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Endostatin level is associated with kidney injury in the elderly: findings from two community based cohorts

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Key words: community, endostatin, glomerular filtration, albumin creatinine ratio, chronic kidney disease, extracellular matrix remodeling, angiogenesis

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Background

We aimed to investigate associations between circulating endostatin and different aspects of renal dysfunction, namely, estimated (Cystatin C) glomerular filtration rate (GFR) and urine albumin-creatinine ratio (ACR).

Methods

Two independent longitudinal community-based cohorts of elderly. Uppsala Longitudinal Study of Adult Men (ULSAM, n=786 men; mean age 78 years; median GFR 74ml/min/1.73 m²; median ACR 0.80mg/mmol); and Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS, n=815; mean age 75 years, 51 % women; median GFR; 67ml/min/1.73 m²; median ACR 1.39mg/mmol). Cross-sectional associations between endostatin levels and GFR as well as ACR, and longitudinal association between endostatin at baseline and incident CKD (defined as GFR <60ml/min/1.73 m²) were assessed.

Results

In cross-sectional regression analyses adjusting for age, gender, inflammation, and cardiovascular risk factors, serum endostatin was negatively associated with GFR (ULSAM: B-coefficient per SD increase -0.51, 95 % CI (-0.57, -0.45), p<0.001; PIVUS -0.47, 95 % CI (-0.54, -0.41), p<0.001) and positively associated with ACR (ULSAM: B-coefficient per SD increase 0.24, 95 % CI (0.15, 0.32), p<0.001; PIVUS 0.13, 95 % CI (0.06-0.20), p<0.001) in both cohorts. Moreover, in longitudinal multivariable analyses, higher endostatin was associated with increased risk for incident CKD defined as GFR< 60 ml/min/1.73 m² at re-investigations in both ULSAM (Odds ratio per SD increase of endostatin 1.39 (95% CI 1.01 – 1.90) and PIVUS 1.68 (95% CI 1.36-2.07)).

Conclusions

Higher circulating endostatin is associated with lower GFR and higher albuminuria and independently predicts incident CKD in elderly subjects. Further studies are warranted to investigate the underlying mechanisms linking endostatin to kidney pathology, and to evaluate the clinical relevance of our findings.

Key words: community, endostatin, glomerular filtration, albumin creatinine ratio, chronic kidney disease, extracellular matrix remodeling, angiogenesis

Introduction

Even mild reductions in glomerular filtration rate (GFR) are associated with an increased risk of cardiovascular disease, end stage renal disease (ESRD), and all-cause mortality [1-4]. In addition to GFR, urinary albumin/creatinine ratio (ACR) is used to diagnose, classify and monitor chronic kidney disease (CKD) and associated risk in clinical practice [5]. However, the molecular pathophysiology underlying progressive loss of kidney function is incompletely understood.

Collagen XVIII is a major component of the basal membranes. Cleavage of collagen XVIII during extracellular matrix (ECM) remodeling gives rise to endostatin, a biologically active fragment with anti-angiogenic activity. Collagen XVIII is highly expressed in the kidney and found in the Bowman's capsule, as well as in the glomerular and tubular basal membranes [6,7]. Alterations of expression of collagen XVIII and endostatin have been observed in various types of renal disease [8-11], and recent animal studies point toward a causal link between endostatin and physiological responses in the kidneys [12,13]. Yet to date, reports on the role of collagen XVIII and endostatin in the development of renal impairment from community-based surveys are limited. We recently reported that serum endostatin was associated with urinary albumin/creatinine-ratio in elderly individuals with hypertension, suggesting that endostatin may be a biomarker of, or causally involved, in the pathophysiology of hypertensive kidney damage [14].

Since endostatin may be involved in the development of CKD we herein aimed to test the following hypothesis in two community-based cohorts of elderly: i) circulating levels of endostatin is associated with clinical markers of kidney dysfunction and kidney damage, namely GFR and ACR; ii) circulating levels of endostatin are predictive of new onset CKD. As a secondary aim, we wanted to study the association between endostatin and specific kidney tubular damage as evaluated by urinary kidney injury molecule (U-KIM)-1).

Methods

Study samples

The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS)

All 70-year old men and women living in Uppsala, Sweden, between 2001 and 2004 were eligible for the PIVUS study (described in detail on <http://www.medsci.uu.se/pivus/pivus.htm>) [15]. Of 2025 invited individuals, 1016 agreed to participate. A second examination cycle of PIVUS was performed 2006-2009 when participants were 75 years old. Of 964 invited participants, 827 participated (86%), and data on endostatin were available in 815 individuals. As no urine was collected at the first examination cycle, the second examination cycle was used for cross-sectional analyses, while the first examination cycle was used as the baseline for longitudinal analyses.

The Uppsala Longitudinal Study of Adult Men (ULSAM)

The ULSAM study was initiated in 1970. All 50-year-old men, born in 1920-24 and living in Uppsala, Sweden, were invited to a health survey, focusing at identifying cardiovascular risk factors (described in detail on <http://www.pubcare.uu.se/ULSAM>) [16]. The present study used the fourth examination cycle as baseline, when participants were approximately 77 years old (1998-2001). Of 1398 invited men, 838 (60%) participated, and data on circulating levels of endostatin was available in 786 individuals. The fourth examination cycle was used in all cross-sectional analyses and as the baseline for the longitudinal analyses. We also used the 5th examination cycle (2003-2005) when participants were 82 years in order to identify those who had progressed to CKD. To this examination, 952 men still living in Uppsala were invited and 530 men (56%) participated in the investigation. Of these, data on GFR was available in 508 participants.

All participants in both studies gave written informed consent and the Ethics Committee of Uppsala University approved the study protocols. Both studies were conducted according to the Declaration of Helsinki.

Baseline investigations

The investigations in PIVUS and ULSAM were performed using similar standardized methods, including anthropometrical measurements, blood pressure, blood sampling, and questionnaires regarding socioeconomic status, medical history, smoking habits, medication and physical activity level [15,16]. Venous blood samples were drawn in the morning after an overnight fast and stored at -70°C until analysis. In PIVUS, a spot sample of morning urine was used for analyses. In ULSAM a 24-hour collection of urine was used.

Cystatin C was measured by latex-enhanced reagent (NLatexCystatin C; Siemens, Deerfield, IL, USA) using a BN ProSpec analyser (Siemens) and used to estimate GFR in ULSAM, [17] and by latex-enhanced reagents (Gentian, Moss, Norway) using an Architect ci8200 (Abbott Laboratories, Abbott Park, IL, USA) in PIVUS [18]. In secondary analyses, we also estimated GFR based on the CKD-EPI creatinine equation in PIVUS [19]. Data on circulating levels of creatinine were not available in the ULSAM cohort.

Urine albumin was measured by nephelometry (Urine albumin, Dade Behring, Deerfield IL, USA) using a Behring BN ProSpec® analyzer (Dade Behring). Urinary creatinine was analyzed with a modified kinetic Jaffe reaction on an Architect Ci8200® analyzer (Abbott, Abbot Park, IL, USA) and creatinine related urine albumin (ACR) was calculated. Urinary KIM-1 was analyzed with the commercial sandwich ELISA kit, (DY1750, R&D Systems, Minneapolis, MN, USA) and adjusted for urinary creatinine.

High-sensitive CRP measurements were performed by latex-enhanced reagent (Siemens) with the use of a BN ProSpec® analyzer (Siemens). Diabetes mellitus was diagnosed as fasting plasma glucose ≥ 7.0 mmol/l (≥ 126 mg/dl), or use of anti-diabetic medication [20].

Statistical analysis

Skewed variables were logarithmically transformed to promote a normal distribution. Pearson correlation coefficients were calculated between endostatin and GFR or ACR. The following multivariable linear regression models were used to further assess cross-sectional associations between endostatin levels and GFR as well as ACR (expressed per standard deviation increase): A-age and sex (PIVUS only), B-established CVD-risk factors (age, sex (PIVUS

only), systolic blood pressure, cholesterol, HDL, BMI, diabetes, lipid lowering and antihypertensive treatment), and CRP.

In secondary analysis, we explored the cross-sectional association between endostatin and proximal tubular damage (as reflected by urinary KIM-1). We also investigated the association between endostatin and creatinine-based GFR in PIVUS.

We also explored whether there were effect modification by diabetes prevalence or gender using multiplicative interaction analyses.

Longitudinal analyses

The longitudinal association between endostatin at baseline and incident CKD (defined as $\text{GFR} < 60 \text{ ml/min/1.73 m}^2$) at a re-examination after 5 years was investigated in both cohorts using the above multivariable models A-B. All individuals with a $\text{GFR} < 60 \text{ ml/min/1.73 m}^2$ at baseline were excluded in these analyses. In separate longitudinal analyses in ULSAM, we also added ACR to multivariable model B.

In the PIVUS cohort, where data on endostatin and GFR was available both at baseline and at follow-up, we investigated the association between the change in endostatin (delta endostatin) and the change in GFR (delta GFR) between examinations using multivariable linear regression models adjusted for age at baseline and follow-up and baseline endostatin and GFR. The statistical software package STATA 12.1 (Stata corp, College Station, TX) was used.

RESULTS

Baseline characteristics

Baseline characteristics of ULSAM and PIVUS are shown in Table 1.

Cross-sectional analyses

Correlations

There was a negative Pearson correlation coefficient between endostatin and GFR in both ULSAM and PIVUS (Figure 1a and 1b), $r=-0.56$ and $r=-0.48$, respectively. The association between endostatin and creatinine based GFR in PIVUS was similar ($r=-0.49$, $p<0.001$). The positive correlation between endostatin and ACR was also highly statistically significant but appeared slightly weaker (Figure 1c and 1d), $r=0.28$ in the ULSAM cohort and $r=0.16$ in the PIVUS cohort. The Pearson correlation between endostatin and creatinine based GFR (CKD-EPI formula) was $r=-0.39$ ($p<0.001$).

Linear regression models

The regression coefficients of the association between endostatin and GFR as well as ACR, respectively, are presented in Table 2. Each SD increment in circulating endostatin level was associated with a 0.5 SD lower GFR in both ULSAM and PIVUS ($p<0.001$ for both).

Additionally, there was a positive association between endostatin and ACR in both cohorts ($p<0.001$). These results remained essentially unaltered after adjustments for age, sex, established CVD risk factors and inflammation (CRP), see table 2. Endostatin was significantly associated with both GFR and ACR in both cohorts even when both of these kidney biomarkers were included in the same multivariable model (data not shown). U-KIM-1 was not significantly associated with endostatin in any model in any of the cohorts (data not shown).

There were no effect modification by diabetes prevalence or gender ($p>0.13$ for all).

Longitudinal analyses

In participants with $\text{GFR} > 60 \text{ ml/min/1.73m}^2$ at baseline, serum endostatin was significantly associated with incident CKD ($\text{GFR} \leq 60 \text{ ml/min/1.73m}^2$) after 5 years follow-up in both cohorts (Table 3). These results remained significant after adjustments for age at baseline and follow-up, sex, cardiovascular risk factors and CRP in both cohorts. In ULSAM, the association between endostatin and the risk for CKD was still significant after adjustment for ACR (OR per SD increase 1.41, 95% CI 1.05-1.89, $p=0.02$). Interestingly, in this model ACR did not predict CKD ($p=0.17$). When baseline GFR was included in the multivariate models, the association between endostatin and CKD was attenuated and no longer statistically significant in any of the cohorts ($p>0.14$, data not shown).

In the PIVUS cohort, 1 unit (ng/ml) increase of serum endostatin levels during the 5 year follow-up (delta endostatin) was associated with 0.25 ml/min/1.73m² decrease of GFR (delta GFR) after adjustment for baseline endostatin, baseline GFR, age at baseline and age at follow-up (Regression coefficient -0.25, 95 % CI -0.30- (-0.21), p<0.001).

In models adjusted for age at baseline and follow-up and baseline GFR, baseline endostatin was not significantly associated with GFR decline (p=0.15 in ULSAM and 0.09 in PIVUS).

Discussion

Main findings

Our data from two independent community-based cohorts of elderly individuals clearly show that higher circulating levels of endostatin parallel decline in kidney function and increasing kidney damage and dysfunction, portrayed by ACR and GFR in cross-sectional analysis. Moreover, in longitudinal analyses, higher circulating endostatin was associated with an increased risk for incident CKD during 5 years follow-up in both cohorts. This association was independent of cardiovascular risk factors and ACR. Also, there was a close association between longitudinal changes in endostatin and changes in GFR over 5 years.

Comparisons with previous studies

Our findings are in accordance with a previous clinical study where, circulating endostatin was shown to be elevated in 201 patients with manifest CKD compared with 201 healthy controls [21]. In this case-control study, endostatin was also inversely associated with urinary albumin excretion. Increased levels of circulating endostatin have also been observed in small studies of patients with CKD [22,23], hemodialysis patients [11], and in kidney transplants recipients [24]. Moreover, we recently reported that circulating endostatin was positively associated with ACR in elderly hypertensive individuals [14]. To our knowledge, the present study is the first to report an association between endostatin and kidney damage/dysfunction in the community. We are aware of no previous study reporting longitudinal data on these associations.

Possible mechanistic explanations for the observed associations

Based on our observational data we cannot establish a causal role for endostatin in the development of kidney disease in the present study; yet, there are several potential mechanisms that may explain the association between circulating endostatin levels and parallel decline in kidney function.

Circulating endostatin has been suggested to reflect extra cellular matrix turnover in patients with malignant diseases, including renal carcinoma [25] and in hypertensive renal disease [14]. Renal fibrosis is, similarly as other fibrotic diseases, largely due to a remodeling of the extra cellular matrix, which can be detected already in early stages of renal disease [7,9,25]. Thus, the association between circulating endostatin and kidney dysfunction could be explained by renal extracellular matrix remodeling. Still, we cannot rule out that the circulating concentrations of endostatin are influenced by extra renal matrix turnover or other processes in renal or extra renal tissues. Yet, recent data from aging mice support the notion that circulating levels of endostatin parallel functional decline in kidney function due to fibrosis [13].

Renal impairment has also been shown to be associated with the loss of peritubular capillaries and tissue hypoxia [26]. As a consequence, a shift in the up- and down regulation of both pro and antiangiogenic factors are present in kidney hypoxia and CKD [27,28]. Endostatin is a potent endogenous antiangiogenic factor [29], and a shift in the angiogenic balance towards an increased level endostatin has been observed in experimental models of renal impairment [30,31], and has been shown to exert protective effects to the kidneys of rats with diabetes [12]. In parallel, a shift in the renal angiogenic balance with an up regulation of trombospodin-1, another strong endogenous antiangiogenic protein, in aged rats with renal impairment has been reported [32]. Our data are in accordance with these experimental observations and suggest a possibility that circulating endostatin to some extent reflects an angiogenic shift in the kidneys.

It is also possible that other factors that are closely associated with endostatin, such as gluco-metabolic disturbance [33,34], and lipids [35], or long term hypertension [14] may mediate the associations between endostatin, and the indices of kidney damage and dysfunction .

Endostatin has also been shown to exert acute blood pressure lowering effects [36]. However,

the fact that the results were essentially unaltered in all multivariable models would argue against confounding by these factors as a major explanation of our findings.

Whether endostatin is merely a marker for pathological processes or a causal factor remains to be established. Previous studies are indeed conflicting; Endostatin infusion caused no kidney dysfunction in mice [37], and similarly, no adverse effect on kidney function could be detected in patients with lung cancer treated with endostatin [38]. Conversely, endostatin have been suggested to have a protective effect in a mouse model of diabetic nephropathy [9], but a possible deleterious role in the progression of kidney disease in other experimental studies have also been put forward [30,31].

It is also possible that higher circulating levels of endostatin are merely due to a decreased renal clearance, similar to that of cystatin C, as urinary excretion is the major elimination route of endostatin [39]. Both cystatin C and endostatin are of similar size and passes the glomerular barrier. To what extent circulating levels of endostatin reflect de novo synthesis, turnaround or a decreased renal clearance remains to be established.

No association was seen between endostatin and urinary KIM-1 indicating that endostatin does not reflect specific damages to the proximal tubuli.

Clinical implications

There has been no major advancement in kidney biomarker identification during the last decades. In clinical practice, GFR and ACR are widely used even though they are limited by impaired sensitivity and specificity and are substantially influenced by a large number of factors such as gender, race, body size, and lifestyle factors. Thus, novel biomarkers for CKD progression and complications are a large unmet medical need. Elucidating such biomarkers can be of great value for individual risk prediction, selection and evaluation of treatment and enrichment of rapid progressors in design of clinical trials. Endostatin may be a promising candidate in this respect. We recently reported that endostatin is a strong independent predictor for cardiovascular mortality, the main cause of death in patients with CKD [40]. However, whether endostatin is a clinically relevant risk factor for cardiovascular events in patients with CKD remains to be established. Clinical studies in patient groups with rapid kidney function decline that follow up on the renoprotective effects of endostatin in rats [12],

are also warranted. A large number of additional studies will be needed to firmly evaluate the utility of using endostatin measurements in clinical practice.

Interestingly, the association between endostatin and GFR appeared strong, with a correlation coefficient of approximately -0.5 in both cohorts. In comparison; the correlation between the two clinical kidney biomarkers GFR and ACR was around -0.15 in both cohorts, and the correlation between creatinine based-GFR and cystatin C-based GFR was 0.43.

Strengths and limitations

Strengths of our investigation include the longitudinal study design, the validation of our findings in an independent cohort and the detailed characterization of study participants. To our knowledge, ULSAM and PIVUS are the largest cohorts with endostatin measurements to date.

Limitations include the unknown generalizability to other age-, and ethnic groups, and that there may be healthy cohort effects at play in population based invited investigations of elderly individuals. Our study was based on single assessments of endostatin, eGFR and albuminuria. However, the potential misclassification at baseline and follow-up due to their short-term variability would if anything, result in conservative estimates. Moreover, the longitudinal association between endostatin and the risk for incident CKD was attenuated after adjusting for baseline GFR which could indicate that endostatin is not an independent CKD risk factor. However, adjustment for baseline GFR may represent an “overadjustment” as GFR is cross-sectionally related to endostatin, and may therefore represent an intermediate state along the causal pathway from endostatin leading to CKD. The fact that the longitudinal changes in endostatin was significantly associated with longitudinal changes in GFR over a 5 year period adds additional support for the theory of a causal association. Further studies are needed to shed light on this issue. Another limitation is the fact that no longitudinal data on ACR was available in any cohort.

Conclusions

Circulating endostatin appears to be a promising biomarker that parallel kidney damage and dysfunction in the community based setting. Further studies are warranted to investigate the

underlying mechanisms linking endostatin to kidney pathology, and to evaluate the clinical relevance of our findings

Author contributions

Author contributions: A.C.C. and T.R. drafted manuscript and researched data. J.Ä. researched data, edited manuscript, contributed to discussion, provided funding. T.E.L., J.-J.C., and L.L. reviewed manuscript, contributed to discussion. L.L collected the PIVUS data. A.L. reviewed manuscript, contributed to discussion and measured endostatin, cystatin C and albuminuria.

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Disclosures

The study was investigator-initiated and -driven. The authors report no conflicts of interests in connection with this study. T.E.L. is a part-time employee of Astellas.

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Table 1 Baseline characteristics of PIVUS and ULSAM shown as numbers (%) or medians (with Bonnet-Price 95% confidence intervals)

Variable	PIVUS	ULSAM
Number of subjects	815	786
Female no. (%)	414 (51%)	0
Age (years)	75.3 (75.3-75.3)	77.8 (77.6-77.9)
CRP (mg/L)	2.1 (2.0-2.3)	2.9 (2.7-3.1)
Endostatin (ng/ml)	55.7 (54.5-56.9)	52.5 (51.5-53.6)
Urinary KIM-1/creatinine (ng/mmol)	101 (96-107)	98 (92-105)
Estimated Glomerular filtration rate (ml/min/1.73m ²)	67 (65-69)	74 (72-75)
CKD (<60ml/min/1.73m ²)	275 (34%)	162 (21%)
Urinary albumin/creatinine ratio (mg/mmol)	1.39 (1.30-1.49)	0.80 (0.70-0.90)
Body mass index (kg/m ²)	26.4 (26.1-26.7)	26.0 (25.7-26.4)
Systolic blood pressure (mmHg)	148 (147-149)	150 (149-151)
Antihypertensive treatment n (%)	394 (48%)	371 (47%)
S-Cholesterol (mmol/l)	5.4 (5.3-5.5)	5.3 (5.3-5.4)
HDL (mmol/l)	1.40 (1.35-1.45)	1.27 (1.25-1.29)
Lipid lowering treatment n (%)	204 (26%)	132 (17%)
Smoking (%)	50 (6%)	59 (8%)
Diabetes n (%)	112 (14)	112 (14)

Table 2 The cross-sectional association between circulating endostatin, glomerular filtration rate and urinary albumin creatinine ratio in the ULSAM and PIVUS cohorts : Multivariable linear regression

	ULSAM		PIVUS	
	Model A	Model B	Model A	Model B
GFR	-0.55 (-0.61-(-0.50))***	-0.51 (-0.57-(-0.45))***	-0.49 (-0.55-(-0.43))***	-0.47 (-0.54-(-0.41))***
ACR	0.28 (0.20-0.35)***	0.24 (0.15- 0.32)***	0.17 (0.10-0.24)***	0.13 (0.06-0.20)***

Data are B-coefficients per standard deviation increment (95% confidence intervals) ***p<0.001, **p<0.01, *p<0.05

Models: A-age and sex (PIVUS), B- established CVD risk factors (age, sex (PIVUS), and BMI, smoking, systolic blood pressure, HDL, Cholesterol, diabetes, and antihypertensive and lipid treatment) and CRP. Significance level GFR-glomerular filtration rate, ACR-urinary albumin creatinine ratio

Table 3 Longitudinal analyses on the association between endostatin and the development of CKD: multivariable logistic regression

	ULSAM	PIVUS
	Odds Ratios with 95% Confidence Intervals	
Model A	1.45 (1.08-1.93) *	1.75 (1.44 – 2.11)***
Model B	1.39 (1.01 – 1.90) *	1.68 (1.36-2.07)***

Significance level ***p<0.001, **p<0.01, *p<0.05. Model A: age (at the baseline and the follow-up examination) and sex (PIVUS only); Model B age at baseline and follow-up, sex (PIVUS), and BMI, smoking, systolic blood pressure, HDL, Cholesterol, diabetes, and antihypertensive and lipid treatment), and CRP.

Number of events/ numbers at risk: ULSAM 213/330, PIVUS 238/537

Figure 1. Scatter plots of linear regression models of natural logarithm (ln) transformed, standard deviation (SD) increments of endostatin and:

a) GFR in ULSAM

b) GFR in PIVUS

c)

ACR in ULSAM

d)

ACR in PIVUS