need for a close monitoring and adjustment of treatment to avoid fluid overload in CKD patients.

Keywords: chronic kidney disease, extracellular fluid volume, volume overload, sodium, joint modelling, trajectory.

†These authors equally contributed to this work.

Abbreviations: BP, blood pressure; CKD, chronic kidney disease; ECF, extracellular fluid volume; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; GFR, glomerular filtration rate; mGFR, measured glomerular filtration rate.

Introduction

An increased extracellular fluid volume (ECF) has been shown to be associated with chronic kidney disease (CKD) progression [1, 2], end-stage kidney disease (ESKD) [1–3] and mortality [1, 4, 5] in patients with nondialysis CKD. Targeting an optimal hydration status is important in the clinical management of patients with CKD and may be achieved by detecting early fluid overload, decreasing sodium intake [6, 7] and adjusting diuretic treatment [8–10]. However, diuretics may be inadequately prescribed, partly because of the fear of their potential detrimental effects on CKD progression [11]. This reinforces the need for a clear evaluation of the impact of ECF variations over time on CKD progression.

So far, studies evaluating the effect of fluid overload during CKD were based on a single measurement of ECF at a given time-point, but not on ECF changes, whilst hydration status may vary over time according to kidney disease progression and diuretic prescription. This may have led to a misevaluation of its exact impact on CKD progression and mortality.

The aim of the present study was to characterize the pattern of ECF changes over time and to evaluate its association with the risks of ESKD and death in a large cohort of patients with CKD.

Patients and methods

Study design and population

The NephroTest study is a prospective hospital-based cohort which enrolled 2084 adult patients with CKD of all stages and of various aetiologies, referred by nephrologists for annual work-up to one of the three renal physiology units (Tenon, Bichat and Georges Pompidou Hospitals) of Paris, France, between January 2000 and December 2012. Pregnancy, a past history of kidney transplantation, and dialysis were exclusion criteria. All patients with missing ECF measurement at baseline ($n = 293$), or with a baseline measured glomerular filtration rate (mGFR) <15 mL min^{-1}/1.73 m^2 ($n = 103$), or who were censored for the events at baseline ($n = 100$), were excluded from the study, leaving 1588 patients for the present analysis (Figure S1).

Data collection

A detailed description of the data collected during each visit has been previously reported [12]. Briefly, patients underwent extensive work-up during a 5-hour in-person visit, including GFR and ECF measurements at baseline and each follow-up visit. 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, treatment, anthropometric data and a large set of clinical and laboratory variables were collected.

GFR and ECF measurements

ECF and GFR were measured using the distribution volume and renal clearance of ^{51}Cr-EDTA (GE Healthcare, Vélizy, France), respectively, as previously described [1, 13]. Briefly, a single dose of 1.8–3.5 MBq of ^{51}Cr-EDTA was injected intravenously. After allowing 1.5 h for equilibration of the tracer in the ECF, patients were asked to void. Urine obtained after this equilibrium time, and all subsequent urine samples were weighed and sent for radioactivity measurement. After the equilibrium period, renal ^{51}Cr-EDTA clearance was determined from the average of six consecutive 30-minute urinary clearance periods. Blood samples were drawn at the midpoint of each period. ECF was calculated as the remaining quantity of the tracer divided by the extrapolated serum concentration of the tracer at the corresponding time-point at each bladder voiding time and averaged [1].

Outcomes

The studied outcomes were ESKD, defined by initiation of maintenance dialysis or pre-emptive kidney transplantation, and death occurring before ESKD. Events were identified either from medical records or through record linkage with the National *Renal Epidemiology and Information Network* (REIN) and death (*National Directory for the Identification of Natural Persons* [RNIPP], maintained by

National Institute for Statistics and Economic Studies [INSEE], France) registries until 31 December 2013.

Statistical analyses

Qualitative and quantitative variables were reported as percentages, and mean ± SD (or median [interquartile range, IQR] as appropriate), respectively. Baseline characteristics between patients with and without available baseline ECF measurement were compared and reported in Table 1. Probabilities of ESKD and death from inclusion into the cohort were estimated using the Aalen–Johansen estimator to account for competing risks [14].

To estimate the effect of ECF trajectory over time on both ESKD and mortality risks, a joint model for competing time to events with shared random effects was used [15]. The joint model was made of a longitudinal part (modelling individual trajectories of ECF) and a survival part (modelling the hazards of ESKD and death simultaneously estimated). A detailed description of the methodology is provided in the Data S1. Briefly, the longitudinal part was modelled using a linear mixed model with a linear function of time since the first ECF measurement. Individual correlated random effects on the intercept, and the slope captured the intra-individual correlation. More flexible functions of ECF trajectories over time were explored but did not provide a better fit to data according to the Akaike information criterion. Three linear mixed-effect models were performed: crude model without any adjustment for covariates (model 0); model 1, in which intercept was adjusted for baseline age, gender, recruitment site, diabetic status (no diabetes, diabetes with or without diabetic nephropathy), body mass index, blood pressure (< or ≥140/90 mmHg), mGFR level (≥45, 30–45, <30 mL min^{-1}/1.73 m^2) and urinary protein-to-creatinine ratio (log-transformed); and model 2 was made of model 1 plus an interaction term between time and mGFR. The linear mixed model requires neither the same number of ECF measurements per patient, nor that these measurements are taken regularly or at the same time-points for all patients. It also allows accounting for patients with only one ECF measurement at baseline (one visit) to reduce selection bias under the missing-at-random assumption. Indeed, because most patients with a single measurement have higher baseline ECF value and are more rapidly dialysis-dependent after inclusion, excluding these patients from the analysis may induce an underestimation of the mean baseline ECF level in the population. Therefore, we analysed data for all 1588 patients, including 584 patients with a single measurement of ECF.

The survival part of the joint model was made of a cause-specific proportional hazard model using the time since inclusion into the cohort as the time axis. Adjustment covariates of the survival model, selected *a priori* as potential confounders, included baseline age, gender, ethnicity (sub-Saharan African origin *versus* others), recruitment site, diabetes status (no diabetes, diabetes with or without diabetic nephropathy), previous cardiovascular events (defined as history of myocardial infarction or percutaneous coronary intervention or coronary artery bypass grafting or heart failure or stroke), body mass index (BMI), blood pressure (< or ≥140/90 mmHg), mGFR (≥60, 45–60, 30–45, <30 mL min^{-1}/1.73 m^2), plasma albumin concentration, urinary protein-to-creatinine ratio (uPCR, log-transformed), 24-h urinary sodium excretion and medication (diuretic and renin–angiotensin system inhibitor). The proportional hazards assumption was checked using Schoenfeld residuals. As missing data were less than 5% (Table 1), single imputations for missing adjustment data (but neither for ECF nor outcomes) were performed.

Subsequently, joint model was performed to relate patient-specific trajectory to his/her prognosis. The effect of the current value of ECF –'current' meaning at the time of hazard assessment – on the hazard of ESKD and death was estimated with and without adjustment for the current slope in ECF. B-spline function with 5 internal nodes was used to approximate the baseline hazard. Estimates from the joint model were then exploited to illustrate the results of the joint model in terms of dynamic prediction of both ESKD and death before ESKD, using four simulated longitudinal profiles of ECF (normal ECF value and low slope, normal ECF value and high slope, high ECF value and low slope, high ECF value and high slope). The goodness of fit of both the longitudinal and survival parts of the joint model was checked (Data S1). Finally, assumption of log-linearity in the relationship between ECF and risks of events was verified. In the absence of software handling nonlinear effect of the longitudinal biomarker on the hazard of events in joint models, we considered two 'standard' cause-specific hazards models (for each outcome) with ECF treated as a time-varying

Table 1. *Comparison of the baseline characteristics of the patients with and without valid baseline extracellular fluid volume measurement*

	Patient with baseline ECF measurement (n = 1588)		Patients without baseline ECF measurement (n = 262)		
	n	Mean ± SD or n (%)	n	Mean ± SD or n (%)	P-value
Demographics and clinical characteristics					
Age (years)	1588	58.74 ± 15.1	262	58.5 ± 16.1	0.78
Gender (men, %)	1588	1058 (66.7)	262	188 (71.8)	0.12
Ethnicity (sub-Saharan African origin, %)	1516	217 (14.3)	250	21 (8.4)	0.02
Weight (kg)	1588	74.6 ± 16.3	262	75.0 ± 16.9	0.66
Height (cm)	1588	167 ± 9.4	262	168 ± 9.8	0.05
Body mass index (kg m^{-2})	1588	26.6 ± 5.1	262	26.4 ± 5.1	0.49
Tobacco consumption	1588		262		0.22
Non smoker (n, %)	–	868 (54.7)	–	130 (49.6)	
Former smoker (n, %)	–	506 (31.9)	–	88 (33.6)	
Current smoker (n, %)	–	214 (13.5)	–	44 (16.8)	
Systolic blood pressure (mmHg)	1532	135 ± 20.2	255	137 ± 20.6	0.36
Diastolic blood pressure (mmHg)	1532	75 ± 11.5	255	74 ± 12.2	0.55
Medical history					
Hypertension (n, %)	1588	1395 (87.8)	262	232 (88.5)	0.82
Diabetes mellitus (n, %)	1587	426 (26.8)	262	77 (29.4)	0.43
No diabetes (n, %)	–	1161 (73.2)	–	185 (70.6)	
Diabetes without diabetic nephropathy (n, %)	–	275 (17.3)	–	44 (16.8)	
Diabetes with diabetic nephropathy (n, %)	–	151 (9.5)	–	33 (12.6)	
Dyslipidaemia (n, %)	1532	276 (18.0)	254	62 (24.4)	0.02
Previous cardiovascular event (n, %)	1558	287 (18.4)	255	44 (17.3)	0.72
Underlying renal disease (n, %)	1588		262		0.12
Diabetic nephropathy (n, %)	–	151 (9.5)	–	33 (12.6)	
Glomerular (n, %)	–	224 (14.1)	–	42 (16.0)	
Vascular (n, %)	–	410 (25.8)	–	68 (26.0)	
Polycystic kidney disease (n, %)	–	100 (6.3)	–	9 (3.4)	
Interstitial (n, %)	–	150 (9.4)	–	16 (6.1)	
Other or unknown conditions (n, %)	–	553 (34.8)	–	94 (35.9)	
Treatment					
Diuretics (n, %)	1586	755 (47.6)	262	113 (43.1)	0.20
Loop diuretic (n, %)	1586	452 (28.5)	262	69 (26.3)	0.52
Thiazide diuretic (n, %)	1586	323 (20.4)	262	49 (18.7)	0.59
Amiloride (n, %)	1586	17 (1.1)	262	3 (1.1)	1.00
Aldosterone antagonist (n, %)	1586	43 (2.7)	262	6 (2.3)	0.85
Number of antihypertensive drugs	1586	2.3 ± 1.6	262	2.2 ± 1.5	0.28
ACEi and/or ARB (n, %)	1588	1182 (74.4)	262	187 (71.4)	0.33

Table 1 (*Continued*)

	Patient with baseline ECF measurement (n = 1588)		Patients without baseline ECF measurement (n = 262)		
	n	Mean ± SD or n (%)	n	Mean ± SD or n (%)	P-value
Biological parameters					
eGFR CKD–EPI (mL min^{-1}/1.73 m^2)	1588	45.9 ± 21.8	262	45.1 ± 22.6	0.59
mGFR (mL min^{-1}/1.73 m^2)	1588	43.6 ± 18.6	262	43.2 ± 19.5	0.72
mGFR (mL min^{-1}/1.73 m^2)	1588		262		0.27
≥60	–	298 (18.8)	–	49 (18.7)	
[45–60[	–	366 (23.0)	–	47 (17.9)	
[30–45[	–	499 (31.4)	–	93 (35.5)	
[15–30[	–	425 (26.8)	–	73 (27.9)	
Measured ECF (L)	1588	16.1 ± 3.6	0	–	–
Measured indexed ECF (L/1.73 m^2)	1588	15.1 ± 2.2	0	–	–
Plasma albumin (g L^{-1})	1546	39.5 ± 4.4	251	38.9 ± 4.8	0.04
24-h urinary sodium excretion (mmol/24 h)	1439	155 ± 73.1	197	164 ± 86.3	0.10
24-h urinary potassium excretion (mmol/24 h)	1438	65 ± 26.2	197	69 ± 31.8	0.09
24-h urinary creatinine excretion (mmol/24 h)	1526	11.6 ± 4.6	247	11.9 ± 4.4	0.27
Protein-to-creatinine ratio (mg mmol^{-1})	1521	24 [11; 80]	242	37 [13; 123]	0.11

Continuous and categorical data are expressed in mean ± SD (or median [IQR]) and n (%), respectively. Diabetes was either self-reported or defined as fasting glycaemia ≥ 7 mmol L^{-1} or antidiabetic drug treatment. Previous cardiovascular event was defined as a history of myocardial infarction or percutaneous coronary intervention or coronary artery bypass grafting or heart failure of stroke. Dyslipidaemia was defined as a total cholesterol >6 mmol L^{-1} or >5 mmol L^{-1} 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.

covariate with penalized spline with four degrees of freedom. Although these hazards models do not account for measurement errors of biomarker, as opposed to the joint model, and assume that the value of biomarker remain constant between two consecutive observed measurements of biomarker [16], they remain relevant for specifically investigating the linearity assumption.

Sensitivity analyses using ECF scaled to body surface area (rather than crude ECF), or using baseline estimated GFR (eGFR, calculated using the Chronic Kidney Disease – Epidemiology Collaboration [CKD-EPI] formula) [17] instead of baseline mGFR were performed.

All statistical analyses were conducted using R 3.4 software. The *jointModel* function of the R package *JM* was used to perform joint models analyses [18].

Ethics statements

All patients signed informed consent before inclusion in the cohort. The NephroTest study was approved by the Ethics 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 9th, 2009).

Results

Baseline characteristics of the patients

Patients' mean age was 58.7 ± 15 years, 66.7% were men, 87.8% had history of hypertension, 26.8% had diabetes, 18.4% had previous cardiovascular event, and less than 4% of them had heart failure (Table 1). At baseline, mean mGFR was

43.6 ± 18.6 mL min^{-1}/1.73 m^2. Measured ECF was 16.1 ± 3.6 L (15.1 ± 2.2 L/1.73 m^2). Diuretic prescription gradually increased from 35.0% to 57.4% in patients with CKD stage 1 to 4. Mean 24-h urinary sodium excretion was 155 ± 73 mmol/24 h and was not significantly different across stages of CKD.

Longitudinal analyses

After a median follow-up of 5.3 [IQR: 3.0;7.4] years, 324 (20.4%) patients reached ESKD and 185 (11.6%) patients died before reaching ESKD. The median number of visits per patient was 2 [IQR: 1–4], and the median time interval between two consecutive visits was 1.1 [1.0; 1.5] year.

Analysis of the longitudinal part of the joint model showed that ECF increased on average by 136 [95%CI 106 to 167] mL per year (Fig. 1, Figure S2), whilst the rate of diuretic prescription and 24-hour urinary sodium excretion remained relatively stable over time (Fig. 1).

Probabilities of ESKD and death before ESKD in the first five years of follow-up were 15.3% [95%CI 13.4 to 17.2] and 7.4% [95%CI 6.1 to 8.9], respectively (Figure S3). A higher current value of ECF was significantly associated with increased hazard of ESKD (adjusted hazard ratio (aHR) per 1 L increase in ECF: 1.12 [95%CI 1.06 to 1.18], $P < 0.001$), and death before ESKD (aHR per 1 L increase in ECF: 1.10 [95%CI 1.04 to 1.17], $P = 0.002$). Associations between the current value of ECF and outcomes persisted after adjustment for the current slope in ECF (Table 2). Current slope of ECF was not significantly associated with the risks of ESKD and death before ESKD (Table 2). Figure S4 suggests that the effect of the current value of ECF on the hazards of both ESKD and death was roughly linear.

Sensitivity analyses

In sensitivity analyses, a higher current value of ECF scaled to body surface area was also significantly associated with increased hazard of ESKD (aHR per 1 L/1.73 m^2 increase in ECF: 1.23 [95% CI 1.13 to 1.33], $P < 0.001$) and death before ESKD (aHR per 1 L/1.73 m^2 increase in ECF: 1.23 [95% CI 1.11 to 1.36], $P < 0.001$), and persisted after adjustment for the slope steepness (Table 3). When joint models were adjusted on baseline eGFR (instead of baseline mGFR), both a higher current value and slope of ECF (scaled to body surface area or not) were significantly associated with increased hazard of ESKD, whereas a higher current value of ECF (scaled to body surface area or not) was associated with the risk of death before ESKD (Tables S1, S2).

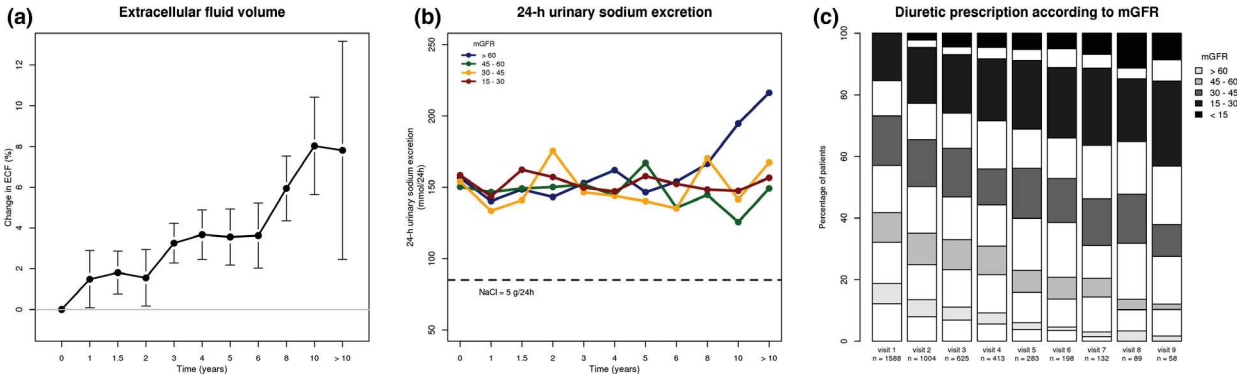

Fig. 1 *Change in extracellular fluid volume, 24-h urinary sodium excretion and diuretic prescription over time. Panel a represents the mean change in extracellular fluid volume (ECF) over time in the total population. ECF is expressed as the percentage change (with 95%CI) between the ECF measured at the visit n and that which was measured at the first visit (baseline). Time since the first visit is expressed in classes: 0, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and > 10 years. Panel b represents mean daily sodium chloride intake (assessed by 24-h urinary sodium excretion) over time. The black dashed line represents the maximal daily sodium chloride intake recommended by the KDIGO for patients with chronic kidney disease. Panel c represents the diuretic prescription across the first nine visits, according to stages of chronic kidney disease. Patients taking any diuretic are represented in grey (with different levels of grey according to mGFR); patients without diuretic prescription are represented in white. The percentage of patients taking any diuretic is indicated above each bar.*

Table 2. *Estimated association between extracellular fluid volume over time and hazards of end-stage kidney disease and death, using joint models*

	Model 0 HR [95%CI]	Model 1 HR [95%CI]	Model 2 HR [95%CI]
End-stage kidney disease (*n* events = 324)			
Considering only current value of ECF			
ECF value (per 1 L increase)	1.12 [1.06; 1.18]***	1.12 [1.06; 1.19]***	1.12 [1.06; 1.19]***
Considering both current ECF value and slope			
ECF value (per 1 L increase)	1.08 [1.01; 1.15]*	1.09 [1.00; 1.18]*	1.05 [0.93; 1.18]
ECF slope (per 100 mL year^{-1} increase)	1.44 [1.01; 2.03]*	1.28 [0.90; 1.82]	1.70 [0.94; 3.04]
Mortality (*n* events = 185)			
Considering only current value of ECF			
ECF value (per 1 L increase)	1.10 [1.04; 1.17]**	1.10 [1.03; 1.18]**	1.10 [1.03; 1.18]**
Considering both current ECF value and slope			
ECF value (per 1 L increase)	1.10 [1.03; 1.18]**	1.12 [1.03; 1.21]**	1.10 [1.01; 1.20]*
ECF slope (per 100 mL/year increase)	0.99 [0.76; 1.31]	0.91 [0.70; 1.18]	1.02 [0.71; 1.46]

P values: *<0.05, **<0.01, ***<0.001.
Extracellular fluid volume was expressed in litres. In all joint models, cause-specific proportional hazard sub-models were adjusted for baseline values of age, gender, ethnicity (sub-Saharan African origin versus others), recruitment site, body mass index, blood pressure (< or ≥140 and/or 90 mmHg), diabetic status (no diabetes, diabetes with or without diabetic nephropathy), previous cardiovascular event (history of myocardial infarction or percutaneous coronary intervention or coronary artery bypass grafting or heart failure or stroke), mGFR (≥60, 45–60, 30–45, <30 mL min^{-1}/1.73 m^2), plasma albumin concentration, urinary protein-to-creatinine ratio (log-transformed), 24-h urinary sodium excretion, diuretic and renin–angiotensin system inhibitor use.
Linear mixed sub-models:
Model 0: crude linear mixed-effect regression model, using both the intercept and the slope fitted as random effects.
Model 1: model 0 + baseline values of age, gender, recruitment site, diabetic status (no diabetes, diabetes with or without diabetic nephropathy), body mass index, blood pressure, protein-to-creatinine ratio (log-transformed) and mGFR (≥ 45, 45-30, <30 mL min^{-1}/1.73 m^2).
Model 2: model 1 + interaction term between time and mGFR.
ECF, extracellular fluid volume; mGFR, measured glomerular filtration rate; ESKD, end-stage kidney disease.

Dynamic prediction

Figures 2 and 3 depict the dynamic predicted estimated probabilities of ESKD and death for four simulated patients without event at 3-years of follow-up and having four different profiles of change in ECF in the first 3 years of follow-up (normal ECF value and low slope, normal ECF value and high slope, high ECF value and low slope, high ECF value and high slope). For example, for a 59-year old white man with CKD stage 3, the predicted probabilities of ESKD (Fig. 2) and death (Fig. 3) in the next five years (thus at eight years after inclusion) are 18.3% [95%CI: 12.2 to 26.8] and 4.0% [2.2 to 7.2] with the first profile, 21.1% [14.0 to 30.7] and 4.6% [2.5 to 8.2] with the second profile, 28.9% [18.7 to 44.2] and 5.9% [2.9 to 11.9] with the third profile, and 33.2% [20.0 to 51.1] and 6.5% [3.1 to 13.6] with the fourth profile of change in ECF over time, respectively.

Discussion

In this large prospective cohort study conducted in patients with CKD stage 1-4 who underwent gold-standard ECF and GFR measurements, we observed that the trajectory of ECF over time was independently associated with a higher risk of ESKD and death, even after adjustment for multiple potential confounding factors.

To our knowledge, the present study is the first one evaluating the association of the change in measured ECF over time with ESKD and mortality in CKD patients. Whilst several studies, including by our team, reported that a higher baseline ECF was

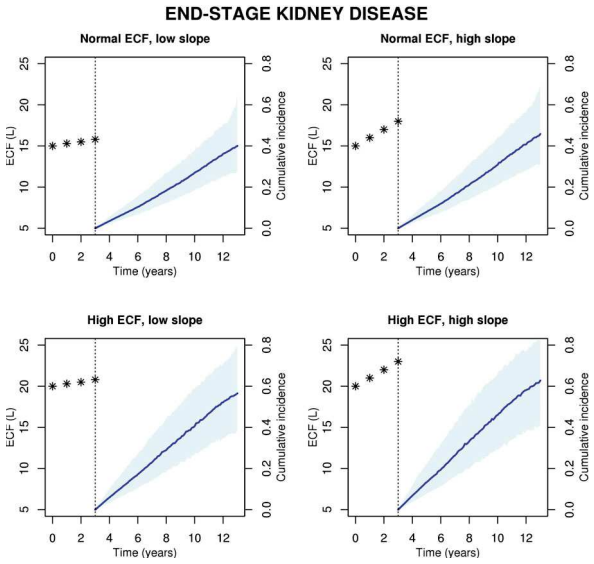

Fig. 2 *Individual dynamic predictions of the risk of end-stage kidney disease, after 3-year follow-up according to profiles of change in extracellular fluid volume over time. The figure represents the predicted individual probabilities (median with 95%CI) of end-stage kidney disease after three years of follow-up, according to four simulated profiles of ECF (represented by stars on the left side of each graph): normal ECF value and low slope, normal ECF value and high slope, high ECF value and low slope, and high ECF value and high slope. The probabilities of events were estimated from 6 months to 10 years after the last ECF measurement, and graphically represented in the right side of each panel.*

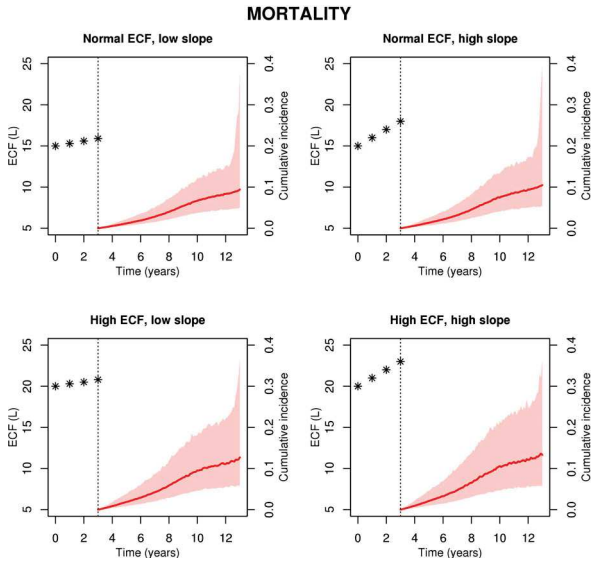

Fig. 3 *Individual dynamic predictions of the risk of death, after 3-year follow-up according to profiles of change in extracellular fluid volume over time. The figure represents the predicted individual probabilities (median with 95%CI) of death before end-stage kidney disease after three years of follow-up, according to four simulated profiles of ECF (represented by stars on the left side of each graph): normal ECF value and low slope, normal ECF value and high slope, high ECF value and low slope, and high ECF value and high slope. The probabilities of events were estimated from 6 months to 10 years after the last ECF measurement, and graphically represented in the right side of each panel.*

associated with mortality [1, 4, 5], CKD progression to ESKD [1–3, 5], and cardiovascular morbidity [4, 5, 19], none of them evaluated the prognostic value of the dynamic change in ECF over time. The repeated measurements better reflect the dynamic pattern of the disease progression than a single baseline assessment [20]. The recently developed joint modelling approach enables to accurately analyse the effect of the trajectory of a biomarker on a time to event by properly taking into account the fact that the biomarker is an internal and intermittently measured variable, potentially with measurement error, resulting in a higher precision in the estimation of the association than a standard survival model considering the biomarker as an external time-varying covariate [15, 20, 21].

Importantly, the observed association between ECF and outcomes persisted after adjustment for baseline mGFR, blood pressure [1, 22–25], proteinuria [26, 27], pre-existing cardiovascular comorbid conditions (including heart failure) [28] and sodium intake [29], which are known to be associated with CKD progression and/or mortality. Our findings suggest that an increase in ECF might lead *per se* to ESKD and death. The first hypothesis to explain the detrimental effects of fluid overload is that overhydration may increase renal venous pressure, alter renal microcirculation and thus decrease renal filtration, leading to sodium retention and initiating a vicious circle resulting in kidney function decline [30]. Furthermore, fluid overload induces an increase in intraglomerular pressure which leads to glomerulosclerosis and kidney disease progression. Another hypothesis is that positive fluid balance may induce pressure-independent alterations of structure and function of large arteries [2, 31–33], which are independently associated with both atherosclerosis and CKD progression [34]. Finally, previous studies

Table 3. *Estimated association between extracellular fluid volume scaled to body surface area over time and hazards of end-stage kidney disease and death, using joint models*

	Model 0 HR [95%CI]	Model 1 HR [95%CI]	Model 2 HR [95%CI]
End-stage kidney disease (*n* events = 324)			
Considering only current value of ECF			
ECF value (per 1 L/1.73 m^2 increase)	1.23 [1.13; 1.33]***	1.25 [1.14; 1.38]***	1.25 [1.14; 1.38]***
Considering both current ECF value and slope			
ECF value (per 1 L/1.73 m^2 increase)	1.21 [1.11; 1.32]***	1.25 [1.14; 1.38]***	1.26 [1.14; 1.40]**
ECF slope (per 100 mL/1.73 m^2 $year^{-1}$ increase)	1.22 [0.96; 1.55]	1.08 [0.83; 1.40]	1.27 [0.95; 1.71]
Mortality (*n* events = 185)			
Considering only current value of ECF			
ECF value (per 1 L/1.73 m^2 increase)	1.23 [1.11; 1.36]***	1.26 [1.13; 1.42]***	1.27 [1.13; 1.42]***
Considering both current ECF value and slope			
ECF value (per 1 L/1.73 m^2 increase)	1.25 [1.12; 1.39]***	1.27 [1.12; 1.43]***	1.27 [1.13; 1.42]***
ECF slope (per 100 mL/1.73 m^2 $year^{-1}$ increase)	0.86 [0.67; 1.10]	0.81 [0.64; 1.01]	0.86 [0.67; 1.11]

P values: *< 0.05, **< 0.01, ***< 0.001.
Extracellular fluid volume was scaled to body surface area and expressed in L/1.73 m^2. In all joint models, cause-specific proportional hazard sub-models were adjusted for baseline values of age, gender, ethnicity (sub-Saharan African origin versus others), recruitment site, body mass index, blood pressure (< or ≥140 and/or 90 mmHg), diabetic status (no diabetes, diabetes with or without diabetic nephropathy), previous cardiovascular event (history of myocardial infarction or percutaneous coronary intervention or coronary artery bypass grafting or heart failure or stroke), mGFR (≥60, 45–60, 30–45, <30 mL min^{-1}/1.73 m^2), plasma albumin concentration, urinary protein-to-creatinine ratio (log-transformed), 24-h urinary sodium excretion, diuretic and renin–angiotensin system inhibitor use.
Linear mixed regression sub-models:
Model 0: crude linear mixed-effect regression model, using both the intercept and the slope fitted as random effects.
Model 1: model 0 + baseline values of age, gender, recruitment site, diabetic status (no diabetes, diabetes with or without diabetic nephropathy), blood pressure, protein-to-creatinine ratio (log-transformed) and mGFR (≥45, 45–30, <30 mL min^{-1}/1.73 m^2).
Model 2: model 1 + interaction term between time and mGFR.
ECF, extracellular fluid volume; mGFR, measured glomerular filtration rate; ESKD, end-stage kidney disease.

suggested that fluid overload may induce inflammatory processes in patients with CKD [32].

In this hospital-based population, carefully followed up by nephrologists and receiving optimized care, ECF increased over time. Albeit significant, this yearly increase in ECF remained limited compared with the expected increase in ECF during CKD. Indeed, Essig *et al.* estimated that a sodium balance disequilibrium related to CKD resulted in a daily gain of 0.5 mmol of sodium in patients with CKD stage 1–3. This would be equivalent to an increase in sodium content of about 185 mmol per year or 1.3 litre per year increase in ECF [33]. In the present study, this modest increase in ECF over time could be explained by the tight control of ECF in this population in which diuretic prescription, although on average of near 50% and stable over time, increased as GFR decreased. Diuretics are often poorly and/or inadequately prescribed with regard to the degree of kidney dysfunction [35–38], with strong differences worldwide from 11% in Asia, 52–78% in Europe, 66–74% in North America, to 79% in Brazil [39]. Up-titration of loop diuretics is required in CKD as GFR decreases because of the reduced number of functioning nephrons, of a lower renal blood flow, of the accumulation of organic acids and increasing proteinuria [35, 40, 41]. Diuretics efficaciously and safely reduce ECF and blood pressure if dosage is carefully adjusted at the onset of the treatment, for instance by monitoring body weight [11, 42], to avoid a clinically relevant increase in serum creatinine [43]. However, the impact of diuretics on death, long-term kidney function and cardiovascular outcomes remains a matter of debate. A *post hoc* analysis of the ALLHAT trial, which enrolled 20 584 patients with high cardiovascular risk and

serum creatinine lower than 176.8 μmol L^{-1}, showed that in each stratum of baseline eGFR (≥90, 60–90, <60 mL min^{-1}/1.73 m^2), a 5-year treatment of chlorthalidone was not superior to lisinopril or amlodipine in preventing cardiovascular events, mortality or ESKD, after 9-year follow-up [44]. However, other interventional studies showed that diuretic treatment slowed down CKD progression [9], reduced left ventricular mass index independently of change in blood pressure in patients with CKD [10] and reduced glomerulosclerosis in rat [5].

As previously reported in other CKD cohorts [29, 36, 37, 45–47], sodium intake (assessed by 24-h urinary sodium excretion) in our study was well above the 5 g of sodium chloride per day (or 2 g of sodium) recommended by the WHO and the KDIGO [22, 48, 49] and, importantly, did not decrease with CKD progression. Of note, patients from this cohort were all followed up by a nephrologist and 685 of them (in Tenon's Hospital) had a dietician visit as part of NephroTest annual work-up. Therapeutic inertia for dietary sodium restriction could explain, at least in part, the increase in ECF observed over time, since the ability to excrete sodium is reduced in patients with CKD [50, 51]. The stability of sodium intake as GFR decreased suggests that dietary counsels might have not been sufficiently provided and/or highlights the difficulties to sustain salt restriction in the long term. This may contribute to blunt the natriuretic effect of diuretic treatment and increase ECF. Dietary salt restriction is all the more important as sodium intake *per se* has been shown to be associated with adverse outcomes. Indeed, several large observational studies conducted in patients with CKD showed that a higher salt intake is associated with mortality and cardiovascular events [29, 52], although the association between salt intake and CKD progression remains more controversial [29, 37]. Conversely, interventional studies of low sodium intake [6, 43, 53–56] showed a reduction of the surrogate markers for CKD progression, such as proteinuria and blood pressure, without significant or clinically relevant short-term modification in GFR.

Our study has several strengths. First, it is a multicentre study including a large number of patients with various underlying nephropathies, a wide range of GFR values and longitudinal follow-up. In addition, our results are unique as they rely on gold-standard methods for both ECF and GFR measurements. Furthermore, compared with proportional hazard model with time-varying covariate, the recently developed joint model approach allows an accurate assessment of the association between the longitudinal trajectory of a biomarker and patient's prognosis and a better handling of noisy and incompletely observed time-varying biomarker information. Such an approach takes also into account the potential informative drop out of the study due to the event when estimating the trajectory of the biomarker over time, leading to unbiased estimates of the relationship between ECF and the two outcomes [15, 21]. Finally, several sensitivity analyses ensure the robustness of our results.

However, we acknowledge some limitations. First, as it is an observational study, despite adjusting for multiple covariates, unidentified potential confounding cannot be fully ruled out. Secondly, GFR and ECF were derived from the same ^{51}Cr-EDTA clearance samples, which may generate a spurious relationship between the two measurements. However, similar results were observed when eGFR was used instead of mGFR, making this hypothesis unlikely. Thirdly, even though our measurements rely on gold-standard methods, sensitivity analyses using another independent ECF measurement method such as bioelectrical impedance spectroscopy and/or biomarkers of ECF would have strengthened our results. Finally, due the tight control of ECF in this population, this observational study may underestimate the 'natural' increase in ECF as the GFR decrease, but also the strength of the association between ECF and adverse outcomes.

Conclusion

In conclusion, in this large cohort of patients phenotyped with reference methods, ECF increased during longitudinal follow-up and current value of ECF over time was associated with both ESKD and death before ESKD in patients with CKD. Monitoring and avoiding excessive fluid overload is of utmost importance for the clinical management of patients with CKD. Large interventional prospective studies are needed to evaluate the benefit of ECF control – independent of change in blood pressure – on CKD progression and to investigate the pathophysiological links between fluid overload and kidney disease progression.

Conflict of interest statement

The authors declared no competing interests.

Funding

The NephroTest CKD cohort study is supported by the following grants: INSERM GIS-IReSP AO 8113LS TGIR, French Ministry of Health AOM 09114, INSERM AO 8022LS, Agence de la Biomédecine R0 8156LL, AURA and Roche 2009-152-447G.

Authors' contributions

ALF, KL, GG and EVP designed the study. ALF, KL and CPL performed the statistical analyses and interpreted the data. ALF, KL, CPL, GG and EVP drafted the manuscript. All authors made critical revision of the manuscript for important intellectual content.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Methods.

Table S1. Estimated association between extracellular fluid volume over time and hazards of end-stage kidney disease and death using joint models (eGFR).

Table S2. Estimated association between extracellular fluid volume scaled to body surface area over time and hazards of end-stage kidney disease and death using joint models (eGFR).

Figure S1. Flow chart of the study.

Figure S2. Change in extracellular fluid volume over time.

Figure S3. Estimated probabilities of end-stage kidney disease and death, using the non-parametric Aalen-Johansen estimator to account for competing risk.

Figure S4. Estimated nonlinear association between extracellular fluid volume, end-stage kidney disease and death, using cause-specific Cox models with time-dependent covariate.

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CLINICAL RESEARCH

Estimating Extracellular Fluid Volume in Healthy Individuals: Evaluation of Existing Formulae and Development of a New Equation

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Introduction: Several clinical settings require an accurate estimation of the physiologically expected extracellular fluid volume (ECFV). We aimed to analyze the performances of existing ECFV-estimating equations and to develop a new equation.

Methods: The performances of 11 ECFV-estimating equations were analyzed in 228 healthy kidney donor candidates (Bichat Hospital, Paris, France) who underwent ECFV measurement using the distribution volume of ^{51}Cr-labeled EDTA (^{51}Cr-EDTA). An equation was developed using a penalized linear modeling approach (elastic net regression) and externally (Tenon Hospital, Paris, France, $N = 142$) validated.

Results: Participants from Bichat (mean age 45.2 ± 12.0 years, 43.0% men) and Tenon (47.8 ± 10.3 years, 29.6% men) hospitals had a mean measured ECFV of 15.4 ± 2.8 l and 15.1 ± 2.1 l, respectively. Available ECFV-estimating formulae have highly variable precision and accuracy. The new equation incorporating body weight, height, sex, and age had better precision and accuracy than all other equations in the external validation cohort, with a median bias of −0.20 (95% CI: −0.35 to −0.05) l versus −2.63 (−2.87 to −2.42) l to −0.57 (− 0.83 to −0.40) l and 0.21 (0.12 to 0.43) l to 2.89 (2.65 to 3.11) l, for underestimating and overestimating equations, respectively, an interquartile range for the bias of 0.88 (0.70 to 1.08) l versus 0.91 (0.71 to 1.20) l to 1.93 (1.67 to 2.25) l, and an accuracy within 10% of 90.9% (83.8 to 94.4) versus 88.0% (81.0 to 92.3) to 8.5% (4.2 to 13.4). These results were consistent across subgroups defined by sex, body mass index (BMI), body surface area (BSA), age, and ethnicity.

Conclusion: We developed and validated a new equation to estimate the individual reference value of ECFV, which is easily usable in clinical practice. Further validation in cohorts including individuals of extreme age and corpulence remains needed.

Kidney Int Rep (2022) **7,** 810–822; https://doi.org/10.1016/j.ekir.2022.01.1057
KEYWORDS: ^{51}Cr-EDTA; equation estimation; extracellular fluid volume; isotope; reference value

Extracellular fluid volume (ECFV) is tightly regulated by the kidneys, through the modulation of urinary sodium excretion. In healthy individuals, ECFV varies markedly with anthropometric parameters and is therefore not readily predictable. In patients, measured ECFV may be higher (overhydration) or lower (dehydration) than the theoretical (physiologically expected) value. Therefore, individual estimation of the theoretical ECFV is of major clinical importance to quantify the magnitude of ECFV deviation from the normal condition. Indeed, if different tools, including bioelectrical impedance spectroscopy, have been

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Received 8 November 2021; revised 2 January 2022; accepted 17 January 2022; published online 26 January 2022

developed to measure ECFV,[1] evaluating the degree of overhydration or dehydration requires accurate estimation of the physiologically expected individual extracellular volume. In addition, in the last decades, new simplified techniques of glomerular filtration rate (GFR) measurement based on single-sample plasma clearance raised attention on the importance of theoretical ECFV evaluation.[2] Finally, several authors have suggested that GFR should be expressed scaled to ECFV rather than to BSA, because this might be more physiologically and clinically relevant for the assessment of renal function.[3–9] Indeed, the ratio GFR/ECFV indicates the fraction of the ECFV that passes the glomerular membranes as an ultrafiltrate of plasma per unit time and thus indicates how often "that which is to be regulated" (i.e., the ECFV) comes into contact with the "regulator" (i.e., the kidneys).[3,4] An accurate prediction of the theoretical ECFV in a given individual is therefore important in many clinical settings.

The historical gold standard for ECFV measurement was established as the volume of distribution of bromide, determined from the total remaining quantity of the tracer divided by its concentration after an equilibrium period.[1,8,10,11] Other tracers have been developed, of which the radioactive ^{51}Cr-labelled ethylenediaminetetraacetic acid (^{51}Cr-EDTA) has been found to yield the most accurate estimation of ECFV, in line with its distribution in the extracellular compartment, which is even more strict than that of bromide.[8,12,13] Nevertheless, such direct measurement of ECFV using isotope dilution requires urine sampling, which is cumbersome in clinical practice, so that other measurement methods have been developed from the analysis of the complete plasma disappearance curve after a single injection of tracers used for GFR measurement, ECFV being calculated as the product of GFR by the mean transit time.[3,14,15] Still, establishing the full plasma disappearance curve including the early phase remains quite cumbersome, so that simplified techniques based on the late disappearance curve (mono-compartment model), and using various correction factors, are preferred in clinical practice.[3,5,13,16–19]

Several equations have been developed to estimate theoretical ECFV from anthropometric parameters.[2,6,9,11,12,19–21] However, these equations were developed in small samples,[2,9,12] in specific patient populations,[2,6] or in mixed populations of both children and adults.[6,9] More importantly, no large-scale study used the above-mentioned gold standard ECFV measurement methods.[1,8,10,11] In addition, to our knowledge, none of these equations have been externally validated.

The aims of our study were, first, to evaluate the performances and the validity of all available ECFV-estimating formulae against a reference measurement and, second, to develop and validate a new equation for the estimation of theoretical ECFV in healthy adults.

METHODS

Study Populations

Data from healthy adults referred for GFR measurement before a potential live kidney donation were used (i) to validate the published formulae and for development and internal validation of the new ECFV-estimating equation (Bichat Hospital, Paris, France, March 2007–September 2018, $N = 411$) and (ii) for external validation of the newly developed equation (Tenon Hospital, Paris, France, January 2006–February 2019, $N = 261$).

Data Collection

Anthropometric data were measured in all participants. Routine laboratory markers were also collected. In both cohorts, GFR was measured from the renal clearance of ^{51}Cr-EDTA.[22,23] As ^{51}Cr-EDTA diffusion is restricted to the extracellular compartment, ECFV was measured during the same procedure, as the distribution volume of the tracer.[23,24] After a bolus i.v. injection of 1.8 to 3.5 megabecquerels (MBq) of ^{51}Cr-EDTA (GE Healthcare, Vélizy, France), patients were asked to void after allowing 90 minutes for equilibration of the tracer in the ECFV and every 30 minutes thereafter until 270 minutes after the injection. Blood samples were drawn in the contralateral arm at midpoint of each 30-minute urine period, and urinary clearance was calculated from the average of the 6 urinary clearances in these 30-minute periods. The equation of the late plasma disappearance curve was determined from the regression of plasma concentration as a function of time and was used to extrapolate the plasma concentration of the tracer at each voiding time. ECFV was calculated at each voiding time as the ratio of the remaining quantity (i.e., the injected minus the cumulative excreted quantity) over the extrapolated plasma concentration of ^{51}Cr-EDTA[23] and expressed in liters. Activity of urinary and plasma samples was measured with the Wallac Wizard 3″ 1480 (PerkinElmer) gamma counter.

$$ECFV(\text{liters})_{(t)} = \frac{Qinjected - Qexcreted_{(t)}}{plasma\ ^{51}CrEDTA\ concentration_{(t)}}$$

Selection of Participants

Individuals with measured GFR < 60 ml/min per 1.73 m^2 or with treated hypertension were excluded from the study. Moreover, although direct measurement of the distribution volume of ^{51}Cr-EDTA is a

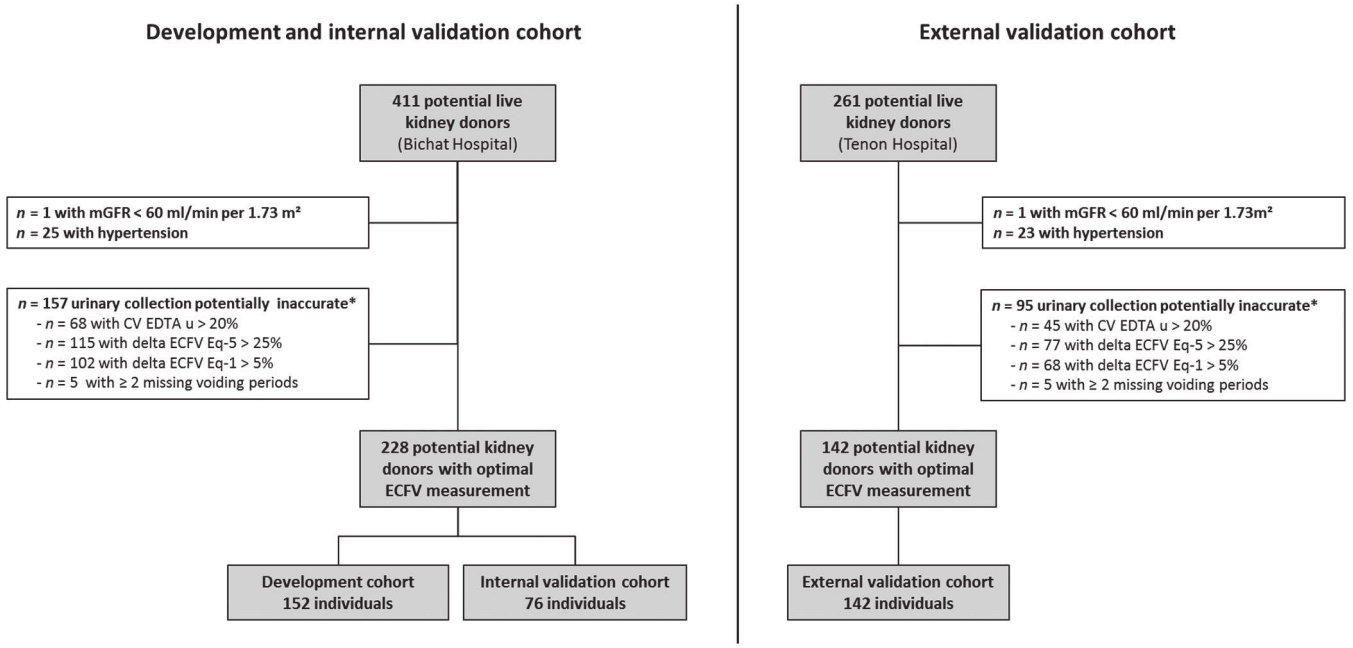

Figure 1. Flowchart. *Participants may have overlapping causes of inaccurate urine collection. CV, coefficient of variation; ECFV, extracellular fluid volume; Eq, equilibrium; mGFR, measured glomerular filtration rate.

reference method for ECFV evaluation, any inaccuracy in voiding completeness compromises the accuracy of the calculated excreted quantity (hence distribution volume) of the tracer. As the reliability of the gold standard measurement was crucial in our study, very stringent selection criteria were used to ascertain the validity of ECFV measurement (Supplementary Figure S1). Data sets from all participants were reviewed by 2 independent experts (ALF and EVP). Individuals with any sign of inaccurate urinary collection, defined by ≥2 missing voiding periods and/or an intra-subject coefficient of variation of the 6 (or 5) fractionated urinary clearances of the tracer > 20%,[25] were excluded from the analyses. In addition, as any urine loss during the procedure leads to cumulative errors in ECFV, although the overall steadiness of consecutive ECFV measurement was used to screen for regular and complete voiding, for this study, the reference ECFV value was considered as the minimum of the first 2 measurements (after equilibrium and after the first 30-minute period), the second one being lower and more accurate than the first when voiding is incomplete at equilibrium. To ensure that no urine was lost during any of these 2 voiding periods, any increase between the first and the second ECFV values > 5% was also an exclusion criterion. Finally, as the combination of urine loss and incomplete voiding at equilibrium could not be detected by this 5% increase criteria (both errors compensating each other at the second void), a subsequent increase between the reference value and the last ECFV value > 25% which could not be explained by a subsequent urine loss after the second void (as analyzed by the corresponding fractionated urinary clearance data) was interpreted as a urine loss during equilibrium and the corresponding data set was also excluded from the present analysis. Importantly, participants may have overlapping causes of inaccurate urine collection. This thorough screening process left a total of 228 subjects (Bichat cohort) with fully validated data sets. The same procedure was applied to the external validation cohort, leaving 142 participants (Tenon cohort) with valid sets of data for the present study (Figure 1).

Statistical Analyses

Evaluation of ECFV-Estimating Equations

ECFV-estimating equations evaluated in this study are reported in Table 1. The "20% of body weight" formula, frequently indicated as an approximation of ECFV in physiology textbooks,[26] was also tested. Their performances were evaluated using the following main parameters: bias (difference between estimated and measured ECFV), precision (interquartile range of the bias), and two metrics of accuracy (root mean square error and percentage of estimated values within 10% of measured ECFV) (Supplementary Method). The 95% CIs were calculated using 10,000 bias-corrected and accelerated bootstrap iterations.[27] Performances of the equations were also graphically analyzed by plotting predicted versus measured ECFV and using the Bland-Altman representation.[28]

Development of a New ECFV-Estimating Equation

Subjects from the Bichat database were randomly divided into 2 of 3 for the development sample (n =

Table 1. Equations used to estimate the theoretical ECFV

Author, journal, ref	Yr	*n*	Tracer	Gold standard	Population	Formula
Moore et al.[11]	1963	17 males 17 females	^{84}Bromide	$\frac{Q(\,^{84}Br_{injected} - Q\,^{84}Br_{excreted})}{\text{plasma } ^{84}Br \text{ concentration}}$	Healthy population	Males: ECFV = 7.35 + 0.135 × weight Females: ECFV = 5.27 + 0.134 × weight
Brøchner-Mortensen et al., *Scand J Clin Lab Invest.*[12]	1982	84	^{51}Cr-EDTA	Plasma disappearance curve (mono-compartment model)	Healthy population Age: 18–70 yr	Males: Log_{10} ECFV = 0.0026 × weight + 3.9510 Females: Log_{10} ECFV = 0.0030 × weight + 3.8657 Males: Log_{10} ECFV = 0.1957 × BSA Dubois + 3.7667 Females: Log_{10} ECFV = 0.2669 × BSA Dubois + 3.6102
Granerus et al., *Swedish Soc Radiol Proc.*[21]	1985	—	^{51}Cr-EDTA	Plasma disappearance curve (mono-compartment model)	—	Males: ECFV = (166 × weight) + 2490 Females: ECFV = (95 × weight) + 6170
Christensen et al., *Clin Physiol.*[2]	1986	45	^{99m}Tc-DTPA	Plasma disappearance curve (bi-compartment model)	Age: 30–79 yr Cancer GFR: 39–126 ml/min	ECFV = (8116.6 × BSA Dubois − 28.2)/1000
Bird et al., *J Nucl Med.*[6]	2003	411	^{51}Cr-EDTA	Plasma disappearance curve (mono-compartment model)	Age: 1–87 yr Nephropathy Cancer	ECFV = weight$^{(0.6469)}$ × height$^{(0.7236)}$ × 0.02154
Silva et al., *Physiol Meas.*[20]	2007	1538	$^{2}H_2O$ and ^{40}K	$\frac{152 \times TBW - TBK}{148}$	Age: 18–98 yr Multiethnic healthy population	Males: ECFV = −12.424 + (0.191 × weight) + (0.0957 × height) + (0.025 × age) Females: ECFV = −4.027 + (0.167 × weight) + (0.05987 × height)
Peters et al., *Nucl Med Commun*[9]	2011	170 (69 children + 101 adults)	^{51}Cr-EDTA	Plasma disappearance curve (mono-compartment model)	Children: nephropathy (age: 0.5–13 yr) Adults: healthy kidney donors (age: 19–76 yr)	ECFV = 6.08 × BSA Haycock$^{1.26}$
Peters et al., *Nephrol Dial Transplant.*[19]	2012	1878	^{51}Cr-EDTA/^{99m}Tc-DTPA	Plasma disappearance curve (mono-compartment model)	Healthy kidney donors Age: 19–77 yr	Males: ECFV = 5.01 + 0.124 × weight Females: ECFV = 4.28 + 0.116 × weight Males: ECFV = (−2.47 + 8.76 × BSA Haycock) Females: ECFV = (−1.96 + 8.05 × BSA Haycock)

^{51}Cr-EDTA, ^{51}Cr-labelled ethylenediaminetetraacetic acid; BrV, bromide volume; BSA, body surface area; ^{99m}Tc-DTPA, ^{99m}Tc-labelled diethylenetriaminepentaacetic acid; ECFV, extracellular fluid volume; PV, plasma volume; Q, quantity; RCV, red blood cell volume; ref, reference; TBK, total body potassium; TBW, total body water.

In the Moore formula, as bromide enters into the red blood cell to a significant degree, a correction of the BrV of distribution for red blood cell (RCV) bromide and PV was carried out by the authors as follows: $\frac{BrV - PV - 0.6RCV}{1.11} + 0.92\,PV$.

In the Silva formula, ECFV was deducted from total body water (calculated as the distribution volume of deuterium, $^{2}H_2O$) and total body potassium. BSA was estimated using the Dubois or Haycock formula; ECFV (expressed in l or ml according to formulae); Q, quantity; TBK mmol; TBW kg. Dubois formula: BSA [m^2] = 0.007184 × height [cm]$^{0.725}$ × weight [kg]$^{0.4255}$; Haycock formula: BSA [m^2] = weight [kg]$^{0.5378}$ × height [cm]$^{0.3964}$ × 0.024265.

152) and 1 of 3 for the internal validation sample ($n = 76$). Equation development process is detailed in the Supplementary Method. Assumption of normality of ECFV was verified. Although this assumption was roughly acceptable to study ECFV linearly, a Box-Cox transformation[29] was also applied on ECFV (function *boxcox* of the R package *MASS*), leading to a natural logarithm transformation of ECFV. Relationships between both ECFV (linear) and log-transformed ECFV and predictors were studied (Supplementary Method). Least-square linear regression was used to relate measured ECFV to clinical and biological characteristics of healthy individuals. ECFV-related variables were defined *a priori* and included body weight, height, age, sex, ethnicity, fasting urinary sodium excretion, and fractional excretions of sodium, uric acid, and urea. Nonlinear relationship between each continuous predictor and ECFV was explored. Then, a combination of clinical guidance and stepwise forward approach was used to select covariates in the adjusted model. Improvement in model performance through addition of new covariates in multivariable linear regression model was evaluated using the Akaike Information Criterion.[30] Adjusted R^2, root mean square error, and absolute bias were also evaluated. Models 1 to 4 (and models 1-log to 4-log) were developed by sequentially adding body weight, sex, height, and age. Models 5 (and 5-log) and 6 (and 6-log) were developed with the same covariates of models 3 (and 3-log) and 4 (and 4-log) but using elastic net regression method[31] (R package *glmnet*) with 5-fold cross-validation, to improve the quality of the prediction (Supplementary Method).

Internal Validation

The most accurate models (models 6 and 6-log) were evaluated in the internal validation data set. Equation obtained from the development cohort was applied in the total population of the internal validation cohort, but also according to subgroups defined by sex, age (<40, 40–60, >60 years), ethnicity (European vs. African origin), BMI (<20, 20–30, >30 kg/m^2), and BSA (<1.73, 1.73–2, >2 m^2). Performances of the predictive models were evaluated graphically and using the same metrics as described previously. Calibration was studied by plotting predicted versus measured ECFV for each quintile of predicted ECFV. Magnitude of the deviation was compared across quintiles using a linear regression model, with bias and quintiles entered as the dependent and independent variables, respectively (the lower the R^2 and the higher the P value, the better the prediction model). Finally, development and internal validation data sets were combined to derive the final coefficients using a penalized elastic net regression.

External Validation of the New ECFV-Estimating Equation

The new ECFV-estimating equation was externally validated in the Tenon cohort ($N = 142$), using the same graphical representation and metrics as for the internal validation. Finally, the new equation was compared with the other formulae.

There were no missing data for any of the covariates used for the development and the internal and external validation of the new equation. All statistical analyses were conducted using R 3.6 software (https://cran.r-project.org/). The transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) statement[32] was followed for reporting the development and validation of the multivariable prediction model (Supplementary Method).

Consent and Ethics

All patients gave their written consent for scientific use of anonymous data. The study was approved by the Local Ethics Committee (Institutional Review Board 00006477, project number 14-051, *Hôpitaux Universitaires Paris-Nord Val de Seine, Assistance Publique–Hôpitaux de Paris*).

RESULTS

Characteristics of the Study Populations

In the 228 participants of the development and internal validation cohorts (Figure 1 and Table 2), mean age was 45.2 ± 12.0 years, 43.0% were men, and 14.2% were of African origin. Mean BMI was 25.9 ± 4.6 kg/m^2. Mean measured GFR was 90 ± 15 ml/min per 1.73 m^2. Mean measured ECFV was 17.0 ± 2.6 and 13.7 ± 2.1 l in males and females, respectively (Supplementary Figure S2). The 142 participants of the external validation cohort were older, more often females, and had a lower measured GFR (Table 2). Overall characteristics of the patients included in the analyses did not differ from those who were excluded because of irregular voiding potentially compromising the validity of ECFV measurement (Supplementary Table S1).

Relationship Between ECFV and Anthropometric Parameters

ECFV was highly correlated with body weight ($r = 0.85$) and BSA ($r = 0.86$) and was on average 21.3 ± 2.1 and 21.0 ± 2.4% of body weight in males and females, respectively. For the lowest and highest values of BMI, ECFV represented >20% and <20% of body weight, respectively, and this finding was similar in males and females (Figure 2).

Performances of ECFV-Estimating Equations

Bias, precision, and accuracy of the ECFV estimation formulae are presented in Figure 3 and Supplementary

Table 2. Clinical characteristics of the study populations

Characteristics	Development and internal validation cohorts (Bichat) $N = 228$	External validation cohort (Tenon) $N = 142$	*P* value	Development data set (Bichat) $n = 152$	Internal validation data set (Bichat) $n = 76$	*P* value
Anthropometric characteristics						
Age (yr)	45.2 ± 12.0	47.8 ± 10.3	0.03	44.9 ± 12.0	45.8 ± 12.0	0.60
Age (%)			0.03			0.77
<40 yr	35 (24.6)			59 (38.8)	26 (34.2)	
40–60 yr	90 (63.4)			76 (50.0)	40 (52.6)	
>60 yr	17 (12.0)			17 (11.2)	10 (13.2)	
Sex (males, %)	98 (43.0)	42 (29.6)	0.01	62 (40.8)	36 (47.4)	0.42
Ethnicity (African origin, %)	32 (14.2)	23 (20.9)	0.16	21 (14.0)	11 (14.7)	1.00
Body weight (kg)	73.5 ± 14.4	71.2 ± 12.6	0.13	73.4 ± 14.6	73.6 ± 14.1	0.93
Height (cm)	168.4 ± 9.8	165.0 ± 8.2	0.001	168.5 ± 10.5	168.4 ± 8.2	0.98
Body mass index (kg/m^2)	25.9 ± 4.6	26.2 ± 4.5	0.51	25.9 ± 4.9	25.8 ± 3.9	0.94
Body mass index (%)			0.92			0.58
<20 kg/m^2	21 (9.2)	12 (8.5)		15 (9.9)	6 (7.9)	
20–30 kg/m^2	164 (71.9)	101 (71.1)		106 (69.7)	58 (76.3)	
>30 kg/m^2	43 (18.9)	29 (20.4)		31 (20.4)	12 (15.8)	
Body surface area (DuBois)[a]	1.83 ± 0.20	1.78 ± 0.17	0.01	1.83 ± 0.21	1.83 ± 0.20	0.93
Body surface area (Haycock)[b]	1.86 ± 0.22	1.81 ± 0.19	0.05	1.86 ± 0.22	1.86 ± 0.22	0.91
Biological parameters						
mGFR (ml/min per 1.73 m^2)	90 ± 15	85 ± 14	0.001	90 ± 15	92 ± 16	0.39
Measured ECFV (l)	15.4 ± 2.8	15.1 ± 2.1	0.33	15.4 ± 2.8	15.5 ± 2.7	0.81
Estimated ECFV (l)						
Moore formula	16.0 ± 2.6	15.5 ± 2.2	0.03	16.0 ± 2.6	16.2 ± 2.6	0.65
Brøchner-Mortensen formula (weight)	13.0 ± 1.8	12.5 ± 1.5	0.02	12.9 ± 1.8	13.1 ± 1.8	0.63
Brøchner-Mortensen formula (BSA)	12.9 ± 1.7	12.4 ± 1.4	0.01	12.9 ± 1.7	12.9 ± 1.6	0.75
Granerus formula	14.0 ± 2.4	13.5 ± 1.9	0.02	14.0 ± 2.3	14.1 ± 2.4	0.69
Christensen formula	14.9 ± 1.7	14.4 ± 1.4	0.01	14.8 ± 1.7	14.9 ± 1.6	0.93
Bird formula	14.2 ± 2.2	13.4 ± 1.8	0.02	14.1 ± 2.2	14.2 ± 2.1	0.93
Silva formula	18.7 ± 3.2	18.0 ± 2.6	0.02	18.7 ± 3.2	18.8 ± 3.2	0.90
Peters formula (BSA 1)	14.0 ± 2.2	13.6 ± 1.9	0.04	14.0 ± 2.3	14.0 ± 2.2	0.92
Peters formula (BSA 2)	13.4 ± 2.1	12.9 ± 1.7	0.02	13.4 ± 2.1	13.5 ± 2.1	0.79
Peters formula (weight)	13.4 ± 2.1	12.9 ± 1.8	0.03	13.7 ± 2.1	13.5 ± 2.1	0.71
20% Body weight	14.7 ± 2.9	14.3 ± 2.5	0.13	14.7 ± 2.9	14.7 ± 2.	0.93

BSA, body surface area; ECFV, extracellular fluid volume, mGFR, measured glomerular filtration rate.
[a]Dubois formula: BSA [m^2] = 0.007184 × height [cm]$^{0.725}$ × weight [kg]$^{0.4255}$.
[b]Haycock formula: BSA [m^2] = weight [kg]$^{0.5378}$ × Height [cm]$^{0.3964}$ × 0.024265.
Continuous data are expressed in mean ± SD and categorical data are expressed in *n* (%). BSA estimated using Dubois or Haycock formula.

Table S2. Median bias of the Christensen formula (−0.47 l, 95% CI [−0.69 to −0.19]) was lower than that of the other formulae. Interquartile range for the difference was close to 2 l for all the equations. The best accuracies within 10% were obtained with the Moore (65.8 [58.8 to 71.5]), Christensen (66.7 [60.1 to 71.9]), and 20% body weight (62.3 [55.3 to 68.0]) formulae. Bland and Altman graphs (Figure 3) revealed that the Moore and 20% body weight formulae were more accurate across the whole ECFV range, whereas for most other formulae, underestimation increased (negative bias) as ECFV increases (Figure 3).

Development of the New ECFV-Estimating Equation

A new equation relating measured ECFV to clinical and biological characteristics of healthy individuals was developed. In univariable analysis, body weight was the strongest predictor of ECFV. Height and age better fitted the data with quadratic and cubic transformations, respectively, compared with no (linear) or spline transformation. Nevertheless, in multivariable analysis, none of the fractional polynomial or spline transformations of the predictors provided a better fit to ECFV (and log-ECFV) compared with a linear model. The β-coefficient for the covariates, statistics for goodness-of-fit, and prediction performance for successive equation modeling in both ECFV and log-ECFV are reported in Supplementary Table S3. In sequential models predicting ECFV and log-ECFV, the adjusted R^2, Akaike Information Criterion, and root mean square error improved with the inclusion of body weight, sex, height, and age. None of the tested interactions were significant. Models 6 and 6-log (i.e., fully adjusted models predicting ECFV and log-ECFV, respectively, using elastic net regularization method) were considered for the internal validation step.

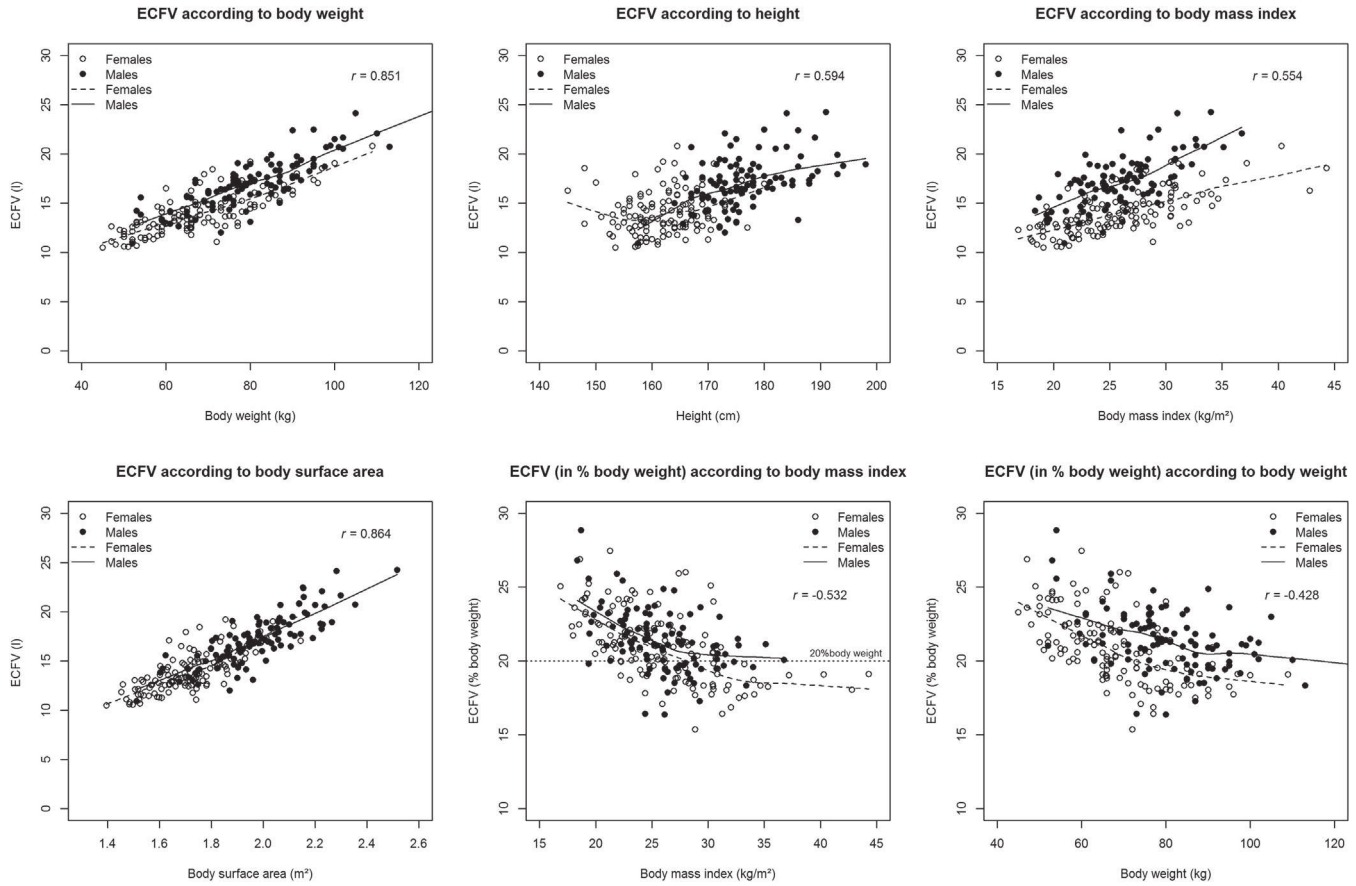

Figure 2. Functional relationship between measured ECFV and anthropometric parameters, according to sex (Bichat cohort, $N = 228$). Smoothed regression lines were computed using the nonparametric LOESS method and are represented as solid (for males) and dashed (for females) black lines. Pearson correlation coefficient r was also calculated for the total population. ECFV, extracellular fluid volume; LOESS, LOcally Estimated Scatterplot Smoothing.

Internal Validation

As equations to estimate both ECFV and log-ECFV from predictors gave similar performances in the internal validation data set (Supplementary Table S4), the simplest model (model 6) was chosen. Prediction and accuracy were consistent across subgroups defined by sex, BMI, BSA, age, and ethnicity (Figure 4 and Supplementary Figure S3). Final coefficients of the selected model 6 were derived from pooled development and internal validation data sets, so that the final equation is:

$$ECF\ (\text{liters}) = \alpha + 0.1393 \times weight\ [kg] + 0.0455 \times height\ [cm] + 0.0125 \times age\ [years]$$

With $\alpha = -2.6631$ *for males and* -3.3407 *for females*

The multiplication factor for sex is incorporated into the intercept, which results in different intercepts for each sex.

External Validation of the New ECFV-Estimating Equation

Figure 4 and Supplementary Figure S4 reveal the predicted versus measured ECFV in the external validation cohort. Metrics for performances of the new equation revealed a median bias of −0.20 l (− 0.35 to −0.05), a median absolute bias of 0.49 l (0.38 to 0.60), an interquartile range for the difference of 0.88 l (0.70 to 1.08), a mean absolute percentage error of 4.19% (3.65 to 4.82). root mean square error of 0.056 (0.050 to 0.064), and percentage of estimated values within 10% of 90.9% (83.8 to 94.4) (Supplementary Table S5). Prediction and accuracy were consistent across subgroups (Figure 4 and Supplementary Figure S5). Compared with all other formulae, the new ECFV-estimating equation displayed the best performances in the external validation cohort (Figure 5a-d, Supplementary Figure S6, and Supplementary Table S5). Although overall performances of the Moore formula were close to those of the new equation in the external validation cohort (Figure 5a-d), the Moore equation suffered from an overestimation of ECFV in males: median bias of 1.046 l (6.4%) versus −0.37 l (−2.3%) in Moore and new equation, respectively (Supplementary Figure S7). In addition, because body weight and gender are the only parameters in the Moore formula, its performances across the range of BMI were not as accurate as ours.

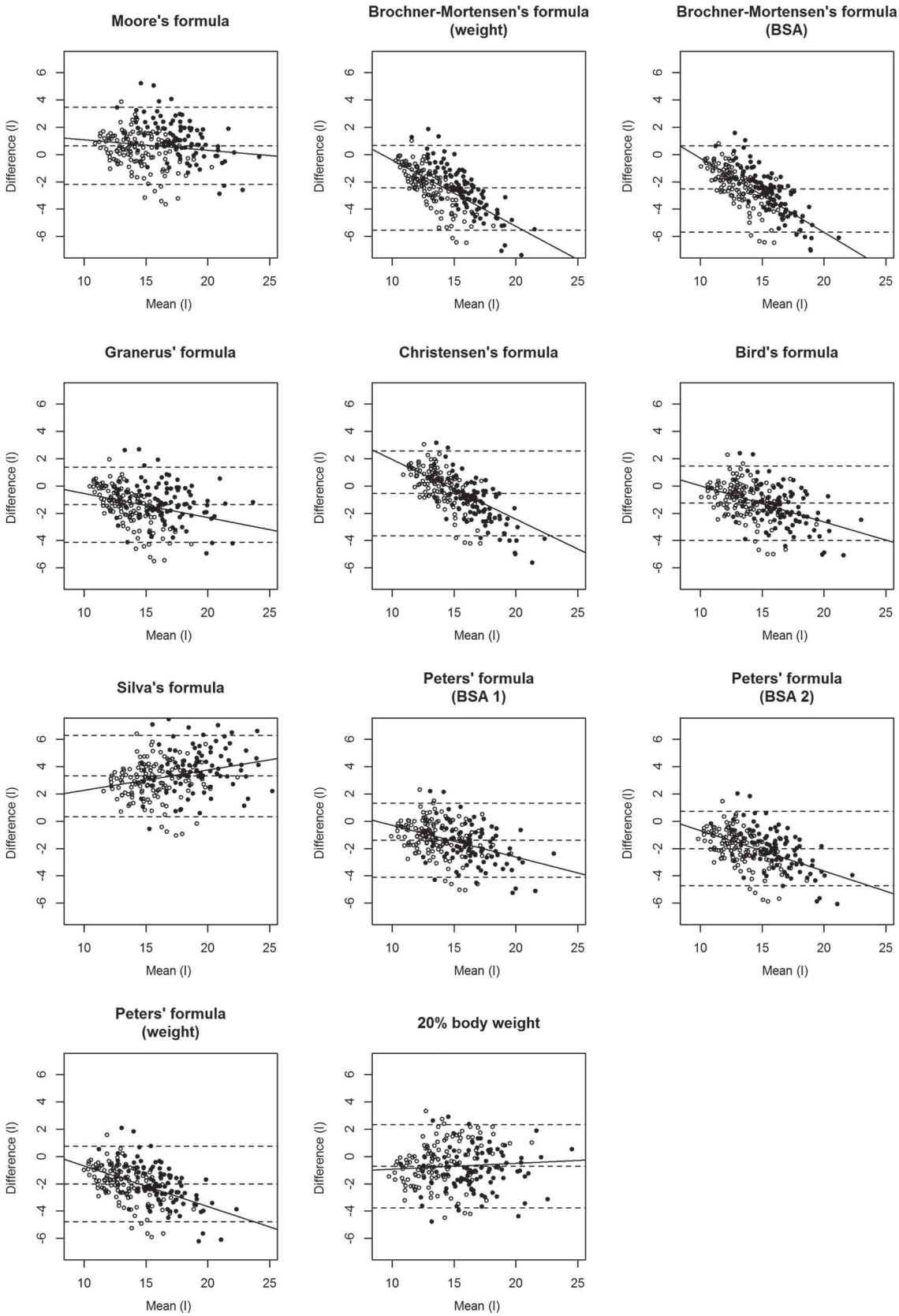

Figure 3. Bland-Altman graphical representations of the estimating equation published in the literature versus measured ECFV (Bichat cohort, $N = 228$). For each ECFV-estimating equation, the difference (estimated − measured ECFV) is plotted versus mean ([estimated + measured ECFV] / 2). Mean bias, upper and lower limits of agreement (mean bias $\pm$ 1.96 $\times$ SD of bias) are represented by the dashed lines. Regression line is represented by the solid black line. BSA, body surface area; ECFV, extracellular fluid volume.

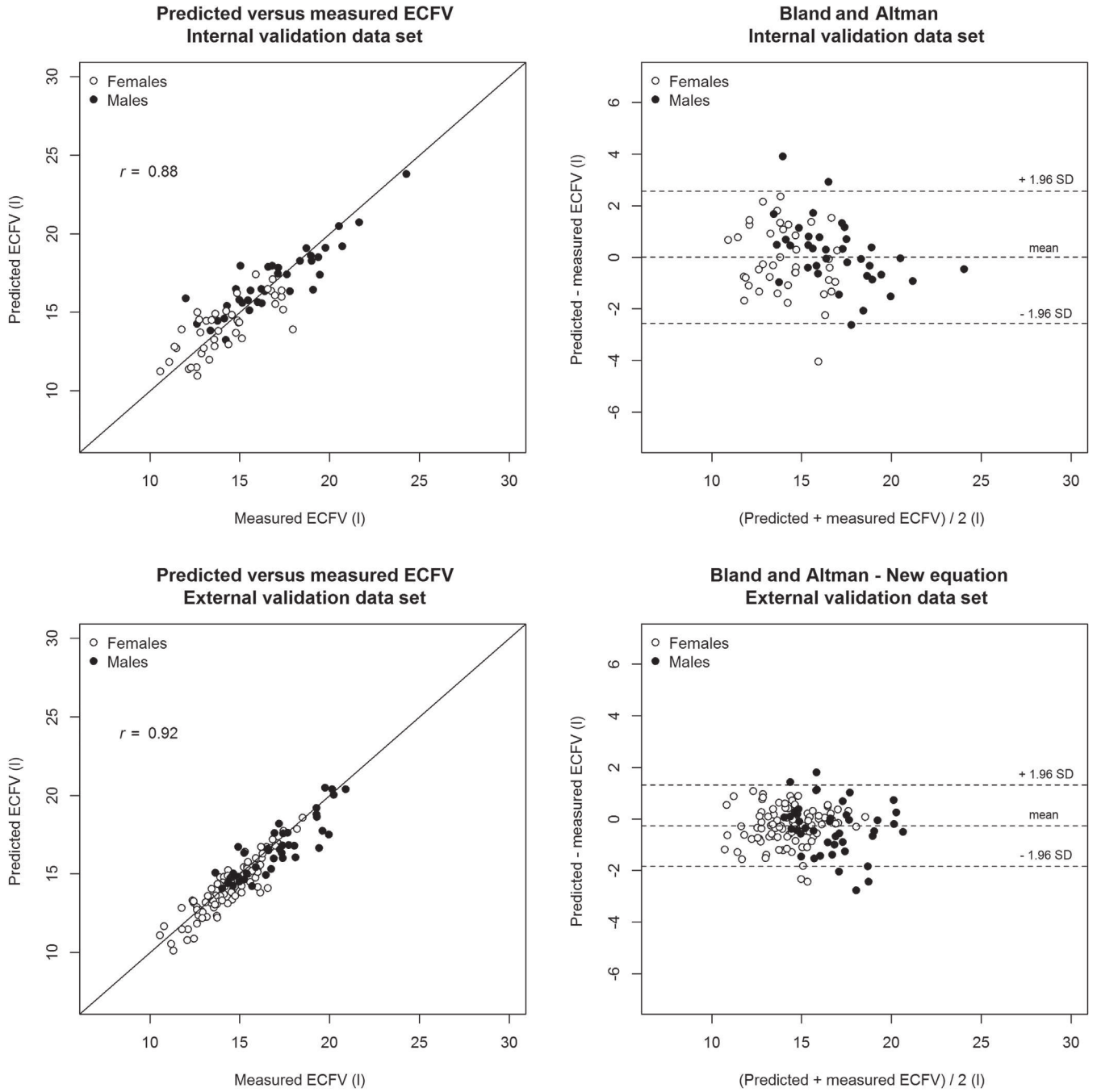

Figure 4. Predicted versus measured ECFV in internal (Bichat cohort, $n = 76$) and external (Tenon cohort, $N = 142$) validation cohorts. Values of ECFV predicted by the model 6 and the new developed equation were plotted against the measured values of ECFV, in the internal (Bichat cohort, $n = 76$) and external (Tenon cohort, $n = 142$) validation cohorts, respectively. In Bland-Altman plots, mean bias, upper and lower limits of agreement (mean bias $\pm$ 1.96 $\times$ SD of bias) are represented by the dashed lines. ECFV, extracellular fluid volume.

DISCUSSION

Our study conducted in healthy individuals with a very thorough screening of ECFV measurement using isotope dilution showed that the precision and accuracy of the ECFV-estimating equations previously published were highly variable and their suitability for routine clinical practice was questionable for most of them. This could be explained at least in part by the fact that they were often developed in small sample size,[2,9,12] in specific patient populations,[2,6] or in mixed populations of children and adults,[6,9] or without distinction between body composition of males and females.[2,6,9] In addition, these equations were not validated in external cohorts.[2,9,11,12,19–21] Moreover, in most cases, the benchmark used to develop the equations was not a gold standard measurement of ECFV.[1,8,10,11,33] Indeed, in one study, ECFV was deducted from total body water and intracellular fluid volume evaluated by total body potassium.[20] In all

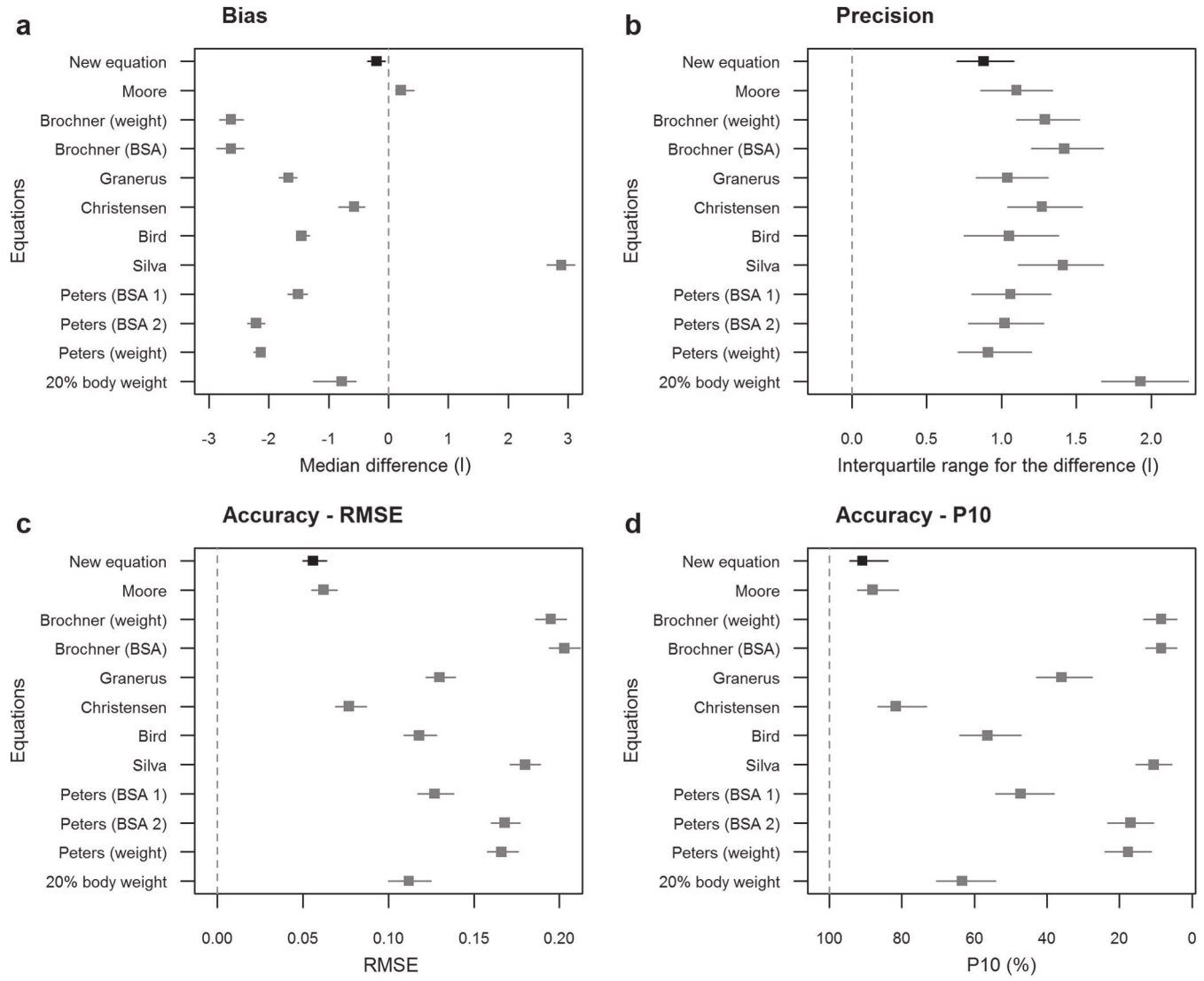

Figure 5. Comparison of the performances of the published and the new ECFV-estimating equations, in the external validation cohort (Tenon cohort, $N = 142$). The newly developed ECFV-estimating equation referred, to as "new equation," is represented in black, and the other equations are represented in gray. (a) Bias was defined as the median difference between estimated and measured ECFV. (b) Precision was evaluated using the IQR of the bias. Accuracy was evaluated using (c) the root mean square error and (d) the P10 of the measured value. Horizontal bars represent 95% CIs computed using 10,000 BCa bootstrap iterations. BCa, bias-corrected and accelerated; BSA, body surface area; ECFV, extracellular fluid volume; IQR, interquartile range; P10, percentage of estimates that were within 10%; RMSE, root mean square error.

other studies, except one[11] in which reference values of ECFV relied on a direct measurement method—quite similar to ours—in 34 subjects, and another[2] based on the plasma disappearance curve of a radioactive tracer using a 2-compartment model in 45 subjects, ECFV was derived from the late plasma disappearance curve of an exogenous tracer with various mathematical corrections meant to better estimate the "true" ECFV. Interestingly, the only equation[11] based on direct ECFV measurement from bromide dilution yielded the best performances. Nevertheless, this formula suffered from an overestimation of ECFV in males and was not as accurate as ours across the range of BMI, because it only integrates body weight and sex. Finally, the accuracy of ECFV assessment is directly affected by the tracer used, and it has been found that the distribution volume of ^{51}Cr-EDTA yields a closer approximation of the ECFV than that of other radioactive and nonradioactive tracers, and even that than of bromide.[8,12–14] Indeed, although it is a historical gold standard for ECFV measurement, bromide may overestimate ECFV owing to a minor leakage in the intracellular space,[11,12] so that a correction factor is used in bromide dilution formulae. A limitation of the isotope dilution method compared with plasma decay-derived methods is that complete and accurate bladder voiding

is mandatory, which we ensured at the cost of reducing our study population after applying very strict selection criteria.

We developed and validated a set of prediction models for ECFV estimation among healthy individuals. Our final model performed better than all other formulae, although it relied on the same simple anthropometric markers. Indeed, the addition of other biological parameters and ethnicity did not improve the model performances. The reliability of our equation can be explained, at least in part, because our reference ECFV value was a direct measurement using isotope dilution in a large population with very stringent criteria to ascertain its technical validity as explained previously, but also because a robust statistical method was used to build prediction models. We found that body weight and BSA were the strongest predictors of ECFV and that mean ECFV was 21.1% ± 2.3% of body weight. Accordingly, Ladegaard-Pedersen *et al.*[34] revealed that the distribution volume of ^{51}Cr-EDTA was on average 21.8% of body weight, and using the same tracer, Brøchner-Mortensen[12] revealed that ECFV represented 19.5% and 18.8% body weight in males and females, respectively. Nevertheless, when ECFV is expressed as a fraction of body weight, a major limitation is that body composition (i.e., lean vs. fat body mass) is not taken into account.[12] Consequently, even if the intersubject comparability of ECFV is better when ECFV is compared as a fraction of BSA than as a fraction of body weight, we chose to include body weight and height separately (instead of BSA or BMI) in the model for a better flexibility in the computation of the coefficients, and thus a better fit of the models. As expected, our results revealed that ECFV was higher in males than in females,[12] but the relationship between sex and ECFV was not affected by body weight, height, or age (i.e., *P* values for interactions between sex, body weight, height, and age were not significant). As previously observed by Silva *et al.*,[20] we did not find a significant association between ECFV and ethnicity. Accuracy of our equation was robust across subgroups.

This new equation, which provides the individual reference (normal) value of ECFV, has important implications for both clinical practice and research. Indeed, several pathologic conditions lead to disturbances of sodium homeostasis and abnormality or modification in fluid distribution. The assessment of the magnitude of overhydration (or dehydration) remains a clinical challenge, as a given measured ECFV may correspond to a marked overhydration in some patients, or to a marked dehydration in others, depending on age, sex, and anthropometric parameters (and therefore on the individual theoretical ECFV value). Our new equation will help appreciate how ECFV may deviate from the normal condition, and thus help optimizing patient management. Indeed, to evaluate the extent of overhydration (or dehydration), the following two pieces of information are needed: first, measured ECFV of the patient (using bedside bioelectrical impedance spectroscopy or even isotope dilution), and second, the individual reference value, the magnitude of over- or dehydration being calculated as the difference between the measured and the reference value of ECFV. Interestingly, although limits of agreements of ECFV measurement using bioelectrical impedance spectroscopy compared with isotope dilution are quite large,[35] the bias between both methods is on average close to 0, so that our equation can be expected to provide appropriate reference values for ECFV measured with bioelectrical impedance spectroscopy.

In addition, to compare hydration status at the population level, ECFV needs to be "normalized" or "indexed" to take into account the variability of ECFV associated with anthropometric parameters. Expressing ECFV as the ratio of measured over individual theoretical ECFV, using our equation, would be helpful in clinical research. Another important clinical application of our results is single-sample GFR measurement, which requires an accurate estimation of theoretical ECFV.[2] Finally, our equation could be used to express GFR scaled to ECFV, rather than scaled to BSA. Indeed, it has been found that assessment of renal function based on GFR indexed to ECFV is more clinically relevant[3–8,33] because ECFV is the compartment filtered by the kidneys. GFR/ECFV reflects the percentage of the ECFV cleared per unit of time.[8,33] Of note, the inverse ratio, ECFV/GFR, reflects the time needed for the kidneys to clear the complete ECFV, the so-called concept of mean transit time or, in other words, the mean residence time of the filtration marker in the ECFV before filtration.[3,14,15] Finally, in line with these considerations, although GFR scaled to BSA differs between males and females, this difference is ironed out when GFR is scaled to ECFV.[8]

Strengths of this study include its design, with separate databases for development and validation of the new equation, a prespecified rigorous statistical analytical plan, and use of the penalized elastic net regression to limit overfitting. Nonetheless, we acknowledge some limitations. The stringent selection criteria diminished the number of subjects included in these analyses. This, however, allowed establishing the validity of the reference measurement better than any previous study, and the number of included individuals was still well above that of most of these

studies. In addition, even if the precision and accuracy of our equation was consistent across subgroups, the equation should be used with caution in patients with extreme values of BMI or anthropometric characteristics and in elderly patients because the present study included few such individuals. Likewise, our equation should not be used in children as only adult patients were included in our study populations. Finally, regarding ethnicity, only data on African origin (required for GFR estimation) was available in the data set; other ethnicities such as Asian origin were not specified. Nevertheless, ethnicity defined as African origin or not did not improve the model performances.

In conclusion, our results showed that precision and accuracy of the previously published ECFV-estimating equations were highly variable. We developed and validated a new ECFV-predicting equation easily usable and which might prove a useful tool for clinical practice and research. External validation in other cohorts including individuals of extreme age and BMI remains needed.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

The authors thank all the individuals for their participation to the study and all the members of the Departments of Physiology from Bichat and Tenon Hospitals, Assistance Publique–Hôpitaux de Paris, France.

DATA STATEMENT

The data that support the findings of this study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

ALF, EVP, MF, and GG designed the study. ALF and EVP reviewed the data sets from all participants. ALF and OL performed the statistical analyses. ALF, EVP, MF, and GG interpreted the data. ALF and EVP drafted the manuscript. All authors made critical revision of the manuscript for important intellectual content.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Table S1. Comparison of the characteristics of the study population with those of the population excluded from the study.

Table S2. Performances of the published formulae used to estimate ECFV compared with measured ECFV (Bichat cohort).

Table S3. Beta-coefficients and goodness-of-fit of the sequential models in the development data set.

Table S4. Comparison of the model performances in the internal and external validation data sets.

Table S5. Performances of the published and the new ECFV-estimating equations in the external validation cohort (Tenon cohort).

Figure S1. Exclusion criteria for the selection of patients with a valid ECFV measurement.

Figure S2. Distribution of measured extracellular fluid volume according to sex (Bichat Cohort).

Figure S3. Predicted versus measured extracellular fluid volume according to subgroups in the internal validation cohort (model 6) (Bichat cohort).

Figure S4. Calibration of the new equation in the external validation cohort (Tenon cohort).

Figure S5. Predicted versus measured extracellular fluid volume according to subgroups in the external validation cohort (new equation) (Tenon cohort).

Figure S6. Bland-Altman graphical representations of the published and new ECFV-estimating equations versus measured ECFV (Tenon cohort).

Figure S7. Comparison of the Moore formula and the new equation, according to sex, weight and body mass index (Tenon cohort).

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A nationwide cohort study comparing the effectiveness of diuretics and calcium channel blockers on top of renin-angiotensin system inhibitors on chronic kidney disease progression and mortality

OPEN

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It is unknown whether initiating diuretics on top of renin-angiotensin system inhibitors (RASi) is superior to alternative antihypertensive agents such as calcium channel blockers (CCBs) in patients with chronic kidney disease (CKD). For this purpose, we emulated a target trial in the Swedish Renal Registry 2007-2022 that included nephrologist-referred patients with moderate-advanced CKD and treated with RASi, who initiated diuretics or CCB. Using propensity score-weighted cause-specific Cox regression, we compared risks of major adverse kidney events (MAKE; composite of kidney replacement therapy [KRT], experiencing over a 40% eGFR decline from baseline, or an eGFR under 15 ml/min per 1.73m^2), major cardiovascular events (MACE; composite of cardiovascular death, myocardial infarction or stroke), and all-cause mortality. We identified 5875 patients (median age 71 years, 64% men, median eGFR 26 ml/min per 1.73m^2), of whom 3165 started a diuretic and 2710 a CCB. After a median follow-up of 6.3 years, 2558 MAKE, 1178 MACE and 2299 deaths occurred. Compared to CCB, diuretic use was associated with a lower risk of MAKE (weighted hazard ratio 0.87 [95% confidence interval: 0.77-0.97]), consistent across single components (KRT: 0.77 [0.66-0.88], over 40% eGFR decline: 0.80 [0.71-0.91] and eGFR under 15ml/min/1.73m^2: 0.84 [0.74-0.96]). The risks of MACE (1.14 [0.96-1.36]) and all-cause mortality (1.07 [0.94-1.23]) did not differ between therapies. Results were consistent when modeling the total time drug exposure, across sub-groups and a broad range of sensitivity analyses. Thus, our observational study suggests that in patients with advanced CKD, using a diuretic rather than a CCB on top of RASi may improve kidney outcomes without compromising cardioprotection.

Kidney International (2023) ■, ■–■; https://doi.org/10.1016/j.kint.2023.05.024

KEYWORDS: calcium channel blockers; chronic kidney disease; diuretics; kidney replacement therapy; renin-angiotensin system inhibitors; salt-sensitive hypertension

Lay Summary

In patients with chronic kidney disease, it is unknown whether initiating a diuretic on top of renin-angiotensin system inhibitors is superior to other alternative antihypertensive agents such as calcium channel blockers. We emulated a target trial in the Swedish Renal Registry 2007 to 2022 including patients with chronic kidney disease stages G3–G5 and hypertension who had good adherence to renin-angiotensin system inhibitors and further initiated either a diuretic (n = 3165) or a calcium channel blocker (n = 2710). Compared with patients initiating a calcium channel blocker, those initiating a diuretic had a significantly lower risk of chronic kidney disease progression and a similar risk of cardiovascular events and all-cause mortality. Our study suggests that in patients with moderate to advanced chronic kidney disease, antihypertensive therapy with diuretics may be associated with further kidney benefits and similar cardioprotection compared with calcium channel blockers.

As chronic kidney disease (CKD) progresses to advanced stages, impaired kidney sodium and water excretion often results in fluid overload and salt-sensitive hypertension, which are independently associated

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Received 2 December 2022; revised 7 May 2023; accepted 18 May 2023

with resistant hypertension,[1] need of kidney replacement therapy (KRT), cardiovascular events, and mortality.[2–7] Targeting optimal extracellular fluid volume status is thus critical to the clinical management of these patients and may be achieved by adjusting diuretic therapy and/or decreasing sodium intake.[8]

The 2021 Kidney Disease: Improving Global Outcomes Guidelines recommend that renin-angiotensin system inhibitors (RASi) be used as the first-line antihypertensive drug in patients with CKD,[9] but there is no clear recommendation for the second-line antihypertensive therapy in CKD, largely because of a lack of trial evidence. The uncertainty about the choice of therapy possibly explains the considerable variation observed in the patterns of use of antihypertensive drugs in persons with CKD worldwide.[10] Some guidelines suggest the use of a calcium channel blocker (CCB) or a diuretic.[11,12] Diuretic therapy may offer additional advantages over CCB therapy: beyond their antihypertensive and natriuretic properties, diuretics are known to potentiate the renoprotective[13–20] and cardioprotective[21] effects of RASi in CKD. They may also decrease blood pressure variability, a factor associated with poor kidney and cardiovascular outcomes.[22] Finally, the kaliuretic effect of diuretics could be of value to patients with CKD and hypertension in whom RAS blockade optimization is hampered by hyperkalemia. On the contrary, dihydropyridine CCB therapy induces an increase in proteinuria[23] and may potentially promote long-term CKD progression.

However, the long-term effects of diuretics in patients with CKD or whether they offer any advantage over CCBs as antihypertensive therapy is essentially unknown. Pivotal randomized trials were often small,[13,17,18,20] did not evaluate KRT, and/or focused on short-term effects of surrogate end points.[13,17,20,24–26] They neither evaluated drug efficacy as a second-line of therapy[24,27,28] nor, in general, failed to include patients with advanced CKD.[24–26,28] Some observational studies have attempted to compare clinical outcomes of diuretics with those of CCBs, but they may be limited by low sample sizes, confounding by indication bias,[29–31] lack of stratification by kidney function,[29,32] or lack of consideration of concomitant use of RASi.[33,34] The ACCOMPLISH (Avoiding Cardiovascular events through COmbination therapy in Patients LIving with Systolic Hypertension) trial, conducted in 11,506 patients at high cardiovascular risk but a low risk of CKD progression (<10% with estimated glomerular filtration rate [eGFR] < 60 ml/min per 1.73 m^2 and <1.5% with albumin-to-creatinine ratio > 30 mg/mmol), showed that compared with RASi/diuretic use, RASi/CCB use was associated with a lower risk of cardiovascular[26] and kidney[25] (i.e., composite of doubling in serum creatinine, eGFR < 15 ml/min per 1.73 m^2, or dialysis) events. However, no difference was observed between treatment groups for all-cause and cardiovascular mortality in the total ACCOMPLISH population, and no clear benefit was observed for kidney events in the subset of 1093 patients with moderate CKD at enrollment, which may be attributed to low power.

With the aim to help inform decisions on the choice of antihypertensive drug for patients with moderate to advanced CKD, we emulated a target trial comparing the risk of long-term outcomes of nephrologist-referred patients who initiated diuretic or CCB therapy on top of RASi therapy.

METHODS

Data source

We used data from the Swedish Renal Registry, a nationwide registry collecting longitudinal information of patients with all-cause CKD attending routine nephrology specialist care in Sweden. According to the registry protocol, patients should be enrolled when reaching an eGFR of <30 ml/min per 1.73 m^2 but encourage enrollment at earlier stages of CKD. Registrations of subsequent outpatient visits to nephrology care are thereafter performed, until death, emigration from the country, or start of KRT. The Swedish Renal Registry collects information on outpatient nephrology visits, including laboratory tests and clinical data. Via each citizen's unique personal identification number, the Swedish Renal Registry was then linked to other national registries, such as the Swedish Prescribed Drug Registry, which provides complete information on prescribed drugs dispensed at any Swedish pharmacies; the National Patient Register, a government-run registry that collects all in- and outpatient specialist diagnoses issued; and the National Death Registry, with virtually no loss to follow-up. The study was approved by the Swedish Ethical Review Authority (project numbers 2018/1591-31/2 and 2022-04594).

Study design and patient selection

We emulated a pragmatic clinical trial comparing the effect of initiating diuretics versus CCBs in patients with moderate to advanced CKD.[35] Explicit emulation of a target trial prevents common biases in pharmacoepidemiology studies,[36] such as immortal time bias and prevalent user bias, and makes the analysis of observational studies more transparent.[37] The protocol of the target trial and its emulation are specified in Supplementary Table S1. Eligible individuals were adult patients with CKD stages G3–G5 (eGFR < 60 ml/min per 1.73 m^2) who, between January 1, 2007, and May 1, 2022, had long-term treatment with good adherence to RASi (i.e., angiotensin-converting enzyme inhibitor or angiotensin receptor blocker) and initiated a diuretic (thiazide, thiazide-like diuretic, or loop diuretic) or a CCB (dihydropyridine or nondihydropyridine; Supplementary Figure S1). *Good adherence to RASi* was defined as a proportion of days covered >75% within the year before the initiation of a diuretic or CCB. To capture therapies that were started because of hypertension management and not because of cardiovascular disease, we excluded patients with any in- or outpatient cardiovascular disease events in the 6 months before therapy initiation. Patients with a history of kidney transplantation or dialysis and those who initiated diuretic and CCB therapy simultaneously were also excluded. Look-back periods for eligibility criteria are specified in Supplementary Figure S2.

Treatment strategies and covariates

In our main analysis, the treatment strategies of interest were "initiation of a diuretic" versus "initiation of a CCB" in an intention-to-treat approach. *New initiation* was defined as the first dispensation recorded without dispensation of either drug in the previous 6 months. The date of the first dispensation constituted the index date and the start of follow-up (Supplementary Figure S2). Because changes in the pattern of antihypertensive therapy are common in the course of CKD, we also

conducted a supporting analysis comparing risks associated with the cumulative drug exposure over time.

Covariates were derived at index date and included age, sex, comorbidities, ongoing medications, clinical assessments, and recent health care use (Supplementary Table S2). Comorbidities considered the underlying cause of CKD,[38] diabetes mellitus, hypertension, coronary artery disease, heart failure, cerebrovascular disease, peripheral vascular disease, arrythmia, and liver disease. Ongoing medications included potassium-sparing diuretics, β-blockers, α-blockers, vasodilators, antidiabetic drugs, lipid-modifying agents, and nonsteroidal anti-inflammatory drugs. Clinical assessments included systolic and diastolic blood pressure, body mass index, GFR estimated with the 2009 creatinine-based Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation, urinary albumin-to-creatinine ratio (ACR), hemoglobin, serum albumin, and serum potassium. Office blood pressure was measured at each outpatient visit, either through automated oscillometric device or manually according to the standard procedure at each nephrology clinic.[39] Because blood pressure was measured for clinical decision making in routine practice, the procedure followed the general guidelines of using an adapted sized cuff in a patient comfortably seated in a quiet room, after 5 minutes of rest. Recent health care use was used as a marker of overall disease burden and included the number of hospitalizations for any cause in the previous year as well as the number of hospitalizations in the previous 6 months for hyperkalemia or acute kidney injury.

Outcomes

The primary study outcome was the occurrence of major adverse kidney events (MAKE),[40] a composite of *initiation of KRT* (defined as start of maintenance dialysis or preemptive kidney transplantation), experiencing a decline in eGFR ≥ 40% from baseline, or experiencing an eGFR of <15 ml/min per 1.73 m^2. Each component of MAKE was also analyzed separately.

The secondary outcomes were all-cause mortality, cardiovascular and noncardiovascular death, and major adverse cardiovascular events (MACE; a composite of cardiovascular death, hospitalization for myocardial infarction, or stroke). We also evaluated repeated blood pressure measurements over the study period in the weighted population and represented them graphically by treatment group.

Safety outcomes were adverse events known to be associated with diuretic therapy, including hospitalizations and outpatient specialist care for acute kidney injury, hyperkalemia, hypokalemia, and hyponatremia. Outcome definitions are detailed in Supplementary Table S2. For each outcome, patients were followed from inclusion to the occurrence of event, death, or end of follow-up (May 1, 2022).

Statistical analyses

Main analyses. To control for baseline confounders, we used propensity score weighting, which targets an average treatment effect on the treated.[41] A multivariable logistic regression model was used to calculate the probability of receiving a diuretic or a CCB as a function of the baseline covariates listed above. Confounders were *a priori* selected on the basis of clinical knowledge and by consensus among study authors. Balance was considered appropriate if the standardized mean difference between treatment groups was <0.1 (10%) after propensity score weighting.

Weighted cumulative incidence curves were estimated using the Aalen-Johansen method. Weighted cause-specific Cox proportional hazards models were used to estimate hazard ratios (HRs) for the association between diuretic or CCB initiation and outcomes, and accounting for competing risks between MAKE and death and between MACE and death. Robust variance estimation was used to calculate 95% confidence intervals (CIs) after propensity score weighting. The proportional hazards assumption was checked using log(–log[S]) plots and Schoenfeld residuals against time. The interpretation of these methods in the presence of the competing risk of death is as follows: the Aalen-Johansen estimator estimates the total effect of the treatment on the outcome. Under strong assumptions, the cause-specific HRs can be interpreted as the direct effect of the treatment on the outcome (i.e., the effect of the treatment on the outcome that is not mediated by death), where death is considered a censoring event.[42,43]

Most study covariates had no missing values, but body mass index, serum potassium, and ACR were missing in ∼30% of patients. Because these clinical assessments are part of the routine monitoring of patients with CKD, we assumed missing to be at random and due to a lack of reporting to the registry. Indeed, characteristics of patients with versus without ACR measurements were not different (Supplementary Table S3). We then performed multiple imputations by chained equations using 50 imputed data sets with 20 iterations.

Subgroup analyses. To evaluate the consistency of our results, we performed prespecified subgroup analyses and tested interactions between treatment and age (≥75 years vs. <75 years), sex, presence versus absence of diabetes, systolic blood pressure (<120, [120–140], [140–160], ≥160 mm Hg), eGFR (≥30 ml/min per 1.73 m^2 vs. <30 ml/min per 1.73 m^2), and ACR (>30 mg/mmol vs. ≤30 mg/mmol).

Supporting analysis. Hypertension problems are common and intrinsic to the progression of CKD, naturally resulting in changes in the pattern of antihypertensive therapy in the course of CKD. To account for temporal and permanent discontinuations of therapy, switches across medication groups, and enrichments, we evaluated the total time drug exposure by modeling the cumulative use of each medication (see details in Supplementary Methods). In short, for each patient and at each dispense, we calculated the cumulative defined daily doses of diuretics and CCBs dispensed since the beginning of therapy. Then, the association between the cumulative use of diuretics, CCBs, and outcomes was analyzed using weighted Cox models and represented graphically. Their relative risk-benefit was compared by calculating the ratio of the HRs ($HR_{diuretics}$/HR_{CCBs}) per 1000 defined daily dose delivered.

Sensitivity analyses. (i) We redefined the window of no dispensation that determines eligibility from 6 to 12 months (n = 2705); (ii) we performed cause-specific Cox models considering the first outcome between MAKE, MACE, and death to assess the direct effect of the exposure and each outcome,[42,43] especially as hypertension and fluid overload are mainly treated by modulating ultrafiltration in dialysis; (iii) we repeated our main analysis in people free of cardiovascular disease at baseline (n = 3656), (iv) with any dispensation of RASi in the 4 months prior without consideration of adherence (n = 6334), (v) as well as with (n = 5799) and without (n = 5555) considering potassium-sparing diuretics in the diuretic group. (vi) Finally, we modeled negative control outcomes (including the most frequent causes of cancer [breast, prostate, lung, and colorectal cancers], gastritis/duodenitis with or without ulcer, cholecystis, and sigmoiditis) to study the influence of potential unmeasured confounders on our effect estimates. Although unmeasured confounders may predict the risk of negative outcomes, we did not expect the initiation of a diuretic or a CCB to cause or prevent them.[44]

We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for reporting of observational studies.[45] All statistical analyses were performed using R 3.6.3 software (https://cran.r-project.org/).

RESULTS

Baseline characteristics of patients with diuretics versus CCBs

We identified 5875 patients with nondialysis CKD stages 3–5 who, under long-term RASi treatment, started a diuretic or a CCB (Supplementary Figure S3). Their median age was 71 [interquartile range (IQR): 60–78] years; 64% ($N = 3779$) were men; eGFR was 26 [IQR: 20–34] ml/min per 1.73 m^2; and ACR was 31 [IQR: 6–116] mg/mmol. Of these, 3165 patients started a diuretic (including 2993, 163, and 9 users of loop diuretics, thiazides, or both, respectively) and 2710 started a CCB (including 2678 users of dihydropyridine CCBs and 32 of non-dyhydropyridine CCBs). Compared with new users of CCBs, patients on diuretics were older, were more often men, and had a higher prevalence of both atheromatous and nonatheromatous cardiovascular diseases (Supplementary Table S4). After weighting, all baseline characteristics were well balanced between the 2 groups (Table 1; Supplementary Figure S4).

Comparative effectiveness of diuretics versus CCBs on study outcomes

During a median follow-up of 6.3 years (IQR: 3.2–9.7 years), blood pressure remained stable and did not differ between the 2 groups of treatment (Supplementary Figure S5). A total of 2549 patients experienced MAKE, 1178 had a MACE, and 2299 patients died. After weighting, diuretic therapy was associated with a lower risk of MAKE (HR for diuretics vs. CCBs use 0.87 [95% CI 0.77–0.97]), which was consistent across each single component: KRT (HR 0.77 [95% CI 0.66–0.88]), ≥40% decline in eGFR (HR 0.80 [95% CI 0.71–0.91]), and eGFR < 15 ml/min per 1.73 m^2 (HR 0.84 [95% CI 0.74–0.96]; Table 2 and Figure 1; Supplementary Figure S6). The 5-year absolute risk of MAKE was lower in diuretic than in CCB users (49.4% [95% CI 47.2%–51.7%] vs. 54.2% [95% CI 50.8%–57.8%]; risk difference −4.80% [95% CI −8.95% to −0.66%]), with higher magnitudes at 8 and 10 years (Supplementary Table S5).

We did not observe any significant difference in the risk of all-cause mortality between diuretic and CCB use (HR 1.07 [95% CI 0.94–1.23]), both for noncardiovascular (HR 1.02 [95% CI 0.87–1.20]) and for cardiovascular (HR 1.19 [95% CI 0.94–1.50]) death. The risk of MACE (HR 1.14 [95% CI 0.96–1.36]) did not differ between therapies (Table 3 and Figure 1; Supplementary Figure S6). In absolute terms, the 5-year risk differences of MACE (4.50% [95% CI 0.84%–8.11%]) and all-cause mortality (4.20% [95% CI 0.192%–8.15%]) favored CCB users, but decreased at 8 and 10 years (Supplementary Table S5).

Supporting analyses

Modeling the total time drug exposure of each treatment provided consistent results with our main analysis. Compared with cumulative CCB use, cumulative diuretic use was associated with a lower risk of CKD progression (ratio of HR per each 1000 defined daily dose delivered: 0.89 [95% CI 0.85–0.94] for MAKE and 0.86 [95% CI 0.81–0.91] for KRT), with a similar risk of all-cause mortality (ratio of HR 1.02 [95% CI 0.98–1.06]) and MACE (ratio of HR 1.02 [95% CI 0.97–1.09]; Supplementary Figure S7).

Subgroup and sensitivity analyses

We observed in general no major differences in HR estimates across subgroups of age, sex, diabetes, systolic blood pressure, eGFR, or ACR (Figure 2; Supplementary Figures S8–S10). However, subgroup analyses might suggest somewhat stronger renoprotection for diuretics in older patients, those with higher blood pressure, or those with eGFR < 30 ml/min per 1.73 m^2.

Results were similar across sensitivity analyses (Supplementary Tables S6 and S7), and we did not observe differences in the risk of negative control outcomes between both therapies (Supplementary Table S8).

Potential adverse drug events, including acute kidney injury, hypokalemia, hyperkalemia, and hyponatremia, were not different between patients initiating diuretic therapy and those initiating CCB therapy (Supplementary Table S9).

DISCUSSION

In this large nationwide observational study of nephrologist-referred patients with CKD stages G3–G5 who initiated antihypertensive therapy on top of guideline-recommended RASi, we observed that compared with CCB therapy, diuretic therapy is associated with a lower risk of CKD progression and a similar risk of death and MACE. The association was consistent across the single components of our composite kidney outcome definition—including the hard end point of KRT—across subgroups of patients when evaluating the total time drug exposure and after considering death as a competing risk.

Our results are in line with the findings from small-scale studies reporting a synergy between diuretics and RASi in renoprotection.[13–20] Our results are novel and cannot be directly compared with preceding trials evaluating the impact of diuretic use, whose characteristics and findings are summarized in Supplementary Table S10. These trials were most often conducted in patients with a low risk of CKD progression[25,26,28,32] and investigated diuretics against no use,[33] as the first-line therapy,[24,34,46] or without cotreatment with RASi,[24,28,33,34,46] and these were not always consistent. We overcame some of the identified limitations of previous studies by selecting nephrologist-referred patients with moderate to advanced CKD, by having a long-term follow-up, and by evaluating a composite kidney outcome that is robust and includes kidney failure. We argue that previous studies have focused on short-term changes in eGFR/albuminuria, which are surrogate end points and may be affected by the reversible hemodynamic increase in serum creatinine often seen at the start of diuretics[17,18,47,48] or by the early vasodilatory effect of CCBs on renal afferent arterioles,[49,50] which may result in a higher initial eGFR,[51] but a higher long-term

Table 1 | Characteristics of the study population by treatment strategy after propensity score weighting

Characteristic	RASi + diuretic (n = 3165)	RASi + CCB (n = 3130)	SMD (%)
Demographics and clinical data			
Age, yr	73 [63–80]	72 [63–78]	2.0
Sex: woman	1229 (38.8)	1294 (41.4)	5.2
Body mass index, kg/m^2	28.4 [24.8–32.4]	28.3 [25.1–32.2]	5.4
Systolic BP, mm Hg	134 [120–148]	137 [125–150]	9.8
Diastolic BP, mm Hg	78 [70–84]	80 [70–85]	6.9
Medical history			
Diabetes mellitus	1312 (41)	1333 (43)	2.3
Myocardial infarction	410 (13)	405 (13)	0.0
Heart failure	614 (19)	580 (19)	2.2
Arrhythmia	511 (16)	476 (15)	2.6
Peripheral vascular disease	208 (7)	198 (6)	1.0
Cerebrovascular disease	212 (7)	195 (6)	2.0
Coronary artery disease	725 (23)	700 (22)	1.3
Primary cause of kidney disease			
Diabetic kidney disease	690 (22)	706 (23)	1.8
Glomerulonephritis	461 (15)	480 (15)	2.1
Nephroangiosclerosis or renovascular nephropathy	754 (24)	722 (23)	1.8
Others or unknown	1260 (40)	1223 (39)	1.5
Liver disease	77 (2)	82 (3)	1.1
Biological values			
Hemoglobin, g/dl	12.4 [11.4–13.5]	12.4 [11.2–13.5]	3.5
eGFR, ml/min per 1.73 m^2	26 [20–33]	26 [19–33]	1.9
eGFR, ml/min per 1.73 m^2			3.4
45–60	240 (8)	226 (7)	
30–45	872 (27)	832 (27)	
15–30	1666 (53)	1663 (53)	
<15	387 (12)	409 (13)	
ACR, mg/mmol	18 [4–95]	30 [6–118]	0.3
ACR, mg/mmol			16.4
A1: <3	645 (21)	522 (17)	
A2: 3–29	1181 (37)	1032 (33)	
A3: ≥30	1339 (42)	1576 (50)	
Serum potassium, mmol/l	4.5 [4.1–4.9]	4.5 [4.2–4.9]	0.9
Serum albumin, g/l	37 [34–40]	37 [34–39]	2.0
Medications			
RASi	3165 (100)	3130 (100)	0.0
β-Blockers	1947 (62)	1932.4 (62)	0.5
Potassium-sparing diuretics	244 (8)	201 (6)	5.0
α-Blockers	278 (9)	320 (10)	4.9
Vasodilators	18 (1)	51 (2)	10.3
NSAIDs	136 (4)	107 (3)	4.6
Lipid-lowering drugs	1886 (60)	1804 (58)	4.0
Health care use			
In the year before the index date			
Hospitalization for any cause	1041 (33)	1035 (33)	0.4
Hospitalization for any cause, no.	0.0 [0.0–1.0]	0.0 [0.0–1.0]	2.1
In the 6 months before the index date			
Hospitalization for hyperkalemia	0 (0)	0 (0)	0.0
Hospitalization for AKI	21 (1)	16 (1)	1.9

ACR, urinary albumin-to-creatinine ratio; AKI, acute kidney injury; BP, blood pressure; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; NSAID, nonsteroidal anti-inflammatory drug; RASi, renin-angiotensin system inhibitor; SMD, standardized mean difference.
Continuous variables are presented as median [interquartile range] and categorical variables as n (%).

increase in albuminuria,[23] and may not result in improved long-term clinical kidney outcomes.[23,24] Interestingly, our evaluation of safety outcomes did not show any increased risk of acute kidney injury or electrolyte disorders, which are adverse effects of diuretics that may be perceived as a barrier to its use. Subgroup analyses suggest somewhat

Table 2 | Primary study outcomes: weighted HRs for the association between diuretic use versus CCB use and adverse kidney outcomes

Kidney outcomes	No. of events	Person-years	Crude IR (95% CI)	Weighted HR[a] (95% CI)
MAKE (composite)				
Overall	2549	16,667	15.3 (14.7–15.9)	
RASi + CCB	1261	7350	17.2 (16.2–18.1)	Reference
RASi + diuretic	1288	9317	13.8 (13.1–14.6)	0.87 (0.77–0.97)
Single components of MAKE				
Kidney replacement therapy				
Overall	1689	20,526	8.2 (7.8–8.6)	
RASi + CCB	862	9062	9.5 (8.9–10.2)	Reference
RASi + diuretic	827	11,464	7.2 (6.7–7.7)	0.77 (0.66–0.88)
≥40% decline in eGFR				
Overall	1902	15,239	12.5 (11.9–13.1)	
RASi + CCB	960	6647	14.4 (13.5–15.4)	Reference
RASi + diuretic	942	8592	11.0 (10.3–11.7)	0.80 (0.71–0.91)
eGFR < 15 ml/min per 1.73 m^2				
Overall	1700	14,203	12.0 (11.4–12.6)	
RASi + CCB	838	6338	13.2 (12.3–14.1)	Reference
RASi + diuretic	862	7865	11.0 (10.2–11.7)	0.84 (0.74–0.96)

CCB, calcium channel blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; IR, incidence rate per 100 patient-year; MAKE, major adverse kidney event; RASi, renin-angiotensin system inhibitor.
[a]Weighted for age, sex, diabetes, hypertension, body mass index, underlying nephropathy, history of ischemic heart disease, heart failure, peripheral vascular disease, cerebrovascular disease, arrythmia, liver disease, systolic and diastolic blood pressure, hemoglobin, estimated glomerular filtration rate, urinary albumin-to-creatinine ratio, serum potassium, serum albumin, β-blockers, potassium-sparing diuretics, α-blockers, vasodilators, statins, hospitalization in the previous 6 months for hyperkalemia, acute kidney injury, and number of hospitalizations for any cause.

stronger renoprotection for diuretics in older patients, those with higher blood pressure, or those with eGFR < 30 ml/min per 1.73 m^2, which may be plausible and explained by a higher salt sensitivity related to a hyporeninism-hypoaldosteronism hormonal profile.

Although the ACCOMPLISH trial also compared RASi/CCB use with RASi/diuretic use,[25,26] the study population was quite different from that of our study (see Supplementary Table S11 for a head-to-head comparison between the ACCOMPLISH trial and our study), which may explain the different findings. ACCOMPLISH was prematurely terminated because of early demonstration of cardiovascular superiority—mainly on coronary disease—of CCBs over thiazides, with risks of all-cause mortality, stroke, and heart failure being not different. This early termination possibly affected the power of secondary kidney outcomes. Although the analysis of kidney events favored CCBs over thiazides,[25] effects were mostly attributed to the single end point of doubling of creatinine and were no longer significant in the <10% of patients with CKD.[25] This leaves a clinical

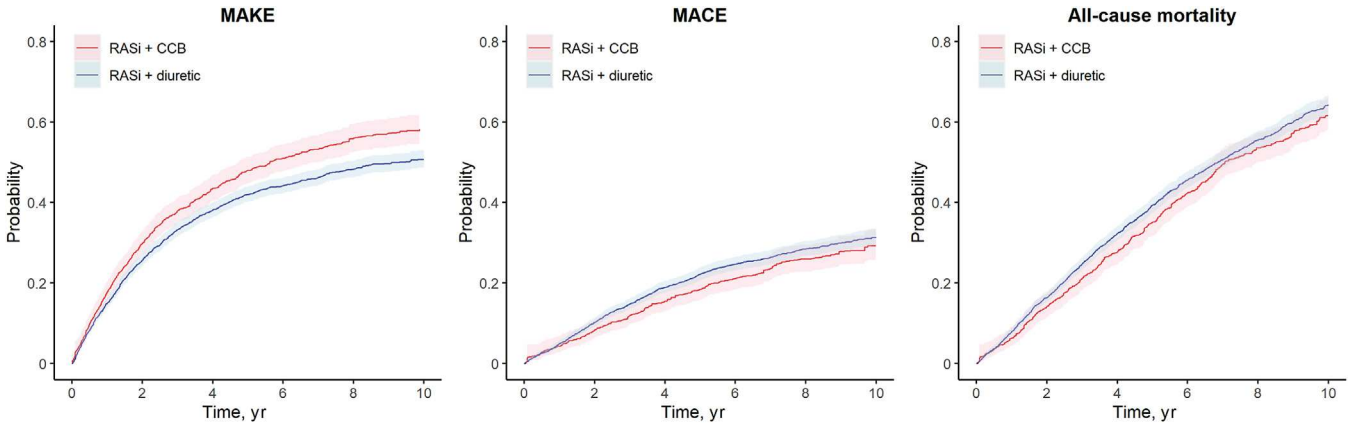

Figure 1 | Weighted cumulative incidence curves for major adverse kidney events (MAKE), major adverse cardiovascular events (MACE), and all-cause mortality according to treatment. Cumulative incidence curves were estimated with the Aalen-Johansen estimator to take into account competing risks between MAKE, MACE, and all-cause mortality. Cumulative incidence curves were weighted for age, sex, diabetes, hypertension, body mass index, underlying nephropathy, history of ischemic heart disease, heart failure, peripheral vascular disease, cerebrovascular disease, arrythmia, liver disease, systolic and diastolic blood pressure, hemoglobin, estimated glomerular filtration rate, urinary albumin-to-creatinine ratio, serum potassium, serum albumin, β-blockers, potassium-sparing diuretics, α-blockers, vasodilators, statins, hospitalization in the previous 6 months for hyperkalemia, acute kidney injury, and number of hospitalizations for any cause. CCB, calcium channel blocker; RASi, renin-angiotensin system inhibitor.

Table 3 | Secondary study outcomes: weighted HRs for the association between diuretic use versus. CCB use, MACE, and death

Outcomes	No. of events	Person-years	Crude IR (95% CI)	Weighted HR[a] (95% CIs)
All-cause death				
Overall	2299	27,927	8.2 (7.9–8.6)	
RASi + CCB	808	12,849	6.3 (5.9–6.7)	Reference
RASi + diuretic	1491	15,078	9.9 (9.4–10.4)	1.07 (0.94–1.23)
Non-CV death				
Overall	1570	27,927	5.6 (5.3–5.9)	
RASi + CCB	584	12,849	4.5 (4.2–4.9)	Reference
RASi + diuretic	986	15,078	6.5 (6.1–7.0)	1.02 (0.87–1.20)
CV death				
Overall	729	27,927	2.6 (2.4–2.8)	
RASi + CCB	224	12,849	1.7 (1.5–2.0)	Reference
RASi + diuretic	505	15,078	3.3 (3.1–3.7)	1.19 (0.94–1.50)
MACE				
Overall	1178	26,408	4.5 (4.2–4.7)	
RASi + CCB	422	12,191	3.5 (3.1–3.8)	Reference
RASi + diuretic	756	14,216	5.3 (4.9–5.7)	1.14 (0.96–1.36)

CCB, calcium channel blocker; CI, confidence interval; CV, cardiovascular; HR, hazard ratio; IR, incidence rate per 100 patient-year; MACE, major adverse cardiovascular event; RASi, renin-angiotensin system inhibitor.
[a]Weighted for age, sex, diabetes, hypertension, body mass index, underlying nephropathy, history of ischemic heart disease, heart failure, peripheral vascular disease, cerebrovascular disease, arrythmia, liver disease, systolic and diastolic blood pressure, hemoglobin, estimated glomerular filtration rate, urinary albumin-to-creatinine ratio, serum potassium, serum albumin, β-blockers, potassium-sparing diuretics, α-blockers, vasodilators, statins, hospitalization in the previous 6 months for hyperkalemia, acute kidney injury, and number of hospitalizations for any cause.

knowledge gap for patients with CKD stages 3–5, where choices of antihypertensive treatment are not defined and that the present study tries to address.

Pathophysiological hypotheses that may explain the observed protective effect of diuretics on CKD progression include the following: (i) a decrease in renal venous pressure, possibly slowing impairment of renal microcirculation and improving renal filtration[52]; (ii) a decrease in intraglomerular pressure slowing glomerulosclerosis and CKD progression; (iii) a decrease in pressure-independent alterations of structure and function of large arteries[4,53–55]; (iv) both thiazides and loop diuretics potentiate anti-albuminuria properties of RASi,[13–19] likely mediated by diuretic-induced volume depletion and hemodynamic changes[18]; and finally, (v) in patients with heart failure, diuretics improve cardiac filling pressures and venous congestion, resulting in better long-term kidney outcome.[56] Furthermore, we cannot exclude the possibility that an adverse effect of CCBs might contribute to explain our results. Indeed, despite a higher eGFR after treatment initiation,[51] CCBs may be associated with an increase in albuminuria[23] and a subsequent faster CKD progression,[23,24] because of an increase in intraglomerular pressure consecutive to afferent arteriole vasodilation and loss of autoregulation.[49,50]

We did not observe any lower risk of death or MACE for any of the treatment strategies, which is consistent with other trials—except ACCOMPLISH—comparing diuretics versus CCBs (with[57] or without[24,28,34,46] cotreatment with RASi) and with a large observational study conducted in patients with diabetes who were treated with a thiazide or a CCB on top of

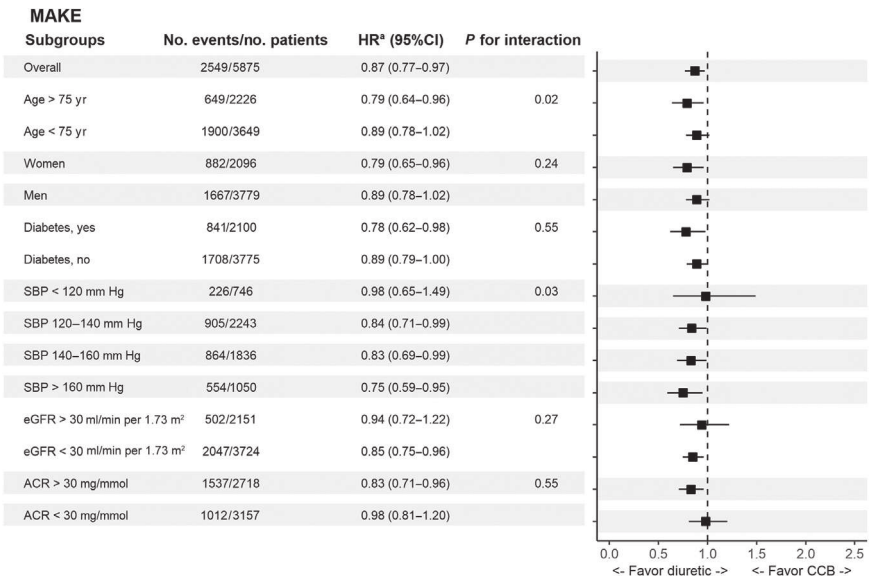

Figure 2 | Subgroup analyses: weighted hazard ratios (HRs) for the association between diuretic use versus calcium channel blocker (CCB) use and major adverse kidney events (MAKE). [a]Weighted for age, sex, diabetes, hypertension, body mass index, underlying nephropathy, history of ischemic heart disease, heart failure, peripheral vascular disease, cerebrovascular disease, arrythmia, liver disease, systolic (SBP) and diastolic blood pressure, hemoglobin, estimated glomerular filtration rate (eGFR), urinary albumin-to-creatinine ratio (ACR), serum potassium, serum albumin, β-blockers, α-blockers, vasodilators, potassium-sparing diuretics, statins, hospitalization in the previous 6 months for hyperkalemia, acute kidney injury, and number of hospitalization for any cause. CI, confidence interval.

a 6-month therapy with RASi.[32] Although not statistically significant, we notice that the risks of MACE and cardiovascular death were somewhat higher in diuretic users than in CCB users whereas the difference in risk was null for non-cardiovascular death. Studying the impact of diuretic prescription on cardiovascular and death outcomes in CKD in observational studies is challenging because of confounding by indication bias. Fluid overload, for instance, may increase the risk of heart failure and subsequent risk of death from cardiovascular disease. A previous study[58] reported a higher risk of heart failure hospitalization in patients with CKD using diuretics versus nonuse (sub-distribution HR 1.83 [95% CI 1.43–2.32]), a counterintuitive finding attributed by the authors to unmeasured confounding by indication. In contrast, we expect this bias to less strongly affect kidney outcomes, given that delay of CKD progression is not an indication for neither therapy.

Our findings have implications for clinical practice and future research, suggesting that a diuretic could be proposed in CKD on top of RASi.[59,60] However, diuretics are often poorly and/or inadequately prescribed, mainly because diuretic prescription is challenging as it may induce an acute decrease in kidney function at start,[61] which may lead to discontinue or reduce the diuretic dose, and in turn cause fluid overload and poor long-term prognosis.[60] Taken together with the strong differences worldwide in nephrology practices for diuretic prescription,[10] this study highlights the need for clearer guidelines for diuretic management in patients with CKD. Diuretics efficaciously and safely reduce extracellular fluid volume and blood pressure if the dosage is carefully adjusted at the onset of the treatment[60,62] to avoid intravascular volume depletion from inadequate plasma refilling, potentially leading to a clinically relevant increase in serum creatinine.[13]

Strengths of our analysis include its large sample size, nationwide capture with long follow-up, careful design, robustness across various supporting and sensitivity analyses, and the unique setting involving real-world patients from a country with universal tax-funded health care, which minimizes selection bias from disparate access to health care. Our study also has limitations, starting by its observational nature, which is prone to residual confounding. The number of patients using thiazides was small, possibly reflecting the guideline-recommended advice not to use thiazides in patients with eGFR < 30 ml/min per 1.73 m^2. This prevented us from analyzing loop diuretics and thiazides separately. Moreover, previous beliefs that thiazide diuretics are not effective in advanced CKD may have influenced the decision to start one or the other medication in our study. We tried to minimize this confounding through propensity score weighting for a large array of identified confounders. However, we cannot rule out the possibility that loop diuretics may have been prescribed for other indications uncontrolled in our analysis, such as clinically evident volume overload, which may explain the magnitude of our cardiovascular disease–related outcomes. We used an intention-to-treat approach and assumed that initiated treatment was continued, which may lead to bias toward the null. Our modeling of total time drug exposure nevertheless shows consistent findings and strengthens our confidence in the results. Another limitation is that adverse events were evaluated by issued diagnoses based on hospitalizations and outpatient specialist care data, but electrolyte disorders not recognized with diagnoses may have been missed. Finally, Sweden has traditionally limited ethnic diversity, which may preclude generalizability of our findings to other ethnicities.

To conclude, results of this large real-world observational study suggest that in patients with CKD stages G3–G5, compared with CCB therapy, diuretic therapy on top of RASi may further slow CKD progression, beyond their antihypertensive effect. Combined with our current understanding of the deleterious effect of volume overload,[1–7] these findings provide the rationale to initiate a clinical trial comparing these 2 antihypertensive treatment strategies in patients with CKD.

DISCLOSURE

ME reports personal honoraria for lectures by AstraZeneca, Astellas Pharma, Vifor Pharma, Fresenius Medical Care, and Baxter Healthcare and being a member of advisory boards for Astellas Pharma, AstraZeneca, and Vifor Pharma. J-JC reports funding to Karolinska Institutet by AstraZeneca, Astellas Pharma, Amgen, Vifor Pharma, and Novo Nordisk; personal honoraria for lectures by Fresenius Kabi, Baxter Healthcare, and Abbott; and being a member of advisory boards for Astellas, AstraZeneca, and GlaxoSmithKline. All the other authors declared no competing interests.

ACKNOWLEDGMENTS

This study was supported by the Swedish Research Council and the Swedish Heart and Lung Foundation. A-LF was supported by Institut National de la Santé et de la Recherche Médicale (Inserm), Paris-Saclay University, France. ELF was supported by a Rubicon grant from the Netherlands Organization for Scientific Research (NWO). ME was supported by funding from Region Stockholm (Center of Innovative MEDicine [CIMED], and ALF Medicine).

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods. Supporting analysis of cumulative drug exposure over time.

Supplementary Table S1. Brief protocol of the pragmatic target trial and its emulation using data from the Swedish Renal Registry.

Supplementary Table S2. Definitions of comorbidities, medications, and outcomes.

Supplementary Table S3. Characteristics of patients with versus without available value of urinary albumin-to-creatinine ratio.

Supplementary Table S4. Characteristics of the study population before propensity score weighting.

Supplementary Table S5. Absolute risks and risk difference of major adverse kidney events (MAKE), major adverse cardiovascular events (MACE), and all-cause mortality.

Supplementary Table S6. Characteristics of new users of diuretics versus calcium channel blockers, new user being redefined as no dispensation of either drug in the previous 12 months.

Supplementary Table S7. Sensitivity analyses: weighted hazard ratios for the association between diuretic use versus calcium channel blocker use and adverse outcomes.
Supplementary Table S8. Negative control outcomes: weighted hazard ratios for the association between diuretic use versus calcium channel blocker use and hospitalization for gastrointestinal diseases or cancer.
Supplementary Table S9. Weighted hazard ratios for the association between diuretic use versus calcium channel blocker use and adverse events.
Supplementary Table S10. Main characteristics and findings from randomized controlled trials and observational studies comparing diuretics and calcium channel blockers as antihypertensive treatment.
Supplementary Table S11. Head-to-head comparison between the patients enrolled and the findings of the ACCOMPLISH trial and the present observational study.
Supplementary Figure S1. Selection of the study population.
Supplementary Figure S2. Study design following the graphical display recommended by the reporting guidelines for observational studies by Schneeweiss *et al*.[63]
Supplementary Figure S3. Flowchart of the study population.
Supplementary Figure S4. Covariates balance.
Supplementary Figure S5. Changes in blood pressure during follow-up according to treatment group.
Supplementary Figure S6. Weighted cumulative incidence curves for kidney replacement therapy (KRT), cardiovascular (CV) mortality, and non-CV mortality according to treatment.
Supplementary Figure S7. Weighted hazard ratios for the association between the cumulative dose use of diuretics and calcium channel blockers (CCBs) and adverse outcomes.
Supplementary Figure S8. Hazard ratios (HRs) for the association between diuretic use (vs. calcium channel blocker use) and all-cause mortality according to subgroups.
Supplementary Figure S9. Hazard ratios (HRs) for the association between diuretic use (vs. calcium channel blocker use) and kidney replacement therapy (KRT) according to subgroups.
Supplementary Figure S10. Hazard ratios (HRs) for the association between diuretic use (vs. calcium channel blocker use) and major adverse cardiovascular events (MACE [composite outcome of the following 3 criteria: cardiovascular death, hospitalization for myocardial infarction, or stroke]) according to subgroups.

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