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Plasma C-terminal agrin fragment and rapid kidney function decline in chronic kidney disease patients

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Abstract

C-terminal agrin fragment (tCAF) is a promising biomarker for glomerular filtration. Data regarding biomarkers that have the ability to predict rapid progression of chronic kidney disease (CKD) are sparse but necessary in order to identify patients at high risk for rapid progression. This study addresses the value of tCAF as a predictor of rapid kidney function decline in CKD patients.

We measured plasma tCAF in a retrospective observational cohort study of 277 prevalent CKD patients stage I-V. Using multivariable Cox proportional hazards regression analysis, we evaluated the association of tCAF with end-stage-renal-disease (ESRD), ≥30%-decline of estimated glomerular filtration rate (eGFR) and the composite endpoint of both, adjusting for eGFR, age, systolic blood pressure, proteinuria and diabetes.

The median age was 58 [interquartile range 47, 71] years, 36% were female. Median tCAF level was 822 [594, 1232] pM, eGFR was 32 [19, 48] ml/min/1.73 m^2. tCAF was correlated to eGFR and proteinuria ($r=-0.76$ and $r=0.49$, $P<.001$ resp.). During a follow-up of 57.1 [42.9, 71.9] weeks, 36 (13%) patients developed ESRD and 13 (5%) had an eGFR decline of ≥30% (composite endpoint: 49 (18%)). In multivariable analysis, each 100 pM higher tCAF was independently associated with ESRD (hazard ratio (HR) 1.05 (95%-CI 1.02-1.08)), ≥30% eGFR decline (HR 1.10 (1.03–1.18)) and the composite endpoint (HR 1.07 (1.04–1.1)).

Plasma tCAF may identify CKD patients at risk for rapid kidney function decline independent of eGFR and other risk factors for eGFR loss such as proteinuria.

Abbreviations: ANOVA = analysis of variance, AUC-ROC = area under the receiver operating characteristic curve, BUN = blood urea nitrogen, CKD = chronic kidney disease, CKD-EPI = Chronic Kidney Disease Epidemiology Initiative, CRP = C-reactive protein, eGFR = estimated glomerular filtration rate, ELISA = enzyme-linked immunosorbent assay, ESRD = end-stage renal disease, HR = hazard ratio, pM = picomolar, SBP = systolic blood pressure, tCAF = total C-terminal agrin fragment, VIF = variance inflation factor.

Keywords: CKD, C-terminal agrin fragment, eGFR, ESRD, phosphate, proteinuria

1. Introduction

Chronic kidney disease (CKD) is a major public health problem with a prevalence of up to 17% in the European population.[1] CKD is associated with increased risk for cardiovascular disease and mortality.[2] Therefore it is important to identify patients with increased risk for rapid kidney function decline not only to attempt to slow down progression, but also to prepare these particular patients for renal replacement therapy and to maximize preventive strategies against cardiovascular disease. However, studies evaluating parameters to identify patients who rapidly develop end-stage renal disease (ESRD) or demonstrate rapid kidney function decline are sparse.

We recently reported that serum C-terminal agrin fragment (tCAF) is a promising new biomarker for kidney function in renal transplant recipients, CKD and ESRD patients.[3–5] Its serum levels reacted faster than creatinine to changes of kidney function in transplant patients and outperformed creatinine in the early detection of delayed graft function. tCAF was also a powerful predictor for doubling of proteinuria as well as for graft loss in kidney transplant recipients.[6] In a recent study of 71 patients with diabetic nephropathy, higher tCAF levels at baseline were associated with more pronounced kidney function decline over a 12-month follow-up, independent from estimated glomerular filtration rate (eGFR) and proteinuria, which are currently viewed as the most important risk factors for CKD progression.[7]

In this study, we evaluated plasma tCAF and its association with rapid kidney function decline in a general CKD cohort.

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2. Patients and methods

2.1. Study population

The cohort consisted of 277 patients at stages I-V of CKD that were routinely seen in the outpatient clinic of our tertiary care university hospital. The study was approved by the local ethics committee of Klinikum rechts der Isar, Technische Universität, Munich, Germany and adheres to the declaration of Helsinki. All patients enrolled in this study provided informed consent. Inclusion criteria followed the definitions for CKD according to the last KDIGO guidelines:[8] "CKD is defined as abnormalities of kidney structure or function, present for >3 months, with implications for health." Therefore, the diagnosis of CKD was made when either eGFR was <60 ml/min/1.73 m^2 and/or there were apparent signs of kidney damage, which included: proteinuria with a cut-off >150 mg/g creatinine on spot-urine specimen and/or histologically proven kidney disease and/or abnormalities detected in imaging techniques (ultrasound, computed tomography, magnetic resonance imaging or nuclear imaging). Calculation of eGFR was based on both serum creatinine and cystatin C concentration using the Chronic Kidney Disease Epidemiology Initiative equation ($CKD\text{-}EPI_{crea\text{-}cystatin\ C}$).[9]

2.2. Study design

The study was performed in an observational retrospective cohort design with exclusively Caucasian patients that were routinely seen in our outpatient clinic. There were no separate outpatient study visits for follow-up only. In order to evaluate the association of tCAF with short-term kidney function decline, we tried to assess the outcomes as close as possible to 1-year follow-up, minimum 6 months after baseline and maximum of 1.5 years. For cases in which more than one follow-up was available within this time frame, the value closest to 1 year was selected. Patients from the original study with no further follow-up visits in our outpatient clinic were excluded from this analysis.

2.3. Exposure

Blood samples were drawn at the baseline visit and centrifuged for 8 minutes at 2000 rpm and supernatant plasma samples obtained. Plasma samples were aliquoted and stored at −80°C until further processing. tCAF concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (NTtotalCAF Elisa Kit, Neurotune, Schlieren, Switzerland), performed as described previously.[5] The other laboratory parameters were assessed by ISO accredited laboratories.

2.4. Outcomes

The outcomes were:

- ESRD, defined as the chronic need for renal replacement therapy (hemodialysis, peritoneal dialysis, kidney transplant) for a period >3 months
- decline in eGFR ≥30% from baseline during or at the end of follow-up. This threshold was chosen based on published literature, demonstrating that eGFR decline of ≥30% is associated with higher incidence of ESRD and mortality[10,11]
- composite endpoint of ESRD and/or ≥30% of eGFR decline

2.5. Covariates

In the multivariable analysis, we adjusted for age, eGFR, proteinuria, systolic blood pressure (SBP) and prevalent diabetes. These co-variables are among the most important risk factors for CKD progression.[12,13]

2.6. Statistical analysis

We described the population overall and across tCAF quartiles using median and interquartile range for continuous variables and percentages for binary and categorical variables. We tested differences between quartiles using analysis of variance (ANOVA) for continuous and Chi-square test for categorical variables. We evaluated the correlation between tCAF and eGFR/proteinuria with Spearman coefficients. We evaluated differences in the incidence of ESRD, eGFR decline and the composite endpoint between the using Kaplan–Meier curves and Log-Rank test. We developed multivariable Cox proportional hazards regression models to examine the association of tCAF (treated as a continuous variable) with ESRD, eGFR decline and the composite endpoint. We tested the assumptions of Cox regression with Schoenfeld residuals (Suppl. Table 2, http://links.lww.com/MD/C978). Collinearity was evaluated using variance inflating factor (VIF). In a separate model, we tested for a potential interaction between tCAF and eGFR. We performed the similar Cox regression model for creatinine, cystatin C and blood urea nitrogen (BUN) adjusting for eGFR, age, SBP, proteinuria and diabetes. We assessed the area under the receiver operating characteristic curve (AUC-ROC), optimal cut-offs using Youden-Index as well as sensitivities/specificities of tCAF, eGFR, creatinine, cystatin C, and BUN for estimating all outcomes, comparing the AUC of tCAF vs creatinine, cystatin C and BUN using DeLong-test and tCAF vs eGFR using bootstrapping.

We conducted all analyses using R, version 3.5.1 (R Core Team (2018), Vienna, Austria).

3. Results

3.1. Patient demographics

There were 372 patients initially included in the study. At the time of follow-up assessment, 95 (25.5%) patients were lost to follow-up since they did not present anymore to our outpatient clinic. The overall cohort and the group of lost to follow-up did not substantially differ from the cohort finally selected for analysis (Suppl. Table 1, http://links.lww.com/MD/C978). In the overall cohort, the median [interquartile range] age was 58 [47, 71] years, and 100 (36.1%) participants were female (Table 1). Median tCAF level was 822 [594, 1232] pM, eGFR was 32 [19, 48] ml/min/1.73 m^2; proteinuria was 262 [84, 1076] mg/g creatinine. Individuals in the higher tCAF quartiles had lower eGFR, higher level of proteinuria, higher C-reactive protein (CRP), and higher levels of potassium, phosphorus, uric acid, and parathyroid hormone, whereas hemoglobin and bicarbonate levels were lower in these quartiles (Table 1). Arterial hypertension and coronary artery disease were more prevalent in quartiles with higher tCAF levels (Table 1). Diabetes and hypertension as the underlying kidney disease were more frequent in the quartiles with higher tCAF concentrations. As expected in view of the median eGFR level, advanced CKD stages were more frequent in

Table 1

Baseline characteristics of participants stratified by quartiles of plasma tCAF.

tCAF range (pM)	Total Cohort (n = 277) 0–6459	Quartile 1 (n = 69) 0–594	Quartile 2 (n = 70) 594–822	Quartile 3 (n = 68) 822–1232	Quartile 4 (n = 70) 1232–6459	*P* value
Demographics						
Age (yr)	58 [47, 71]	52 [43, 67]	62 [48, 71]	64 [52, 73]	57 [48, 71]	.020
Female	100 (36.1)	31 (44.9)	25 (35.7)	25 (36.8)	19 (27.1)	.188
Body-mass-index	26.0 [23.2, 30.7]	26.7 [22.9, 31.10]	26.2 [23.3, 29.1]	25.6 [23.2, 29.9]	26.8 [24.1, 31.0]	.733
Laboratory measures						
tCAF (pM)	822 [594, 1232]	501 [474, 547]	693 [633, 765]	997 [875, 1141]	1714 [1387, 2249]	<.001
eGFR (ml/min/1.73 m^2)	32 [19, 48]	57 [45, 68]	36 [27, 48]	26 [20, 34]	12 [8, 19]	<.001
Creatinine (mg/dl)	1.7 [1.2, 2.6]	1.1 [0.9, 1.3]	1.5 [1.2, 2.0]	2.0 [1.5, 2.6]	3.7 [2.6, 5.2]	<.001
Cystatin C (mg/l)	1.4 [1.0, 2.3]	0.8 [0.6, 1.0]	1.3 [1.3, 1.5]	1.8 [1.4, 2.4]	2.6 [2.1, 3.4]	<.001
Urea-nitrogen (mg/dl)	27 [19, 41]	17 [14, 21]	24 [20, 33]	32 [26, 42]	50 [33, 60]	<.001
Proteinuria (mg/g creatinine)	262 [84, 1076]	112 [66, 236]	146 [80, 509]	367 [98, 760]	1265 [566, 2746]	<.001
C-reactive Protein (mg/dl)	0.2 [0.1, 0.7]	0.2 [0, 0.4]	0.2 [0, 0.5]	0.2 [0.1, 0.8]	0.40 [0.2, 1.1]	.007
Sodium (mmol/l)	140 [138, 142]	140 [138, 141]	141 [139, 142]	141 [139, 142]	140 [138, 142]	0.217
Potassium (mmol/l)	4.7 [4.4, 5.1]	4.6 [4.3, 4.9]	4.7 [4.3, 5.0]	4.7 [4.5, 5.0]	5.0 [4.5, 5.4]	<.001
Serum Protein (mg/dl)	7.0 [6.5, 7.3]	7.1 [6.6, 7.3]	7.1 [6.6, 7.4]	7.0 [6.5, 7.3]	6.9 [6.4, 7.3]	.319
Phosphorus (mg/dl)	3.5 [3.1, 4.0]	3.1 [2.8, 3.6]	3.3 [2.9, 3.7]	3.5 [3.3, 4.0]	4.1 [3.8, 4.7]	<.001
Calcium (mmol/l)	2.4 [2.3, 2.4]	2.4 [2.3, 2.5]	2.4 [2.3, 2.7]	2.4 [2.3, 2.5]	2.3 [2.2, 2.4]	.003
Uric Acid (mg/dl)	6.9 [5.8, 8.5]	5.9 [5.0, 7.0]	7.1 [5.8, 8.5]	7.1 [6.0, 8.4]	7.8 [6.7, 9.1]	<.001
Magnesium (mmol/l)	0.8 [0.8, 0.9]	0.8 [0.8, 0.9]	0.8 [0.8, 0.9]	0.8 [0.8, 0.9]	0.9 [0.8, 1.0]	.018
Hemoglobin (g/dl)	13.2 [11.5, 14.4]	14.4 [13.4, 15.0]	13.2 [12.2, 14.6]	12.5 [11.1, 14.0]	11.6 [10.4, 12.9]	<.001
Bicarbonate (mmol/l)	26.3 [24.2, 28.3]	27.5 [24.8, 28.8]	27.1 [25.0, 29.0]	25.7 [24.0, 27.5]	24.9 [23.6, 27.5]	<.001
Parathyroid hormone (pg/ml)	68 [42, 114]	50 [36, 68]	55 [39, 74]	74 [51, 132]	136 [79, 238]	<.001
CKD risk factors						
Systolic BP (mmHg)	133 [120, 150]	130 [120, 142]	130 [120, 138]	138 [125, 150]	140 [130, 153]	.001
Diastolic BP (mmHg)	80 [76, 90]	80 [76, 90]	80 [78, 88]	80 [70, 90]	81 [77, 90]	.585
Diabetes mellitus	58 (20.9)	5 (7.2)	14 (20.0)	19 (27.9)	20 (28.6)	.006
Arterial hypertension	200 (72.2)	37 (53.6)	53 (75.7)	50 (73.5)	60 (85.7)	<.001
Coronary artery disease	36 (13.0)	4 (5.8)	8 (11.4)	9 (13.2)	15 (21.4)	.052
Peripheral artery disease	14 (5.1)	1 (1.4)	6 (8.6)	4 (5.9)	3 (4.3)	.277
Active tumoral disease	37 (13.4)	3 (4.3)	8 (11.4)	14 (20.6)	12 (17.1)	.029
Connective tissue disease	11 (4.0)	3 (4.3)	3 (4.3)	1 (1.5)	4 (5.7)	.633
Medication use						
ACE-inhibitor /ARB	203 (73.3)	47 (68.1)	57 (81.4)	53 (77.9)	46 (65.7)	.106
Underlying disease						.014
Diabetic nephropathy	18 (6.5)	1 (1.4)	4 (5.7)	5 (7.4)	8 (11.4)	
Hypertensive nephropathy	37 (13.4)	2 (2.9)	4 (5.7)	14 (20.6)	17 (24.3)	
Glomerular disease	105 (37.9)	35 (50.7)	30 (42.9)	21 (30.9)	19 (27.1)	
Postrenal disease	18 (6.5)	6 (8.7)	6 (8.6)	2 (2.9)	4 (5.7)	
Tubulointerstitial disease	27 (9.7)	6 (8.7)	10 (14.3)	8 (11.8)	3 (4.3)	
Single kidney	23 (8.3)	7 (10.1)	7 (10.0)	5 (7.4)	4 (5.7)	
Others/unknown	49 (17.7)	12 (17.4)	9 (12.9)	13 (19.1)	15 (21.4)	
CKD stage						<.001
I	34 (12.3)	25 (15.1)	5 (7.1)	3 (4.4)	1 (1.4)	
II	54 (19.5)	29 (42.0)	15 (21.4)	7 (10.3)	3 (4.3)	
III	96 (34.7)	14 (20.3)	46 (65.7)	28 (41.2)	8 (11.4)	
IV	60 (21.7)	1 (1.4)	4 (5.7)	27 (39.7)	28 (40.0)	
V	33 (11.9)	0 (0.0)	0 (0.0)	3 (4.4)	30 (42.9)	

Continuous variables are presented as median [interquartile range], categorical variables in percentage of total cohort. Significance of differences between the quartiles were tested using analysis of variance (ANOVA) for continuous and chi-square-test for categorical variables.

ACE = angiotensin-converting-enzyme; ARB = Angiotensin-II-receptor-blocker, BP = blood pressure, CKD = chronic kidney disease, eGFR = estimated glomerular filtration rate, tCAF = total C-terminal Agrin Fragment.

quartiles with higher tCAF levels. tCAF was strongly correlated to eGFR (Spearman rho (r) = −0.76, P < .001) and proteinuria (r = 0.49, P < .001). In multivariable Cox regression, we did not detect departure from proportional hazards over time for any of the variables in all three models (Suppl. Table 2, http://links.lww.com/MD/C978). Therefore, all variables were entered into the models on raw scale. The VIFs for all covariables was below 2 in all models (except for eGFR in the model with outcome eGFR decline ≥30%, in which it was 2.4), ruling out collinearity. The interaction term tCAF*eGFR was not significant in any of the multivariable models.

3.2. Kaplan–Meier curves

There were 36 (13%) patients who developed ESRD over a median follow-up of 57.1 [42.9, 71.9] weeks, 16 (44%) of these

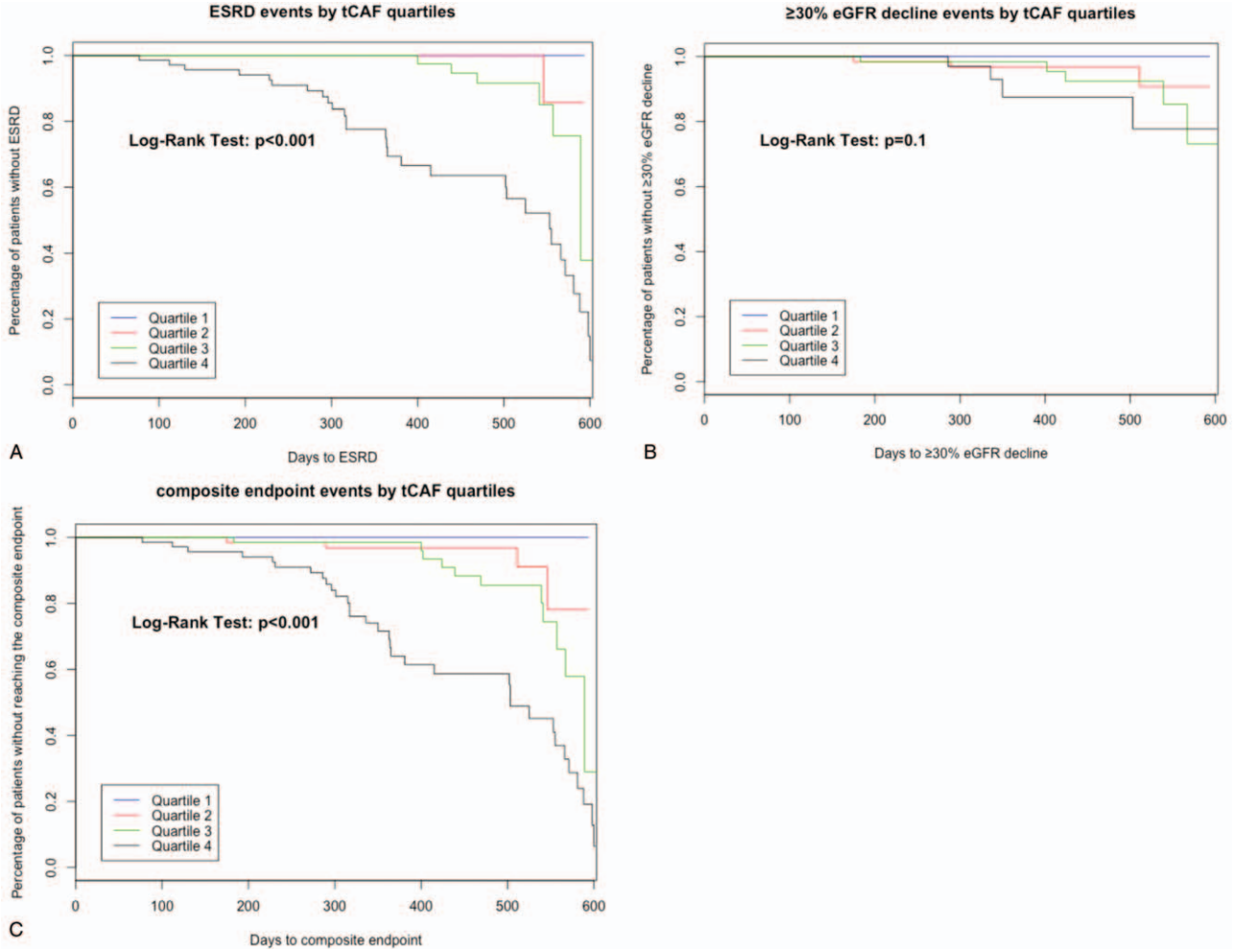

Figure 1. Kaplan–Meier curves for tCAF quartiles with respect to percent of patients not reaching (A) ESRD, (B) eGFR decline ≥30%, (C) the composite endpoint. Statistical comparison was done using Log-Rank test. eGFR = estimated glomerular filtration rate, ESRD = end-stage renal disease, tCAF = total C-terminal agrin fragment.

also developed ≥30% eGFR decline. Of the ESRD events, 29 occurred in the quartile with the highest tCAF levels, followed by 6 in the second-highest and 1 in the second-lowest (Fig. 1, $P < .001$). Overall, 13 (5%) patients had a ≥30%eGFR decline during follow-up, of whom 4 were in the highest tCAF quartile, 6 in the second highest and 3 in the second-lowest (Fig, 1, $P = .1$). In total, 49 (18%) patients reached the composite endpoint, 33 of the highest tCAF quartile, 12 of the second-highest and 4 of the second-lowest (Fig. 1, $P < .001$).

3.3. tCAF and outcomes

In univariable analysis, each 100 pM higher tCAF was significantly associated with a 9% increased risk for ESRD (hazard ratio (HR) 1.09 (95%-confidence interval (CI) 1.07–1.11, Table 2), a 6% increased risk for eGFR decline (HR 1.06 (1.00–1.12)) and 8% increased risk for the composite endpoint (HR 1.08 (1.06–1.10)). After adjustment for eGFR, proteinuria, age, SBP and diabetes, the association remained significant for all outcomes (HR for ESRD 1.05 (1.02–1.08), HR for ≥30% eGFR decline 1.09 (1.02–1.17), HR for the composite endpoint 1.07 (1.04–1.09), Table 2).

Concerning the covariables, eGFR and proteinuria were significantly associated with ESRD and ≥30% eGFR loss in multivariable analysis (Table 2). However, only proteinuria but not eGFR was independently associated with the composite endpoint. Neither age, SBP nor diabetes were associated with any of the endpoints.

In univariable analysis, higher creatinine, cystatin C and BUN were associated with increased hazard for ESRD and the composite endpoint, but we did not detect an association for eGFR decline (Suppl. Table 3, http://links.lww.com/MD/C978). In multivariable analysis of these variables, only BUN was independently associated with all three endpoints.

3.4. ROC-analysis

All markers had similar excellent AUC for predicting ESRD during follow-up, ranging from 0.899 (BUN) to 0.930 (creatinine, Fig. 2, Suppl. Table 4, http://links.lww.com/MD/C978). tCAF had numerically the highest AUC for detecting ≥30% eGFR decline (0.706, Fig. 2) at an optimal cut-off of 1180 pM with a sensitivity of 100% and a specificity of 52%. The AUC was significantly higher compared to eGFR (0.526, $P = .034$,

Table 2

Associations of plasma tCAF and co-variables with outcomes in Cox proportional hazards regression analysis.

Outcome/Variable	Cases	HR (95%-CI) in univariable analysis	HR (95%-CI) in multivariable analysis
ESRD			
per 100 pM higher tCAF	36/277	1.09 (1.07–1.11)	1.05 (1.02–1.08)
per 1 ml/min/1.73 m^2 higher eGFR			0.91 (0.87–0.96)
per 1 yr of age older			0.99 (0.97–1.01)
per 10 mmHg SBP higher			1.14 (0.95–1.37)
per 100 mg/g creatinine higher proteinuria			1.01 (1.00–1.03)*
for prevalent diabetes mellitus			1.03 (0.46–2.27)
≥30% eGFR decline			
per 100 pM higher tCAF	13/277	1.06 (1.00–1.12)	1.10 (1.03–1.18)
per 1 ml/min/1.73 m^2 higher eGFR			1.03 (1.01–1.06)
per 1 yr of age older			0.99 (0.94–1.04)
per 10 mmHg SBP higher			0.88 (0.58–1.34)
per 100 mg/g creatinine higher proteinuria			1.03 (1.01–1.06)
for prevalent diabetes mellitus			3.03 (0.79–11.61)
Composite endpoint ESRD/≥30% eGFR decline			
per 100 pM higher tCAF	49/277	1.08 (1.06–1.10)	1.07 (1.04–1.09)
per 1 ml/min/1.73 m^2 higher eGFR			1.00 (0.98–1.02)
per 1 yr of age older			0.99 (0.98–1.02)
per 10 mmHg SBP higher			1.08 (0.92–1.27)
per 100 mg/g creatinine higher proteinuria			1.02 (1.01–1.03)
for prevalent diabetes mellitus			1.30 (0.66–2.56)

95%-confidence intervals (95%-CI) for the hazards ratios (HR) are given in parentheses.
eGFR = estimated glomerular filtration rate, ESRD = end-stage renal disease, SBP = systolic blood pressure tCAF = total C-terminal agrin fragment,
* 95%-CI significant (lower value 1.003 when reported with three decimal digits).

Suppl. Table 4, http://links.lww.com/MD/C978) and creatinine (0.519, $P = .036$). tCAF also had the numerically highest AUC for detecting the composite endpoint (0.857, Fig. 2) at an optimal cut-off of 1067 pM (sensitivity 84%, specificity 77%). However, there was no statistically significant difference to the other markers.

4. Discussion

Our results demonstrate that in a European cohort of CKD patients, higher tCAF is associated with rapid development of ESRD, eGFR loss and the composite of both endpoints, independent of baseline eGFR, proteinuria, age and SBP. In contrast, neither creatinine nor cystatin C was independently associated with ESRD and eGFR decline.

tCAF is expressed in many tissues, with the main source of serum tCAF coming from the central nervous system.[14] After its release to the circulation, it is filtered by the glomerulus, reabsorbed and degraded by the proximal tubule and can, therefore, be recognized as a glomerular filtration marker.[15] This hypothesis is supported by data showing a high correlation between tCAF with eGFR in different cohorts.[3–5,16,17] Our results of the predictive value of tCAF for short-term deteriorations of kidney function is supported by previously published data, which also demonstrated an association of tCAF with eGFR decline independent from baseline eGFR and proteinuria over a follow-up period of 12 months in 71 patients with diabetes mellitus.[7] Despite tCAF being associated with eGFR decline over the 12-month period, it is interesting to note that baseline eGFR and baseline proteinuria were not. Furthermore, tCAF was also found to be a stronger predictor for graft loss and doubling of proteinuria in a study of transplants patients, independent of eGFR and proteinuria.[6]

There are a few possible explanations as to why tCAF is associated with ESRD and eGFR decline in different populations, independent from eGFR as well as proteinuria. Most likely the GFR-determinants of tCAF differ from those of creatinine and cystatin C. While creatinine levels tend to be lower in patients with lower muscle mass (sarcopenia), tCAF tends to be higher in sarcopenic patients compared to non-sarcopenic patients.[18,19] However, the differences were small in the studies investigating this association, therefore, the influence of muscle mass on serum tCAF levels in CKD patients appear to be negligible. Cystatin C is influenced by various clinical states such as inflammation, which has not been detected for tCAF.[5] In theory, the association of tCAF with kidney function decline could be due to nephrotoxicity mediated by tCAF. However, to our knowledge, no nephrotoxic effects of tCAF have been demonstrated so far. Furthermore, tCAF serum levels might react faster on changes of kidney function. In the early phase after renal transplantation, tCAF levels dropped significantly faster than creatinine. Finally, the range of tCAF levels is broader when compared to creatinine or cystatin C. This might enable the clinician to detect more subtle changes of kidney function, which in turn improves the predictive value of the marker.

Our study has limitations: as we focused on short-term outcomes, we cannot provide information regarding long-term outcomes. Secondly, the study included mainly Caucasians from a single European center, so the generalizability to other ethnicities needs to be evaluated. We had a higher proportion of patients with chronic glomerulonephritis than in the general CKD population, a fact that might impede the transfer to other general CKD cohorts. However, since tCAF has already been studied in diabetic patients, our cohort adds data dealing with non-diabetic CKD cohorts, increasing the generalizability of tCAF. Plasma samples were stored at −80°C before tCAF

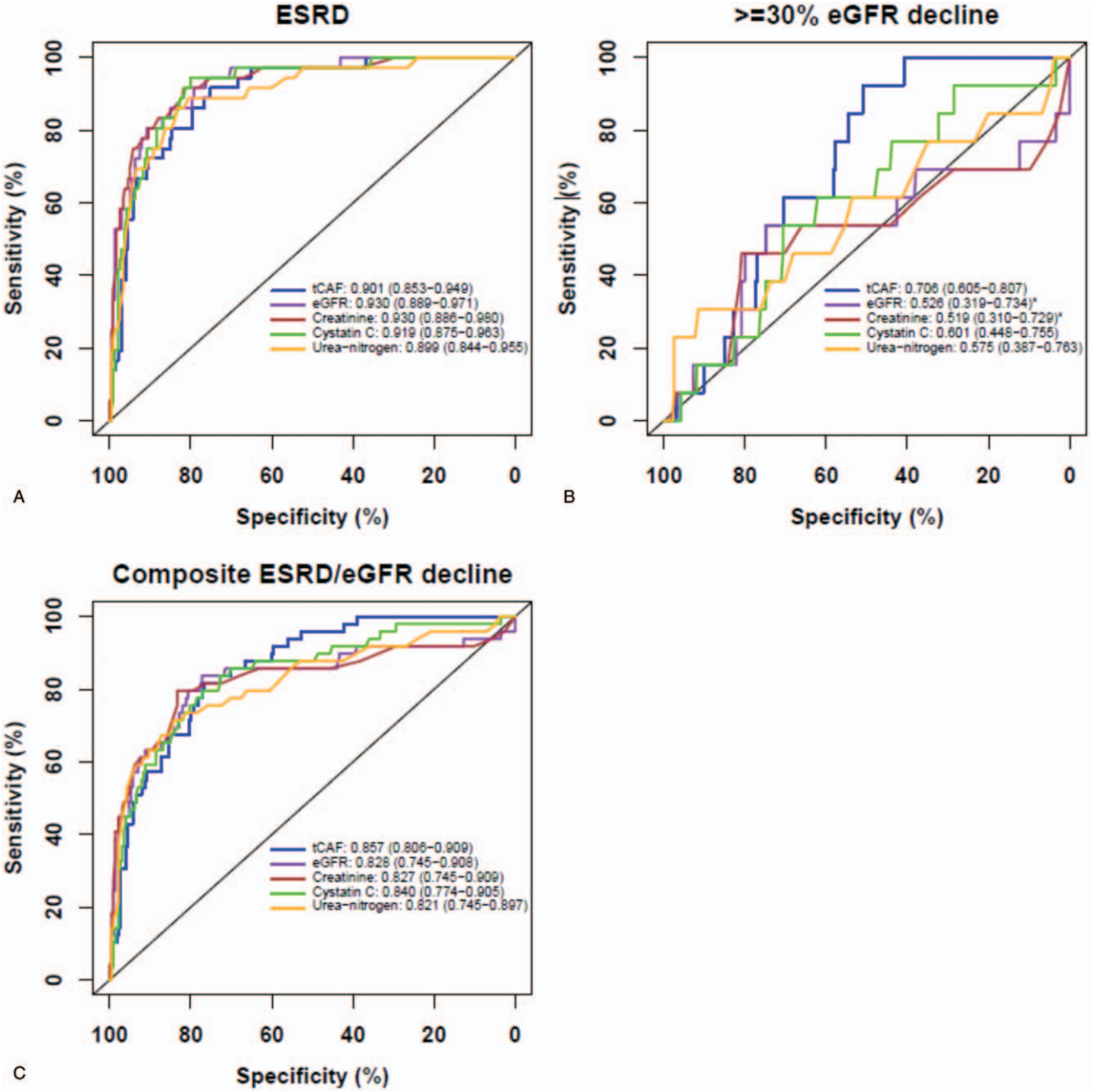

Figure 2. ROC curves for detecting (A) ESRD, (B) eGFR decline and the (C) composite outcome. Sensitivity is presented on the y-axis, specificity on the *x*-axis. The area-under-the-curve results for every marker are presented with confidence intervals in parentheses. $^{*}P < .05$ for difference between corresponding marker and plasma tCAF for the same outcome. eGFR = estimated glomerular filtration rate; ESRD = end-stage renal disease, ROC = Receiver operating characteristics.

measurements were performed. However, from the current knowledge, this should not significantly affect the validity of the measurements. Finally, patients were not evenly distributed among CKD stages but stage III° was predominant, contributing 35% of patients. Our study has several strengths. It is the largest non-selected CKD cohort in which the predictive value of tCAF for kidney outcomes has been evaluated. The endpoints ESRD and ≥30% eGFR decline have not been studied so far in an analysis adjusting for baseline eGFR and proteinuria, 2 of the strongest predictors of adverse renal events. We applied eGFR calculations using both creatinine and cystatin C, the most accurate GFR estimation method currently available.

As far as clinical implications, these results further support the potential value of tCAF measurements in routine clinical care to better assess glomerular kidney function in addition to eGFR calculated from creatinine and/or cystatin C. Many management decisions would benefit greatly from an ability to predict patients at risk of rapid progression to better accuracy than the current standard of biomarkers. However, whether the potential additional benefit justifies the higher laboratory costs needs to be evaluated in future studies with larger cohorts.

In conclusion, plasma tCAF appears to be a promising biomarker to assess the risk for rapid CKD progression independent from eGFR and proteinuria. Due to the higher

costs of measurement, it is unlikely to replace creatinine as the mainstay of GFR assessment in the near future, however, we suggest its use as a complimentary marker in situations when the reliability of creatinine has to be questioned due to non-GFR determinants. This finding needs to be validated in larger cohorts, different ethnicities, and other clinical settings.

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