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To cite this article: Toralph Ruge, Axel C. Carlsson, Erik Ingelsson, Ulf Risérus, Johan Sundström, Anders Larsson, Lars Lind & Johan Ärnlöv (2018): Circulating endostatin and the incidence of heart failure, Scandinavian Cardiovascular Journal, DOI: [10.1080/14017431.2018.1483080](https://doi.org/10.1080/14017431.2018.1483080)

To link to this article: <https://doi.org/10.1080/14017431.2018.1483080>



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Published online: 12 Jun 2018.



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## Circulating endostatin and the incidence of heart failure

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### ABSTRACT

**Objective.** Circulating levels of endostatin are elevated in many underlying conditions leading to heart failure such as hypertension, diabetes, chronic kidney disease and ischemic heart disease. Yet, the association between endostatin and the incidence of heart failure has not been reported previously in the community. **Design.** We investigated the longitudinal association between serum endostatin levels and incident heart failure in two community-based cohorts of elderly: Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS,  $n = 966$ ; mean age 70 years, 51% women, 81 events, mean follow-up 10 years) and Uppsala Longitudinal Study of Adult Men (ULSAM,  $n = 747$  men; mean age 78 years, 98 heart failure events, mean follow-up 8 years). We also investigated the cross-sectional association between endostatin and echocardiographic left ventricular systolic function and diastolic function (ejection fraction and E/A-ratio, respectively). **Results.** Higher serum endostatin was associated with an increased risk for heart failure in both cohorts after adjustment for established heart failure risk factors, glomerular filtration rate and N-terminal pro-brain natriuretic peptide (NT-proBNP) (PIVUS: multivariable hazard ratio (HR) per 1-standard deviation (SD) increase, HR 1.46 (95%CI, 1.17-1.82,  $p < .001$ ); ULSAM: HR 1.29 (95%CI, 1.00-1.68,  $p < .05$ ). In cross-sectional analyses at baseline, higher endostatin was significantly associated with both worsened left ventricular systolic and diastolic function in both cohorts. **Conclusion** Higher serum endostatin was associated with left ventricular dysfunction and an increased heart failure risk in two community-based cohorts of elderly. Our findings encourage further experimental studies that investigate the role of endostatin in the development of heart failure.

### ARTICLE HISTORY

Received 8 January 2018  
Revised 21 May 2018  
Accepted 26 May 2018

### KEYWORDS

Heart failure; left ventricular systolic function; remodelling of extracellular matrix; angiogenesis; anti-angiogenesis; population based studies; epidemiology

## Introduction

Circulating levels of endostatin, a potent endogenous angiogenesis inhibitor generated from collagen XVIII in the basal membranes, has in several previous studies been shown to be closely associated with many of the key underlying conditions leading to heart failure such as: hypertension, diabetes, left ventricular hypertrophy, and prevalent ischemic heart disease, stroke, or peripheral arterial disease [1]. Endostatin has also been put forward as a promising risk marker for kidney disease progression in patients with diabetes [2] or chronic kidney disease [3], as well as in the community based setting [4]. Despite several associations between circulating endostatin and underlying causes of heart failure, the role of endostatin in the development of heart failure is poorly understood [5,6] and data on the associations between circulating endostatin and heart failure incidence in the community are lacking.

We hypothesized that higher endostatin levels would be associated with an increased risk of heart failure. Therefore, we aimed to investigate the longitudinal association between endostatin levels and the incidence of heart failure in two independent community-based cohorts of elderly and whether endostatin might improve the prediction of heart failure beyond established heart failure risk factors. As a secondary aim we wanted to investigate the cross-sectional associations between endostatin and echocardiographic indices of left ventricular systolic and diastolic function.

## Methods

### Study populations

#### *The prospective investigation of the vasculature in Uppsala seniors (PIVUS)*

All 70-year old men and women, living in Uppsala Sweden, between 2001-2004, were eligible for the PIVUS study [7]

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 Supplemental data for this article can be accessed [here](#).

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(described in detail on <http://www.medsci.uu.se/pivus/pivus.htm>). Of 2025 invited individuals, 1016 agreed to participate. After exclusion of individuals with prevalent heart failure (defined as a previous hospitalization for heart failure based on the Swedish hospital discharge register) and missing endostatin measurements at baseline, 966 individuals comprised the present study sample.

### 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 [8] (described in detail on <http://www.pub-care.uu.se/ULSAM>). These present analyses are based on the fourth examination cycle of ULSAM, when participants were approximately 77 years old (1998-2001). Of 1398 invited men, 838 participated. After exclusion of individuals with prevalent heart failure (defined as a previous hospitalization for heart failure based on the Swedish hospital discharge register) and missing endostatin measurements at baseline, 747 individuals comprised the present study sample.

All participants in both studies gave written informed consent and the Ethics Committee of Uppsala University approved the study protocols.

### Baseline investigations

The investigations in PIVUS and ULSAM were performed using the same standardized methods, which included anthropometrical measurements, blood pressure, fasting blood samples and a questionnaire regarding their medical history, smoking habits and regular medication [7], [8]. Venous blood samples were drawn in the morning after an overnight fast and stored at  $-70^{\circ}\text{C}$  until analysis. Body mass index (BMI) was calculated as the ratio of the weight to the height squared ( $\text{kg}/\text{m}^2$ ). Blood pressure was measured by a calibrated mercury sphygmomanometer to the nearest even mmHg after at least 10 min of rest and the average of three (PIVUS) or two (ULSAM) recordings were used. Heart rate was recorded at rest during a standard electrocardiogram. Lipid variables and fasting blood glucose were measured by standard laboratory techniques. Diabetes was defined as a fasting plasma glucose  $\geq 7.0$  mmol/L or use of insulin or oral hypoglycaemic agents. Estimations of glomerular filtration rate (eGFR) were based on creatinine using the CKD-epi formula [9]. In PIVUS, plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) was analysed using a sandwich immunoassay (Roche Diagnostics) on an Elecsys 2010 instrument. In ULSAM NT-proBNP was analysed using a multiplex proteomics assay (Proseek CVD 1, OLINK Biosciences) as previously described [10].

Serum levels of endostatin were analysed 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  $\text{Na}_2\text{HPO}_4$ , 0.15 M NaCl, pH 7.2 containing 10 g/L of bovine serum albumin prior to analysis. The calibrators were run in duplicates in all plates while the samples were analyzed as singletons. The intra-assay

**Table 1.** Baseline characteristics in the PIVUS and ULSAM cohorts.

Variable	PIVUS	ULSAM
Number of subjects	966	747
Female (%)	51	n.a
Age (years)	70.1 $\pm$ 0.1	77.5 $\pm$ 0.8
Body mass index ( $\text{kg}/\text{m}^2$ )	27 $\pm$ 4	26 $\pm$ 3
Resting heart rate (beats/minute)	60 $\pm$ 9	72 $\pm$ 12
Systolic blood pressure (mmHg)	150 $\pm$ 23	151 $\pm$ 20
Serum endostatin (ng/ml)	47 $\pm$ 13	55 $\pm$ 17
Glomerular filtration rate ( $\text{ml}/\text{min}/1.73 \text{ m}^2$ )	80 $\pm$ 14	77 $\pm$ 13
Left ventricular ejection fraction*	0.67 $\pm$ 0.10	0.64 $\pm$ 0.11
E/A-ratio†	0.93 $\pm$ 0.21	0.88 $\pm$ 0.23
Endothelial dependent vasodilation (%)	527 $\pm$ 313	n.a.
Smoking (%)	11	8
Diabetes (%)	12	14
Previous cardiovascular disease (%)	6	10
Anti hypertensive medication (%)	30	40
N-terminal pro brain natriuretic peptide (pg/ml)	176 $\pm$ 293	‡

Data are mean  $\pm$  standard deviation for continuous variables and % for categorical variables \* Ejection fraction data were available in 792 participants in PIVUS and 138 participants in ULSAM. †E/A-ratio data were available in 852 participants in PIVUS and 293 participants in ULSAM ‡Measured with a proteomics assay that does not provide absolute concentrations.

coefficient of variation was 4.6% and the total CV was 7%. All samples were analyzed in batch mode with the same endostatin kit lot number to minimize assay differences due to lot to lot variation.

Diabetes mellitus was diagnosed as fasting plasma glucose  $\geq 7.0$  mmol/l or use of anti-diabetic medication. History of myocardial infarction at baseline was assessed using the Swedish national hospital discharge register.

In ULSAM (fourth examination cycle) urine albumin was measured by nephelometry (Urine albumin, Dade Behring, Deerfield IL, USA) using a Behring BN ProSpec<sup>®</sup> analyzer (Dade Behring). Urine creatinine was analyzed with a modified kinetic Jaffe reaction on an Architect Ci8200<sup>®</sup> analyzer (Abbott, Abbot Park, IL, USA) and creatinine related urine albumin (ACR) was calculated. No urine samples were collected at baseline in PIVUS.

An echocardiographic examination was performed at baseline in most participants of PIVUS, but in ULSAM, the echocardiographic investigation was performed in a subset of participants approximately 2 years prior to the baseline investigation of examination cycle 4 (Table 1). Left ventricular dimensions were measured with M-mode. Left ventricular volumes (LVEDV, LVESV) were calculated according to the Teichholz M-mode formula;  $\text{volume} = 7D^3 / (2.4 + D)$ , D = diameter. Left ventricular ejection fraction, reflecting left ventricular systolic function, was calculated as left ventricular diastolic volume – left ventricular systolic volume/left ventricular diastolic volume. Ventricular diastolic function was assessed by the E/A-ratio. The transmitral Doppler amplitudes of the E and A waves were assessed in the apical projection and the E/A-ratio was calculated. In the analyses of the E/A-ratio, all individuals with suspected pseudo-normalization were excluded (ejection fraction  $< 0.5$  or E/A-ratio  $> 1.5$ ).

### Outcome

Medical records for all individuals hospitalized for heart failure according to the Swedish National Hospital Discharge Register heart failure codes International Classification of

Diseases tenth revision (ICD-10) I50, and hypertensive heart disease with heart failure, ICD-10 I11, during follow-up were reviewed by physicians blinded to the baseline data [11]. They classified the heart failure events as definite, questionable, or miscoded according to the European Society of Cardiology definitions. [12] We included all definite cases of heart failure in our analyses.

### Statistical analysis

Mean values and standard deviations were calculated for all continuous variables at baseline.

The association between standard deviation increments of endostatin and the incidence of heart failure hospitalizations was investigated using Cox proportional hazard regression. Proportional hazards assumptions were confirmed by Schoenfeld's tests. Adjustments were made using the following multivariable models:

- a. Adjusted by sex (PIVUS only) and age (modelled as a timeline)
- b. Model A + eGFR
- c. Model B + heart failure risk factors included in the Atherosclerosis Risk in Communities Study (ARIC) heart failure risk score [13] (age, BMI, systolic blood pressure, sex, antihypertensive treatment, heart rate, diabetes mellitus, history of myocardial infarction, current smoker and former smoker status). The ARIC factor regarding ethnicity did not apply in these homogeneous samples of European descent.
- d. Adjusted for Model C + Nt-proBNP

In secondary analyses in PIVUS, we also performed the following additional multivariable model:

Adjusted for Model D + echocardiographic left ventricular mass and endothelial function (as endostatin has previously been shown to be cross-sectionally associated with these factors [14]).

In secondary analyses in ULSAM, we also performed the following additional multivariable models:

- A. Adjusted for Model D + urinary albumin/creatinine ratio (as endostatin has been suggested to be a previously been shown to be cross-sectionally associated with this marker of kidney damage [4]). No data on urinary albumin/creatinine ratio was available in PIVUS.

We also performed subgroup analyses in individuals without a myocardial infarction at baseline ( $n = 912$  for the PIVUS cohort and  $n = 669$  in the ULSAM cohort).

Differences in C statistics after the addition of endostatin to the ARIC heart failure risk score model [13] with and without NT-proBNP were estimated in order to evaluate improvement in model discrimination (Harrel's C). These analyses were performed after merging the two samples in order to increase the statistical power.

Age and sex-adjusted linear regression analyses were used to assess the cross-sectional associations of endostatin levels (independent variable) with left ventricular systolic function (ejection fraction) and diastolic function (E/A-ratio) as dependent variables in separate models were conducted in the both cohorts. We also assessed the cross-sectional association between endostatin and NT-proBNP at baseline using Spearman correlation.

A two-sided  $p$  value  $< .05$  was regarded as significant in all analyses. The statistical software package STATA 14 (Stata Corp, College Station, TX, USA) was used for all analyses.

### Results

Baseline characteristics of both cohorts are shown in Table 1.

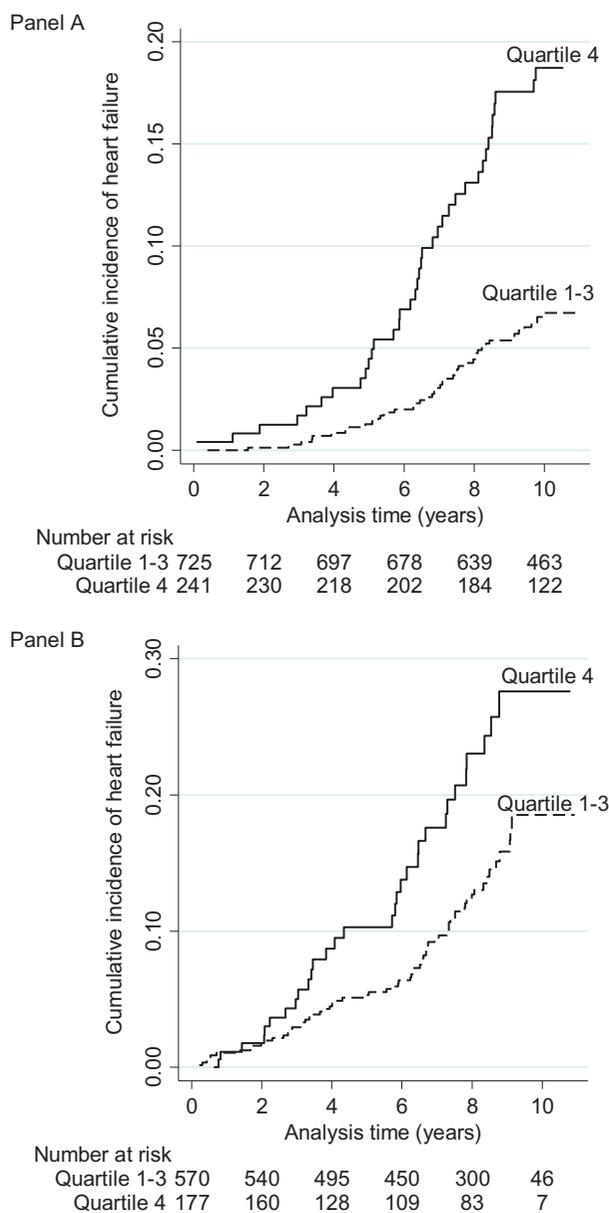
#### Endostatin and heart failure incidence

During follow-up in the PIVUS cohort (median 10.0 years, range 0.1–10.9 years), 81 participants were hospitalized for heart failure (incidence rate 0.91/100 person-years at risk), and in ULSAM, 98 participants were hospitalized for heart failure (median follow-up 8.0 years, range 0.2–10.9 years, incidence rate 1.83/100 person-years at risk). The cumulative incidence curves in individuals with upper quartile levels of endostatin versus the lower three quartiles are shown in Figure 1. As shown in Table 2, there was a significant association between higher levels of circulating endostatin and higher risk of incident heart failure in both PIVUS and ULSAM in age and sex adjusted models (Model A), after further adjustment for glomerular filtration rate (Model B), or heart failure risk factors (Model C). The addition of NT-proBNP to model C (Model D) attenuated the associations somewhat, albeit still significant in both cohorts (Table 2) or when merging the two cohorts (Model C HR 1.32, 95% CI 1.10–1.58,  $p = .003$ ). In secondary analyses in PIVUS, higher endostatin was still significantly associated with higher heart failure risk after further adjustment for endothelial function and echocardiographic left ventricular mass (hazard ratio per standard deviation increase 1.48, 95% CI, 1.18–1.87,  $p < .001$ ). In ULSAM, higher endostatin was still significantly associated with higher heart failure risk with the addition of urinary albumin/creatinine ratio to model D (hazard ratio per standard deviation increase 1.43, 95% CI, 1.08–1.89,  $p = .01$ ).

Associations were similar in subgroup analyses of individuals without a previous myocardial infarction at baseline (Supplementary Table 1).

#### Risk prediction

After merging the two samples, the C statistic increased significantly for the prediction of heart failure incidence when endostatin was incorporated into a model with the ARIC heart failure risk score (C-statistics ARIC score 0.700 and for ARIC score + endostatin 0.716,  $p < .05$ ) but not when



**Figure 1.** Cumulative incidence curves in individuals with upper quartile levels of endostatin versus the lower three quartiles. Panel A: PIVUS, Panel B ULSAM.

endostatin was added to a model that included both the ARIC score and NT-proBNP (C-statistics ARIC score/NT-proBNP 0.758 and for ARIC score/NT-proBNP + endostatin 0.761,  $p = .44$ )

### Endostatin and echocardiographic left ventricular function

The cross-sectional association between endostatin and echocardiographic indices of left ventricular function in the PIVUS cohort at baseline are shown in Table 3. In age adjusted linear regression models, higher endostatin was associated with both worsened left ventricular systolic function (ejection fraction) and worsened diastolic function (E/A-ratio) in both cohorts.

Also, there was a significant cross-sectional correlation between endostatin and baseline NT-proBNP in both cohorts (PIVUS  $r = 0.13$ ,  $p < .001$ ; ULSAM  $r = 0.24$ ,  $p < .001$ )

**Table 2.** The association between serum endostatin and heart failure incidence: Multivariable cox proportional hazard regression analyses.

	The PIVUS cohort Hazard ratio (95% CI)	The ULSAM cohort Hazard ratio (95% CI)
Continuous models: per SD higher endostatin		
Model A	1.67 (1.44-1.95)***	1.32 (1.12-1.56)***
Model B	1.55 (1.28-1.88)***	1.40 (1.11-1.79)**
Model C	1.52 (1.24-1.87)***	1.35 (1.05-1.73)*
Model D	1.46 (1.17-1.82)***	1.29 (1.00-1.68)*

CI: confidence intervals.

Model A: Adjusted by sex (PIVUS only) and age (modelled as a timeline); Model B: Model A + eGFR; Model C: Model B + heart failure risk factors included in the Atherosclerosis Risk in Communities Study (ARIC) heart failure risk score [13] (age, BMI, systolic blood pressure, sex, antihypertensive treatment, heart rate, diabetes mellitus, history of myocardial infarction, current smoker and former smoker status); Model D: Adjusted for Model C + NT-proBNP; \* $p < .05$ , \*\* $p < .01$  and \*\*\* $p < .001$ .

**Table 3.** The cross-sectional association between serum endostatin and indices of echocardiographic left ventricular systolic and diastolic function in ULSAM and PIVUS: Multivariable linear regression models.

	Systolic function Ejection fraction B-coefficient (95% CI)	Diastolic function E/A-ratio B-coefficient (95% CI)
PIVUS	-0.14 (-0.21, -0.07)***	-0.06 (-0.1, -0.003)*
ULSAM	-0.29 (-0.51, -0.11)**	-0.22 (-0.36, -0.08)**

Data are linear regression coefficients per SD increase of endostatin adjusting for age and sex (PIVUS only). The echocardiographic variables are also expressed per SD increase. In PIVUS data on ejection fraction was available in 792 and E/A-ratio in 852 participants. In ULSAM data on ejection fraction was available in 138, and E/A-ratio in 293 participants. \* $p < .05$ , \*\* $p < .01$  and \*\*\* $p < .001$ .

## Discussion

### Main findings

In two community-based cohort studies of elderly individuals without heart failure at baseline, higher levels of endostatin were associated with increased incidence of heart failure independently of established heart failure risk factors, left ventricular mass, endothelial function, urinary albumin/creatinine ratio, glomerular filtration rate and NT-proBNP. Even though endostatin improved the model discrimination for the prediction of heart failure beyond the ARIC heart failure risk score, no improvement was seen when NT-proBNP was included in the base model. In cross-sectional analyses, higher endostatin levels were associated with both worsened left ventricular systolic and diastolic function.

### Comparison with previous studies

Previous studies on the association between endostatin and heart failure has essentially been performed in patients with prevalent heart failure. For instance, increased levels of circulating endostatin were associated with higher NT-proBNP [15] and increased mortality in patients with heart failure [5]. Furthermore, increased levels of endostatin were observed in patients with pulmonary arterial hypertension and were linked to heart failure severity and increased circulating NT-proBNP [16]. Conversely, no direct association between endostatin and indices of heart failure was observed in a recent study [17] and no association with

adverse outcomes was found in another study in heart failure patients [6]. To our knowledge, our study is the first to report the association between elevated levels of endostatin and incident heart failure in the community.

### **Possible mechanisms for observed associations**

Our observational data precludes any firm conclusions regarding whether the present associations are causal or not. One explanation for the association between endostatin and heart failure risk could be that the circulating endostatin mirrors an increased extra cellular matrix turnover in the myocardium. The prolonged hypertrophy observed in heart failure leads to substantial pathological extra cellular matrix remodelling, deposition of extra cellular matrix proteins and cardiac fibrosis. This process can be initiated for example by hypertension, cardiac stress and valve dysfunction [18]. Specifically, matrix metalloproteinase 9, is activated in hypertrophied myocardium and associated with increased levels of endostatin [19]. In contrast, inhibition of endostatin in an animal model resulted in severe fibrosis and heart failure implying an acute protective role of endostatin [20].

Another explanation could be that circulating endostatin reflects an activated angiogenic milieu in the hypertrophic heart. Cardiac hypertrophy is suggested to be associated with a reduction in local capillary density and a subsequent reduced capillary perfusion resulting in hypoxia [21]. The initial local adaptation, compensatory hypertrophy, results in increased expression of angiogenic factors such as vascular endothelial growth factor. Later during the de-compensatory cardiac hypertrophy, antioangiogenic factors including endostatin are expressed [22]. In addition, expression of endostatin is increased in rat cardiomyocytes exposed to hypoxia and after induction of a myocardial infarct and several groups have observed an association between endostatin and reduced collateral circulation [23]. But there is also conflicting findings. In a previous study in NYHA II heart failure patients, increased serum concentration of endostatin was associated with an increased collateral formation [24].

Thirdly, endostatin has in several previous studies been put forward as a relevant marker for kidney damage and dysfunction both in patients with diabetes [2] and in the general population [4]. Moreover, endostatin levels has been shown to be associated with underlying factors that predispose to both chronic kidney disease and to heart failure such as diabetes [25], ischemic heart disease [26,27] and hypertension [14]. Thus speculatively, it is possible that endostatin could be a marker for the cardiorenal interplay. Yet, it should be noted that all associations were independent of baseline eGFR. The fact that endostatin predicted heart failure also in individuals without ischemic heart disease indicate that the associations are not primarily mediated via coronary atherosclerosis.

Fourthly, preliminary data suggest that there also may be acute hemodynamic effects of endostatin; recombinant infused endostatin has been shown to induce acute heart failure [28], but also to induce vasorelaxation and to acutely

lower blood pressure [29]. It is thus possible that the increased circulating endostatin seen in patients in heart failure causes additional detrimental effects on the hemodynamic state in the heart and so aggravates the development of heart failure.

Finally, given the cross-sectional association between endostatin and indices of left ventricular function and NT-proBNP, and the fact that adjustment for NT-proBNP attenuated the longitudinal association between endostatin and heart failure incidence suggests that a feasible explanation of the present findings is that higher endostatin levels to some degree reflects an impaired left ventricular function that predisposes to an increased heart failure risk.

### **Clinical implications**

Even though a statistically significant improvement in risk discrimination was seen when adding information on endostatin levels to the ARIC heart failure score no improvement was seen if NT-proBNP was added to the baseline model. This suggest that there may be limited utility of using endostatin as a heart failure risk marker in clinical practice if NT-proBNP data is available. Our cross-sectional analyses show that higher endostatin levels are associated with both left ventricular systolic and diastolic dysfunction, but whether endostatin assessment could be useful in estimating the risk of the two major subtypes of heart failure - reduced or preserved ejection fraction - remains to be established.

### **Strengths and limitations**

Strengths of our investigation include the longitudinal study design, the use of two community based cohorts of elderly and the detailed characterization of study participants. Limitations include the unknown generalizability to other age-, and ethnic groups, the inability to differentiate between heart failure with reduced or preserved ejection fraction and the relatively low participation rate in this study. Also, even though we excluded individuals that had previously been hospitalized for heart failure it is possible that some had unrecognised heart failure at baseline.

### **Conclusions**

In two community based cohorts of elderly individuals, higher circulating endostatin was associated with worsened left ventricular dysfunction and an increased risk of heart failure incidence, independently of both established heart failure risk factors and NT-proBNP. Yet, endostatin did not substantially improve the prediction of heart failure incidence if data on NT-proBNP was available. Our findings encourage further experimental studies that investigate the role of endostatin in the development of heart failure but also further clinical studies evaluating the utility of endostatin measurements in clinical practice.

## Acknowledgements

This study was supported by The Swedish Research Council, Swedish Heart-Lung Foundation, the European Union Horizon 2020 (grant number 634869), the Marianne and Marcus Wallenberg Foundation, the Thuréus foundation, Dalarna University and Uppsala University. The funding sources did not play any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. Dr. Ärnlov is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of data and the accuracy of data analysis.

## Disclosure statement

Erik Ingelsson is an advisor and consultant for Precision Wellness, Inc., and advisor for Cellink and Olink Proteomics. Johan Sundström has an advisory board membership for Itrim. Johan Ärnlov has received lecturing fees from AstraZeneca.

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