Cystatin C is associated with risk of venous thromboembolism in subjects with normal kidney function – the Tromsø study

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ABSTRACT

Background
Previous studies have shown an association between impaired kidney function, assessed by cystatin C-based estimated glomerular filtration rate, and venous thromboembolism. The aim of this study was to investigate whether serum cystatin C was associated with a risk of venous thromboembolism among subjects with normal kidney function in a prospective population-based study.

Design and Methods
Cystatin C was measured in serum from 3251 men and women with normal kidney function, aged 25-84 years, who participated in the Tromsø study in 1994-1995. Normal kidney function was defined as a creatinine-based estimated glomerular filtration rate greater than 90 mL/min/1.73 m^2 and absence of microalbuminuria. Incident venous thromboembolism was registered from the date of inclusion through to the end of follow-up, September 1, 2007. Cox-regression models were used to calculate hazard ratios with 95% confidence intervals for venous thromboembolism.

Results
There were 83 incident venous thromboembolic events, of which 53 (63.9 %) were provoked, during a median of 12.3 years of follow-up. A one standard deviation (0.11 mg/L) increase in serum cystatin C levels was associated with a 43% (hazard ratio 1.43; 95% confidence interval 1.17-1.72) increased risk of total venous thromboembolism. Subjects with cystatin C levels in the top quartile (≥0.87 mg/L) had a 2.5-fold (hazard ratio 2.51; 95% confidence interval 1.27-4.96) increased risk of venous thromboembolism compared to those with levels in the bottom quartile (≤0.72 mg/L) in adjusted analysis. The risk estimates were even higher for provoked venous thromboembolism (hazard ratio 3.11; 95% confidence interval 1.23-7.86).

Conclusions
Serum cystatin C levels were associated with the risk of venous thromboembolism in subjects with normal kidney function. Our findings suggest that elevated serum cystatin C levels may promote venous thrombosis beyond reflecting impaired kidney function.

Key words: cystatin C, venous thromboembolism, VTE, risk.

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Introduction

Venous thromboembolism (VTE) including deep vein thrombosis and pulmonary embolism is a common disease with serious short- and long-term complications and a potentially fatal outcome.[1,2] The incidence of VTE is 1 to 3 per 1000 person-years with a steep incline with age.[1,2] Even though many environmental and inherited predisposing factors have been associated with VTE,[1-5] 30–50% of the events have no obvious provoking factors.[6-8]

Cystatin C is a non-glycosylated cysteine protease inhibitor with a low molecular weight of 13 kDa synthesized at a constant rate (housekeeping gene product) by most nucleated cells.[9] Although its levels were previously reported to be unaffected by gender, age or muscle mass,[10,11] recent studies have shown higher concentrations of cystatin C in men and that the levels are associated with increasing height, weight, and age.[12-14] Impaired renal function, assessed by estimated glomerular filtration rate (eGFR) based on serum cystatin C concentrations, is associated with increased risk of future arterial cardiovascular disease.[15] Observational studies also suggest that serum cystatin C levels predict arterial cardiovascular disease in subjects with normal kidney function.[16]

Previous studies have yielded diverging results on the relation between mildly impaired kidney function determined on the basis of cystatin C and risk of VTE.[17,18] Serum cystatin C was not associated with a risk of VTE in the Cardiovascular Health Study (CHS).[17] However, eGFR based on serum cystatin C was associated with a 1.6-fold increased risk of total VTE in patients with severe kidney disease in the Atherosclerosis Risk in Communities (ARIC) study.[18] Although renal dysfunction appears to be the most plausible link between increased cystatin C and VTE, the predictive value of cystatin C for VTE in a population with normal kidney function has not been elucidated. The aim of our study was to investigate whether serum cystatin C was associated with risk of VTE among subjects with normal kidney function in a prospective population-based study.

Design and Methods

Study population

Participants were recruited from the fourth survey of the Tromsø study (conducted in 1994-1995), a single-center, prospective, population-based study involving repeated health surveys of the inhabitants of Tromsø, Norway. All inhabitants aged over 24 years old were invited to participate, and 27,158 (77% of the eligible population) did so. The participants aged 55–74 years and 5–10% of the other birth cohorts (n=9,057) were invited to a more extensive visit 3–12 weeks later, and 75% (n=6,889) attended. Subjects who did not consent to medical research (n=23), subjects not officially registered inhabitants of the municipality of Tromsø at baseline (n=16), subjects with a previous history of VTE (n=18), subjects with missing values of cystatin C or serum creatinine (n=210) and subjects with a GFR <90 mL/min and microalbuminuria (n=3,321) were excluded from the study. Thus, 3251 subjects were included in the study, and incident VTE events among the study participants were recorded from the date of enrollment to the end of follow up, September 1, 2007. The study was approved by the regional committee for research ethics, and all participants gave written informed consent to their participation.

Measurements

Height and weight were measured with subjects wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms, divided by the square of the subject's height in meters (kg/m^2). Self-reported information on diabetes, current daily smoking (pipe/cigar/cigarettes), current hormone therapy and prior cardiovascular disease (myocardial infarction, angina pectoris or stroke) was collected through a self-administered questionnaire. Hormone therapy was defined as self-reported current use of estrogen supplementation (tablets or patches) or current use of oral contraceptives. Blood pressure and non-fasting serum lipids were measured as previously described.[19] Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or the use of antihypertensives drugs. Cystatin C was measured by particle-enhanced turbidimetric immunoassay using reagents from Gentian (Gentian, Moss, Norway) on a Modular E analyzer (Roche Diagnostics). The inter-assay coefficient of variation was 5.0%. High sensitivity C-reactive protein (hs-CRP) was measured by a particle-enhanced turbidimetric immunoassay on a Modular P autoanalyzer (Roche/Hitachi), using reagents from Roche Diagnostics GmbH, Mannheim, Germany.

Assessment of renal function

Plasma creatinine was analyzed by a modified Jaffe reaction, but a subsample was reanalyzed with an enzymatic method, and recalculated creatinine values[20] were used for estimation of the glomerular filtration rate (eGFRcrea). eGFRcrea was calculated using the recalibrated four-variable Modification of Diet in Renal Disease (MDRD) study equation; eGFRcrea = 175 x (s-creatinine (μmol/l)/88.4)$^{-1.154}$ x age$^{-0.203}$ x (0.742 if female).[21] Chronic kidney disease was categorized based on the National Kidney Foundation guidelines[22] using eGFRcrea; eGFR ≥90 mL/min/1.73 m^2 for normal kidney function, eGFR between 60 and 89 mL/min/1.73 m^2 for mildly impaired kidney function, and eGFR between 15 and 59 mL/min/1.73 m^2 for stage 3/4 chronic kidney disease.

Three samples of morning spot-urine, collected on consecutive days, were tested with a dipstick and analyzed immediately for albumin and creatinine, using commercial kits (ABX Diagnostics; Montpellier, France). One urine sample was cultured. The albumin–creatinine ratio was calculated for each urine specimen, and the mean value of this ratio (mg/mmol) was used in the analyses. Normal kidney function was defined as an eGFRcrea >90 mL/min/1.73 m^2 and the absence of microalbuminuria (albumin-creatinine ratio ≥1.92 mg/mmol in women and ≥2.83 mg/mmol in men).

Ascertainment of venous thromboembolism

All first lifetime events of VTE during follow-up were identified as previously described[19] by searching the hospital discharge diagnosis registry, the autopsy registry, and the radiology procedure registry at the University Hospital of North Norway.

The medical records for each potential case of VTE were reviewed by trained personnel. For subjects derived from the hospital discharge diagnosis registry and the radiology procedure registry, an episode of VTE was verified and recorded as a validated outcome when all four of the following criteria were fulfilled: (i) objectively confirmed by diagnostic procedures [compression ultrasonography, venography, spiral-computed tomography (CT), perfusion-ventilation scan, pulmonary angiography or autopsy]; (ii) the medical record indicated that a physician had made a diagnosis of deep vein thrombosis or pulmonary embolism; (iii) signs and symptoms consistent with deep vein thrombosis or pulmonary embolism were present; (iv) therapy with anticoagulants (heparin, warfarin, or similar agent), thrombolytics, or vascular

surgery was required. For subjects derived from the autopsy registry, a VTE event was recorded as an outcome when the autopsy record indicated VTE as a cause of death or as a significant condition.

Based on the presence of provoking factors at the time of diagnosis, the VTE event was classified as unprovoked (no provoking factors) or provoked (≥1 provoking factors). Major surgery, trauma, or an acute medical condition (acute myocardial infarction, ischemic stroke, or major infectious disease) within the 8 weeks preceding the event, active cancer at the time of the event and marked immobilization (bed rest for longer than 3 days, confinement to a wheelchair, or long distance travel exceeding 4 hours within the last 14 days prior to the event) were considered provoking factors.

Statistical analyses

Statistical analyses were carried out using SPSS version 17.0 (SPSS Inc. Chicago, IL, USA). The baseline characteristics of participants across quartiles of serum cystatin C level were compared using a χ^2 test for categorical variables and one-way ANOVA for continuous variables.

Cox-proportional hazards regression models were used to estimate hazard ratios (HR), with 95% confidence intervals (CI), for unprovoked, provoked and total VTE by quartiles of cystatin C concentration. In the Cox models, the lowest quartile of cystatin C was used as the reference group. Hazard ratios for the associations between cystatin C and risk of VTE were first adjusted for age and sex and then for additional potential confounders such as BMI, hs-CRP, diabetes and hypertension. The proportional hazard assumption was verified by evaluating the parallelism between the curves of the log-log survivor function for quartiles of cystatin C.

Results

There were 83 incident VTE events during a median of 12.3 years of follow-up. The overall crude incidence rate of VTE was 2.6 per 1,000 person-years. The baseline characteristics of participants across quartiles of cystatin C are shown in Table 1. Age, BMI, blood pressure, hs-CRP, and the proportions of males and smokers increased across quartiles of cystatin C (all *P*-values for trend <0.001) (Table 1).

Among the patients who had a VTE, 61.4% had deep vein thrombosis and 38.6% had pulmonary embolism with or without concurrent deep vein thrombosis (Table 2). Thirty (36.1%) events were unprovoked (Table 2). Cancer was the most common provoking factor (26.5% of the VTE patients had a cancer-related VTE event), followed by surgery (22.9%) (Table 2).

The risk of total VTE increased significantly across quartiles of cystatin C (*P* for trend across categories 0.002) in analyses adjusted for age and sex (Table 3), and was only moderately attenuated by further adjustment for BMI, smoking, diabetes and hs-CRP (*P* for trend =0.001). Subjects with cystatin C in the top quartile (≥0.87 mg/L) had a 2.5-fold increased risk of VTE compared to those with cystatin C levels in the lowest quartile (≤0.72 mg/L) (HR 2.51; 95% CI: 1.27-4.96) adjusted for age, sex, BMI, smoking, diabetes and hs-CRP. The risk estimates were even higher for provoked VTE (HR 3.11; 95% CI: 1.23-7.86). Moreover, when analyzing cystatin C as a continuous variable, an increase of one standard deviation (SD) (0.11 mg/L) in cystatin C concentration was associated with a 46% increased risk of total VTE, a 45% increased risk of provoked VTE, and a 48% increased risk of unprovoked VTE. The differences in risk estimates for unprovoked VTE across quartiles of cystatin C were not statistically significantly different (Table 3).

The cumulative incidences of VTE by quartiles of cystatin C are shown in Figure 1. The curves diverged progressively over the entire observation period. During a maximum of 12.3 years of follow-up, 1% of participants in the lowest quartiles (quartiles 1 and 2), 1.5 % of those in quartile 3 and 5% of those in the highest quartile (quartile 4) developed VTE (Figure 1).

Discussion

To the best of our knowledge, this study is the first to identify cystatin C as a risk factor for VTE in subjects with normal kidney function in a prospective, population-based study. The association between serum cystatin C and risk of VTE held true in statistical models treating cystatin C levels both as continuous and categorized variables, and after adjustment for potential confounders. During follow-up, there was a stepwise increase in incident events with increasing levels of cystatin C. The cumulative incidence

Table 1. Baseline characteristics across quartiles of cystatin C concentration. The Tromsø study 1994-2007. Values are means with standard deviations (SD) in brackets for continuous variables and percentages with numbers in brackets for dichotomized variables.

	Quartiles of Cystatin C (mg/L)				
	≤ 0.72	0.73-0.79	0.80-0.86	≥ 0.87	*P*
Participants, n.	830	821	789	811	
Cystatin C, mg/L (SD)	0.66(0.046)	0.76(0.019)	0.82(0.019)	0.94(0.075)	<0.001
Age, years (SD)	54(11)	57(11)	58(10)	61(9)	<0.001
Male sex, % (n.)	41.8(347)	53.7(441)	62.0(489)	66.8(542)	<0.001
Smoking, % (n.)	28(232)	34(279)	39(308)	42(342)	<0.001
Body mass index, kg/m^2 (SD)	25.3(3.7)	25.8(3.9)	26.0(4.0)	26.2(4.2)	<0.001
Diabetes, % (n.)	2.3(20)	2.2(18)	2.4(19)	2.3(23)	0.86
Systolic blood pressure, mmHg(SD)	136(20)	139(20)	139(20)	143(22)	0.001
Diastolic blood pressure, mmHg (SD)	79(11)	80(12)	80(12)	82(13)	0.001
High sensitivity C-reactive protein, mg/L (SD)	1.88(64.8)	1.94(4.14)	2.30(5.0)	3.41(6.61)	0.001

was 5% in the highest quartile (cystatin C ≥0.87 mg/L) compared with 1% in the lowest quartile (cystatin C <0.72 mg/L). Our findings suggest that elevated serum cystatin C levels may promote venous thrombosis independently of impaired kidney function.

Increased levels of cystatin C are found in patients with coronary artery disease,[15,23,24] and cystatin C has been identified as a risk factor for myocardial infarction and

Table 2. Characteristics of VTE patients (n= 83), at the time of the VTE event. The Tromsø study, 1994-2007. Values are percentages with numbers in brackets.

Women	44 (1432)
Deep vein thrombosis	61.4 (51)
Pulmonary embolism	38.6 (32)
Unprovoked VTE	36.1 (30)
Clinical risk factors:	
Estrogens*	12.9(4
Heredity†	3.6 (3)
Pregnancy	0
Other medical condition‡	25.3 (21)
Provoking factors:	
Surgery	22.9 (19)
Trauma	3.6 (3)
Acute medical condition	21.7 (18)
Cancer	26.5 (22)
Immobilization (bed rest>3 days, wheelchair)	9.6 (8)
Other§	3.6 (3)

*Hormone replacement therapy/oral contraceptives. †Heredity: family history of VTE in first degree relative before the age of 60 years. ‡Other diseases within the previous year (myocardial infarction, ischemic stroke, heart failure, inflammatory bowel disease, chronic infections, chronic obstructive pulmonary disease or myeloproliferative disorders). §Other factor specifically described as provoking in the medical record (e.g intravascular catheter)

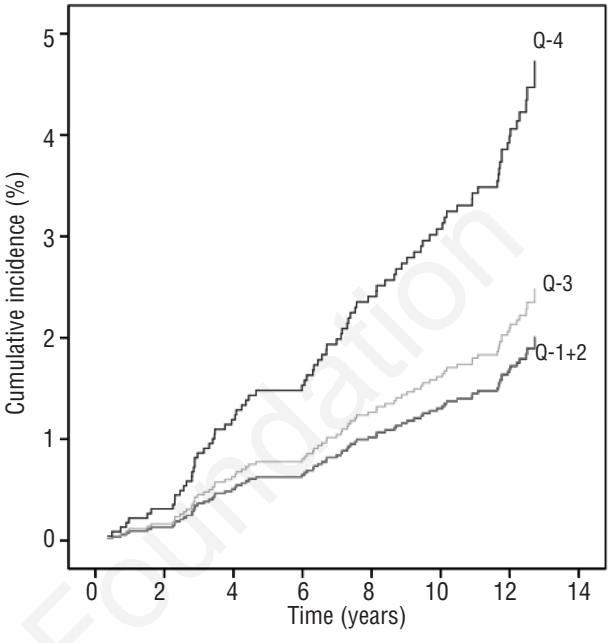

Figure 1. Cumulative incidence of venous thromboembolism by quartiles of cystatin C level plotted against time. Data were adjusted for sex, age, smoking, BMI, diabetes and hs-CRP.

Table 3. Age and sex adjusted incidence rates (IR) and hazard ratios (HR) with 95% confidence intervals (95% CI) for VTE by quartiles of cystatin C. The Tromsø study 1994-2007.

Quartile range Cystatin C (mg/L)	Subjects	VTE events	IR (95 % CI)*	HR (95 % CI)*	HR (95 % CI)**
Total VTE					
≤ 0.72	830	12	1.26 (0.69-2.32)	1.00 (reference)	1.00 (reference)
0.73-0.79	821	14	1.28 (0.71-2.32)	1.01 (0.46-2.20)	1.02 (0.47-2.22)
0.80-0.86	789	18	1.60 (0.91-2.81)	1.26 (0.60-2.05)	1.30 (0.61-2.71)
≥ 0.87	811	39	3.11 (1.91-5.04)	2.46 (1.26-4.83)	2.51 (1.27-4.96)
P for trend				*0.002*	*0.001*
Per one SD increase in cystatin C		83		1.45 (1.20-1.76)	1.46 (1.20-1.77)
Provoked VTE					
≤ 0.72	824	6	0.59 (0.25-1.39)	1.00 (reference)	1.00 (reference)
0.73-0.79	816	9	0.76 (0.36-1.62)	1.28 (0.52-3.50)	1.27 (0.45-3.60)
0.80-0.86	783	12	0.97 (0.47-1.98)	1.62 (0.60-4.39)	1.63 (0.60-4.41)
≥ 0.87	789	26	1.88 (1.01-3.49)	3.15 (1.26-7.89)	3.11 (1.23-7.86)
P for trend				*0.003*	*0.004*
Per one SD increase in cystatin C		53		1.39 (1.11-1.74)	1.45 (1.14-1.84)
Unprovoked VTE					
≤ 0.72	824	6	0.69 (0.29-1.64)	1.00 (reference)	1.00 (reference)
0.73-0.79	812	5	0.52 (0.19-1.37)	0.7 (0.22-2.48)	0.77 (0.23-2.54)
0.80-0.86	777	6	0.62 (0.24-1.58)	0.89 (0.28-2.81)	0.94 (0.29-3.01)
≥ 0.87	785	13	1.24 (0.57-2.72)	1.80 (0.65-4.95)	1.95 (0.69-5.46)
P for trend				*0.12*	*0.14*
Per one SD increase in cystatin C		30		1.43 (1.04-1.97)	1.48 (1.08-2.05)

*One SD=0.11 mg/L. *Age- and sex-adjusted. **Multivariable model adjusted for age, sex, BMI, smoking, diabetes and hs-CRP.*

stroke.[25-27] Moreover, increased cystatin C was associated with all-cause, cardiovascular, and even non-cardiovascular mortality in a population-based cohort of subjects with normal kidney function.[28] However, whether cystatin C merely reflects the association of mildly impaired kidney function with increased risk of cardiovascular disease, or is an independent risk factor involved in the pathogenesis of atherosclerosis, has not been fully elucidated. Cystatin C is an endogenous inhibitor of potentially destructive proteases such as cathepsins, and is severely reduced and associated with abundant levels of cathepsins in atherosclerotic lesions.[29] The positive association between serum cystatin C and risk of cardiovascular diseases is suggested to represent a compensatory mechanism to reduce proatherogenic cathepsin activity.[30]

Although the mechanisms through which elevated cystatin C is related to venous thrombosis remains unclear, it is tempting to speculate that other pathophysiological pathways independent of impaired glomerular filtration rate are involved. Besides reflecting impaired kidney function, cystatin C has been associated with chronic low-grade inflammation and atherosclerosis.[31] Accordingly, we found a linear increase in hs-CRP across categories of elevated cystatin C (Table 1). Adjustment for hs-CRP in the statistical models did not affect the risk estimates for VTE by cystatin C, indicating that low-grade downstream inflammation, assessed by hs-CRP, is not a substantial contributor to risk mediated by cystatin C. Cystatin C is also abundantly secreted by human adipose tissue explants *in vitro*,[32] and according to our findings (Table 1) is positively associated with BMI. Adjustment for BMI did not affect the risk estimates for VTE by cystatin C, suggesting that the association between VTE and high cystatin C levels was not mediated by BMI. Recently, neutrophil proteases, in concert with externalized nucleosomes, were shown to promote thrombus formation inside blood vessels.[33] Cystatin C is known to modulate neutrophil chemotactic activity[34] and may inhibit prothrombotic activity of proteolytic substances secreted by activated neutrophils. Thus, it may be hypothesized that increased serum levels of cystatin C represent an inadequate counterbalancing mechanism to avoid thrombosis formation.

The main strengths of our study are the large number of participants and validated VTE events, the prospective design, and the long-term follow-up. The study was performed in a population generally without a previous diagnosis of chronic kidney disease. The study does, however, have some limitations. First, there are potential sources of misclassification. Renal function estimated by the Modification of Diet in Renal Disease (MDRD) formula and serum creatinine is not as accurate as a direct measurement from iothalamate or creatinine clearance using a 24-h urine collection. However, direct measurement of glomerular filtration rate is not feasible in a large epidemiological study. Furthermore, estimation of renal function was based on only one measure of serum creatinine, and may be subject to intraindividual variation. A possible change in kidney function during the study period could have resulted in misclassification of chronic kidney disease status, and thereby underestimation of our risk estimates due to regression towards the null hypothesis.

In conclusion, our prospective population-based study showed that cystatin C levels were associated with risk of VTE among subjects with normal kidney function. Our findings suggest that elevated serum Cystatin C levels may predict venous thrombosis beyond reflecting impaired kidney function.

Authorship and Disclosures

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