ORIGINAL ARTICLE

Collagen type III formation but not degradation is associated with risk of kidney disease progression and mortality after acute kidney injury

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ABSTRACT

Background. Acute kidney injury (AKI), a rapid decrease in kidney function, is associated with increased risk of adverse outcomes including development and progression of CKD. Kidney fibrosis is one of the pathological processes central to this AKI-to-CKD transition. Here we investigate the association of biomarkers of collagen type III turnover with adverse outcome following AKI.

Methods. We measured three biomarkers reflecting collagen type III (PRO-C3) formation and degradation (C3M and C3C) in plasma samples collected 1 year after an episode of AKI in 800 patients (392 patients with AKI and 408 non-AKI controls) from the prospective AKI Risk in Derby (ARID) study. Patients were followed until 3 years after the episode of AKI and the following outcomes were assessed: kidney disease progression, mortality, heart failure, cardiovascular events, and hospital readmission.

Results. PRO-C3 levels were elevated in the AKI group compared with the controls ($P < .001$), whereas C3M and C3C levels were not different between groups. In multivariate models including common risk factors, PRO-C3 was prognostic for kidney disease progression and mortality in the AKI group and for heart failure in the control group. C3M and C3C were not prognostic for any of the investigated outcomes.

Conclusions. Circulating PRO-C3, a biomarker of fibroblast activity, was prognostic for kidney disease progression and mortality when measured 1 year after an episode of AKI. Biomarkers of fibroblast activity may help patient stratification after an episode of AKI by identifying patients at higher risk of kidney disease progression.

Keywords: acute kidney injury, biomarkers, collagen type III, fibroblasts, PRO-C3

KEY LEARNING POINTS

What was known:
- Kidney fibrosis is a pathological mechanism involved in the progression from acute kidney injury to chronic kidney disease
- Circulating PRO-C6, a biomarker of collagen type VI formation, was previously associated with risk of kidney disease progression in the ARID cohort of patients that experienced AKI.

This study adds:
- Here we investigated three biomarkers of collagen type III formation and degradation in the ARID cohort. Collagen type III is one of the most abundant interstitial collagens in the kidneys.
- The biomarker of collagen type III formation PRO-C3 was significantly associated with kidney disease progression and mortality over 2 years' follow-up, when measured 1 year after the AKI episode. The degradation markers were not associated with outcome.
- We confirmed that fibroblast activity, described by levels of circulating PRO-C3 and PRO-C6, is a crucial process in kidney disease progression after AKI

Potential impact:
- Collagen formation biomarkers could be implemented in clinical biochemistry to identify endotypes of patients at increased risk of mortality and disease progression, that may benefit from timely antifibrotic treatment.

INTRODUCTION

Acute kidney injury (AKI) is characterized by a rapid decrease in kidney function [1]. Many patients experience reduced kidney function after AKI, which has a bidirectional risk relationship with chronic kidney disease (CKD). AKI occurs in up to 20% of hospital admissions and is associated with in-hospital morbidity and mortality. AKI is also associated with risk of hospital readmission [2, 3], development or progression of CKD [2, 4], recurrent AKI [5–8], and cardiovascular events [9]. Recently there have been different initiatives to improve post-AKI care, for example in the USA the number of Medicare beneficiaries with post-AKI kidney function evaluation has increased, and health authorities are restructuring discharge care after AKI [10].

Even though several early biomarkers predicting AKI have been described and are still emerging, biomarkers that can assess patients' long-term health risks after AKI are still lacking. One of the hallmarks for progression of AKI to CKD is the development of kidney fibrosis, characterized by an imbalanced turnover of extracellular matrix (ECM) components. Hence, the ECM could be an important reservoir of new biomarkers associated with AKI-to-CKD progression [11].

Previous data in IgA nephropathy patients suggested that measuring different fragments of the same ECM protein can give different information about the burden and activity of fibrosis in the kidney [12]. PRO-C3, C3M, and C3C are biomarkers derived from different processing of collagen type III. PRO-C3 reflects collagen type III formation by targeting the N-terminal pro-peptide released by ADAMTS-2 during collagen maturation [13, 14]. C3M and C3C reflect collagen type III degradation, by targeting the epitope generated by MMP-9 and cathepsin B, L, and S cleavage, respectively [14–16]. These biomarkers have previously been evaluated in patients with CKD [12, 17–19]. While most evidence has been collected on urinary C3M as a biomarker of kidney disease progression in CKD [18] that is associated with levels of histologically proven tubulointerstitial fibrosis [12, 17], serum levels of C3M were associated with CKD progression in a population with diabetic kidney disease [20].

In this study, we investigated the association of circulating PRO-C3, C3M, and C3C after AKI with long-term adverse outcomes. In addition, we compared the prognostic ability of the biomarkers in the AKI group to a matched non-AKI control group. To our knowledge, this is the first time that these biomarkers of collagen type III turnover have been investigated in an AKI cohort.

MATERIALS AND METHODS

Study cohort

The AKI Risk in Derby (ARID) study (ISRCTN25405995) is a prospective parallel-group cohort study designed to report long-term outcomes following AKI [21]. The study recruited people who had been hospitalized at the Royal Derby Hospital, UK, between May 2013 and May 2016, and had survived to at least 90 days after hospital admission. The study was divided into two cohorts: one cohort consisted of people who had an episode of AKI during hospital admission (the AKI group, $n = 433$), and one cohort had not (the control group, $n = 433$). The patients in the two groups were matched 1:1 for baseline eGFR stage (eGFR >60 ml/min/1.73 m^2, eGFR stage 3A, 3B, or 4), age (± 5 years) and presence of diabetes. In addition, there were no significant differences in the proportion of patients with heart failure, ischaemic heart disease or cancer between groups.

In this study, we analysed 800 patients from the ARID study who had been successfully matched, were alive and under active follow-up at year 1, and had plasma samples collected at year 1 available for biomarker measurements. We selected the year 1 samples for biomarker measurements as we aimed to investigate sustained fibrotic processes separate from the changes that happen at the time of and immediately after AKI [22]. AKI was determined according to the serum creatinine (sCr) component of the Kidney Disease: Improving Global Outcomes (KDIGO) criteria [23] using the most recent stable sCr prior to hospital admission as baseline. Exclusion criteria were lack of a baseline sCr value in the 12 months preceding hospital admission (urine output was not used due to its inaccurate recording in a general hospitalized population), total or partial nephrectomy during index admission, pre-existing CKD stage 5, and receiving palliative care or AKI after renal transplantation. Study approvals were obtained from Derbyshire Research Ethics Committee and the National Information Governance Board. All patients provided written informed consent, and the study was conducted in compliance with the Declaration of Helsinki.

Table 1: Patient characteristics.

	Baseline			Y1			Y3		
	AKI group	Control group	P-value	AKI group	Control group	P-value	AKI group	Control group	P-value
n	392	408	.57	392	408	.57	312	351	.13
Sex (% female)	44	50	.11	44	50	.11	44	51	.06
Age (years)	71 (64–78)	71 (64–76)	.43	72 (66–79)	72 (65–78)	.45	74 (67–80)	74 (67–80)	.75
sCr (µmol/L)	89 (76–106)	87 (74–105)	.23	100 (83–122)	84 (72–105)	<0.0001	100 (83–122)	86 (71–102)	<.0001
eGFR (mL/min/1.73 m^2)	70 (57–82)	72 (56–87)	.30	60 (46–74)	73 (58–86)	<.0001	60 (45–75)	71 (57–84)	<.0001
ACR (mg/mmol)	NA	NA		1.4 (0.5–6.0)	0.8 (0.0–3.2)	<.0001	1.3 (0.3–5.2)	0.6 (0.0–2.3)	<.0001
CRP (mg/L)	NA	NA		3.0 (1.6–7.0)	3.0 (1.1–6.0)	<.05	3.0 (1.4–7.0)	2.0 (1.0–6.0)	.06
DM at BL (% yes)	22	22	0.83	NA	NA		NA	NA	
CKD at BL (% yes)	29	29	.90	NA	NA		NA	NA	
CKD stage (%)									
1	16	17	.79	8	16	<.0001	6	17	<.0001
2	55	54		43	56		44	54	
3A	20	19		25	18		25	18	
3B	7	7		18	7		18	6	
4	2	2		5	3		7	5	
5	0	0		1	0		0	0	
AKI stage (%)									
1	60	NA		NA	NA		NA	NA	
2	24	NA		NA	NA		NA	NA	
3	16	NA		NA	NA		NA	NA	
Recurrent AKI (%)				12	2	<.0001	13	7	<.05

Data are presented as median (IQR).
Recurrent AKI for Y1 is from BL to Y1 and recurrent AKI for Y3 is from Y1 to Y3.
Statistical difference between the AKI and the control group was assessed by the Mann–Whitney or χ^2 test. p-values <0.05 are marked in bold.
BL, baseline; NA, not applicable.

Data collection

At year 1 and 3 after AKI onset, sCr, eGFR, calculated using the CKD Epidemiology Collaboration Equation [23], proteinuria:creatinine ratio (PCR), and urinary albumin:creatinine ratio (ACR) were measured. Kidney disease progression from year 1 to 3 was defined as a decline in eGFR of ≥25% associated with a change in CKD stage, as previously described [24]. The patients included in the study were asked not to eat meat for at least 12 hours before blood samples were collected and to provide an early morning urine specimen. Levels of sCr and urinary albumin were measured on the Roche Cobas 702 module (Roche Diagnostics Limited, Burgess Hill, UK) using an enzymatic assay and an immunoturbidimetric assay (Tina-quant Albumin Generation 2), respectively. Blood samples were collected in BD Vacutainer EDTA tubes, transported to the laboratory at ambient temperature and centrifuged within 6 hours of collection at 3000 RPM/1508 RCF for 10 minutes. Plasma was then removed, aliquoted and stored at −80°C for biomarker measurements. Hospital admission data, Charlson score, inpatient laboratory test results, coded comorbidities and mortality were extracted from the hospital electronic medical record. Cross-referencing with the local renal database was used to track commencement of long-term kidney replacement therapy.

Collagen type III biomarker measurements

The biomarkers of collagen type III turnover PRO-C3, C3M, and C3C were measured in plasma collected at year 1 (Y1) using the competitive enzyme-linked immunosorbent assays, nordicPRO-C3^{TM}, nordicC3M^{TM}, and nordicC3C^{TM}, according to manufacturer instructions (Nordic Bioscience, Herlev, Denmark). The monoclonal antibodies used in the PRO-C3, C3M, and C3C assays specifically detect 10 amino acids before or after the cleavage site of the N-terminal pro-peptide [13, 14] and 2 internal fragments [14–16], respectively.

Statistical analysis

Spearman rank correlations were used to analyse associations between biomarkers and clinical variables. Differences between groups were analysed with Mann–Whitney or χ^2 tests. Logistic regression for kidney disease progression and Cox proportional hazards regression for mortality, heart failure, cardiovascular events, and readmission were used to evaluate different uni- and multivariable models. The multivariable model included sex, age, eGFR, ACR, presence of diabetes, baseline CKD, PRO-C3, C3M, and C3C. A backward elimination logistic and Cox proportional hazards regression analysis was used. The follow-up time was 2 years (Y1 to Y3). Comparison of Kaplan–Meier curves for PRO-C3 tertiles was done with the Mantel–Cox test. Statistical analysis was performed by R software (2022, R Core Team) and GraphPad Prism (version 7.05, GraphPad Software, La Jolla, CA, USA), and P-values <.05 were considered significant.

RESULTS

PRO-C3, C3M, and C3C were measured in the Y1 plasma samples of 800 patients (392 AKI and 408 controls). At baseline the two study groups were similar with regard to sex distribution in addition to the 1:1 matched criteria (eGFR, age, and presence of diabetes). At both Y1 and Y3 the AKI group had significantly lower eGFR and higher levels of urine ACR and C-reactive protein (CRP). More patients in the AKI group had further AKI episodes during follow-up as compared with the control group (Table 1).

At Y1, PRO-C3 levels correlated with sCr, ACR, CRP and negatively correlated with eGFR in the AKI group, and with age, sCr,

Table 2: Correlations of ECM biomarkers at Y1 with variables measured at Y1 and at Y3.

		Biomarker Y1 and variables Y1				Biomarker Y1 and variables Y3			
		AKI group		Control group		AKI group		Control group	
Biomarker	Variable	ρ	P-value	ρ	P-value	ρ	P-value	ρ	P-value
PRO-C3	Age	0.08	.11	0.13	**<.01**	0.05	.35	0.16	**<.01**
	sCr	0.15	**<.01**	0.24	**<.0001**	0.13	**<.05**	0.25	**<.0001**
	eGFR	−0.13	**<.01**	−0.21	**<.0001**	−0.12	**<.05**	−0.21	**<.0001**
	ACR	0.13	**<.01**	0.09	.07	0.14	**<.05**	0.08	.14
	CRP	0.21	**<.0001**	0.20	**<.0001**	0.16	**<.01**	0.13	**<.05**
C3M	Age	0.03	.61	0.05	.33	0.03	.62	0.04	.50
	sCr	−0.02	.63	0.02	.76	−0.01	.89	−0.02	.64
	eGFR	−0.03	.55	0.01	.80	−0.04	.49	0.05	.37
	ACR	−0.01	.83	0.09	.08	0.00	.98	0.01	.89
	CRP	0.17	**<.001**	0.16	**<.01**	0.14	**<.05**	0.07	.21
C3C	Age	−0.07	.19	−0.08	.09	−0.10	.06	−0.07	.17
	sCr	0.2	.70	−0.03	.50	−0.05	.36	−0.08	.12
	eGFR	−0.05	.31	0.04	.41	0.03	.62	0.07	.20
	ACR	0.05	.30	0.05	.35	0.05	.41	0.07	.21
	CRP	0.11	**<.05**	0.10	**<.05**	0.02	.71	0.11	**<.05**

Spearman rank correlation. Delta variables were defined as measurements at Y3 minus measurements at Y1. p-values <0.05 are marked in bold.

CRP and negatively with eGFR in the control group (Table 2). C3M and C3C levels only correlated with CRP in both the AKI and the control groups (Table 2). There were no differences in PRO-C3 levels between different categories of AKI aetiology (data not shown).

PRO-C3 levels at year 1 were significantly elevated in the AKI group [median (interquartile range, IQR) = 66.5 (56.5–78.9) ng/mL] compared with the control group [median (IQR) = 62.8 (52.4–73.8) ng/mL; P < .001; Fig. 1A]. When patients in the AKI and the control groups were further subdivided into patients with and without CKD at baseline, PRO-C3 was significantly higher in the AKI + CKD [median (IQR) = 68.9 (58.4–81.2) ng/mL] group than in patients with neither AKI nor CKD [median (IQR) = 59.8 (51.4–70.5) ng/mL] (P < .0001; Fig. 1B). PRO-C3 was also higher in patients with AKI and diabetes mellitus (DM) [median (IQR) = 68.3 (60.5–90.6) ng/mL] than in patients with neither AKI nor DM [median (IQR) = 62.3 (51.9–72.2) ng/mL]. C3M and C3C levels were not significantly different in any of the groups (data not shown).

Patients in the AKI group were grouped according to AKI stages (AKI 1–3) at baseline and all patients were grouped according to CKD stages (CKD 1–5) at Y1. PRO-C3, C3M, and C3C levels were not different in different AKI stages (Fig. 1C and data not shown). PRO-C3 levels increased progressively with increasing CKD stages (Fig. 1D), whereas C3M and C3C levels were not significantly different between CKD stages (data not shown).

From Y1 to Y3, 70 out of the 800 patients died: 43 in the AKI group (11%) and 27 in the control group (7%; P < .05). PRO-C3 levels were significantly higher in patients who died from year 1 to 3 compared with survivors in both the AKI [median (IQR) = 77.9 (63.5–91.5) ng/mL vs median (IQR) = 66.0 (56.1–77.2) ng/mL; P < .01] and control [median (IQR) = 71.1 (54.1–84.4) ng/mL vs median (IQR) = 62.5 (52.4–73.5) ng/mL; P = .12] groups. In both the AKI and control groups, C3M and C3C levels were not significantly different between non-survivors and survivors (P > .05, data not shown). Patients were stratified into tertiles based on collagen type III biomarker levels. Patients in the highest PRO-C3 tertile were more likely to die in both the AKI [tertile 1 (T1) = (16.6–60.0); T2 = (60.0–73.1); T3 = (73.1–281.0) ng/mL; P < .01;

Fig. 2A] and the control group [T1 = (11.7–55.7); T2 = (55.7–69.4); T3 = (69.4–269.0) ng/mL; P < .05; Fig. 2D]. Tertiles of C3M and C3C were not significantly associated with risk of mortality (Fig. 2B, E, C, and F).

In the AKI group, PRO-C3 was significantly associated with mortality in the univariable analysis [hazard ratio (HR) =2.48; P < .001; Fig. 3A] and in the multivariable Cox proportional hazards regression with backward elimination together with age (HR = 3.15; P < .0001; Table 3). In the control group, none of the biomarkers were associated with mortality (Fig. 3B and C and Table 3).

At Y3, 42 patients had kidney disease progression (from the 705 patients with complete data). Of these, 25 out of 336 were in the AKI group (7%) and 17 out of 369 were in the control group (5%; P = .11). Logistic regression was used to assess the univariate and multivariate association of the biomarkers with kidney disease progression. In the univariable analysis, only PRO-C3 [odds ratio (OR) = 2.46; P < .05, Fig. 3A–C] was significantly associated with kidney disease progression from year 1 to 3 in the AKI group. In the control group, none of the biomarkers were associated with kidney disease progression (Fig. 3A–C). After adjustment for the confounding factors, PRO-C3 was the only biomarker retained in the AKI group (OR = 3.13; P < .05), but not in the control group (Table 3).

A total of 103 (13%), 193 (24%), and 531 (66%) out of the 800 patients had heart failure events [62 (16%) in the AKI and 41 (10%) in the control group; P < .05], cardiovascular (CV) events [104 (26%) in the AKI and 89 (22%) in the control group; P = .12] or were readmitted to the hospital [267 (68%) in the AKI and 264 (65%) in the control group; P = .31], respectively. In univariable analysis, PRO-C3 was the only collagen type III biomarker associated with heart failure in both the AKI (HR = 1.68; P < .05; Fig. 3A) and the control (HR = 2.31; P < .001; Fig. 3A) group. The significance was maintained in the multivariable analysis together with eGFR (HR = 1.99; P < .01; Table 3) in the control group but not in the AKI group. PRO-C3 was also associated with CV events in the control group (HR = 1.68; P < .01; Fig. 3A) and hospital readmission in the AKI group (HR = 1.32; P < .05; Fig. 3A) but the association was not maintained in the multivariable model.
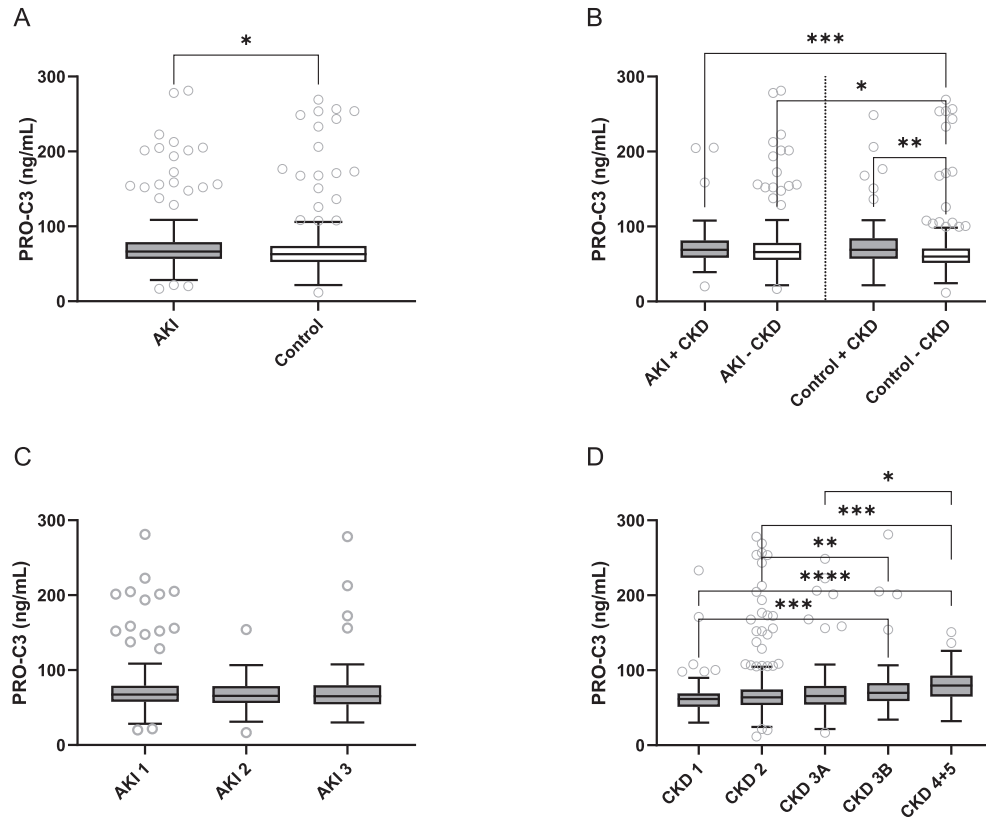
Figure 1: PRO-C3 levels in patients with and without AKI (**A**), in AKI and control patients with and without CKD (**B**), at different AKI stages (**C**) and at different CKD stages (**D**). Data are presented as Tukey box plots. Statistical differences between groups were assessed by Kruskal–Wallis test with Dunn's multiple comparison test; *P < .05, **P < .01, ***P < .001, ****P < .0001.

Table 3: Multivariate outcome analyses (backward elimination, BE).

Outcome	Y1 variables Multivariate model with BE	AKI group OR	95% CI	P-value	Control group HR	95% CI	P-value
Kidney disease progression	Age	1.08	1.02–1.15	<.01			
	ACR	1.01	1.00–1.02	<.05	1.03	1.01–1.05	<.0001
	BL CKD (yes)	5.35	1.61–19.18	<.01			
	eGFR	1.05	1.02–1.09	<.01			
	Log$_2$ (PRO-C3)	**3.13**	**1.25–7.95**	**<.05**			
	Multivariate models with BE	**HR**	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
Mortality	Age	1.08	1.04–1.12	<.001			
	BL CKD (yes)						
	eGFR				0.98	0.96–0.99	<.01
	Log$_2$ (PRO-C3)	**3.15**	**1.81–5.50**	**<.0001**			
Heart failure	Age	1.05	1.02–1.09	<.01			
	BL DM (yes)	2.42	1.45–4.03	<.001			
	eGFR				0.96	0.95–0.98	<.0001
	Log$_2$ (PRO-C3)				**1.99**	**1.19–3.33**	**<.01**
Cardiovascular event	Age	1.05	1.03–1.08	<.0001	1.03	1.00–1.06	<.05
	ACR	1.01	1.00–1.01	<.001	1.01	1.00–1.01	<.01
	BL DM (yes)	1.92	1.27–2.901	<.01			
	eGFR				0.98	.97–0.99	<.001
Readmission	ACR				1.00	1.00–1.01	<.01
	BL DM (yes)				1.44	1.09–1.91	<.01
	eGFR	0.98	0.98–1.00	<.001			

Data are based on multivariate logistic regression (kidney disease progression) and Cox proportional hazards regression (mortality, heart failure, cardiovascular event, and readmission) analyses from Y1 to Y3 with backward elimination.
Kidney disease progression was defined as ≥25% decline in eGFR and a decline in CKD stage. The HR of PRO-C3 in the different models is marked in bold.
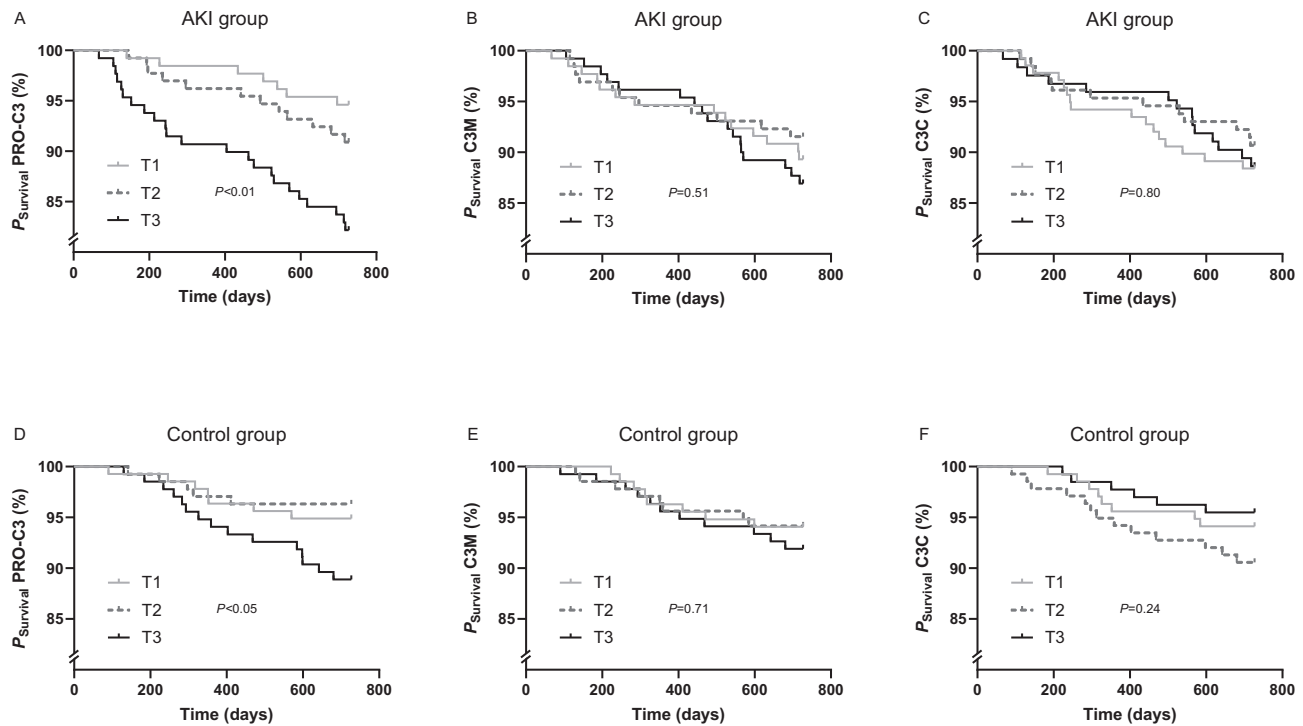BL, baseline.

Figure 2: Survival curves for tertiles of ECM biomarker levels in the AKI and control groups. Survival data are based on tertiles of the ECM biomarker levels in the AKI (**A–C**) and control groups (**D–F**) (tertiles are labelled T1, T2, and T3). Statistical differences between curves were assessed by the Mantel–Cox test. $P_{survival}$, probability of survival.

DISCUSSION

In the current study, we investigated the association of three circulating biomarkers of collagen type III turnover with risk of adverse outcomes in patients who previously experienced an episode of AKI and compared it with a matched non-AKI control group. We have shown that out of the three investigated biomarkers, PRO-C3, reflecting collagen type III formation, was associated with kidney disease progression and mortality after AKI, but C3M and C3C, reflecting collagen type III degradation mediated by MMPs and cathepsins, were not. These results highlight the importance of measuring the right epitope in a protein to describe the pathological process leading to disease progression.

Tubulointerstitial fibrosis is associated with kidney disease severity and is due to an increased deposition of ECM components, including collagens. Collagen type III is expressed in small amounts in the tubulointerstitium and is not detected in the glomeruli of a healthy kidney. In contrast, a scarred kidney has increased collagen type III expression in the tubulointerstitium and glomeruli [25, 26]. During fibrogenesis the N-terminal propeptide of collagen type III (PRO-C3) is released and can enter the circulation and be excreted in the urine. This biomarker reflects the activity of fibroblasts, the main collagen-producing cells.

In a previous investigation in IgA nephropathy patients, measuring the same three collagen type III biomarkers in serum and urine provided different information regarding pathological kidney tissue alterations. Serum levels of PRO-C3 and C3C correlated directly with the degree of fibrosis, whereas urinary C3M correlated inversely with the degree of fibrosis [12]. PRO-C3 is a very well described risk biomarker in liver disease [27–30]. The results from this work suggest that the production of one of the main interstitial collagens, collagen type III, may be a common mechanism across different organ diseases, given the promising prognostic indications in patients that experienced an AKI. Since collagen type III is ubiquitously expressed, PRO-C3 may reflect fibrosis in other organs, and AKI may be a predisposing event not only for kidney diseases but also for a systemic profibrotic environment. It is interesting to note that PRO-C3 levels in the AKI population seem to be most indicative of kidney disease progression and mortality, while in the control population of patients with CKD but that did not experience AKI, it seems to be mostly related to cardiovascular outcomes.

C3M and C3C are mostly related to inflammation as they are produced by proteases released by inflammatory cells cleaving collagen type III. In this study, these fragments were not informative on the risk of progression after AKI. We can speculate that this may suggest that the activation of fibroblasts is a more dominant pathological process after the acute event than the inflammatory response. Alternatively, the time point at which we measured the biomarkers may be relevant, when a predominantly fibrotic rather than inflammatory picture is more likely. In any case, these data emphasize the importance of measuring the right epitope, as different parts of the same molecule can harbour very different biological information regarding pathogenetic processes.

In a previous investigation in the ARID cohort, a biomarker of collagen type VI formation, PRO-C6, was also associated with kidney disease progression and mortality [11].

Similar to PRO-C3, PRO-C6 is a biomarker of fibroblast activity. This confirms that the process of fibroblast activation and collagen production and deposition is an important one in the progression from AKI to CKD and in the further progression to kidney failure and death.
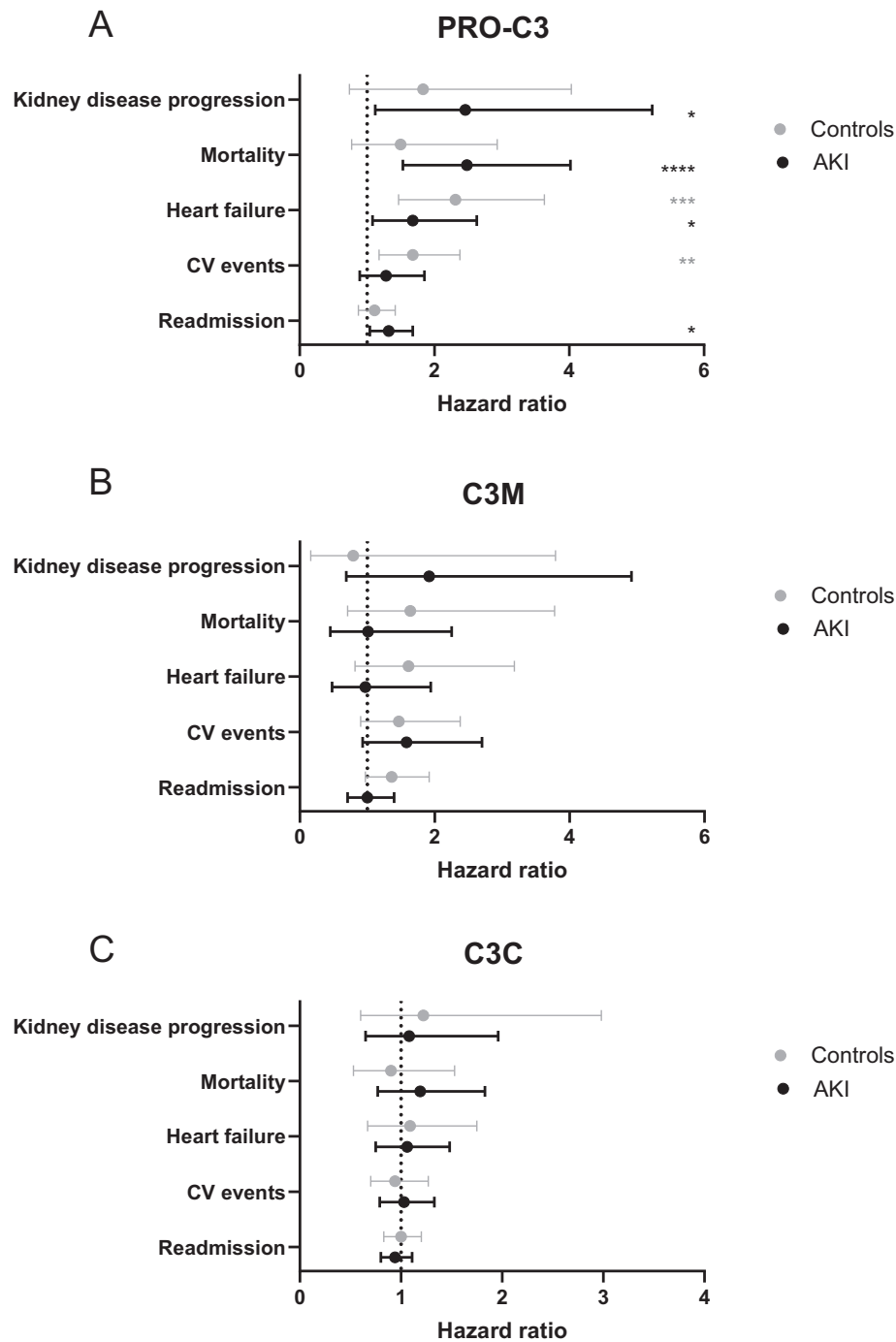
Figure 3: Forest plot of odds ratio (for kidney disease progression) and hazard ratio (for the rest of the outcomes) for outcome for doubling ($\log_2$) of PRO-C3 in univariate analysis. *P < .05, **P < .01, ***P < .001, ****P < .0001.

Biomarkers of fibroblast activity such as PRO-C3 and PRO-C6 can provide important mechanistic information about CKD progression after AKI, can inform future research and be potentially used for trial enrichment to identify patients with high fibroblast activity that would benefit from antifibrotic therapy.

The main limitation of this study is that we chose to measure biomarkers at 1 year after AKI. This was done deliberately to investigate established fibrosis post-AKI, but we were therefore unable to study the utility of biomarkers at earlier time points. Patients who died or progressed to kidney failure before the one-year time point were therefore not studied. Future direction of this investigation could be to shed light on the timeline of these pathological alterations, by measuring these biomarkers at earlier time points after AKI, where markers of inflammatory-driven tissue degradation may also find a utility.

In conclusion, we have demonstrated that circulating PRO-C3, a biomarker of fibroblast activity, provides prognostic information in terms of kidney disease progression and mortality

when measured 1 year after an episode of AKI. Together with previous studies, our results suggest that biomarkers of fibroblast activity (PRO-C3 and PRO-C6) may have a role in stratifying patients after an episode of AKI, in particular to identify patients at higher risk of subsequent kidney disease progression, a process that is characterized by fibrotic change. Future work to validate these findings and explore the utility of these biomarkers at earlier time points is now warranted.

AUTHORS' CONTRIBUTIONS

F.G. and N.M.S. conceptualized the study; N.S. performed the work and wrote the first draft of the manuscript; N.S., F.G., N.M.S., and M.A.K. provided intellectual contributions to the interpretation of the analyses and contributed to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

N.S. F.G., and M.A.K. were full-time employees at Nordic Bioscience at the time the work was performed. Nordic Bioscience is a privately owned, small to medium-size enterprise partly focused on the development of biomarkers. None of the authors received fees, bonuses or other benefits for the work described in this article. F.G. and M.A.K. hold stock in Nordic Bioscience. The patent for the ELISAs used in this work is owned by Nordic Bioscience. The funder provided support in the form of salaries for authors N.S., F.G.E. and M.A.K., but did not have any additional role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. All other authors report no competing financial interests relevant to this article. The results presented in this article have not been published previously in whole or part, except in abstract format.

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