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Research Article

Overhydration Measured by Bioimpedance Spectroscopy and Urinary Serine Protease Activity Are Risk Factors for Progression of Chronic Kidney Disease

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Keywords
Bioimpedance spectroscopy · Chronic kidney disease · Overhydration · Progression · Proteasuria

Abstract
Background: Overhydration (OH) is common in chronic kidney disease (CKD) and might be related to the excretion of urinary serine proteases. Progression of CKD is associated with proteinuria; however, the interrelations of urinary serine proteases, OH, and progression of CKD remain unclear. ***Methods:*** In *n* = 179 patients with stable nondialysis-dependent CKD of all stages, OH was measured using bioimpedance spectroscopy (Body Composition Monitor; Fresenius), and urinary serine protease activity was determined using the peptide substrate S-2302. After a median follow-up of 5.9 (IQR: 3.9–6.5) years, progression to end-stage renal disease (ESRD) was analyzed retrospectively. ***Results:*** OH correlated with baseline MDRD-eGFR, urinary albumin creatinine ratio (ACR), and urinary aprotinin-sensitive serine protease activity. Progression to ESRD occurred in *n* = 33 patients (19%) and correlated with OH and urinary serine protease activity as well as MDRD-eGFR and ACR. Patients were divided into 2 groups determined by cutoff values from receiver operating characteristics for MDRD-eGFR (32 mL/min/1.73 m^2), ACR (43 mg/g creatinine), urinary serine protease activity (0.9 RU/g creatinine), and OH (1 L/1.73 m^2). Across these cutoff values, Kaplan-Meier curves for renal survival showed significant separations of the groups. In Cox regression adjusted for MDRD-eGFR, ACR, P-NT-pro-BNP, systolic blood pressure, and diabetes mellitus, patients with OH >1 L/1.73 m^2 had a 3.32 (95% CI: 1.26–8.76)-fold higher risk for progression to ESRD. ***Conclusions:***

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Our results corroborate that OH detected by bioimpedance spectroscopy in CKD patients is an independent risk factor for progression to ESRD in addition to GFR and albuminuria. Urinary serine protease activity is associated with OH and progression of CKD and provides a possible underlying mechanism.

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Introduction

Identification of patients at risk for progression of chronic kidney disease (CKD) and prevention of progression to end-stage renal disease (ESRD) is a challenge for the treating nephrologist. Besides GFR, albuminuria has been recognized as an important risk factor for progression of CKD, leading to a reclassification of CKD staging by the KDIGO guidelines in 2012 [1]. Addition of the albuminuria category independently and strongly predicted major clinical endpoints such as mortality, risk of CKD progression, and development of ESRD or acute kidney injury [2]. Up to now, the mechanisms underlying these strong associations remain unclear.

In addition to being a marker of renal and overall endothelial damage, albuminuria may also indicate a pathophysiological role of proteinuria in CKD by promoting tubular damage, salt sensitivity, and sodium retention [3]. Proteinuria was found to be a significant and independent predictor of fluid overload compatible with a pathophysiological role in mediating sodium retention through urinary excretion of active serine proteases, described by the term proteasuria [3, 4]. Of note, proteinuria was in the nonnephrotic range, and excretion of active plasmin or plasma kallikrein as surrogates for proteasuria was associated with fluid overload [4, 5]. Extracellular volume expansion as a consequence of disturbed sodium homeostasis has been observed in several studies in patients with nondialysis-dependent CKD and was associated with progression of CKD [6–8]. Our group has shown that overhydration (OH) measured by bioimpedance spectroscopy is present in a latent form in CKD patients, which escapes clinical examination [4]. Proteasuria could provide an underlying mechanism for the strong association of proteinuria and progression of CKD, as it could affect renal function by increasing volume overload and by proteolytic destruction and tubular integrity [9]. However, the interrelations of urinary serine protease activity and latent fluid overload discovered by bioimpedance spectroscopy with progression of CKD remain ill defined. In this study, we attempted to close this gap of knowledge by investigating the prognostic impact of OH and proteasuria on progression of CKD from a retrospective data analysis of a previously phenotyped CKD cohort [4].

Materials and Methods

Patients

This study is a retrospective outcome analysis of a cross-sectional phenotyped cohort of patients with CKD. Patients with CKD who presented at the outpatient clinic of the department of Internal Medicine IV of the University Hospital of Tuebingen between September 2012 and April 2013 had been consecutively included in the primary study, if they gave their informed consent. The primary study included among others the measurement of body fluid status using bioimpedance spectroscopy and collection of blood and spot urine samples [4]. Patients were classified into the GFR category (G1 eGFR >90 mL/min/1.73 m^2; G2 eGFR 60–89 mL/min/1.73 m^2; G3a eGFR 45–59 mL/min/1.73 m^2; G3b eGFR 30–44 mL/min/1.73 m^2; G4 15–29 mL/min/1.73 m^2; and G5 eGFR <15 mL/min/1.73 m^2) and the albuminuria category (A1 albumin

creatinine ratio [ACR] <30 mg/g creatinine; A2 ACR 30–300 mg/g creatinine; and A3 ACR >300 mg/g creatinine) according to the Kidney Disease Improving Global Outcomes classification of CKD [1]. The primary study and analysis of the follow-up (FU) were approved by the local ethics committee of the University of Tuebingen (259/2012MPG23 and 732/2019BO2), and all participants gave their informed consent prior to their inclusion in the study [4].

Collection of Clinical Data

From all patients, data on demographics, diagnoses, and medication were collected. Body weight and height, edema, and office blood pressure were determined. Body composition and fluid status were measured using bioimpedance spectroscopy (Body Composition Monitor [BCM]; Fresenius Medical Care) as described previously [4]. BCM measurement was performed in a lying position on the right side of the body with placement of the first electrode adhesive over the wrist or ankle joint and the second electrode adhesive at least 3 cm distal to it. Results of the measurement were accepted when quality estimate *Q* was >90%. The BCM enables to distinguish between intracellular and extracellular water by measuring the conductivity of the body at different measuring frequencies [10]. On the basis of physiological models, the device calculates the parameters for assessing fluid balance and body composition [11]. OH measured by BCM in healthy individuals was reported to be −1.1 to +1.1 L [10]. Parameters of fluid status were normalized to a body surface area of 1.73 m^2.

A single blood and spot urine sample was collected from all patients. Laboratory measurements were performed using automated Siemens autoanalyzers (Advia 1800, Immulite 2000, and BN ProSpec Analyzer). Serum aldosterone and plasma renin activity were measured manually using RIA methods (Immunotech, Prague, Czech Republic and Zentech, Angleur, Belgium), with results expressed as pg/mL for serum aldosterone and ng Ang I/mL/h for plasma renin activity.

As a surrogate for proteasuria, aprotinin-sensitive proteolytic activity in urine samples was measured manually using a chromogenic tripeptide substrate (S-2302, H-D-Pro-Phe-Arg-pNA·2HCl, 2 mM; Haemochrom Diagnostica, Essen, Germany) as difference of absorption without and with addition of the broad-spectrum serine protease inhibitor aprotinin (1 mg/mL; Loxo, Heidelberg, Germany) in vitro. In nephrotic mice, aprotinin has prevented sodium retention pointing to an essential role of trypsin-like serine proteases excreted in the urine [6, 12]. Reactions took place in a total volume of 63 µL (10-µL urine +50-µL S-2302 + 3-µL aprotinin or Ampuwa) at 37°C, and absorption was measured at 405 nm after an incubation time of 8 h (EL800 Microplate Reader; BioTek Instruments Inc.,Winooski, VT, USA). Values were normalized for urinary creatinine concentration and expressed as relative units (1 RU = 1,000 relative fluorescence units/mg creatinine).

FU and Outcome

FU data were collected from patients' records of the University Hospital Tuebingen in October 2019. Diagnoses, medication, and laboratory parameters from the last presentation of each patient were screened, and the following data were documented: date of the documented data, eGFR estimated by the MDRD formula or in case of progression to ESRD, the date of start of renal replacement therapy (RRT), and date of death.

The primary outcome investigated was progression of CKD, defined as progression to ESRD with start of RRT or decline by 30% of baseline eGFR. Start of RRT was determined as the date of the first hemodialysis or peritoneal dialysis treatment or the date of transplantation which was realized as preemptive living kidney donor transplantation. Decline by 30% of baseline MDRD-eGFR was defined as progression of CKD as proposed by Levey et al. [13].

Patients who died during FU were included in the analysis of progression of CKD. Patients with no available FU data were not included in the analysis.

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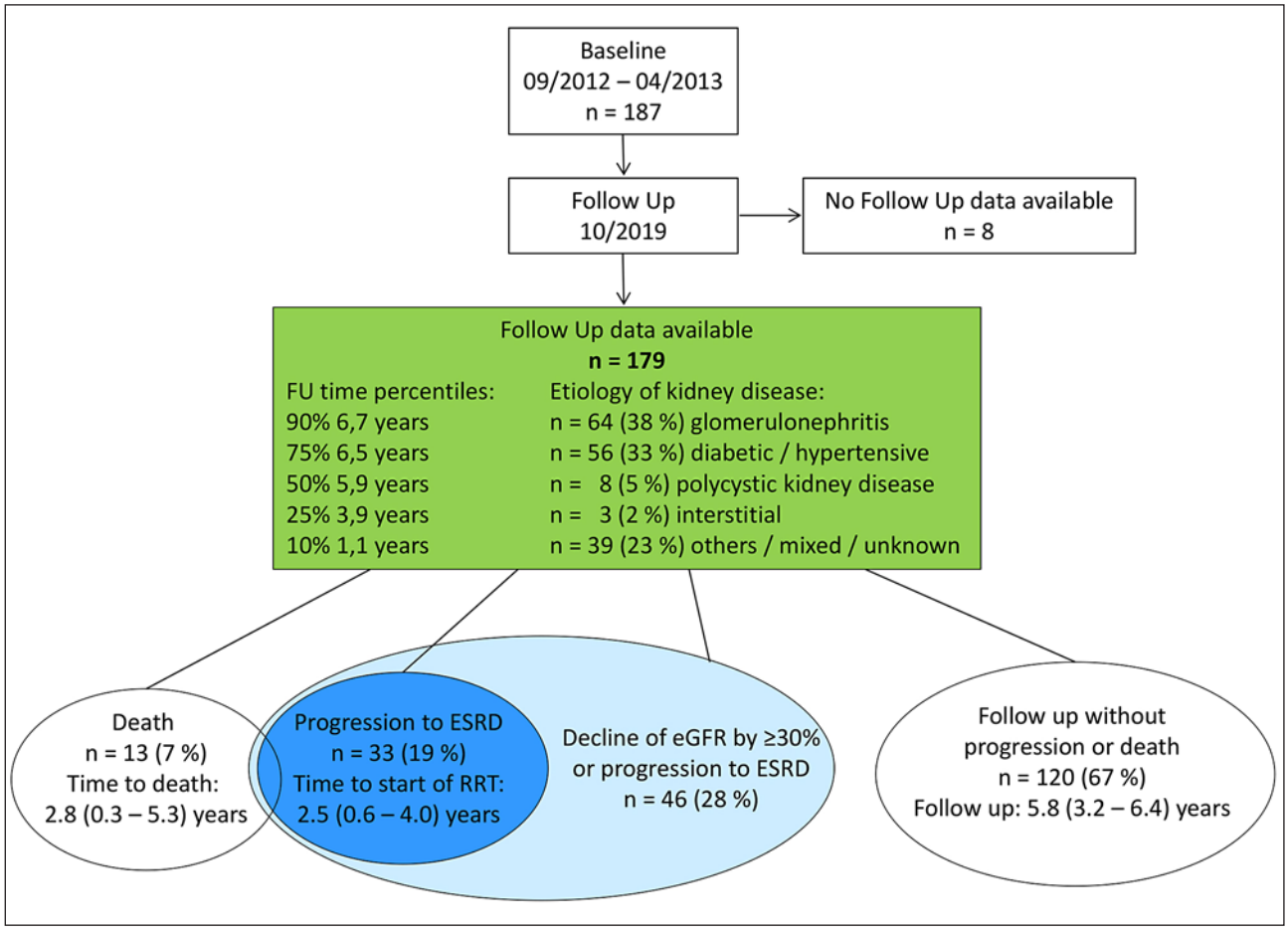

Fig. 1. Flowchart of patients. In *n* = 2 cases, progression to ESRD and death occurred during FU. ESRD, end-stage renal disease; FU, follow-up; RRT, renal replacement therapy; eGFR, estimated glomerular filtration rate.

Statistical Analysis

Continuous parameters were tested for normal distribution. Distributions are reported as number (*n*) and percent for categorical parameters and median and interquartile range (IQR) for continuous parameters. Differences between groups were tested using the Wilcoxon (continuous parameters) or χ^2 or ANOVA (categorical parameters) test. Univariate correlations with endpoints were tested using the χ^2 test for categorical parameters and receiver operating characteristic (ROC) for continuous parameters. Where ROC analysis was performed, area under the curve (AUC) and cutoff values determined by the Youden index (J = sensitivity + [specificity – 1]), with associated sensitivity and specificity, are reported. For multivariate analysis, parameters that correlated significantly in univariate analysis were included in a stepwise model with forward and backward direction modes, probability to enter $p = 0.25$, and probability to leave $p = 0.1$ to select parameters that were included into the final nominal logistic model. Patients were divided into groups on the basis of the cutoff values of the ROC analysis, and Kaplan-Meier curves were constructed for different groups; the log-rank test was performed to test for differences of Kaplan-Meier curves of different groups. Cox regression was performed to analyze proportional hazard ratios (HRs); HRs for continuous parameters are reported per increase of 1 unit or of 1 standard deviation. Data analysis was performed using the statistical software packages (SAS Institute Inc.) JMP 14.2.0 and MedCalc 19.1.

Table 1. Baseline characteristics of the study cohort

Baseline parameters	All patients with available FU (*n* = 179)	Patients with progression to ESRD (*n* = 33)	Patients without progression to ESRD (*n* = 144)	*p* value
Age, years	60 (48–71)	59 (48–65)	60 (48–74)	0.3310
Gender, male, *n* (%)	88 (55)	17 (52)	81 (56)	0.6216
Hypertension, *n* (%)	145 (81)	30 (90)	114 (79)	0.1182
Diabetes mellitus, *n* (%)	37 (21)	8 (24)	29 (20)	0.6010
Presence of edema, *n* (%)	65 (36)	15 (45)	49 (34)	0.2178
Systolic BP, mm Hg	134 (125–149)	139 (130–153)	134 (124–147)	0.0769
Diastolic BP, mm Hg	80 (70–87)	80 (70–89)	80 (71–86)	0.9085
Diuretic therapy, *n* (%)	110 (61)	22 (67)	86 (60)	0.4606
RAAS blocker, *n* (%)	140 (78)	24 (72)	115 (80)	0.3680
>4 BP medications, *n* (%)	30 (17)	9 (27)	21 (15)	0.0797
OH, L/1.73 m^2	0.2 (−0.5 to 1.2)	1.4 (0.0–2.2)	0.0 (−0.6 to 0.7)	**0.0003**
ECW, L/1.73 m^2	15.8 (14.7–16.9)	16.3 (15.6–17.2)	15.7 (14.6–16.7)	0.0754
ICW, L/1.73 m^2	17.7 (16.3–19.5)	17.9 (16.6–18.6)	17.7 (16.2 (19.7)	0.6191
ECW/ICW	0.89 (0.82–0.98)	0.9 (0.9–1.0)	0.88 (0.81–0.96)	**0.0140**
BMI, kg/m^2	28.4 (25.5–32.1)	28.7 (23.6–31.1)	28.0 (25.6–32.2)	0.6704
P-creatinine, mg/dL	1.4 (0.9–2.1)	3.0 (2.2–4.2)	1.3 (0.9–1.7)	**<0.0001**
MDRD-eGFR, mL/min/1.73 m^2	47 (30–71)	18 (12–30)	56 (37–76)	**<0.0001**
S-aldosterone, pg/mL	119 (86–166)	118 (98–178)	118 (82–164)	0.6035
P-renin, ng Ang I/mL/h	2.9 (1.2–9.4)	2.5 (0.9–8.4)	3.0 (1.3–9.5)	0.6020
P-NT-pro-BNP, pg/mL	182 (68–613)	536 (309–2,463)	126 (65–388)	**<0.0001**
U-Na, mmol/g Crea	162 (98–244)	151 (90–248)	164 (100–249)	0.6285
PCR, mg/g Crea	441 (146–1,639)	2,176 (620–4,003)	310 (136–1,027)	**<0.0001**
ACR, mg/g Crea	43 (5–198)	411 (60–1,490)	31 (5–189)	**<0.0001**
U-aprotinin-sensitive activity, RU/g Crea	0.75 (0.21–3.24)	5.23 (0.95–16.03)	0.61 (0.19–2.06)	**0.0014**

Values reported are *n* (%) for categorical variables and median (IQR) for continuous variables. Differences between groups of patients with and without progression to ESRD were tested. *p* values are from the χ^2 test for categorical variables and Wilcoxon test for continuous variables and marked in bold if significant ($p < 0.05$). FU, follow-up; ESRD, end-stage renal disease; BP, blood pressure; RAAS, renin angiotensin aldosterone system; OH, overhydration; ECW, extracellular water; ICW, intracellular water; BMI, body mass index; U, urine; Na, natrium; Crea, creatinine; P, plasma; MDRD-eGFR, calculated glomerular filtration rate by MDRD formula; S, serum; BNP, brain natriuretic peptide; PCR, urinary protein creatinine ratio; ACR, urinary albumin creatinine ratio; RU, relative units.

Results

Characterization of the Study Cohort and Baseline Parameters

The initial study included consecutively *n* = 187 patients with CKD presenting at the outpatient clinic of the department of Internal Medicine IV at the University Hospital Tuebingen [4]. From these patients, FU was available in *n* = 179 patients, and overall FU time was 5.9 years (IQR: 3.9–6.5). The flowchart and baseline characteristics of the study cohort are provided in Figure 1 and Table 1. Information on progression of CKD to ESRD was available in *n* = 177 patients, and progression to ESRD occurred in *n* = 33 patients (19%) with median start of RRT after 2.5 years (IQR: 0.6–4.0, Fig. 1). A decline of eGFR by ≥30% of baseline eGFR without progression to ESRD occurred in *n* = 13 (10%) of *n* = 133 patients with available laboratory parameters at FU, leading to *n* = 46 (28%) patients with the combined endpoint (*n* = 166). Mortality was only observed in *n* = 13 (7%) patients in this cohort and too low to perform statistical analyses.

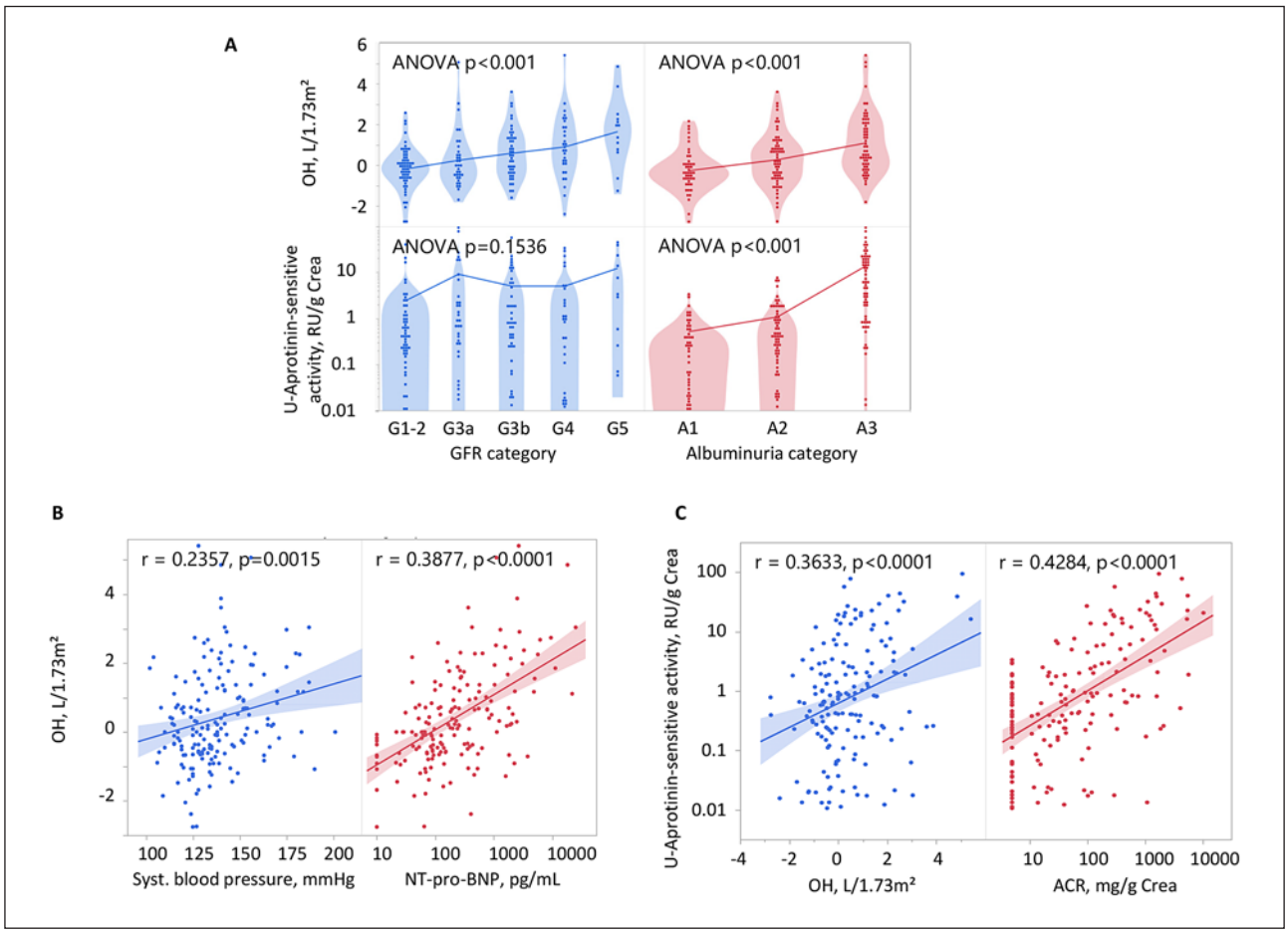

Fig. 2. Correlations of OH and urinary aprotinin-sensitive proteolytic activity with the eGFR and albuminuria category of CKD (**A**), OH with systolic blood pressure and NT-pro-BNP (**B**), and urinary aprotinin-sensitive proteolytic activity with OH and albuminuria (**C**) at baseline. GFR and albuminuria categories of CKD were classified according to the Kidney Disease Improving Global Outcomes classification of CKD as follows: G1 eGFR >90 mL/min/1.73 m^2; G2 eGFR 60–89 mL/min/1.73 m^2; G3a eGFR 45–59 mL/min/1.73 m^2; G3b eGFR 30–44 mL/min/1.73 m^2; G4 15–29 mL/min/1.73 m^2; and G5 eGFR <15 mL/min/1.73 m^2 and A1 ACR <30 mg/g creatinine; A2 ACR 30–300 mg/g creatinine; and A3 ACR >300 mg/g creatinine. OH, overhydration measured by bioimpedance spectroscopy; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; BNP, brain natriuretic peptide; U, urine; RU, relative units; Crea, creatinine; ACR, urinary albumin creatinine ratio.

In patients with available FU, baseline age was 60 years (IQR: 48–71), 55% of patients were male, and 36% of patients had edema at physical examination (Table 1). eGFR at baseline was 47 mL/min/1.73 m^2 (IQR: 30–71), and ACR was 43 mg/g creatinine (IQR: 5–198). Baseline OH measured by bioimpedance spectroscopy was 0.2 L/1.73 m^2 (IQR: −0.5 to 1.2) and significantly increased with the higher GFR category and albuminuria category of CKD (Fig. 2A). Baseline OH showed significant correlations with systolic blood pressure and NT-pro-BNP (Fig. 2B). Baseline aprotinin-sensitive urinary proteolytic activity was 0.75 RU (IQR: 0.21–3.24) and increased with the higher albuminuria category but not GFR category of CKD (Fig. 2A). Urinary aprotinin-sensitive proteolytic activity significantly correlated with baseline OH ($r = 0.3633$, $p < 0.0001$) and ACR ($r = 0.4284$, $p < 0.0001$; Fig. 2C).

Table 2. Univariate analysis of baseline parameters and progression of CKD

Baseline parameters	Progression to ESRD (n = 33/177)		Progression to ESRD or decline of GFR by 30% (n = 46/166)	
	AUC, p value	cutoff (sens., spec.)	AUC, p value	cutoff (sens., spec.)
OH, L/1.73 m^2 (BCM)	0.705, p = 0.0001	0.98 (0.61, 0.79)	0.675, p = 0.0005	0.69 (0.59, 0.71)
ECW, L/1.73 m^2 (BCM)	0.599, p = 0.0998	15.6 (0.79, 0.45)	0.592, p = 0.0458	15.6 (0.70, 0.50)
ECW/ICW (BCM)	0.637, p = 0.0251	0.89 (0.76, 0.52)	0.643, p = 0.0172	0.89 (0.74, 0.57)
P-creatinine, mg/dL	0.922, p < 0.0001	1.8 (0.94, 0.79)	0.888, p < 0.0001	1.8 (0.83, 0.86)
MDRD-eGFR, mL/min/1.73 m^2	0.930, p < 0.0001	31.8 (0.88, 0.86)	0.885, p < 0.0001	39.3 (0.89, 0.80)
P-NT-pro-BNP, pg/mL	0.760, p = 0.0168	281.0 (0.81, 0.71)	0.713, p = 0.0417	253 (0.68, 0.70)
PCR, mg/g Crea	0.779, p < 0.0001	994.1 (0.71, 0.74)	0.771, p < 0.0001	763.5 (0.73, 0.73)
ACR, mg/g Crea	0.747, p = 0.0131	43.1 (0.84, 0.59)	0.731, p = 0.0162	43.1 (0.77, 0.61)
U-aprotinin-sensitive activity, RU/g Crea	0.690, p = 0.0438	0.92 (0.76, 0.63)	0.695, p = 0.0343	0.92 (0.71, 0.66)

p values are from the χ^2 test. AUC values are from ROC analysis performed for continuous variables; cutoff values were determined by the Youden index (J = sensitivity + [specificity – 1]). CKD, chronic kidney disease; ESRD, end-stage renal disease; GFR, glomerular filtration rate; sens., sensitivity; spec., specificity; AUC, area under the curve; OH, overhydration; BCM, Body Composition Monitor; ECW, extracellular water; ICW, intracellular water; P, plasma; MDRD-eGFR, calculated glomerular filtration rate by the MDRD formula; BNP, brain natriuretic peptide; U, urine; PCR, urinary protein creatinine ratio; Crea, creatinine; ACR, urinary albumin creatinine ratio; RU, relative units; ROC, receiver operating characteristic.

Univariate Correlations

Bioimpedance spectroscopy-measured OH and aprotinin-sensitive urinary protease activity were significantly associated with progression of CKD to ESRD, as it was the case for plasma creatinine concentration, eGFR calculated by the MDRD formula, proteinuria and albuminuria, and extracellular water and ratio of extra- to intracellular water measured by bioimpedance spectroscopy (Table 2). Plasma NT-pro-BNP reflecting cardiovascular risk was also significantly correlated with progression of CKD (Table 2), whereas parameters reflecting traditional risk factors such as blood pressure, known hypertension, diabetes mellitus, or medication with diuretics or RAAS blockers did not show significant correlations with progression of CKD (therefore, not shown in Table 2). The combined endpoint of progression to ESRD and decline of eGFR by >30% of baseline were associated significantly with the same parameters (Table 2). The proportion of progression to ESRD was higher in patients with higher values for OH and aprotinin-sensitive urinary protease activity (Table 3). Other parameters such as age, gender, urinary sodium concentration, renin activity, and aldosterone concentration or presence of edema did not show any significant correlations with progression of CKD in this cohort (p > 0.05).

Multivariate Models and ROC

To identify parameters independently associated with progression of CKD, all parameters significantly associated with progression of CKD in univariate analysis were included in a nominal logistic model, with selection of parameters entering the final model by a stepwise approach. In the stepwise selection model, eGFR estimated by the MDRD formula and ACR were determined as the only independent determinants of progression of CKD and entered the final nominal logistic model (Table 4, models 1 and 2). Entering OH as the variable of interest and OH crossed with MDRD-eGFR and ACR for controlling interaction lead to a slight increase of r^2 and showed a significant influence of OH on progression of CKD (Table 4, model 3). OH crossed with MDRD-eGFR was borderline significant (Table 4, model 3), which, with the correlation of OH with MDRD-eGFR at baseline, could indicate a partly joint influence on

Table 3. Contingency table of progression to ESRD by OH and aprotinin-sensitive urinary proteolytic activity

Count (row %)	Progression to ESRD			χ^2 test *p* value
	no, *n* (%)	yes, *n* (%)	total	
OH				
≤1 L/1.73 m^2	114 (89)	14 (11)	128	<0.0001
>1 L/1.73 m^2	30 (61)	19 (39)	49	
1–2 L/1.73 m^2	17 (65)	9 (35)	26	0.0003
2–3 L/1.73 m^2	9 (56)	7 (44)	16	
>3 L/1.73 m^2	4 (57)	3 (43)	7	
U-aprotinin-sensitive activity				
≤0.9 RU/g Crea	82 (92)	7 (8)	89	0.0002
>0.9 RU/g Crea	50 (69)	22 (31)	72	

ESRD, end-stage renal disease; OH, overhydration; U, urine; RU, relative units; Crea, creatinine.

Table 4. Multivariate nominal logistic models for progression of CKD

	Model 1: progression to ESRD (*n* = 167)	Model 2: progression to ESRD or decline of eGFR by ≥30% (*n* = 156)	Model 3: progression to ESRD, inclusion of OH (*n* = 167)
r^2	0.5072, $p < 0.0001$	0.3680, $p < 0.0001$	0.5344, $p < 0.0001$
Independent variables	estimate ± SE, *p* value	estimate ± SE, *p* value	estimate ± SE, *p* value
MDRD-eGFR, mL/min/1.73 m^2	0.0.14±0.027, $p < 0.0001$	0.084±0.014, $p < 0.0001$	0.169±0.037, $p = 0.0001$
ACR, mg/g Crea	−0.0.0007±0.0003, $p = 0.0141$	−0.0005±0.0002, $p = 0.0040$	−0.0009±0.0004, $p = 0.0172$
OH, L/1.73 m^2	–	–	−0.8161±0.4059, $p = 0.0444$
MDRD-eGFR × OH	–	–	−0.0294±0.0152, $p = 0.0535$
ACR × OH	–	–	0.0001±0.0001, $p = 0.2463$
(constant)	−2.746±0.779, $p = 0.0004$	−2.101±0.539, $p < 0.0001$	−3.158±0.919, $p = 0.0006$

All parameters with significant correlation in univariate analysis were included in a stepwise model (forward and backward direction mode, probability to enter $p = 0.25$, probability to leave $p = 0.1$) to select parameters of the final nominal logistic model. In model 3, OH as parameter of interest was added, and crossed with MDRD-eGFR and ACR for controlling interaction. CKD, chronic kidney disease; OH, overhydration; MDRD-eGFR, calculated glomerular filtration rate by the MDRD formula; ACR, urinary albumin creatinine ratio; ESRD, end-stage renal disease; SE, standard error; Crea, creatinine.

progression, with OH being excluded by the MDRD-eGFR in the stepwise model. In these multivariate models, aprotinin-sensitive proteolytic activity could not replace albuminuria.

In multivariate ROC with the endpoint progression to ESRD, eGFR estimated by the MDRD formula showed the highest AUC with AUC 0.924 ($p < 0.001$), followed by ACR (AUC: 0.747, $p < 0.001$) and OH (AUC: 0.729, $p < 0.001$, Fig. 3A). Combination of MDRD-eGFR, ACR, and OH increased the AUC to 0.941 ($p < 0.001$, Fig. 3A); however, the combined AUC was not significantly different from the AUC of MDRD-eGFR in pairwise comparison. Urinary aprotinin-sensitive proteolytic activity could replace albuminuria in multivariate ROC (AUC: 0.7696, $p < 0.001$, Fig. 3B).

Time-Dependent Analysis: Kaplan-Meier Curves and Cox Regression

Patients were divided into 2 groups for each eGFR, ACR, OH, and urinary aprotinin-sensitive proteolytic activity according to the cutoff values determined from ROC analysis

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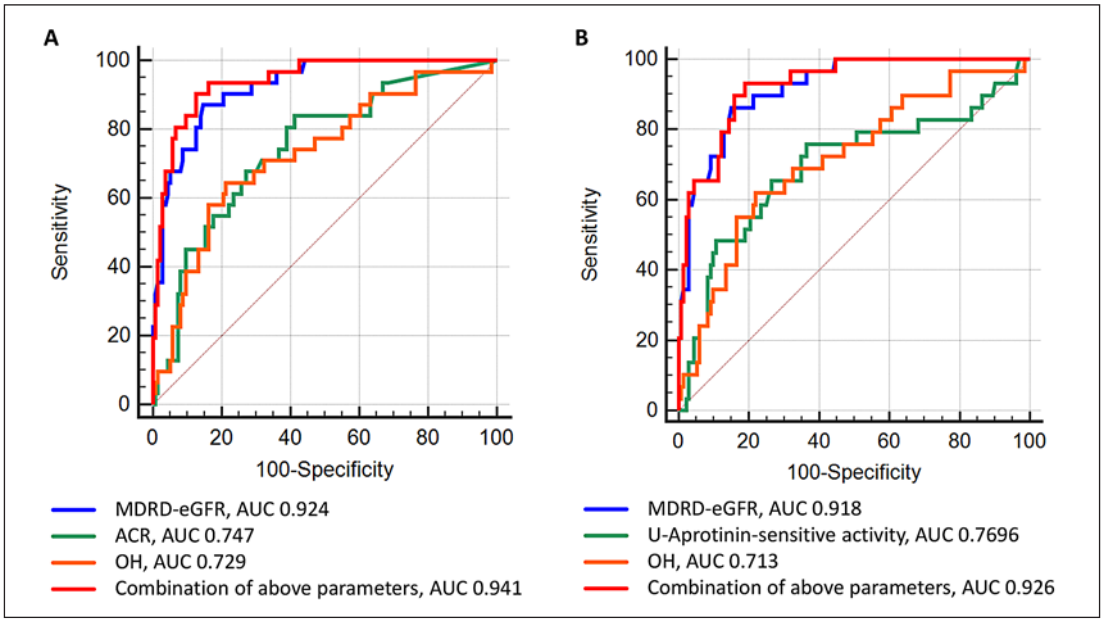

Fig. 3. Multivariate ROC analysis for progression to ESRD by baseline MDRD-eGFR, albuminuria (**A**) or urinary proteolytic activity (**B**), and OH. Pairwise comparison of ROC curves: (**A**) MDRD-eGFR and ACR, p = 0.0010; MDRD-eGFR and OH, p = 0.0009; MDRD-eGFR and combined parameters, p = 0.1098; ACR and OH, p = 0.7509; ACR and combined parameters, p < 0.0001; OH and combined parameters, p = 0.0001. **B** MDRD-eGFR and urinary proteolytic activity, p = 0.0010; MDRD-eGFR and OH, p = 0.0010; MDRD-eGFR and combined parameters, p = 0.2840; urinary proteolytic activity and OH, p = 0.7698; urinary proteolytic activity and combined parameters, p = 0.0003; OH and combined parameters, p = 0.0003; ROC, receiver operating characteristic; ESRD, end-stage renal disease; MDRD-eGFR, estimated glomerular filtration rate by the MDRD formula; AUC, area under the curve; U, urine; ACR, urinary albumin creatinine ratio; OH, overhydration measured by bioimpedance spectroscopy.

(Table 2). Kaplan-Meier curves demonstrated an early and clear separation of the different strata for the probability to start RRT (Fig. 4). Freedom of RRT was significantly lower in patients with eGFR <32 mL/min/1.73 m^2 (n = 51), ACR >43 mg/g creatinine (n = 82), OH >1 L/1.73 m^2 (n = 48), and urinary aprotinin-sensitive proteolytic activity >0.9 RU/g creatinine (n = 72) as analyzed by the log-rank test (p < 0.0001 each, Fig. 4).

Cox regression was performed to determine the HRs for progression to ESRD. Patients with OH >1 L/1.73 m^2 had a 4.58 (95% confidence interval: 2.27–9.25, p < 0.0001)-fold higher risk for progression to ESRD, which remained statistically significant when adjusted for MDRD-eGFR, ACR, NT-pro-BNP, systolic blood pressure, and diabetes mellitus (p = 0.0128, Table 5). Cox regression for aprotinin-sensitive urinary protease activity showed an HR of 1.25 (CI: 0.97–1.50, p = 0.0712) per standard deviation for progression to ESRD and remained significant with adjustment for MDRD-eGFR, ACR, NT-pro-BNP, systolic blood pressure, and diabetes mellitus (HR 1.50 [CI: 1.05–2.16], p = 0.0483, Table 5).

Cox regression also showed a significantly elevated risk for progression to ESRD for plasma creatinine and eGFR estimated by the MDRD formula, plasma NT-pro-BNP, proteinuria, albuminuria, and quotient of extra- and intracellular water (Table 5), analogous to significant associations in univariate analysis reported above. Cox regression showed no significant change of the HR for age, gender, diabetes, edema, BMI, systolic or diastolic blood pressure, serum aldosterone concentration, plasma renin activity, extracellular water and intracellular water, and fat tissue index and lean tissue index measured by bioimpedance spectroscopy.

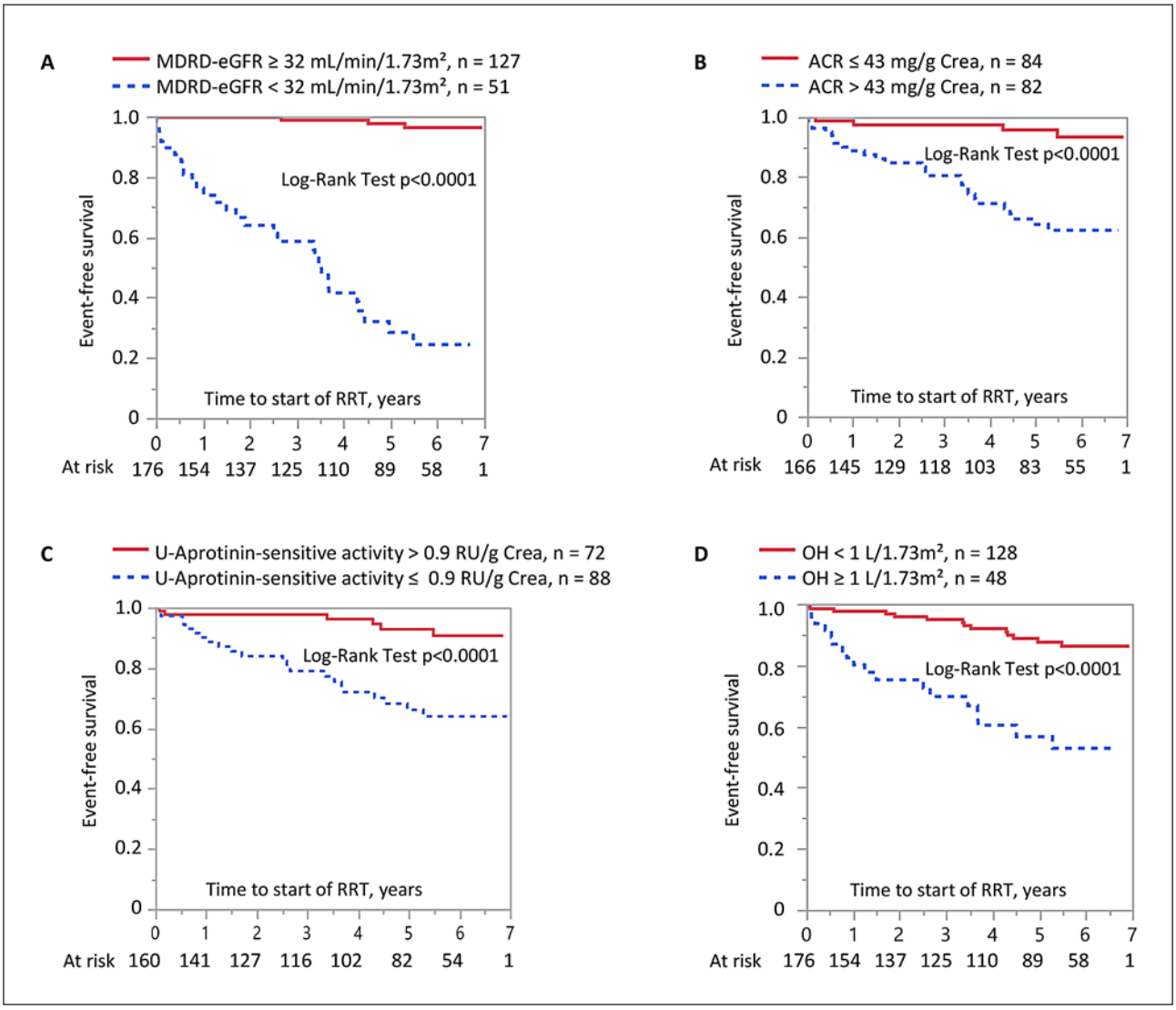

Fig. 4. Kaplan-Meier curves for progression to ESRD by group of MDRD-eGFR (**A**), albuminuria (**B**), urinary aprotinin-sensitive proteolytic activity (**C**), and OH (**D**). Patients were divided into 2 groups for each GFR, albuminuria, urinary aprotinin-sensitive proteolytic activity, and OH according to the cutoff values determined by the Youden Index in ROC analysis. At risk is the number of subjects at risk (subjects that have not had an event and have not been censored) immediately before the respective time point. ESRD, end-stage renal disease; MDRD-eGFR, estimated glomerular filtration rate by the MDRD formula; U, urine; RU, relative units; Crea, creatinine; ACR, urinary albumin creatinine ratio; OH, overhydration measured by bioimpedance spectroscopy; ROC, receiver operating characteristic; RRT, renal replacement therapy.

Discussion

Our analyses revealed a prognostic significance of bioimpedance spectroscopy-measured OH and aprotinin-sensitive urinary protease activity with progression of CKD. This correlation was already detectable in single measurements at any time during the presentation of the patients in the outpatient clinic. The detrimental effects of OH were also reported by Hung et al. [14], who found a strong correlation of volume overload with progression of CKD, cardiovascular morbidity, or mortality in a cohort of advanced CKD stage 3–5 after an FU time of 2.1

Table 5. Proportional HRs for progression to ESRD

Baseline parameter	Unadjusted			Adjusted for MDRD-eGFR, ACR, P-NT-pro-BNP, systolic blood pressure, and diabetes mellitus	
	SD	HR (95% CI)	*p* value	HR (95% CI)	*p* value
OH, L/1.73 m^2	1.381	1.75 (1.32–2.28)	0.0002	1.24 (0.83–1.90)	0.2898
OH > 1 vs. ≤1 L/1.73 m		4.58 (2.27–9.25)	<0.0001	3.32 (1.26–8.76)	0.0128
ECW/ICW	0.117	1.47 (1.06–1.98)	0.0233	1.20 (0.69–2.08)	0.5204
P-creatinine, mg/dL	1.311	4.25 (3.02–6.23)	<0.0001	2.87 (1.10–7.93)	0.0310
MDRD-eGFR, mL/min/1.73 m^2	28.69	0.01 (0.004–0.04)	<0.0001	0.01 (0.002–0.04)	0.0001
P-NT-pro-BNP, pg/mL	3,141	1.58 (1.27–1.89)	<0.0001	1.40 (1.03–1.78)	0.0340
PCR, mg/g Crea	1,841	1.54 (1.25–1.85)	0.0002	1.88 (1.11–3.05)	0.0211
ACR, mg/g Crea	1,282	1.47 (1.15–1.79)	0.0044	1.82 (1.33–2.41)	0.0006
U-aprotinin-sensitive activity, RU/g Crea	12.81	1.25 (0.97–1.50)	0.0712	1.50 (1.05–2.16)	0.0483

HRs for continuous variables are per increase of 1 SD. ESRD, end-stage renal disease; MDRD-eGFR, glomerular filtration rate calculated by the MDRD formula; U, urine; ACR, urinary albumin creatinine ratio; P, plasma; BNP, brain natriuretic peptide; SD, standard deviation; HR, hazard ratio; CI, confidence interval; OH, overhydration; ECW, extracellular water; ICW, intracellular water; PCR, urinary protein creatinine ratio; Crea, creatinine; RU, relative units.

years. Tsai et al. [15, 16] described fluid overload as an independent risk factor for progression to ESRD and all-cause mortality in stage 4–5 CKD. Tai et al. [17] found a higher risk of progression of CKD in the highest tertile of extracellular water measured by bioimpedance analysis and normalized to calculate total body water in CKD patients. Faucon et al. [18] found that extracellular fluid measured using a tracer dilution method was associated with progression to end-stage kidney disease and mortality in patients with stage 1–4 CKD. Strict volume control with use of diuretics showed a positive effect on progression of CKD and delayed initiation of RRT in advanced nondialysis CKD patients [19]. In our cohort, other parameters indicating fluid overload, such as blood pressure and plasma NT-pro-BNP, were significantly associated with OH and with progression of CKD, which confirms the robustness of the relationship between volume overload and progression. Our results corroborate that fluid overload as substantiated by bioimpedance spectroscopy is associated with progression of CKD with the novel aspect that the association is valid in a cohort of patients of all stages of CKD and after a longer FU period. Bioimpedance spectroscopy was superior to clinical assessment of fluid status based on edema, and in contrast to dilution methods, it is usable in clinical routine.

It remains to be discussed if fluid overload itself is a risk factor for progression of CKD or if both, fluid overload and progression, are predicted by lower GFR and albuminuria. Corresponding statistical analyses are difficult because of the dominance of the correlation of MDRD-eGFR with progression to ESRD in multivariate models. Crossing of OH with MDRD-eGFR in multivariate analysis revealed a tendency of an interaction and joint influence of OH and MDRD-eGFR on progression to ESRD. However, patients with OH >1 L/1.73 m^2 showed markedly higher risk of progression to ESRD, even after adjustment for MDRD-eGFR and albuminuria, indicating that OH might be an additional risk factor for progression to ESRD.

Albuminuria represents glomerular proteinuria in the KDIGO staging of CKD, but other urinary proteins and their mechanistic value for progression of CKD should also be considered. Proteasuria has lately been investigated as a mechanism for edema formation and OH in proteinuric kidney disease [3,12,20]. Serine proteases that reach the urine with proteinuria have been shown to cleave the gamma unit of the epithelial sodium channel and lead to

sodium and fluid retention [21]. Proteasuria could additionally affect tubular integrity and propose a mechanism for risk of progression associated with higher proteinuria in CKD [9]. Our results show that aprotinin-sensitive proteolytic activity is detectable in urine from CKD patients of all stages and is associated with OH and with progression of CKD, along with proteinuria and albuminuria, even after adjustment for other risk factors such as blood pressure, diabetes mellitus, or NT-pro-BNP as markers for cardiovascular risk. Kaplan-Meier curve for progression to ESRD of patients with high versus low aprotinin-sensitive proteolytic activity showed clear and significant separation. However, statistical analysis of the significance of urinary proteolytic activity for progression is complicated by the high correlation of urinary proteins with each other and the co-correlation with progression of CKD. In a cohort of type 1 diabetes mellitus patients, the urinary serine protease plasmin was associated with hypertension and mortality, with analogous difficulty to statistically distinguish effects of plasmin from effects of albuminuria [22]. A current proteomic study of our group has characterized the sum of all urinary proteases or the "urinary proteasome" in the healthy and nephrotic state of humans and mice [23]. To analyze serine protease activity as a possible therapeutic target for prevention of progression of CKD, further investigation of the role of urinary serine proteases in CKD is needed.

The study is limited by its observational character, small size, lack of serial measurements, and low mortality rate which excluded more extensive statistical analysis. The influence of confounding factors on the relationships investigated, such as blood pressure, heart function, or medication, was taken into account as much as possible but cannot be excluded with final certainty. However, the analyses with regard to the endpoint start of RRT were consistent in different statistical approaches. The prognostic performance of GFR and albuminuria, both cornerstones of CKD staging, was clearly reproduced in this medium-sized cohort, indicating a high validity of the results. The cutoff values with prognostic relevance were 32 mL/min/1.73 m^2 for eGFR and 43 mg/g creatinine for ACR corresponding to the transition from the G3 and A1 categories to the G4 and A2 categories, respectively. Other strengths of the study are the long FU time and the observation of the start of RRT as a hard clinical endpoint.

In conclusion, our study confirms that fluid overload determined as OH by bioimpedance spectroscopy in CKD is an independent risk factor for progression to ESRD and reveals urinary serine protease activity as a possible underlying mechanism.

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Statement of Ethics

The primary study and analysis of the follow-up were approved by the local ethics committee of the University of Tuebingen (259/2012MPG23 and 732/2019BO2), and all participants gave their informed consent prior to their inclusion in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.S. obtained the ethics vote, carried out the clinical measurements and the collection of the follow-up data, analyzed and interpreted the data, and drafted the manuscript. B.N.B., N.H., and A.L.B. helped analyzing and interpreting the data and revised the manuscript. F.A. planned the study and helped analyzing and interpreting the data and revised the manuscript. All authors approved the final version of the manuscript submitted.

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