

The Association between Serum Endostatin, Kidney Disease Progression, and Mortality in Patients with Chronic Kidney Disease in the Salford Kidney Study

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Key Learning Points

What was known?

- People with chronic kidney disease have an increased risk of cardiovascular morbidity.
- Circulating endostatin is associated with both cardiovascular morbidity and impaired kidney function in the general population.
- The utility of endostatin as a prognostic marker for CKD progression and mortality in patients with CKD is not well studied.

This study adds the following:

- Cox regression models revealed an association between endostatin and mortality with adjustments for established cardiovascular risk factors.
- The association was attenuated and nonsignificant after adjustments for baseline eGFR and uPCR.
- Changes of endostatin concentrations during the study were significantly associated with eGFR decline in all models.

Potential impact includes the following:

- The clinical utility of plasma endostatin for risk prediction purposes in patients with CKD seems limited.
- The clinical relevance of the association between decline in kidney function and endostatin warrants further studies.

Johanna Helmersson-Karlqvist and Rajkumar Chinnadurai contributed equally to this work and are joint first authors.

Keywords

Endostatin · Glomerular filtration rate · Mortality · Chronic kidney disease

Abstract

Introduction: Patients with chronic kidney disease (CKD) have an increased risk of cardiovascular morbidity. Circulating endostatin is associated with both cardiovascular morbidity and impaired kidney function in the general population, but the utility of endostatin as a prognostic marker for CKD progression and mortality in patients with CKD is not well studied. The aim was to study association between serum endostatin and mortality, and also kidney function decline in a cohort of CKD patients (Salford Kidney Study [SKS]). **Methods:** Analyses were performed on baseline and annual follow-up samples from 970 adults in the SKS cohort with CKD stage 3–5. Association with mortality was studied using Cox proportional hazard models adjusted for age, gender, systolic and diastolic blood pressure, smoking status, diabetes mellitus, prior cardiovascular disease, creatinine-based estimated glomerular filtration rate (eGFR), and urine protein-to-creatinine ratio (uPCR). Associations between endostatin and eGFR decline were studied with linear regression analyses. eGFR decline was defined as the percentage difference between baseline eGFR and follow-up eGFR (median follow-up, 6.2 years). **Results:** Median age of the cohort was 66 years, with a median eGFR of 30 mL/min/1.73 m². Multivariate Cox regression models revealed an association between higher endostatin levels and mortality with adjustments for established cardiovascular risk factors (HR: 1.14; CI: 1.02–1.28; $p = 0.02$) but was attenuated and nonsignificant after adjustments for baseline eGFR and uPCR. Baseline levels of endostatin were associated with eGFR decline but were nonsignificant after adjustments for baseline eGFR and uPCR. Changes of endostatin concentrations during the study were significantly associated with eGFR decline in all models (regression coefficient 0.0023% decrease per month [95% confidence intervals 0.0012–0.0034, $p < 0.001$]). **Conclusion:** The clinical utility of plasma endostatin for risk prediction in CKD patients seems limited. Importantly, longitudinal changes of endostatin were significantly associated with eGFR decline. The clinical relevance of this warrants further studies.

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Introduction

Patients with chronic kidney disease (CKD) have an increased frequency of cardiovascular disease compared to many other patient groups [1]. Beyond the increased incidence, the cardiovascular comorbidity is also more often severe and not recognized and undertreated [2], and the mortality is high [3, 4]. The underlying cardiovascular risk is not completely understood, multifactorial in its cause and only partly due to specific kidney diseases [5]. CKD presents, in its initial phase of disease, with only a few clinical symptoms. Biomarkers that are sensitive enough to detect the disease initiation as well as a more rapid progression of CKD, and those more likely to develop cardiovascular events, are warranted.

Although numerous biomarkers have been proposed to reflect inflammation, fibrosis, tubular injury, or cardiovascular stress in CKD, few capture the combined processes of vascular injury, extracellular matrix remodeling, and microvascular rarefaction - mechanisms central to CKD progression. Endostatin, a C-terminal fragment of type XVIII collagen, is mechanistically linked to anti-angiogenic activity and extracellular matrix turnover [6], and circulating concentrations correlate with cardiovascular injury [7–11], diabetic kidney disease severity [12, 13], and CKD progression risk in experimental and population-based studies [14–19].

More recently, there has been growing interest in dynamic or longitudinal biomarkers that better reflect ongoing pathophysiological processes than single baseline measurements. Several large studies have shown that serial changes in biomarkers - including tubular injury markers, proteomic signatures, and mineral metabolism factors - predict estimated glomerular filtration rate (eGFR) decline more accurately than baseline levels alone. For example, recent analyses of CKD and diabetes cohorts demonstrate that trajectories of biomarkers such as KIM-1, TNF-receptors, and multi-omics profiles provide improved risk stratification compared with static measurements, underscoring the importance of monitoring biomarker evolution over time rather than relying solely on a single measurement [20–23].

In light of the limited evidence regarding endostatin as a prognostic biomarker in advanced CKD, our aim was to examine the associations between circulating endostatin and two key outcomes: all-cause mortality and kidney function decline. We also sought to evaluate whether longitudinal changes in endostatin provide additional information beyond baseline levels. We hypothesized that higher endostatin concentrations, particularly increases

over time, would be associated with more rapid deterioration of kidney function and increased mortality risk in patients with established CKD.

Methods

Study Population

The Salford Kidney Study is an ongoing prospective study, initiated in 2002, and at the time of this study, it only included patients with CKD stages 3–5 but not on dialysis therapy, with the intention to investigate the progression and outcomes of kidney disease and associated comorbidities [24]. Subsequent amendments have been undertaken, and recruitment of patients receiving renal replacement therapy (RRT) is also ongoing now.

In short, patients with eGFR >10 and <60 mL/min/1.73 m² referred for renal opinions and investigations to the Salford Royal Hospital renal services were asked if they wanted to participate in the study. Out of the patients recruited between 2004 and 2013 (*n* = 2,171), endostatin measurements were available in 970 (44.6%) randomly selected patients who were included in the present study. Out of them, 458 patients were followed up annually and had multiple [2–7, 9, 11] measurements of eGFR and endostatin. Patients with previous evidence of RRT, i.e., dialysis or kidney transplant, were excluded from the study.

Clinical Data

At enrollment and at annual follow-up, variables such as demographic, clinical, and laboratory data were obtained from the patient records, which is a continually updated electronic healthcare system. Patient interviews and detailed clinical examination were also undertaken. The demographic variables available for analysis were age, sex, ethnicity, blood pressure, and prescribing data (by medication class). Self-reported smoking status was classified as smokers (current and former) and non-smokers. Comorbidity variables that were available for analysis were hypertension, diabetes mellitus, ischemic heart disease, myocardial infarction, congestive cardiac failure, cerebrovascular accident, and peripheral vascular disease. Any cardiovascular event was defined as a history of any of the following at baseline: myocardial infarction, congestive cardiac failure, cerebrovascular accident, and peripheral vascular disease. Blood pressure was recorded by an automated sphygmomanometer after at least 5 min of seated rest, with an appropriately sized cuff. A minimum of two readings was obtained, and the average of the two results was recorded [25]. The follow-up time stretched from the time of entry into the SKS

cohort to death or reaching end-stage kidney disease (commencing RRT or reaching an eGFR of 10 mL/min/1.73 m² in patients who opted for conservative care) or end of follow-up (February 23, 2018).

Laboratory Measurements

Biosamples were stored at patient enrollment at –80°C. Serum levels of endostatin were analyzed using a commercially available ELISA kit for endostatin (DY1098, R&D Systems, Minneapolis, MN). The patient samples were all diluted 1:40 in 0.02 M Na₂HPO₄, 0.15 M NaCl, pH 7.2 containing 10 g/L of bovine serum albumin. The assay had a total coefficient of variation of approximately 7% and an intra-assay variation of 4.5%. Endostatin values were analyzed in stored samples. Change in endostatin during follow-up was calculated as the difference between last visit before exiting the study and baseline visit and expressed as 1 SD increase; thus, an endostatin difference >0 indicated an endostatin increase. Urine protein was analyzed.

All serum creatinine measurements were performed using a blank rated and compensated Jaffé reaction with a Roche Modular analyzer (Roche Diagnostics, Rotkreuz, Switzerland), which was isotope dilution mass spectrometry-calibrated. In addition to internal quality control, the laboratory participates in the UK National External Quality Assessment Scheme. eGFR creatinine was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation [26].

Follow-Up Outcomes

All-cause mortality was the primary outcome. Delta eGFR was calculated as the difference between eGFR creatinine at baseline and at the last visit before exiting the study or last follow-up and expressed as % decrease per month; thus, a delta eGFR >0 indicated an eGFR decline [27]. Rapid eGFR decliner was defined as individuals in quartile 4 of delta eGFR.

Statistical Analysis

The cohort was split into four quartiles (Q1 lowest to Q4 highest) based on the endostatin levels and analyzed. In the descriptive part of the analysis, continuous variables were expressed as median (interquartile range) and the *p* value of difference between Q1 and Q4 given by Mann-Whitney U test. The categorical variables are expressed as number (percentage) and the *p* values given by Chi-square test.

The associations of baseline endostatin and total mortality were analyzed with Cox proportional hazard regression in an unadjusted model and in five multivariable models (model A: adjusted for age, gender; model B: age, gender, systolic blood pressure, diastolic blood pressure,

Table 1. Baseline characteristics of the study population with the quartiles of endostatin values and the subsample who were followed up annually

Variable	Quartiles					p value (Q1 vs. Q4)
	total	1 (lowest)	2	3	4 (highest)	
Patients, <i>n</i>	<i>n</i> = 970	<i>n</i> = 242	<i>n</i> = 243	<i>n</i> = 242	<i>n</i> = 243	
Age, years	66 (54–74)	66 (55–74)	65 (54–72)	65 (53–74)	69 (57–76)	0.062
Sex, male	597 (61.5)	146 (60.3)	152 (62.6)	150 (62)	149 (61.3)	0.824
Ethnicity, white, <i>n</i> (%)	929 (95.8)	235 (97.1)	231 (95.1)	230 (95)	233 (95.9)	0.464
Smoking history, <i>n</i> (%)	652 (67.2)	155 (64)	167 (68.7)	161 (66.5)	169 (69.5)	0.199
Alcohol history, <i>n</i> (%)	494 (50.9)	132 (54.5)	130 (53.5)	122 (50.4)	110 (45.3)	0.041
Weight, kg	79 (70–91)	79 (70–91)	79 (69–90)	80 (69–91)	79 (69–92)	0.887
Height, m	1.7 (1.62–1.75)	1.7 (1.5–1.7)	1.7 (1.6–1.75)	1.7 (1.5–1.7)	1.7 (1.6–1.8)	0.964
Systolic blood pressure, mm Hg	136 (123–150)	138 (122–148)	131 (122–148)	136 (124–152)	138 (126–152)	0.143
Diastolic blood pressure, mm Hg	76 (68–82)	78 (70–83)	76 (68–83)	75 (66–82)	75 (66–82)	0.071
Diabetes mellitus, <i>n</i> (%)	286 (29.5)	68 (28.1)	70 (28.8)	79 (32.6)	69 (28.4)	0.942
Hypertension, <i>n</i> (%)	908 (93.6)	218 (90.1)	225 (92.6)	231 (95.5)	234 (96.3)	0.007
Ischemic heart disease, <i>n</i> (%)	206 (21.2)	54 (22.3)	52 (21.4)	50 (20.7)	50 (20.6)	0.641
MI, <i>n</i> (%)	167 (17.2)	36 (14.9)	37 (15.2)	42 (17.4)	52 (21.4)	0.062
CCF, <i>n</i> (%)	158 (16.3)	36 (14.9)	38 (15.6)	34 (14)	50 (20.6)	0.100
CVA, <i>n</i> (%)	105 (10.8)	17 (7)	21 (8.6)	27 (11.2)	40 (16.5)	0.001
PVD, <i>n</i> (%)	145 (14.9)	32 (13.2)	34 (14)	36 (14.9)	43 (17.7)	0.173
Any cardiovascular event, <i>n</i> (%)	381 (39.3)	86 (35.5)	89 (36.6)	88 (36.4)	118 (48.6)	0.004
Renin-angiotensin system inhibitors, <i>n</i> (%)	624 (64.3)	150 (62)	155 (63.8)	159 (65.7)	160 (65.8)	0.376
Beta blockers, <i>n</i> (%)	318 (32.8)	74 (30.6)	79 (32.5)	85 (35.1)	80 (32.9)	0.579
Statin, <i>n</i> (%)	596 (61.4)	141 (58.3)	156 (64.2)	156 (64.5)	143 (61.4)	0.896
Oral diabetic medications, <i>n</i> (%)	115 (11.9)	33 (13.6)	31 (12.8)	19 (7.9)	32 (13.2)	0.880
Insulin <i>n</i> (%)	85 (8.8)	18 (7.4)	20 (8.2)	29 (12)	18 (7.4)	0.990
Endostatin, pg/mL	118,218 (79,222–171309)	59,419 (45,926–67115)	97,219 (87,316–108024)	141,232 (129,263–155415)	214,316 (190,956–261958)	<0.001
Creatinine, μmol/L	190 (144–271)	145 (121–174)	169 (136–226)	208 (161–280)	296 (218–404)	<0.001
eGFR creatinine, mL/min/1.73 m ²	30 (20–42)	42 (33–52)	34 (24–44)	27 (20–37)	18 (13–25)	<0.001
Cystatin C, mg/L	2.52 (1.86–3.35)	1.76 (1.5–2.34)	2.4 (1.9–3.2)	2.7 (2.2–3.4)	3.3 (2.7–4.1)	<0.001

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Table 1 (continued)

Variable	Quartiles					<i>p</i> value (Q1 vs. Q4)
	total	1 (lowest)	2	3	4 (highest)	
eGFR cystatin C, mL/min/1.73 m ²	22 (15–32)	35 (24–46)	24 (15–33)	20 (14–27)	15 (12–19)	< 0.001
uPCR, mg/mmol ^a	36 (16–103)	20 (12.5–52)	25.5 (15–100)	46,918–129)	58 (24–144)	< 0.001
Outcomes on follow-up						
All-cause mortality, <i>n</i> (%)	424 (43.7)	109 (45)	101 (41.6)	100 (41.3)	114 (46.9)	0.679
Renal replacement therapy, <i>n</i> (%)	263 (27.1)	35 (14.5)	52 (21.4)	78 (32.2)	98 (40.3)	< 0.001
Follow-up, years	6.2 (3.6–10)	8.5 (4.6–11)	6.7 (4–10)	6.2 (3.5–9.4)	4.6 (2.7–7.5)	< 0.001

Any cardiovascular event: MI/CVA/CCF/PVD. Continuous variables were expressed as median (interquartile range), *p* value by Mann-Whitney U test; categorical variables were expressed as number (percentage) and *p* value by chi-square test. uPCR, urine protein creatinine ratio; PVD, peripheral vascular disease; CVA, cerebrovascular accident; CCF, congestive cardiac failure; MI, myocardial infarction. ^aMissing uPCR values in 130 patients.

Table 2. Associations between endostatin and mortality risk in Cox regression models

Cox models	All-cause mortality prior to RRT	<i>p</i> value
	HR (95% CI)	
Univariate model	Reference	–
Quartile 1	1.08 (0.83–1.42)	0.563
Quartile 2	1.06 (0.93–1.22)	0.349
Quartile 3	1.24 (1.14–1.36)	< 0.001
Quartile 4	1.01 (1.01–1.02)	< 0.001
Continuous (1 SD increase)		
Multivariate model A	1.19 (1.09–1.30)	< 0.001
Multivariate model B	1.18 (1.08–1.29)	< 0.001
Multivariate model C	1.14 (1.02–1.28)	0.02
Multivariate model D	1.17 (1.05–1.29)	0.002
Multivariate model E	1.12 (0.98–1.27)	0.080

Multivariate models compared quartile 4 and quartile 1 (reference). Model A: adjusted for age and gender. Model B: age, gender, systolic blood pressure, diastolic blood pressure, smoking status, diabetes, and prior cardiovascular event. Model C: model B + baseline eGFR (MDRD). Model D: model B + urine protein creatinine ratio (model excluded 75 patients without uPCR values). Model E: model B + baseline eGFR (MDRD) and urine protein creatinine ratio (model excluded 75 patients without uPCR values). HR, hazard ratio; CI, confidence interval; RRT, renal replacement therapy.

smoking status, diabetes, and prior cardiovascular event; model C: model B + baseline eGFR (MDRD); model D: model B + urine protein creatinine ratio; model E: model B + baseline eGFR (MDRD) and urine protein creatinine ratio). No imputation was made for missing values (regarding urine protein values).

Associations between baseline endostatin, endostatin increase, and eGFR difference were analyzed in linear regression models A–E. Associations between endostatin at baseline and endostatin difference and the risk of being a rapid eGFR decliner (defined as quartile 4 versus quartiles 1–3 of delta eGFR over follow-up) were analyzed in logistic

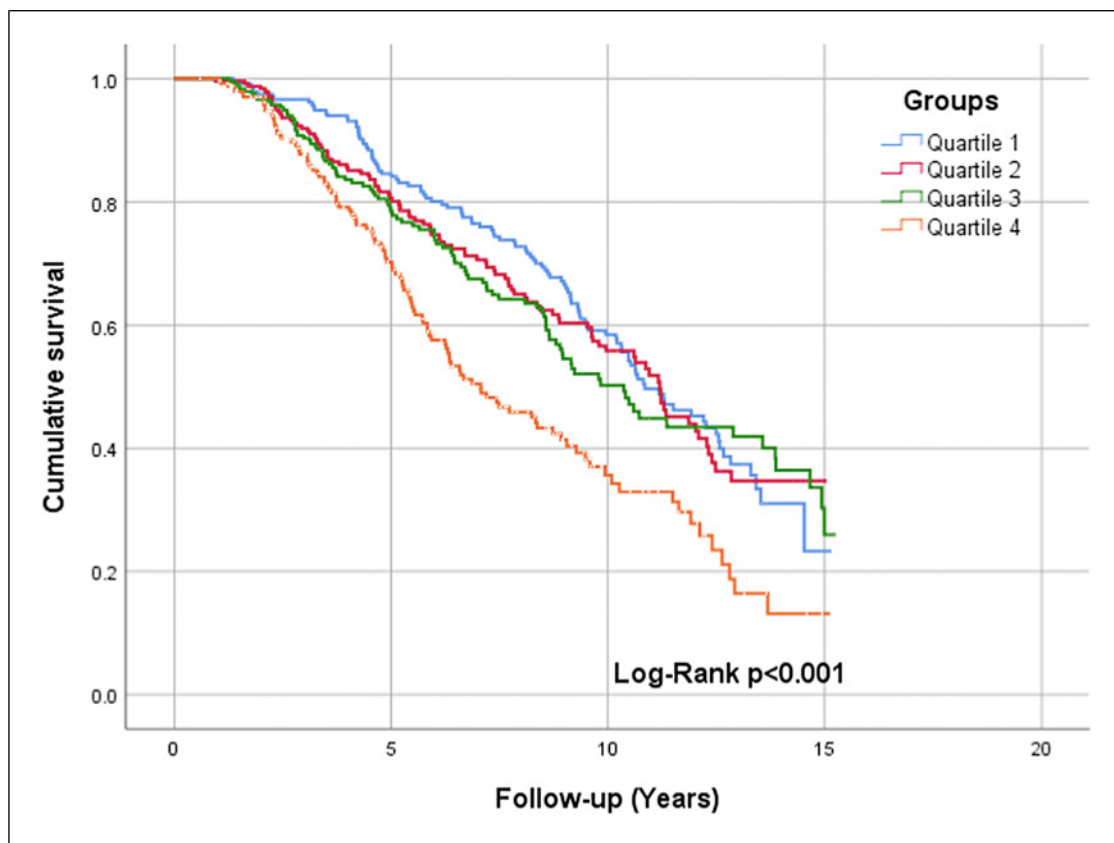


Fig. 1. Kaplan-Meier chart for all-cause mortality across the quartiles of endostatin.

regression models A–E (model A: adjusted for age, gender; model B: age, gender, and C-reactive protein; model C: age, gender, systolic blood pressure, diastolic blood pressure, smoking status, heart failure, diabetes, and prior cardiovascular events; model D: model A + B + C combined; model E: model A + B + C + baseline eGFR and urine protein). Baseline endostatin concentration was entered into the models as a continuous variable and in quartiles to explore the possible nonlinear associations.

A p value of <0.05 was considered statistically significant. The dataset was handled, and calculations were performed with Stata 16 (Stata Corp., College Station, TX, USA) and SPSS version 26.

Results

Baseline characteristics of the total study population and of the subsample with annual follow-ups are shown in Table 1. The median age of the cohort was 65 years with a predominance of males ($n = 597$, 61.5%) and white ethnic background ($n = 929$, 96%). A higher proportion

of patients in Q4 had a history of any cardiovascular event compared to Q1 (48.6 vs. 35.5%, $p = 0.004$). Compared to Q1, patients in Q4 had a significantly higher urine protein creatinine ratio (58 vs. 20 mg/mmol, $p < 0.001$) and a lower eGFR (15 vs. 35 mL/min/1.73 m², $p < 0.001$). Over a median follow-up of 6.2 years, a higher proportion of patients in Q4 reached RRT (40.3 vs. 14.5%, $p < 0.001$). In the stepwise multivariate Cox regression models comparing Q4 and Q1, the higher endostatin quartile (Q4) was significantly associated with all-cause mortality in models A to D and this significance was lost in model E which included both uPCR and eGFR (Table 2).

Kaplan-Meier charts illustrate a significant difference in cumulative survival, RRT-free survival, and combined outcome across the endostatin quartiles (log-rank $p < 0.001$) (Fig. 1–3). We also wanted to study the association between baseline endostatin and the change of eGFR during the study. The delta eGFR in mean % per month for all study participants and for each quartile is presented in Table 3 (mean follow-up time 4.4 years). We here observed that the patients with higher baseline

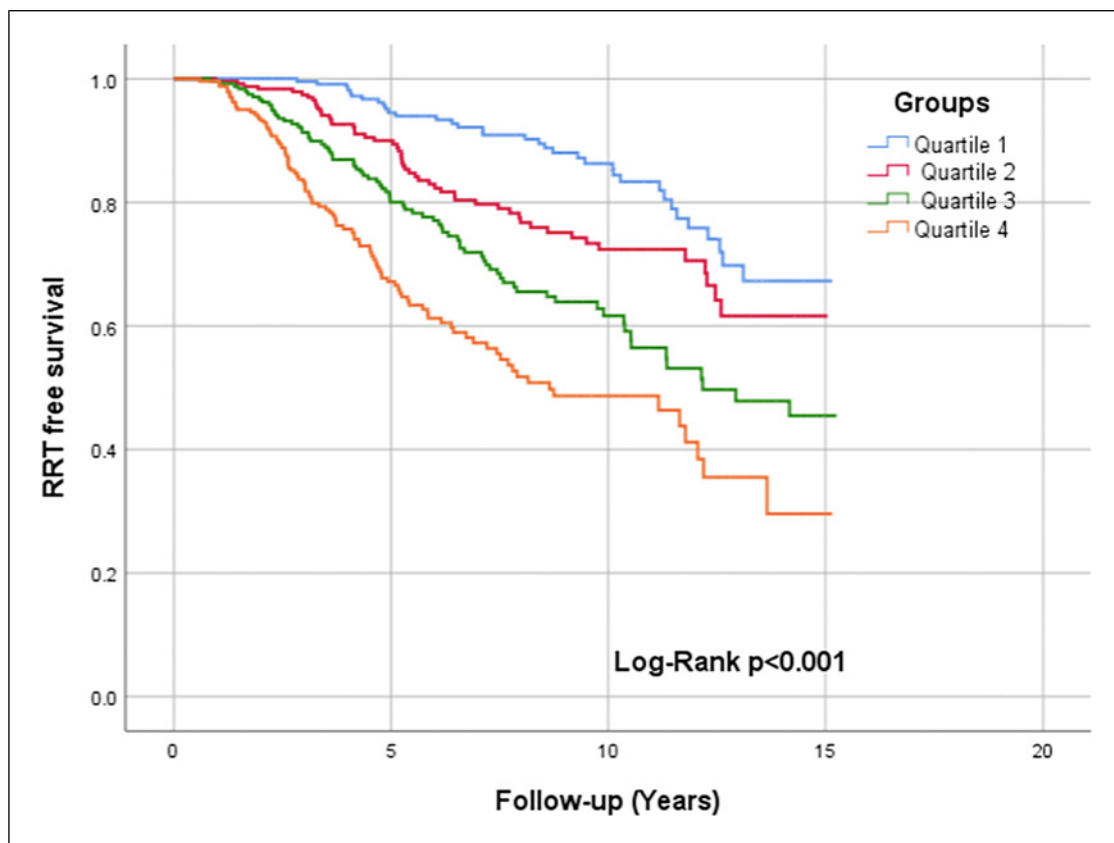


Fig. 2. Kaplan-Meier chart for RRT-free survival across the quartiles of endostatin.

endostatin had higher risk of being in quartile 4 of delta eGFR, i.e., being rapid eGFR decliners in model A–D (Table 4). However, when baseline eGFR and urine protein was added to the model, this association was abolished and no longer significant (model E, Table 4). Patients with a larger endostatin increment at the follow-up examination had higher risks of being rapid eGFR decliners (Table 5), and these associations were still significant when adjusting for cardiovascular risk factors and when including baseline eGFR and urine protein concentration) (model E, Table 5).

Discussion

The main finding of this study was that higher serum endostatin concentrations were associated with mortality and kidney function decline when adjusting for cardiovascular risk factors, but these associations were no longer independent once baseline eGFR and uPCR were included. In contrast, longitudinal increases in

endostatin remained robustly associated with subsequent eGFR decline.

Circulating endostatin reflects anti-angiogenic activity and extracellular matrix turnover, both central to microvascular integrity and fibrotic remodeling in the kidney. Experimental studies demonstrate that endostatin modulates pathological vascular remodeling by reducing neovascularization and plaque progression [28–30], inhibiting vasa vasorum angiogenesis [31, 32], and increasing in response to myocardial injury. In renal ischemia and fibrotic models, endostatin expression is upregulated at both the transcriptional and protein levels [18, 19], and elevated renal endostatin is strongly linked to microvascular rarefaction, tubulointerstitial fibrosis, and impaired angiogenic repair [15]. Anti-angiogenic interventions can attenuate interstitial fibrosis and albuminuria in models of early diabetes or diabetic nephropathy [33, 34]. These findings support a mechanistic framework in which rising endostatin levels reflect progressive microvascular compromise and extracellular matrix dysregulation – pathological features characteristic of accelerating CKD.

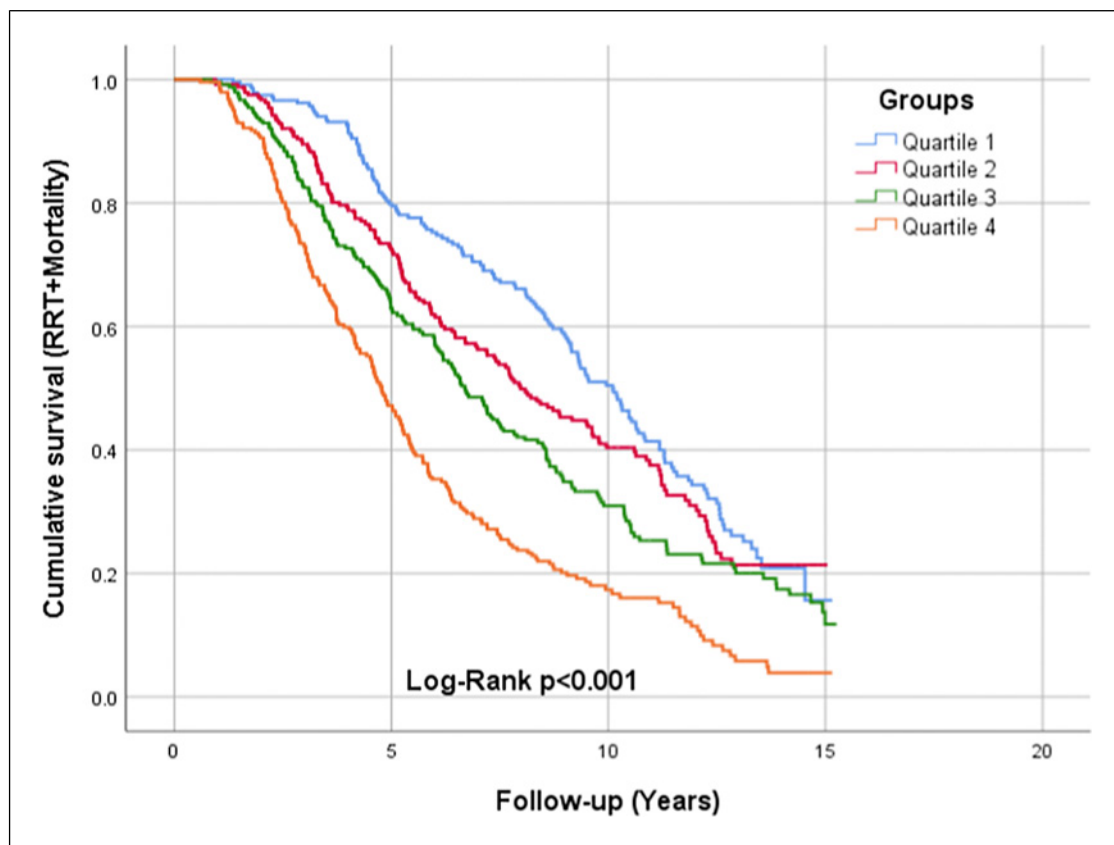


Fig. 3. Kaplan-Meier chart for combined outcome (RRT or mortality) across the quartiles of endostatin.

Table 3. Mean eGFR difference in all participants (in % per month) and in quartiles of eGFR difference

	N	Mean (% per month)	Interval (min–max)	Comment
All	455	0.40	–7.34 to 10.99	7.34% eGFR increase to 10.99% eGFR decline
Quartile 1	114	–0.72	–7.34 to –0.008	7.34 to 0.008% eGFR increase
Quartile 2	114	0.18	–0.007 to 0.34	0.007% eGFR increase to 0.34% eGFR decline
Quartile 3	114	0.55	0.34 to 0.77	0.34 to 0.77% eGFR decline
Quartile 4 (“rapid decliner”)	113	1.60	0.78 to 10.99	0.78 to 10.99% eGFR decline
Quartiles 1–3	342	0.0051	–7.34 to 0.77	7.34% eGFR increase to 0.77% eGFR decline

Clinical investigations have similarly shown that elevated circulating endostatin is associated with impaired kidney function and lower eGFR in CKD [14, 19], predicts incident CKD in community cohorts [16], correlates with CKD severity and cardiovascular comorbidity [14], and is linked to cardiovascular events [17] and mortality in hemodialysis patients [35]. Together, these studies indicate that endostatin is closely

connected to vascular pathology and structural injury in CKD, although few have examined whether changes in endostatin over time track with kidney function decline. Our findings add to this literature by demonstrating that longitudinal increases in endostatin, rather than absolute baseline levels, may capture ongoing renal injury.

Endostatin is partly cleared by renal filtration and tubular uptake [36], and tubular cells secrete endostatin

Table 4. Associations between endostatin at baseline and risk of being a rapid eGFR decliner (defined as quartile 4 of eGFR difference), respectively

	eGFR difference				
	continuous	<i>p</i> value	quartiles 1–3	quartile 4	<i>p</i> value
	regression coefficient (95% CI)		ref	OR (95% CI)	
<i>n</i>	455		340	115	
Unadjusted model	0.0011 (–0.00007 to 0.0023)	0.064	Ref	1.52 (1.18 to 1.96)	0.001
Model A	0.0014 (0.00019 to 0.0025)	0.023	Ref	1.59 (1.23 to 2.05)	<0.001
Model B	0.0013 (0.00016 to 0.0025)	0.026	Ref	1.57 (1.22 to 2.04)	0.001
Model C	0.0012 (0.000050 to 0.0024)	0.041	Ref	1.60 (1.23 to 2.08)	<0.001
Model D	0.0012 (3.5e–06 to 0.0024)	0.049	Ref	1.58 (1.22 to 2.06)	0.001
Model E	0.0007 (–0.0007 to 0.0021)	0.330	Ref	1.29 (0.94 to 1.76)	0.114

The differences in time between the measurements of eGFR were 4.37 years. Model A: adjusted for age and gender. Model B: age, gender, and C-reactive protein. Model C: age, gender, systolic blood pressure, diastolic blood pressure, smoking status, heart failure, diabetes, and prior cardiovascular events. Model D: model A + B + C combined. Model E: model A + B + C + baseline eGFR and urine protein.

Table 5. Associations between endostatin difference (1 SD increments) and eGFR difference (in % decrease per month) and risk of being a rapid eGFR decliner (defined as quartile 4 of eGFR difference), respectively

	eGFR difference				
	continuous	<i>p</i> value	quartiles 1–3	quartile 4	<i>p</i> value
	regression coefficient (95% CI)		ref	OR (95% CI)	
<i>n</i>	455		340	115	
Unadjusted model	0.0027 (0.0016–0.0038)	<0.001	Ref	1.98 (1.55–2.52)	<0.001
Model A	0.0026 (0.0015–0.0037)	<0.001	Ref	1.98 (1.55–2.52)	<0.001
Model B	0.0026 (0.0015–0.0037)	<0.001	Ref	1.97 (1.54–2.51)	<0.001
Model C	0.0023 (0.0012–0.0035)	<0.001	Ref	1.92 (1.50–2.46)	<0.001
Model D	0.0023 (0.0012–0.0034)	<0.001	Ref	1.91 (1.49–2.45)	<0.001
Model E	0.0023 (0.0012–0.0034)	<0.001	Ref	1.85 (1.44–2.37)	<0.001

The mean differences in time between the measurements of eGFR were 4.37 years. Model A: adjusted for age, gender. Model B: age, gender, and C-reactive protein. Model C: age, gender, systolic blood pressure, diastolic blood pressure, smoking status, heart failure, diabetes, and prior cardiovascular events. Model D: model A + B + C combined. Model E: model A + B + C + baseline eGFR and urine protein.

during inflammation and fibrosis [37]. Thus, elevated concentrations may reflect both impaired clearance and increased production. Rising endostatin during the transition from AKI to CKD and during established CKD progression has been linked to ischemia-driven microvascular injury and interstitial fibrosis [38]. This biological

context aligns with our observation that increasing endostatin levels track with subsequent eGFR decline.

Previous biomarker work shows that baseline eGFR exerts a dominant influence on future kidney decline, often attenuating associations with single baseline biomarker measurements. Enoksen et al. [27] and Carlsson

et al. [39] found that several protein biomarkers predicted eGFR decline, but most lost significance after adjustment for baseline eGFR. Our findings are consistent with these observations: baseline endostatin did not provide information beyond what was already captured by eGFR and proteinuria. However, the persistence of associations for longitudinal endostatin changes highlights the potential value of dynamic biomarker assessment in advanced CKD.

From a clinical perspective, identifying early signals of accelerated kidney decline is increasingly important given the expanding use of nephroprotective treatments, particularly SGLT2 inhibitors [40]. Although baseline endostatin does not outperform eGFR or proteinuria, rising levels may indicate active disease processes such as microvascular rarefaction or fibrotic expansion. Dynamic biomarkers may therefore complement traditional markers in stratifying risk or identifying patients who may benefit from earlier or intensified interventions. Experimental evidence showing that therapies targeting endothelial oxidative stress can reduce circulating endostatin [41] further supports the relevance of exploring endostatin as part of a broader biomarker-guided approach.

This study has limitations. Its observational design precludes causal inference, and residual confounding is likely despite adjustment for multiple covariates. Medication adherence and dosing data were unavailable and may influence biomarker dynamics. The cohort consisted of referred patients with advanced CKD, limiting generalizability to milder disease stages. Although endostatin was measured in stored samples, standardized conditions reduce the likelihood of measurement bias. Replication in independent cohorts with more frequent sampling is needed to better define the temporal relationship between endostatin changes and CKD progression.

In summary, longitudinal increases in circulating endostatin were consistently associated with subsequent eGFR decline, supporting the importance of dynamic biomarker evaluation in advanced CKD. Baseline endostatin was associated with mortality only before adjusting for kidney disease severity, limiting its value as a static risk marker. These findings motivate further investigation into the biological pathways reflected by rising endostatin and the potential role of dynamic endostatin measurement in clinical risk stratification.

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Statement of Ethics

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Ethical approval for this study was obtained from ethical approval was granted by the North West – Greater Manchester South Ethics Committee (15/NW/0818) and from the Ethical Authority in Sweden to perform statistical analyses in Sweden (Ref No. 2023-01854-01). Written informed consent was obtained from the participants prior to the study.

Conflict of Interest Statement

J.Ä. has served on advisory boards for Astellas, AstraZeneca, and Boehringer Ingelheim and has received lecturing fees from AstraZeneca and Boehringer Ingelheim, all of which are unrelated to the present work. P.A.K. has served on advisory boards for AstraZeneca and CSL Vifor and has received lecture fees from Napp, Bayer, Novartis, CSL Vifor Pharma, Pharmacosmos, Boehringer Ingelheim, and Lilly, all of which are unrelated to the present work. Grants from CSL Vifor, Evotec, and BerGenBio have helped sustain the Salford Kidney Study. J.H.-K., R.C., P.W., A.C.C., A.O.L., T.F., and T.R. have no conflicts of interest to report.

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Author Contributions

J.H.-K.: statistical analysis and review and edits. R.C.: complementary statistical analyses and review and revisions. P.W.: original draft and revisions. P.A.K.: collected the data and review. A.O.L.: expert advice and analysis of biomarkers and review. T.F.: statistical analysis and review. A.C.C., J.Ä., and T.R.: study design and review.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Research data are not publicly available on ethical grounds. Further inquiries can be directed to the corresponding author.

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