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 m². 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 (1.04-1.11)).

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]

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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.
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 m² 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-EPIcrea-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 (NTotalCAF Elisa Kit, Neurotune, Schlieren, Switzerland), performed as described previously.[3] 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 m²; 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...
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/Med). Therefore, all variables were entered into the models on raw scale. The VIFs for all covariates 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...
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, \( P = .034 \)).
Suppl. Table 4, http://links.lww.com/MD/C978) and creatinine (0.519, P = 0.036), 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.[11] This hypothesis is supported by data showing a high correlation between tCAF and 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.[16]

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.[14] 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
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.

**Author contributions**

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**References**

[1] Bruck K, Stel VS, Gambaro G, et al. CKD prevalence varies across the European general population. J Am Soc Nephrol: JASN 2016;27:2135-47.
[2] Astor BC, Matsushita K, Gansevoort RT, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. Kidney Int 2011;79:1331-40.
[3] Steubl D, Hettwer S, Dahinden P, et al. C-terminal agrin fragment (CAF) as a serum biomarker for residual renal function in peritoneal dialysis patients. Int Urol Nephrol 2015;47:391-6.
[4] Steubl D, Hettwer S, Vrijbloed W, et al. C-terminal agrin fragment—a new fast biomarker for kidney function in renal transplant recipients. Am J Nephrol 2015;38:501-8.
[5] Steubl D, Roos M, Hettwer S, et al. Plasma total C-terminal agrin fragment (tCAF) as a marker for kidney function in patients with chronic kidney disease. Clin Chem Lab Med 2016;54:1487-95.
[6] Steubl D, Vogel A, Hettwer S, et al. Early postoperative C-terminal agrin fragment (CAF) serum levels predict graft loss and proteinuria in renal transplant recipients. Clin Chem Lab Med 2016;54:63-72.
[7] Devetzis V, Daryadel A, Roumeliotis S, et al. C-Terminal fragment of agrin (CAF): a novel marker for progression of kidney disease in type 2 diabetics. PLoS One 2015;10:e0143524.
[8] KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl. 2013;3:128-133.
[9] Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med 2012;367:20-9.
[10] Coresh J, Tarn TC, Matsushita K, et al. Decline in estimated glomerular filtration rate and subsequent risk of end-stage renal disease and mortality. Jama 2014;311:2518–31.
[11] Levey AS, Inker LA, Matsushita K, et al. GFR decline as an end point for clinical trials in CKD: a scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration. Am J Kidney Dis: the official journal of the National Kidney Foundation 2014;64:821–35.
[12] Taal MW, Brenner BM. Predicting initiation and progression of chronic kidney disease: developing renal risk scores. Kidney Int 2006;70:1694-705.
[13] Tsai WC, Wu HY, Peng YS, et al. Risk factors for development and progression of chronic kidney disease: a systematic review and exploratory meta-analysis. Medicine 2016;95:e3013.
[14] Stephan A, Mateos JM, Kozlov SV, et al. Neutrophil extracellular traps are associated with sarcopenia in older multimorbid hip fractured patients. Exp Gerontol 2016;79:31-6.
[15] Daryadel A, Hauibitz M, Figueredó M, et al. The C-terminal fragment of agrin (CAF), a novel marker of renal function, is filtered by the kidney and reabsorbed by the proximal tubule. PLoS One 2016;11:e0157905.
[16] Drey M, Behnes M, Kob R, et al. C-terminal agrin fragment (CAF) reflects renal function in patients suffering from severe sepsis or septic shock. Clin Lab 2015;61:69-76.
[17] Yu D, Li HX, Liu Y, et al. The reference intervals for serum C-terminal agrin (CAF): a novel marker for progression of kidney disease in type 2 diabetics. Clin Chem 2014;60:821-35.
[18] Landi F, Calvani R, Lorenzi M, et al. Serum levels of C-terminal agrin fragment (CAF) are associated with sarcopenia in older multimorbid community-dwellers: Results from the diSIRENTE study. Exp Gerontol 2016;79:31-6.
[19] Marzetti E, Calvani R, Lorenzi M, et al. Serum levels of C-terminal agrin fragment (CAF) are associated with sarcopenia in older hip fractured patients. Exp Gerontol 2014;60:79-82.