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Cystatin C as risk factor for cardiovascular events and all-cause mortality in the general population. The Tromsø Study

Ingrid Toft[1,2], Marit Solbu[1], Jens Kronborg[3], Ulla D. Mathisen[1,2], Bjørn O. Eriksen[1,2], Hilde Storhaug[2], Toralf Melsom[1,2], Maja-Lisa Løchen[4], Ellisiv B. Mathiesen[2,5], Inger Njølstad[4], Tom Wilsgaard[4] and Jan Brox[6,7]

[1]Department of Nephrology, University Hospital of North Norway, Tromsø, Norway, [2]Department of Clinical Medicine, University of Tromsø, Tromsø, Norway, [3]Department of Laboratory Medicine, Innlandet Hospital Trust, Lillehammer, Norway, [4]Department of Community Medicine, University of Tromsø, Tromsø, Norway, [5]Department of Neurology, University Hospital of North Norway, Tromsø, Norway, [6]Department of Medical Biochemistry, University Hospital of North Norway, Tromsø, Norway and [7]Department of Medical Biology, University of Tromsø, Tromsø, Norway

Correspondence and offprint requests to: Ingrid Toft; E-mail: Ingrid.Toft@unn.no

Abstract

Background. Glomerular filtration rate <60 mL/min/1.73m^2 is associated with increased cardiovascular risk. Cystatin C is believed to be a better tool than creatinine for detection of mild renal dysfunction (>60 mL/min/1.73m^2) and possibly a more sensitive marker for cardiovascular risk and all-cause mortality. We examined the association of cystatin C with cardiovascular morbidity and all-cause mortality in a prospective population-based study.

Methods. Cystatin C was measured in 2852 men and 3153 women in the Tromsø Study 1994/95. Gender-specific associations during 12 years of follow-up for all-cause mortality and 9.5 years for myocardial infarction (MI) and ischaemic stroke were assessed (Cox proportional hazard ratios, HRs).

Results. During follow-up, 591 MIs, 293 ischaemic strokes and 1262 deaths occurred. In women, HR for all-cause mortality was increased in the upper cystatin C quartile (≥0.93 mg/L) compared with the lowest quartile (≤0.73 mg/L); 1.38, 95% confidence interval 1.04–1.84. A significant interaction with gender was observed. One SD (0.17 mg/L) increase in cystatin C was associated with 9% higher risk of death in women, also when persons with a cancer history were excluded. Crude HRs for MI and ischaemic stroke were increased in both genders, but the associations did not persist after multi-variable adjustments. No independent associations with end points were observed in non-gender-specific analyses.
Conclusions. Cystatin C was not independently associated with fatal and non-fatal MI or ischaemic stroke in the general population. However, cystatin C was a risk factor for all-cause mortality in women.

Introduction

Chronic kidney disease (CKD) [1] as well as mild renal dysfunction [2, 3] are associated with increased risk of cardiovascular disease (CVD). It is not clear if the risk is mediated by mechanisms secondary to renal dysfunction or by risk factors common to both CVD and decreased glomerular filtration rate (GFR). Creatinine, most commonly used for estimation of renal function, is influenced by non-renal factors such as age, gender and muscle mass [4, 5].

Cystatin C is a marker of renal function [6, 7] that is regarded as less sensitive for extrarenal influence [8], although associations with cancer [9], hyperthyroidism [10] and C-reactive protein (CRP) have been reported [11]. Cystatin C may detect mild renal dysfunction better than creatinine [12, 13], and in elderly men, cystatin C was found to be superior to creatinine in predicting adverse cardiovascular events and total mortality [13, 14]. Similar findings were observed in prospective studies of selected cohorts such as elderly persons (>65 years) [13–17], exclusively men [15, 18], persons with known coronary disease [19, 20] and persons with established chronic kidney disease [21]. The first prospective study of a general population was published by the The third National Health and Nutritional Examination Survey (NHANES III) group [22]. They reported that in a US population, cystatin C was more strongly associated with cardiovascular and all-cause mortality than creatinine-based estimation of renal function [22]. However, the role of cystatin C in predicting cardiovascular outcomes and all-cause mortality may not have been sufficiently addressed in women, as gender-specific analyses were not reported in most studies that included women [11, 13, 19, 20].

Our aim was to examine if cystatin C predicted cardiovascular outcomes and all-cause mortality in men and women, as well as in a mixed population, in a European, general population. Secondly, we wanted to investigate whether cystatin C represented a more sensitive CVD risk marker than creatinine and the GFR$_{CKD-EPI}$ equation.

Materials and methods

Study population

The Tromsø Study is a population-based, repetitive prospective study of inhabitants of the municipality of Tromsø, northern Norway [23]. Cystatin C measurements from frozen blood samples obtained in the fourth survey in 1994/95 were available from 6738 persons. We excluded subjects with self-reported myocardial infarction (MI, $n = 378$), ischaemic stroke ($n = 99$) or both ($n = 20$) and subjects with diabetes ($n = 299$), defined as self-reported diabetes, use of antidiabetic medication, HbA1c ≥6.5% or non-fasting plasma glucose ≥10.0 mmol/L. This left 6005 persons (2852 men and 3153 women) who were eligible for analyses.

The surveys were conducted by the University of Tromsø in cooperation with the National Health Screening Service. The Regional Committee for Medical Research Ethics approved the study, and all participants gave their informed written consent.

Measurements

All measurements and information on risk factors were obtained from baseline data from the fourth survey. Information about presence of diabetes, present or previous diagnosis of cancer, smoking habits and physical activity was obtained from a self-administered questionnaire. Blood pressure was recorded in triplet (Dinamap) and the mean of the second and third measurement was used. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic pressure (DBP) ≥90 mmHg and/or current use of anti-hypertensive medication. Leisure physical activity was dichotomized as active (>1 h physical activity/week) or inactive (all others). Smoking habits were classified as non-smokers or current smokers. Lipids were measured as previously described [23].

Cystatin C and high-sensitivity CRP (hs-CRP) were measured in 2007 and 2009 by identical methods, from frozen serum stored at −70° C. Cystatin C was analysed in a Modular E analyser (Roche Diagnostics, Mannheim, Germany) with turbidimetric immunoassay (Gentian AS, Moss, Norway), reference range: 0.50–1.00 mg/L, intra-assay coefficient of variation (CV): 4.0%. Sixty cystatin C samples that were analysed in 2007 were reanalysed in 2009. The inter-assay CV was 1.2%. hs-CRP was measured by ultra sensitive CRP method (immunoturbidimetric assay) in Modular P auto-analyser (CV 3%). Creatinine was originally analysed by a modified Jaffe reaction. Since the formula used in creatinine-based estimation of GFR is better validated for enzymatic creatinine measurements, 111 plasma samples from the 1994/95 survey were thawed and reanalysed with an enzymatic method (Modular P/Roche). Values were fitted to a linear regression model, and recalibrated creatinine values were calculated for all participants. Creatinine-based GFR was calculated from the CKD Epidemiology Collaboration (CKD-EPI) formula [24]: estimated GFR (eGFR) $= 141 \times \min (S_{Cr}/k,1)^a \times \max(S_{Cr}/k,1)^{-1.209} \times 0.993^{age} \times ([1.018$ if female] × [1.159 if black]), where S_{Cr} is serum creatinine (mg/dL), k is 0.7 for females and −0.411 for males, min indicates the minimum of S_{Cr}/k and max indicates the maximum of S_{Cr}/k. Cystatin C-based GFR estimation (eGFR$_{CYST C}$) was calculated according to Stevens formula [25], which was the cystatin C equation that showed highest accuracy, when compared with GFR measured as iohexol clearance in a recent study [26] [Stevens formula: $127.7 \times (S_{cystatin C})^{-1.17} \times (age^{-0.13}) \times (0.91$ if female, 1.06 if black)]. For comparison, eGFR$_{CYST C Arnal–Dade}$ was also calculated according to the Arnal–Dada formula $74.835/(cystatin C)^{1.333}$ [27, 28].

Outcomes

Cardiovascular events were defined as first-ever non-fatal or fatal MI and ischaemic stroke. Adjudication of hospitalized and out-of-hospital events was performed by an independent end point committee and based on data from hospital and out-of-hospital medical records, autopsy records and death certificates. Event ascertainment followed a detailed protocol, according to established diagnostic criteria [29, 30]. Each case was reviewed separately by trained physicians. Stroke was defined according to the World Health Organization definition [30] and classified as ischaemic only when computed tomography or magnetic resonance imaging scans had ruled out haemorrhage. Individuals who had died or emigrated from Tromsø were identified through the Population Registry of Norway.

Table 1. Baseline characteristics by gender and cystatin C quartiles: the Tromsø Study 1994/95[a]

Cystatin C quartiles	I (≤0.76 mg/L)	II (0.77–0.84 mg/L)	III (0.85–0.94 mg/L)	IV (≥0.95 mg/L)	P-value for linear trend
Men					
N	681	711	728	732	
Age, years	55 ± 10	57 ± 10	59 ± 10	64 ± 9	<0.001
BMI, kg/m^2	25.8 ± 3.1	25.9 ± 3.3	25.9 ± 3.3	26.0 ± 3.5	0.30
SBP, mmHg	141 ± 18	144 ± 19	144 ± 20	148 ± 22	<0.001
Hypertension, n (%)	364 (53)	416 (59)	418 (57)	495 (68)	<0.001
HDL cholesterol, mmol/L	1.45 ± 0.40	1.42 ± 0.41	1.37 ± 0.40	1.32 ± 0.38	<0.001
Cholesterol, mmol/L	6.59 ± 1.22	6.53 ± 1.22	6.43 ± 1.15	6.42 ± 1.22	0.03
hs-CRP, mg/L	1.02 (0.53–2.18)	1.10 (0.61–2.27)	1.36 (0.70–2.87)	1.64 (0.83–3.45)	<0.001
Cystatin C, mg/L	0.71 (0.67–0.74)	0.81 (0.79–0.83)	0.89 (0.87–0.91)	1.03 (0.98–1.11)	<0.001
Creatinine, μmol/L	67 (62–73)	71 (66–78)	74 (68–81)	82 (75–91)	<0.001
eGFR$_{CKD-EPI}$, mL/min/m^{2b}	102 (96–107)	98 (92–103)	94 (89–100)	86 (76–93)	<0.001
eGFR$^b_{cystatin\ C,\ Stevens\ equation}$	113 (107–121)	97 (94–100)	86 (83–89)	72 (66–77)	<0.001
eGFR$^b_{cystatin\ C,\ Arnal–Dade\ equation}$	118 (112–127)	99 (96–103)	87 (85–90)	72 (65–77)	<0.001
Current smoker, n (%)	199 (29)	243 (34)	284 (39)	290 (40)	<0.001
Physically active, n (%)	212 (31)	246 (35)	209 (29)	190 (26)	0.005

Cystatin C quartiles	I (≤0.73 mg/L)	II (0.74–0.82 mg/L)	III (0.83–0.92 mg/L)	IV (≥0.93 mg/L)	P-value for linear trend
Women					
N	760	815	773	805	
Age, years	54 ± 12*	59 ± 10*	62 ± 8**	66 ± 7**	<0.001
BMI, kg/m^2	24.9 ± 4.0**	25.3 ± 3.8**	25.9 ± 4.4	27.0 ± 4.7**	<0.001
SBP, mmHg	138 ± 23*	142 ± 23	145 ± 23	151 ± 25	<0.001
Hypertension, n (%)	336 (44)**	430 (53)*	440 (57)	546 (68)	<0.001
HDL cholesterol, mmol/L	1.72 ± 0.45**	1.72 ± 0.44**	1.64 ± 0.43**	1.57 ± 0.41**	<0.001
Cholesterol, mmol/L	6.54 ± 1.42	6.83 ± 1.34**	6.91 ± 1.28**	7.11 ± 1.30**	<0.001
hs-CRP, mg/L	0.79 (0.43–1.75)*	0.93 (0.51–1.97)*	1.19 (0.59–2.28)*	1.62 (0.89–3.11)	<0.001
Cystatin C, mg/L	0.68 (0.65–0.71)**	0.78 (0.76–0.80)**	0.87 (0.85–0.89)**	1.02 (0.97–1.10)*	<0.001
Creatinine, μmol/L	55 (50–60)**	57 (53–62)**	61 (56–67)^v	67 (61–73)**	<0.001
eGFR$_{CKD-EPI}$, mL/min/m^{2b}	101 (96–109)	96 (91–101)**	92 (85–97)**	83 (74–91)**	<0.001
eGFR$^b_{cystatin\ C,\ Stevens\ equation}$	108 (103–116)**	92 (88–95)**	80 (77–83)**	66 (60–71)**	<0.001
eGFR$^b_{cystatin\ C,\ Arnal–Dade\ equation}$	125 (118–132)	104 (101–108)	90 (87–93)	72 (66–77)	<0.001
Current smoker, n (%)	219 (29)	265 (33)	236 (31)*	261 (32)*	0.23
Physically active, n (%)	137 (18)**	146 (18)**	120 (16)**	77 (10)**	<0.001

[a]Gender-specific quartiles. Values are mean (SD), median (interquartile range) or number (%). Hypertension: SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or self-reported use of anti-hypertensive medication. Physical active: self-reported hard physical activity ≥1 h/week. Significant differences between men and women within corresponding cystatin C quartiles (*, P < 0.05; **, P < 0.001; two-tailed *t*-test or Pearson chi-square).
[b]Cystatin-based GFR; Stevens equation: $127.7 \times (S^-_{cystatin\ C}{}^{1.17}) \times (age^{-0.13})$ if male, $127.7 \times [(S^-_{cystatin\ C}{}^{1.17}) \times (age^{-0.13}) \times 0.91]$ if female [25]. eGFR$_{CKD-EPI}$: creatinine-based GFR estimate, CKD EPI formula: eGFR $= 141 \times \min(S_{Cr}/k,1)^a \times \max(S_{Cr}/k,1)^{-1.209} \times 0.993^{age} \times ([1.018$ if female] and $\times [1.159$ if black]) where S_{Cr} is serum creatinine (mg/dL), k is 0.7 for females and −0.411 for males, min indicates the minimum of S_{Cr}/k and max indicates the maximum of S_{Cr}/k [24]. eGFR$_{cystatin\ C,\ Arnal–Dade\ equation}$: $74.835/(cystatin\ C^{1.333})$ [27, 28].

Causes of deaths were obtained from the Causes of Death Registry at Statistics Norway. The national 11-digit identification number allowed a linkage to the Population Registry of Norway and ensured a complete follow-up status for all-cause mortality until 1 March 2009. Since the cardiovascular end point register was completed only until 31 December 2005, follow-up time for MI and ischaemic stroke was assigned from the date of screening in 1994/95 until 31 December 2005. Data were censored for date of registered emigration or death from causes other than MI or ischaemic stroke.

Statistical analysis

Data are given as mean ± SD or median and interquartile range and logarithmically transformed if appropriate. Cystatin C was categorized into gender-specific quartiles. Crude- and age-adjusted incidence rates were calculated as events per 1000 person-years at risk. Age adjustment of incidence rates was performed on 10-year age groups with the population of Tromsø in 1995 as the standard population. Two-sided *t*-test or chi-square test was used for gender comparisons. Analysis of variance or chi-square test with tests for linear trend was used for comparison of baseline characteristics across cystatin C quartiles. Influence of age was examined by analysis of covariance (ANCOVA). Pearson correlation coefficients were used for univariate associations. Cox proportional hazard models were used to compare the associations of cystatin C quartiles with end points, with the lowest quartile as reference, and to compare associations of cystatin C, creatinine and eGFR$_{CKD-EPI}$ with cardiovascular outcome and mortality, calculated per 1 SD change in each measure of kidney function. Covariates in the multivariable analyses were SBP, body mass index (BMI), high-density lipoprotein (HDL), total cholesterol, hs-CRP, smoking status and physical activity. Tests of two-way interactions were assessed in separate models. The proportional hazard assumption was checked by visual inspection of the −log(−log (survival)) curves. P-values <0.05 were considered statistically significant. Analyses were run using SPSS software version 18.0 (SPSS, Inc., Chicago, IL). The authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Baseline characteristics

Mean age was 59 ± 10 years for men and 60 ± 10 years for women, P < 0.001. Only 2.7% of the population had

Table 2. Crude- and age-adjusted incidence rates (95% CI) of all-cause mortality and CVD[a,b] by gender and cystatin C quartiles: the Tromsø Study, 1994/95

Cystatin C quartiles	No. at risk	Events (n)	Crude incidence rate[c]	Age-adjusted incidence rate[c]
All-cause mortality				
Men (33 723 person-years)				
I (≤0.76 mg/L)	681	140	17.27 (14.41–20.13)	16.27 (14.80–17.70)
II (0.77–0.84 mg/L)	711	152	17.94 (15.09–20.81)	13.19 (12.06–14.36)**
III (0.85–0.94 mg/L)	728	171	18.29 (16.39–22.18)	12.08 (10.96–13.03)**
IV (≥0.95 mg/L)	732	263	31.78 (27.94–35.62)**	18.01 (15.95–21.26)
Women (39 024 person-years)				
I (≤0.73 mg/L)	760	68	7.17 (5.46–8.87)	5.96 (5.25–6.70)
II (0.74–0.82 mg/L)	815	116	11.35 (9.29–13.42)**	8.10 (7.00–9.01)*
III (0.83–0.92 mg/L	773	124	12.82 (10.57–15.08)**	6.52 (5.93–7.12)
IV (≥0.93 mg/L)	805	228	23.63 (20.57–26.70)**	9.06 (8.30–9.83)**
MI				
Men (26 960 person-years)				
I (≤0.76 mg/L)	681	57	8.99 (6.66–11.32)	7.10 (6.10–8.62)
II (0.77–0.84 mg/L)	711	87	13.41 (10.59–16.23)**	9.30 (8.18–10.23)
III (0.85–0.94 mg/L)	728	108	16.07 (13.04–19.10)**	10.63 (9.20–11.85)**
IV (≥0.95 mg/L)	732	126	19.65 (16.22–23.08)**	9.36 (8.45–10.00)*
Women (30 350 person-years)				
I (≤0.73 mg/L)	760	27	3.64 (2.26–5.01)	3.61 (3.05–4.16)
II (0.74–0.82 mg/L)	815	41	5.17 (3.59–6.75)	3.04 (2.58–3.52)
II (0.83–0.92 mg/L)	773	64	8.52 (6.43–10.60)**	5.28 (4.39–6.08)
IV (≥0.93 mg/L)	805	81	10.84 (8.48–13.20)**	4.21 (3.18–4.31)
Ischaemic stroke				
Men (25 961 person-years)				
I (≤0.76 mg/L)	681	34	5.36 (3.56–7.17)	4.60 (3.56–5.52)
II (0.77–0.84 mg/L)	711	35	5.40 (3.61–7.18)	3.50 (2.89–4.10)
III (0.85–0.94 mg/L)	728	30	4.46 (2.87–6.06)	2.26 (1.95–2.91)*
IV (≥0.95 mg/L)	732	71	11.07 (8.50–13.65)**	4.69 (4.19–5.40)
Women (30 350 person-years)				
I (≤0.73 mg/L)	760	15	2.02 (1.00–3.04)	1.59 (1.12–2.10)
II (0.74–0.82 mg/L)	815	28	3.53 (2.22–4.84)	2.52 (1.86–3.28)
III (0.83–0.92 mg/L	773	36	4.86 (3.27–6.44)*	3.52 (2.694.44)
IV (≥0.93 mg/L)	805	44	5.89 (4.15–7.63)**	2.21 (1.77–2.65)

[a]Gender-specific quartiles.
[b]MI and ischaemic stroke: first-ever non-fatal or fatal event.
[c]Incidence rates; events per 1000 person-years at risk.
*P < 0.05 (P-value for differences in incidence rates; lowest versus the other cystatin C quartiles, tested for by normal test with continuity correction).
**P < 0.001 (P-values for differences in incidence rates; lowest versus the other cystatin C quartiles, tested for by normal test with continuity correction).

$eGFR_{CKD-EPI}$ <60 mL/min/1.73m^2, and 5.1% reported having had a previous or present cancer diagnosis. Cystatin C levels were slightly higher in men (median [interquartile range]: 0.85 [0.77–0.95 mg/L]) compared with women (0.83 [0.74–0.93 mg/L], P < 0.001). Median $eGFR_{CKD-EPI}$ in men was 96 (88–102) mL/min/1.73m^2 and 93 (86–100) mL/min/1.73m^2 in women, P < 0.001. Baseline characteristics according to cystatin C quartiles are shown in Table 1. Compared to $eGFR_{CKD-EPI}$, cystatin C-based GFR estimates showed slightly higher values in the lowest cystatin C quartiles but lower values in the upper quartiles. GFR estimates according to Stevens and Arnal–Dade showed similar patterns in men; however, Arnal–Dade GFR estimates were higher in women compared to Stevens formula (Table 1). Increasing cystatin C levels were associated with older age and more unfavourable cardiovascular risk profile. After controlling for age (ANCOVA), the interquartile differences persisted for HDL cholesterol (P < 0.001) and hs-CRP (P < 0.001) in men and HDL cholesterol (P < 0.001), hs-CRP (P < 0.001) and BMI (P < 0.001) in women.

Correlations

Correlations of cystatin C with $eGFR_{CKD-EPI}$ were r = −0.60 for men, r = −0.58 for women, P < 0.001 for both. Correlation coefficients for cystatin C and age were r = 0.28 in men, r = 0.38 in women and r = 0.33 overall (men and women). Corresponding correlations of $eGFR_{CKD-EPI}$ with age were r = −0.55 (men), r = −0.58 (women) and r = −0.56 (men and women). Cystatin C was also correlated with hs-CRP (men: r = 0.17 and women: r = 0.21) and with previous or present cancer diagnosis (men: r = 0.11, women: r = 0.09 and P < 0.001 for all).

Significant interactions with sex in their associations with cystatin C were found for age, BMI and SBP.

Event rates

Numbers of events during follow-up were 591 (9.8%) first-ever fatal or non-fatal MI, 293 (4.9%) fatal or non-fatal ischaemic strokes and 1262 (21%) deaths from all causes. Median observation time was 10.6 years for MI and ischaemic stroke (for both men and women) and 13.7

Table 3. HRs[a,b] for all-cause mortality, first-ever MI and ischaemic stroke by gender and cystatin C quartiles: the Tromsø Study, 1994/95

Cystatin C quartiles	*N*	Incidence rate[c]	HR (95% CI)		
			Unadjusted	Age adjusted	Multivariable adjusted[d]
All-cause mortality					
Men (726 events)					
I (≤0.76 mg/L)	681	17.4	1 (reference)	1	1
II (0.77–0.84 mg/L)	711	17.8	1.04 (0.83–1.31)	0.87 (0.69–1.09)	0.82 (0.65–1.04)
III (0.85–0.94 mg/L)	728	18.8	1.12 (1.89–1.40)	0.79 (0.63–0.99)	0.68 (0.54–0.86)
IV (≥0.95 mg/L)	732	32.4	1.87 (1.53–2.30)	0.93 (0.75–1.15)	0.79 (0.63–0.99)
P for trend			<0.001	0.56	0.04
Women (536 events)					
I (≤0.73 mg/L)	760	7.2	1	1	1
II (0.74–0.82 mg/L)	815	11.8	1.59 (1.18–2.14)	1.15 (0.85–1.56)	1.13 (0.83–1.53)
III (0.83–0.92 mg/L	773	12.8	1.80 (1.34–2.42)	1.11 (0.83–1.50)	1.07 (0.79–1.45)
IV (≥0.93 mg/L)	805	23.7	3.38 (2.58–4.44)	1.53 (1.16–2.02)	1.38 (1.04–1.84)
P for trend			<0.001	0.001	0.03
MI					
Men (378 events)					
I (≤0.76 mg/L)	681	9.0	1	1	1
II (0.77–0.84 mg/L)	711	13.6	1.50 (1.07–2.09)	1.35 (0.97–1.89)	1.26 (0.89–1.77)
III (0.85–0.94 mg/L)	728	15.8	1.79 (1.30–2.47)	1.49 (1.08–2.05)	1.35 (0.96–1.87)
IV (≥0.95 mg/L)	732	20.2	2.21 (1.61–3.01)	1.48 (1.07–2.05)	1.18 (0.85–1.66)
P for trend			<0.001	0.02	0.55
Women (213 events)					
I (≤0.73 mg/L)	760	3.6	1	1	1
II (0.74–0.82 mg/L)	815	5.2	1.43 (0.88–2.32)	1.07 (0.66–1.75)	0.98 (0.60–1.59)
III (0.83–0.92 mg/L)	773	8.5	2.36 (1.50–3.70)	1.53 (0.97–2.40)	1.43 (0.91–2.26)
IV (≥0.93 mg/L)	805	11.4	3.02 (1.95–4.66)	1.49 (0.95–2.34)	1.20 (0.76–1.90)
P for trend			<0.001	0.03	0.25
Ischaemic stroke					
Men (170 events)					
I (≤0.76 mg/L)	681	5.4	1	1	1
II (0.77–0.84 mg/L)	711	4.9	0.99 (0.62–1.58)	0.84 (0.53–1.35)	0.76 (0.48–1.23)
III (0.85–0.94 mg/L)	728	4.3	0.80 (0.49–1.31)	0.60 (0.37–0.98)	0.52 (0.31–0.85)
IV (≥0.95 mg/L)	732	11.1	2.09 (1.39–3.14)	1.15 (0.75–1.78)	0.90 (0.57–1.40)
P for trend			<0.001	0.49	0.99
Women (123 events)					
I (≤0.73 mg/L)	760	2.2	1	1	1
II (0.74–0.82 mg/L)	815	3.7	1.75 (0.94–3.28)	1.29 (0.69–2.41)	1.42 (0.75–2.71)
III (0.83–0.92 mg/L	773	4.7	2.36 (1.29–4.13)	1.46 (0.80–2.67)	1.59 (0.85–2.97)
IV (≥0.93 mg/L)	805	5.9	2.88 (1.61–5.18)	1.27 (0.70–2.33)	1.29 (0.69–2.41)
P for trend			<0.001	0.51	0.56

[a]Gender-specific quartiles.
[b]Median observation time was 13.6 years for all-cause mortality and 10.6 years for fatal or non-fatal MI and ischaemic stroke.
[c]Events per 1000 person-years at risk.
[d]Multivariable adjustment; adjusted for age, BMI, hs-CRP, SBP, hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or self-reported use of anti-hypertensive medication), HDL-cholesterol, total cholesterol, smoking habits and physical activity.

years (men) and 13.8 years (women) for all-cause mortality. In women, age-adjusted incidence rate for all-cause mortality was increased in the upper cystatin C quartile (≥0.93 mg/L) compared to the lower quartile (≤0.73 mg/L) (Table 2). In contrast, age-adjusted mortality rate in men was not significantly elevated in the fourth cystatin C quartile (≥0.95 mg/L), and the mortality rates were lower in the second and third quartiles compared with the first quartile (≤0.76 mg/L). For MI, age-adjusted incidence rates were elevated in the third and fourth quartiles in men, not in women. Incidence rates for ischaemic stroke did not increase across cystatin C quartiles in any genders after age adjustment.

Associations between cystatin C quartiles and outcome variables

Cystatin C levels in the upper quartile were associated with increased all-cause mortality in women, and there was a significant linear trend across the quartiles (Table 3). Cystatin C in the fourth quartile was associated with 53% increased risk of death as compared to the lowest quartile, after adjustment for age, and 38% increased risk after multivariable adjustments. These associations were not observed in men. Significant interaction between gender and the dichotomized variable 'upper versus other cystatin C quartiles' was observed for all-cause mortality (P = 0.04). Exclusion of persons who reported a present or previous cancer diagnosis did not alter the associations observed for all-cause mortality. Cystatin C was not associated with MI in either gender after adjustments for age or cardiovascular risk factors. No associations were observed between the upper cystatin C quartiles and stroke in multivariable analyses. In pooled analyses of the mixed population, no significant associations between cystatin C and any of the end points were observed after multivariable adjustments [MI: hazard ratio (HR), 95% confidence interval (CI) 1.14, 0.88–1.49;

Table 4. Associations of cystatin C and creatinine-based estimation of renal function with adverse cardiovascular events[a] and all-cause mortality in men ($n = 2852$) and Women ($n = 3153$): the Tromsø Study 1994/95

	HR (95% CI)[b]					
	Cystatin C		Creatinine		Estimated GFR$_{CKD-EPI}^{c}$	
	Men	Women	Men	Women	Men	Women
All-cause mortality						
Unadjusted	1.23 (1.18–1.29)	1.36 (1.29–1.43)	0.98 (0.91–1.06)	1.08 (0.99–1.18)	1.30 (1.25–1.33)	1.32 (1.28–1.38)
Age adjusted	1.07 (1.01–1.14)	1.15 (1.07–1.23)	0.89 (0.70–0.96)	1.00 (0.91–1.07)	0.86 (0.75–0.97)	1.01 (0.90–1.11)
Multivariable adjustment[d]	1.02 (0.96–1.09)	1.09 (1.01–1.18)	0.93 (0.86–0.99)	1.01 (0.93–1.09)	0.90 (0.80–1.01)	1.03 (0.93–1.12)
MI						
Unadjusted	1.20 (1.14–1.27)	1.28 (1.17–1.41)	1.13 (1.03–1.24)	1.07 (0.93–1.22)	1.27 (1.20–1.33)	1.30 (1.22–1.39)
Age adjusted	1.13 (1.05–1.22)	1.06 (0.93–1.20)	1.06 (0.97–1.17)	0.98 (0.86–1.12)	1.07 (0.95–1.18)	1.03 (0.84–1.17)
Multivariable adjustment[d]	1.07 (0.99–1.17)	0.97 (0.85–1.11)	1.04 (0.94–1.14)	0.97 (0.86–1.11)	1.03 (0.89–1.14)	1.00 (0.81–1.10)
Ischaemic stroke						
Unadjusted	1.21 (1.11–1.32)	1.28 (1.13–1.45)	1.09 (0.95–1.27)	1.01 (0.85–1.22)	1.27 (1.15–1.38)	1.12 (0.95–1.26)
Age adjusted	1.07 (0.95–1.22)	1.02 (0.86–1.20)	1.01 (0.87–1.17)	0.93 (0.78–1.11)	1.10 (0.92–1.25)	0.84 (0.52–1.09)
Multivariable adjustment[d]	1.02 (0.89–1.17)	1.00 (0.84–1.19)	1.01 (0.87–1.16)	0.95 (0.79–1.13)	1.08 (0.90–1.22)	0.86 (0.56–1.09)

[a]Cardiovascular events: first-ever non-fatal and fatal MI or ischaemic stroke.
[b]Cox proportional HRs per SD increase in cystatin C (0.17 mg/L) and creatinine (15.7 µmol/L) concentrations and per SD (13.2 mL/min/1.73m^2) fall into creatinine-based estimation of GFR.
[c]eGFR CKD-EPI: estimated glomerular filtration rate (CKD-EPI formula [24]).
[d]Multivariable adjustment: adjusted for age, BMI, hs-CRP, SBP, hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg and/or self-reported use of anti-hypertensive medication), HDL-cholesterol, total cholesterol, smoking habits and physical activity.

ischaemic stroke: HR, 95% CI 0.91, 0.65–1.29; all-cause mortality: HR, 95% CI 1.02, 0.86–1.22, highest versus lowest cystatin C quartiles].

Associations between different markers of renal function and outcome variables

In age-adjusted analyses, an SD increase in cystatin C (SD = 0.17 mg/L) was associated with 15% increased risk of mortality in women (Table 4). Significance persisted after multivariable adjustments (HR, 95% CI 1.09, 1.01–1.18) and the HR was unchanged in reanalysis where persons with self-reported cancer diagnosis were excluded. No independent associations could be detected for cystatin C and MI or ischaemic stroke after multivariable adjustments. An inverse association with all-cause mortality was observed in men when renal function was measured as creatinine. One SD (16 µmol/L) fall in creatinine was associated with a ~7% higher risk of death, with a significant quadratic term of creatinine (P < 0.001). Other association between creatinine and creatinine-based eGFR with end points was all non-significant after multivariable adjustments.

In pooled analyses of both genders, HRs and 95% CI after multivariable adjustments for 1 SD increase in cystatin C (0.17 mg/L) were 1.05, 0.97–1.13 for MI; 1.00, 0.90–1.13 for ischaemic stoke and 1.05, 0.99–1.10 for all-cause mortality (all non-significant). Corresponding values for 1 SD fall in eGFR$_{CKD-EPI}$ (13 mL/min/1.73m^2) were 0.97, 0.94–1.03 for MI; 1.02, 0.81–1.12 for ischaemic stroke and 1.03, 0.90–1.04 for all-cause mortality.

Discussion

In the present study of a general population where persons with diabetes or previously known CVD were excluded and where cystatin C levels were close to normal range, we found that in women, cystatin C levels in the upper quartile (≥0.93 mg/L) were associated with a 38% increased risk for all-cause mortality as compared with the lowest quartile after adjustments for traditional cardiovascular risk factors. This was not observed in men or in overall analyses of men and women together.

In both genders, the associations between cystatin C levels and MI or ischaemic stroke were not significant after multivariable adjustments. In women, an increase in cystatin C of 0.17 mg/L was associated with a 15% increased risk of death from all causes after age adjustment and 9% increased risk after multivariable adjustments. This could not be detected by creatinine-based estimation of renal function.

The earliest cystatin C studies were based on selected cohorts and suggested no gender differences in cystatin C levels [7, 13], and cystatin C was reported to be associated with both all-cause mortality and cardiovascular risk [13, 19, 20, 22]. In the present study, a slightly lower cystatin C level was observed in women compared with men, similar to the finding in a general population of US adults (NHANES III) [31]. This gender difference may partly relate to higher cystatin C production rate with increasing body mass, but other factors probably also play a role since the gender difference persisted after adjustment for height and weight. The finding that cystatin C, even at concentrations within the reference range, was associated with increased mortality risk in women, but not in men, is not obvious. A significant interaction with gender was observed for the association of cystatin C with age, SBP and BMI. Gender differences in colinearity of cystatin C and other factors linked with pathophysiological processes that influence the mortality risk, possibly independent of GFR, probably play a role. The cardiovascular risk profile worsened across cystatin C quartiles in both men and

women. Processes linked either with renal function or with non-GFR mechanisms, such as chronic inflammation influencing cystatin C turnover rate, could be of prognostic importance in women at an earlier stage than in men. In a recent study [32] where cystatin C-based GFR estimation was adjusted for by iohexol-measured GFR preformed in 1620 persons from the Tromsø Study, we found that $eGFR_{CYST\ C}$, in addition to true GFR, depended on several cardiovascular risk factors, especially in women. Cystatin C may mirror these risk factors differently in men and women, especially in persons with GFR levels close to normal, as in the present study.

Cystatin C was reported to be an independent predictor of both all-cause mortality as well as CVD in elderly persons [13] and among persons with known CVD [19, 20] as well as in the general population (NHANES III, US population) [22, 33]. In the present study, no independent association between cystatin C and CVD was observed, although cystatin C was a strong predictor in unadjusted models. But the effect depended on age, traditional cardiovascular risk factors and hs-CRP. This finding was unexpected and in contrast to a large number of previous studies [13–15, 17–19, 22]. The discrepancy may be related to differences in the study populations. Previous studies are mostly based on cohorts from the US population [14, 17, 19, 21, 22, 33], and in these studies, cystatin C predicted unfavourable outcome in multivariable analyses at levels between 1.00 [13, 22] and 1.20–1.40 mg/L [15, 19]. In the present study, only 15% had cystatin C levels >1.00 mg/L and 7% had levels >1.10 mg/L. In contrast to another Norwegian population study where GFR <60 mL/min/1.73m^2 was observed in 4.4% of the population [34], only 2.7% had CKD Stage 3 or higher in the present study. Reasons for this may be that persons with known previous CVD and diabetes were excluded in the present study and that Tromsø is a University City, with a high number of academics among the inhabitants. This may have influenced GFR levels and the clustering of cardiovascular risk factors. We have studied the role of cystatin C at levels mainly within the normal range (0.40–1.00 mg/L), and this may explain why our results differ from previous studies. The narrow variation in cystatin C may also have weakened the associations with end points. However, risk factors did become more unfavourable with increasing cystatin C levels in the present study, also after controlling for age. This implies that the development of low-grade renal dysfunction and increased cardiovascular risk are mediated through common pathophysiological processes, which is in line with the finding that cystatin C-related risk for future CV events depended on traditional cardiovascular risk factors or chronic inflammation, common for the development of both atherosclerosis and renal dysfunction. For clinical purpose, cystatin C may still be a useful marker for cardiovascular risk estimation.

We observed no increased risk of ischaemic stroke with increasing cystatin C levels. On the contrary, a risk reduction seemed to be present in the third quartile among men. This finding was probably due to lower event rates for this end point. In elderly cohorts, cystatin C has been associated with stroke [13, 35], but HRs were lower than those for MI.

It has previously been shown that $eGFR_{CKD-EPI}$ is predictive for CVD only at levels <60 mL/min/1.73m^2 [2, 13, 22], and most studies find that cystatin C is a more sensitive and stronger predictor than are creatinine and $eGFR_{CKD-EPI}$ for cardiovascular events and all-cause mortality [13, 21, 22]. In the present study where renal function was close to normal, no independent associations were observed between creatinine/$eGFR_{CKD-EPI}$ and CVD, as expected. An inverse relationship was observed between creatinine and all-cause mortality in men, with significant test of non-linearity.

This was also seen when the Modification of Diet and Renal Disease (MDRD) Study formula ($eGFR_{MDRD}$) [36] was used for GFR estimation (data not shown), but the inverse relation between renal function and all-cause mortality in men was weakened and not significant (P = 0.09) when eGFR was calculated according to the CKD-EPI formula. Astor *et al.* [22] reported a U-shaped relationship between $eGFR_{MDRD}$ and all-cause mortality, where eGFR >120 mL/min/1.73m^2 was associated with a 36% increment in mortality risk. Shlipak *et al.* [13] reported similar findings. A J-shaped relation was observed between creatinine levels and all-cause mortality in elderly men, where levels <75 μmol/L were associated with higher mortality rates. This phenomenon is likely due to high degree of co-morbidity, associated with muscle wasting and reduced generation of creatinine and false over-estimation of GFR [4].

In contrast to creatinine-based assessment of renal function, a modest increase in cystatin C of 0.17 mg/L was associated with a 15% increased all-cause mortality risk in women (age-adjusted data). Cystatin C may capture subtle changes in GFR earlier than creatinine-based estimates of renal function, or alternatively, cystatin C may also be a marker of chronic inflammation, independently of GFR, due to the effects of various risk factors.

The strength of the present study is the prospective population-based design with longer observation time than previous studies of cystatin C. Gender-specific analyses are performed in a population without pre-existing CVD or other conditions known to influence cardiovascular risk and mortality, such as diabetes. A major strength is also, in contrast to other studies where events were obtained from national cause-of-death registries, that cardiovascular end points in the present study were obtained through thorough review of medical records where each event was confirmed separately by an independent end point committee consisting of clinical specialists. The long observation time in the present study could possibly be an explanation for the discrepancy of our results compared with previous studies, regarding the role of cystatin C as marker of future CVD [13–15, 7–9, 22, 33]. However, this was not the case, as subanalyses of the periods from 1994/95 to 2000 and 2001 to 2005 showed similar results as observed in the total time period, with independent associations of cystatin C only with all-cause mortality in women (data not shown).

In conclusion, we found that in a general population, with mainly normal renal function, cystatin C was associated with death from all causes in women but not in men or in the total study population. Neither cystatin C nor creatinine-based estimation of renal function was

independently associated with fatal and non-fatal MI or ischaemic stroke in either gender or in the complete cohort from the general population where persons with diabetes or previous CVD were excluded.

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