The cystatin C/creatinine ratio, a marker of glomerular filtration quality: associated factors, reference intervals, and prediction of morbidity and mortality in healthy seniors

METTE-TRIIN PURDE, STEFAN NOCK, LORENZ RISCH, PEDRO MEDINA ESCOBAR, CHRIS GREBHARDT, URS E. NYDEGGER, ZENO STANGA, and MARTIN RISCH

SCHAAN AND TRIESEN, LIECHTENSTEIN; TALLINN, ESTONIA; INNSBRUCK, AUSTRIA; AND LIEBEFELD, BERN, AND CHUR, SWITZERLAND

The ratio of cystatin C (cysC) to creatinine (crea) is regarded as a marker of glomerular filtration quality associated with cardiovascular morbidities. We sought to determine reference intervals for serum cysC-crea ratio in seniors. Furthermore, we sought to determine whether other low–molecular weight molecules exhibit a similar behavior in individuals with altered glomerular filtration quality. Finally, we investigated associations with adverse outcomes. A total of 1382 subjectively healthy Swiss volunteers aged 60 years or older were enrolled in the study. Reference intervals were calculated according to Clinical & Laboratory Standards Institute (CLSI) guideline EP28-A3c. After a baseline exam, a 4-year follow-up survey recorded information about overall morbidity and mortality. The cysC-crea ratio (mean 0.0124 ± 0.0026 mg/μmol) was significantly higher in women and increased progressively with age. Other associated factors were hemoglobin A1c, mean arterial pressure, and C-reactive protein ($P < 0.05$ for all). Participants exhibiting shrunken pore syndrome had significantly higher ratios of 3.5–66.5 kDa molecules (brain natriuretic peptide, parathyroid hormone, β_2-microglobulin, cystatin C, retinol-binding protein, thyroid-stimulating hormone, α_1-acid glycoprotein, lipase, amylase, prealbumin, and albumin) and creatinine. There was no such difference in the ratios of very low–molecular weight molecules (urea, uric acid) to creatinine or in the ratios of molecules larger than 66.5 kDa (transferrin, haptoglobin) to creatinine. The cysC-crea ratio was significantly predictive of mortality and subjective overall morbidity at follow-up in logistic regression models adjusting for several factors. The cysC-crea ratio exhibits age- and sex-specific reference intervals in seniors. In conclusion, the cysC-crea ratio may indicate the relative retention of biologically active low–molecular weight compounds and can independently predict the risk for overall mortality and morbidity in the elderly. (Translational Research 2016;169:80–90)

Abbreviations: BMI = body mass index; BNP = brain natriuretic peptide; BUN = blood urea nitrogen; CAPA = Caucasian, asian, pediatric and adult cohort; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration; CLSI = Clinical & Laboratory Standards Institute; Crea = creatinine; CysC = cystatin C; GFR = glomerular filtration rate; HDL = high density lipoprotein; IDMS

From the Labormedizinisches Zentrum Dr. Risch, Schaan, Liechtenstein; Tallinn University of Technology, Faculty of Science, Tallinn, Estonia; Private University, Triesen, Liechtenstein; Division of Clinical Biochemistry, Medical University Innsbruck, Innsbruck, Austria; Labormedizinisches Zentrum Dr. Risch, Liebefeld, Switzerland; Department of Endocrinology, Diabetes and Clinical Nutrition, Bern University Hospital, and University of Bern, Bern, Switzerland; Kantonsspital Graubünden, Zentrallabor, Chur, Switzerland.

Mette-Triin Purde and Stefan Nock contributed equally to this work.

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Reprint requests: Martin Risch, Kantonsspital Graubünden, Zentrallabor, Loëstrasse 170, 7000 Chur, Switzerland; e-mail: martin.risch@ksgr.ch.

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= isotope dilution mass spectrometry; LDL = low density lipoprotein; LM = Lund-Malmö; PTH = parathyroid hormone; SPS = shrunken pore syndrome; TSH = thyroid-stimulating hormone

AT A GLANCE COMMENTARY

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Background

The cystatin C/creatinine ratio has been suggested to capture alterations in glomerular filtration quality. Such changes occur due to the shrinking of glomerular pores, which leaves filtration of very small molecules such as creatinine unimpeded, whereas low–molecular weight proteins are retained in circulation because of their diameter.

Translational Significance

Not only renal function markers but also hormonally active proteins known to be associated with mortality are selectively retained in a disorder called ''shrunken pore syndrome''. An alteration in glomerular filtration quality represents an independent risk factor for morbidity and mortality. The reference intervals for the cystatin C/creatinine ratio reported in this article will allow for a potential clinical use of the investigated ratio.

INTRODUCTION

The human body clears the blood of substances 66 kDa and smaller by glomerular filtration. The structure of the glomerular filtration barrier is complex, and there is no generally accepted 3-D model available.[1] Creatinine is the most readily used endogenous marker for estimating the glomerular filtration rate (GFR), but serum creatinine is influenced by a number of nonrenal factors, such as age, sex, muscle mass, and diet.[2-4] Creatinine is a 113 Da breakdown product of creatine phosphate in muscle and is produced at a fairly constant rate; it is freely filtered across the glomerular membrane, actively secreted in tubules, and is not reabsorbed.[5] When GFR decreases, the tubular secretion of creatinine increases; therefore, a mild degree of kidney dysfunction (<50% reduction in GFR) will not increase the serum creatinine concentration above the upper limit of normal values.[6]

Compared with creatinine, cystatin C is better correlated with GFR and is a more accurate predictor of several clinical outcomes.[7-9] Cystatin C is a 13.3 kDa cysteine protease inhibitor produced by all nucleated cells.[10] It is freely filtered across the glomerular membrane, reabsorbed, and catabolized by the tubules.[11-13] The serum level of cystatin C is also influenced by nonrenal factors, such as steroid medication and thyroid dysfunction.[14,15] However, the cystatin C concentration is considered a better predictor of adverse outcomes compared with directly measured GFR, and this may be because these nonrenal influences enable a superior estimate of actual kidney function.[16]

Because the levels of the 2 markers are affected by different nonrenal factors, their ratio varies. The ratio of cystatin C and creatinine in serum (cysC-crea ratio) is substantially increased in a condition called "shrunken pore syndrome", which is characterized by very different GFR estimates based on the 2 markers ($eGFR_{Cys} \leq 60\%\ eGFR_{Crea}$).[1] Shrinking of the glomerular pores is thought to occur in certain conditions, for example, pregnancy, and is particularly pronounced in cases of preeclampsia.[1,17] Although GFR initially remains normal (and even increases in normal pregnancy), the composition of the glomerular filtrate changes because larger molecules can no longer pass through the pores.[18] Consequently, the serum concentrations of larger molecules increase. Because cystatin C is larger than creatinine, its plasma levels begin to increase first.[19,20] Therefore, an increased cysC-crea ratio indicates a change in glomerular filtration quality that suggests early-stage kidney dysfunction.

The present work aimed to define factors associated with the cysC-crea ratio. We also evaluated reference intervals for this ratio in the elderly. Furthermore, we aimed to explore whether other low–molecular weight molecules display a similar behavior to cystatin C relative to creatinine. Finally, we investigated the cysC-crea ratio as a predictor of morbidity and mortality in healthy elderly individuals.

METHODS

Study population. The present study was conducted within the framework of the SENIORLAB study, the primary aim of which is to establish reference intervals for several laboratory parameters for the elderly (www.seniorlabor.ch). The study consecutively enrolled subjectively healthy elderly volunteers from May 2009 to December 2011. Potential study participants were contacted through newspaper advertisements, various associations with high proportions of healthy

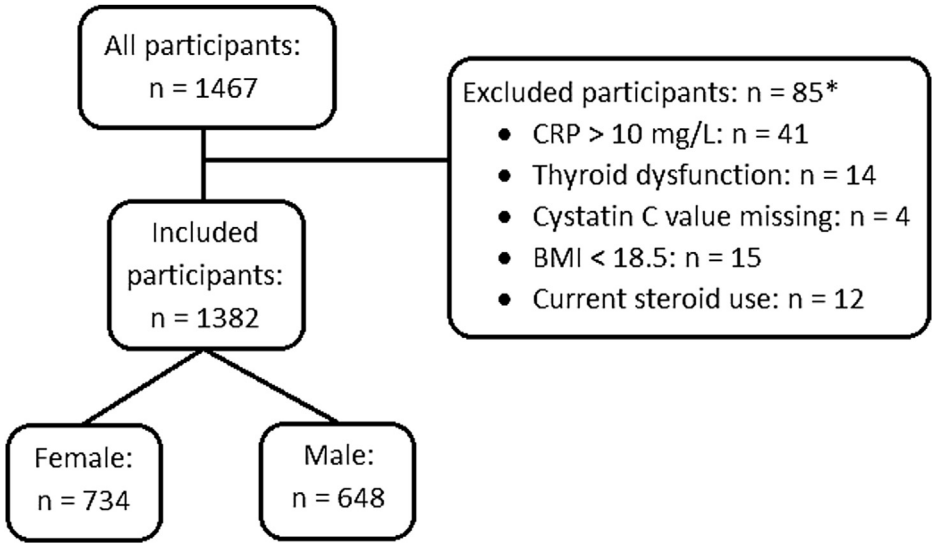

Fig 1. Chart representing all recruited participants, including those excluded from the study (n = number of participants). *One participant fulfilled 2 exclusion criteria. BMI, body mass index; CRP, C-reactive protein.

elderly members (eg, alpine clubs and sports clubs), and personal contacts of the study collaborators. Inclusion criteria were as follows: age of at least 60 years, residence in Switzerland, and a subjective perception of health. The exclusion criteria for the present study were as follows: current steroid use, thyroid dysfunction (free-thyroxin level <9 or >19 pmol/L), C-reactive protein (CRP) concentration above 10 mg/L, and underweight (body mass index [BMI] <18.5 kg/m^2). The patient inclusion process is illustrated in Fig 1. The exclusion criteria were applied to reduce confounding nonrenal influences on creatinine and cystatin C.[21] We considered underweight a surrogate marker for low–muscle mass and/or inadequate nutrition. The study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the responsible institutional review board (Kantonale Ethikkommission Bern). Written informed consent was obtained from all patients participating in the study.

Data collection. The participants' personal histories and anthropometric measurements, such as height and weight, were collected at the baseline exam. Blood pressure was measured in a sitting position after a 10-minute rest. Venous blood was drawn into S-Monovettes (Sarstedt, Sevelen, Switzerland) after an overnight fasting period. Blood samples were processed (centrifuged, aliquoted, and analyzed or frozen at −80°C) immediately to enable standardized preanalytics. A follow-up survey recorded information about subjective well-being and mortality. Overall morbidity was defined as a negative answer to the question of whether the patient was feeling healthy. For the follow-up examination, study participants were contacted by mail and telephone. In case of a lack of response, official communal authorities, relatives, and/or neighbors were contacted.

Laboratory methods. Laboratory parameters were determined using various analytical platforms. Serum cystatin C levels were assessed using a nephelometric method on a Siemens ProSpec (Siemens, Zurich, Switzerland) and standardized to the International Federation of Clinical Chemistry and Laboratory Medicine standard, as described by Inker et al[22,23] The isotope dilution mass spectrometry (IDMS)-standardized serum creatinine concentration was determined by a modified Jaffe method on a Cobas Integra 800 instrument (Roche Diagnostics, Risch-Rotkreuz, Switzerland). Commercially available materials were used for quality control. The interday coefficients of variation were as follows: 3.75% at 0.37 mg/L and 3.43% at 0.44 mg/L for cystatin C and 4.27% at 42 μmol/L and 1.96% at 556 μmol/L for creatinine. The levels of high-sensitivity CRP, retinol-binding protein, α_1-acid glycoprotein, and haptoglobin were determined on a Siemens ProSpec (Siemens). The hemoglobin A1c level was assessed by high-performance liquid chromatography (Bio-Rad D-10; Pratteln, Switzerland). Total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, blood urea nitrogen (BUN), uric acid, lipase, pancreatic amylase, prealbumin, and transferrin were measured on a Cobas Integra 800, whereas brain natriuretic peptide (BNP) and thyroid-stimulating hormone (TSH) were assessed using an Architect i4000 instrument (Abbott, Baar,

Switzerland). Parathyroid hormone (PTH) and β_2-microglobulin were assayed by an Immulite 2000 analyzer (Diagnostics Products Corporation, DPC; Bühlmann Laboratories, Allschwil, Switzerland).

Estimated GFR (eGFR) was calculated according to Nyman et al by calculating the arithmetic mean between $eGFR_{Cys}$ (as obtained from the Caucasian, Asian, Pediatrics, and Adults cohort's, CAPA equation) and $eGFR_{Crea}$ (as obtained from the Lund-Malmö revised equation, i.e. LM_{rev}; $eGFR_{CAPA+LM_{rev}}$).[24-26] This average $eGFR_{CAPA+LM_{rev}}$ has been shown to display a better diagnostic performance in a European population than the combined Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI 2012) equation.[27] In the article by Björk et al,[27,28] it has also been shown that even when $eGFR_{Cys}$ and $eGFR_{Crea}$ differ considerably, the average generally remains a very good estimate of measured GFR. In addition, to estimate the prevalence and sequelae of shrunken pore syndrome, $eGFR_{Cys}$ and $eGFR_{Crea}$ were calculated separately according to the CKD-EPI equations and according to the LM_{rev} equation (for $eGFR_{Crea}$) and the CAPA (for $eGFR_{Cys}$) equation.[25,26,29,30]

Statistical analysis. We used several descriptive and inferential statistical methods. To evaluate the univariate associations between continuous variables, Spearman rank correlations were used. Continuous variables were compared using the Mann-Whitney U test for correlations between 2 groups and using the Kruskal-Wallis test for correlations among 3 or more groups. Proportions were compared using the χ^2 test.

Double-sided 95% reference intervals for the cysC-crea ratio were calculated for participants with eGFR >60 mL/min/1.73 m^2 and $eGFR_{Cys}$ differing by less than 40% from $eGFR_{Crea}$ by using the robust method, as recommended by the Clinical & Laboratory Standards Institute (CLSI) guideline EP28-A3c.[31] The criteria of eGFR <60 mL/min/1.73 m^2 and the presence of shrunken pore syndrome were applied to exclude participants with overt kidney disease from reference interval determination.[1,21] Outliers were eliminated according to Dixon and Reed et al.[31-33]

To determine whether molecules with different molecular weights are handled differently in patients with shrunken pore syndrome, the ratios of BUN (60 Da), uric acid (168 Da), BNP (3.5 kDa), PTH (9.4 kDa), β_2-microglobulin (11.7 kDa), cystatin C (13.3 kDa), retinol-binding protein (20.6 kDa), TSH (28 kDa), α_1-acid glycoprotein (40 kDa), lipase (47 kDa), prealbumin (55 kDa), pancreatic amylase (55.4 kDa), albumin (66.5 kDa), transferrin (78 kDa), and haptoglobin (>100 kDa) to creatinine were compared between participants with shrunken pore syndrome and controls matched for age (±2 years), sex, and eGFR (±8 mL/min/1.73 m^2).[34]

Linear regression models were fitted to evaluate the factors associated with the cysC-crea ratio. Here, several factors known to carry a cardiovascular risk were included as covariates. Finally, logistic regression models were fitted to evaluate predictors of overall morbidity at follow-up. Cox multivariate proportional hazards models were used to determine independent predictors of mortality. Finally, survival rates during follow-up were estimated by Kaplan-Meier curves for participants with and without shrunken pore syndrome, and comparisons were made by log-rank test. A sensitivity analysis using different methods of $eGFR_{Cys}/eGFR_{Crea}$ estimation (CKD-EPI vs CAPA/LM_{rev}) and different cutoffs (60%, 70%, and 80%) was used; $P < 0.05$ was considered significant. All calculations were performed with MedCalc version 14.12.0 (Mariakerke, Belgium) and Excel 2010 (Microsoft, Seattle, Wash). Figures were drawn with PowerPoint (Microsoft) and GraphPad Prism 5.04 (GraphPad Software, La Jolla, Calif).

RESULTS

Baseline characteristics. A total of 1382 participants with a mean age of 71.9 ± 7.8 years and a female proportion of 53.1% (734 females, 648 males) were included in the present analysis. The detailed characteristics of the study population are presented in Table I and reflect the expected spectrum of subjectively healthy seniors. Follow-up for survival after a mean of 3.7 ± 0.7 years was available for all participants (100%), and follow-up for morbidity was missing for only 0.4% (n = 5) of the surviving participants. At follow-up, 129 participants (9.3%) reported overall morbidity, and 58 participants (4.2%) died within the follow-up period after 2.2 ± 1.2 years.

The mean cysC-crea ratio was 0.0124 ± 0.0026 mg/μmol. There was a significant correlation between the cysC-crea ratio and age ($r = 0.33$; $P < 0.001$). Males had significantly lower ratios than females (0.0111 ± 0.0019 vs 0.0136 ± 0.0024 mg/μmol; $P < 0.001$). The ratio of cystatin C–based eGFR to creatinine-based eGFR ($eGFR_{Cys}/eGFR_{Crea}$) was higher in males (1.07 ± 0.18) than that in females (1.04 ± 0.17; $P = 0.001$) when CKD-EPI equations were used. The sex difference was different when using the CAPA and LM_{rev} equations (1.18 ± 0.19 in males vs 1.21 ± 0.19 in females; $P = 0.01$).

Shrunken pore syndrome, defined by Grubb et al[1] as an $eGFR_{Cys}$ <60% of $eGFR_{Crea}$, was observed in 10 participants (0.7%) by using the CKD-EPI equations. The participants with $eGFR_{Cys}/eGFR_{Crea}$ <0.6 were significantly older than those with $eGFR_{Cys}/eGFR_{Crea}$

Table I. Detailed characteristics of the investigated cohort

Characteristic	Mean ± SD or % (n)
Female	53.1 (734)
Age (y)	71.9 ± 7.8
BMI (kg/m^2)	25.5 ± 3.7
Systolic blood pressure (mmHg)	149 ± 23
Diastolic blood pressure (mmHg)	90 ± 13
Mean arterial pressure (mmHg)	110 ± 15
Pulse pressure (mmHg)	58 ± 16
Hemoglobin A1c (%)	5.9 ± 0.6
Antihypertensive use	37.8 (523)
Current smoker	6.7 (93)
Cholesterol (mmol/L)	5.75 ± 1.13
LDL cholesterol (mmol/L)	3.77 ± 1.04
HDL cholesterol (mmol/L)	1.66 ± 0.46
Creatinine (μmol/L)	77 ± 19
Cystatin C (mg/L)	0.93 ± 0.22
Cystatin C/creatinine (mg/μmol)	0.0124 ± 0.0026
CKD-EPI $eGFR_{Cys\ +\ Crea}$ (mL/min/1.73 m^2)	81 ± 16
CKD-EPI $eGFR_{Cys}$ (mL/min/1.73 m^2)	81 ± 19
CKD-EPI $eGFR_{Crea}$ (mL/min/1.73 m^2)	78 ± 14
$eGFR_{CAPA+}LM_{rev}$ (mL/min/1.73 m^2)	76 ± 14
CAPA $eGFR_{Cys}$ (mL/min/1.73 m^2)	82 ± 18
LM_{rev} $eGFR_{Crea}$ (mL/min/1.73 m^2)	69 ± 13
BNP (pg/mL)	72 ± 112
CRP (mg/L)	1.86 ± 1.83

Abbreviations: BMI, body mass index; *BNP*, brain natriuretic peptide; *CRP*, C-reactive protein; *eGFR*, estimated glomerular filtration rate; *LM_{rev}*, Lund-Malmö revised equation; *SD*, standard deviation. The mean ± SD is given when appropriate.

>0.6 (82.3 ± 8.7 vs 71.9 ± 7.7 years; $P < 0.001$). Of these 10 participants, 3 (30%) died, and 5 of the remaining 6 (83%; 1 with missing information) reported overall morbidity. Compared with participants with $eGFR_{Cys}/eGFR_{Crea}$ >0.6, participants with shrunken pore syndrome showed significantly higher morbidity and mortality ($P < 0.01$). When the CAPA/LM_{rev} equations were used, the prevalence of shrunken pore syndrome was lower than estimated with the CKD-EPI equations (3/1382; 0.2%). The distributions of the $eGFR_{Cys}/eGFR_{Crea}$, as estimated by CKD-EPI and CAPA/LM_{rev} equations, are given in Supplementary Fig 1 together with the prevalence of shrunken pore syndrome at different cutoffs for $eGFR_{Cys}/eGFR_{Crea}$. Furthermore, the distributions of the ratios between cystatin C and creatinine are also given.

Reference intervals. Reference intervals for the cysC-crea ratio were stratified according to age and sex. Females displayed higher values than those of males, and the lower and upper limits of the reference intervals increased with advancing age. Detailed reference intervals are presented in Table II. Respective reference intervals for CKD-EPI $eGFR_{Cys}/eGFR_{Crea}$ and CAPA/LM_{rev} estimates of $eGFR_{Cys}/eGFR_{Crea}$ are provided in Supplementary Tables I and II.

Associated factors. Several risk factors for cardiovascular disease were investigated for univariate associations with the cysC-crea ratio (Table III). There were no significant differences in the cysC-crea ratio because of current smoking ($P = 0.24$), anamnestic hypertension ($P = 0.73$), or antihypertensive drug use ($P = 0.11$). Conventional cardiovascular risk factors, with the exception of hemoglobin A1c and current smoking, were associated with the cysC-crea ratio in univariate analysis.

A linear regression model including the cysC-crea ratio as the dependent variable and several cardiovascular risk factors (age, sex, BMI, mean arterial pressure, pulse pressure, hemoglobin A1c, current smoking, HDL cholesterol, LDL cholesterol, eGFR, BNP, and CRP) as independent variables explained 37% of the variability (adjusted R^2) of the ratio. All predictors had a low variance inflation factor (<2), indicating the absence of collinearity in this model. Age ($P < 0.001$), male sex ($P < .001$), hemoglobin A1c ($P = 0.002$), mean arterial pressure ($P = 0.048$), and CRP ($P < 0.001$) were significant predictors of the cysC-crea ratio. HDL cholesterol ($P = 0.058$), BNP ($P = 0.06$), and pulse pressure ($P = 0.08$) were borderline significant predictors, whereas BMI ($P = 0.55$), $eGFR_{CAPA+LM_{rev}}$ ($P = 0.49$), current smoking ($P = 0.13$), and LDL cholesterol ($P = 0.28$) were not predictive.

Behavior of other low–molecular weight molecules. The 10 participants with shrunken pore syndrome had significantly higher molecule-to-creatinine ratios compared with the 40 controls matched for age, sex, and kidney function for molecules with a molecular weight between 3.5 and 66.5 kDa (Fig 2). There were no significant differences regarding age (85; interquartile range, [78–89] vs 85.5 [78–89] years), sex (20/40 males vs 5/10 males), BMI (24.9 [22.8–27.5] vs 24.3 [22.0–27.2] kg/m^2), CRP (1.93 [0.77–3.70] vs 1.86 [1.47–2.46] mg/L), or eGFR (54 [46–61] vs 53 [44–60] mL/min/1.73 m^2) between the controls and the cases. Interestingly, there were no such differences in these ratios for molecules with a molecular weight less than 200 Da or greater than 66.5 kDa (Table IV). The proteins displaying higher ratios relative to creatinine belong to the functional families of hormones, transport proteins, enzymes, kidney function markers, and acute phase reactants.

Predictivity for overall morbidity. Participants reporting morbidity after a mean of 3.7 ± 0.7 years of follow-up after reporting well-being at baseline had significantly higher cysC-crea ratios than those who remained well (0.0130 ± 0.003 vs 0.01231 ± 0.0024 mg/μmol; $P = 0.004$). A logistic regression model with morbidity at follow-up as the outcome variable and

Table II. Reference intervals for the serum cysC-crea ratio (mg/μmol) stratified according to age and sex

	Females			Males		
Age	Subjects	Lower limit	Upper limit	Subjects	Lower limit	Upper limit
60–64	130	0.009 (0.0087–0.0094)	0.0157 (0.0153–0.0161)	126	0.0072 (0.0068–0.0076)	0.0131 (0.0127–0.0135)
65–69	173	0.0088 (0.0084–0.0092)	0.0168 (0.0163–0.0173)	154	0.0073 (0.0069–0.0077)	0.0137 (0.0132–0.0141)
70–74	147	0.0092 (0.0087–0.0098)	0.0173 (0.0167–0.0179)	139	0.0082 (0.0079–0.0086)	0.0135 (0.0132–0.0139)
75–79	106	0.0093 (0.0085–0.0102)	0.0181 (0.0173–0.0189)	87	0.0071 (0.0063–0.0078)	0.0147 (0.0140–0.0155)
80–84	66	0.0105 (0.0097–0.0113)	0.0193 (0.0183–0.0202)	61	0.0085 (0.0079–0.0092)	0.0155 (0.0149–0.0161)
≥85	29	0.0090 (0.0075–0.0107)	0.0199 (0.0179–0.0217)	16	0.0079 (0.0067–0.0095)	0.0167 (0.0148–0.0182)

Upper and lower limits are provided with 90% confidence intervals in parentheses.

Table III. Spearman correlation coefficients (ρ) for the serum cysC-crea ratio and several cardiovascular risk factors

Parameter	ρ	*P* value
Age (y)	0.33	<0.001
BMI (kg/m^2)	−0.092	<0.001
Systolic blood pressure (mmHg)	0.08	0.005
Diastolic blood pressure (mmHg)	0.06	0.02
Mean arterial pressure (mmHg)	0.07	0.006
Pulse pressure (mmHg)	0.05	0.08
Hemoglobin A1c (%)	0.01	0.60
Cholesterol (mmol/L)	0.11	<0.001
LDL cholesterol (mmol/L)	0.09	0.001
HDL cholesterol (mmol/L)	0.16	<0.001
$eGFR_{CAPA+LM_{rev}}$ (mL/min/1.73 m^2)	−0.15	<0.001
BNP (pg/mL)	0.32	<0.001
CRP (mg/L)	0.15	<0.001

Abbreviations: BMI, body mass index; *BNP,* brain natriuretic peptide; *CRP,* C-reactive protein; *eGFR,* estimated glomerular filtration rate.

age (years), sex, BMI (kg/m^2), cysC-crea ratio (g/μmol), systolic as well as diastolic blood pressure (mm Hg), hemoglobin A1c (%), total cholesterol (mmol/L), current smoking, CRP (mg/L), BNP (mg/mL), and $eGFR_{CAPA+LM_{rev}}$ (mL/min/1.73 m^2) as the predictor variables exhibited a c-statistic of 0.66 (95% confidence interval [CI], 0.64–0.69). In this model, hemoglobin A1c ($P = 0.005$) and the cysC-crea ratio ($P = 0.046$) were significantly predictive of overall morbidity at follow-up. The odds ratios for these significant predictors were 1.41 (95% CI, 1.11–1.81) for hemoglobin A1c and 1.09 (95% CI, 1.001–1.19) for the cysC-crea ratio.

Analogous models were fit with $eGFR_{CysC}/eGFR_{Crea}$ instead of the cysC-crea ratio. For these 2 models, CKD-EPI equations and the CAPA and LM_{rev} equations, respectively, were used. Both models revealed only hemoglobin A1c as a significant predictor for overall morbidity ($P = 0.008$ in both) with an odds ratio of 1.40 (95% CI, 1.09–1.79) for the ratio between the CKD-EPI equations and an odds ratio of 1.39 (95% CI, 1.09–1.78) for the ratio between CAPA and LM_{rev} equations. The odds ratios for the $eGFR_{CysC}/eGFR_{Crea}$ were 1.0 (95% CI, 0.98–1.01) for CKD-EPI and 1.0 (95% CI, 0.99–1.01) for CAPA/LM_{rev}.

Predictivity for overall mortality. The 58 participants who died within the follow-up period had significantly higher cysC-crea ratios at baseline compared with the surviving participants (0.0138 ± 0.0035 vs 0.0124 ± 0.0025 mg/μmol; $P < 0.001$). A Cox proportional hazards regression model with age (years), sex, $eGFR_{CAPA+LM_{rev}}$ (mL/min/1.73 m^2), and the cysC-crea ratio (g/μmol) as predictor variables was significantly predictive of mortality ($P < 0.001$). In this model, the cysC-crea ratio (hazard ratio 1.11; 95% CI, 1.03–1.20; $P = 0.007$), age (hazard ratio 1.13; 95% CI, 1.08–1.18; $P < 0.001$), and male gender (hazard ratio 2.56; 95% CI, 1.46–4.48; $P = 0.001$) were significant independent predictors of mortality, whereas there was no such association with $eGFR_{CAPA+LM_{rev}}$ (hazard ratio 0.99; 95% CI, 0.97–1.01; $P = 0.4$).

Adding 1 additional cardiovascular risk factor to the age, sex, and eGFR-adjusted model for the prediction of death by the cysC-crea ratio in several models revealed that the significant relationship between the cysC-crea ratio and overall mortality remained intact when adjusting for BMI, mean arterial pressure, systolic blood pressure, diastolic blood pressure, BNP, hemoglobin A1c, known diabetes, CRP, pulse pressure, HDL, LDL, or current smoking. A Kaplan-Meier survival analysis for participants exhibiting shrunken pore syndrome defined at <60%, 70%, and 80% $eGFR_{Cys}/eGFR_{Crea}$ showed significantly lower survival in participants with shrunken pore syndrome, regardless of whether the ratio of $eGFR_{Cys}/eGFR_{Crea}$ was assessed by CKD-EPI (n = 10, $P < 0.001$ at <60%; n = 52, $P < 0.001$ at 70%; n = 92, $P < 0.001$ at 80%) or the CAPA and LM_{rev} equations (n = 3, $P = 0.004$ at

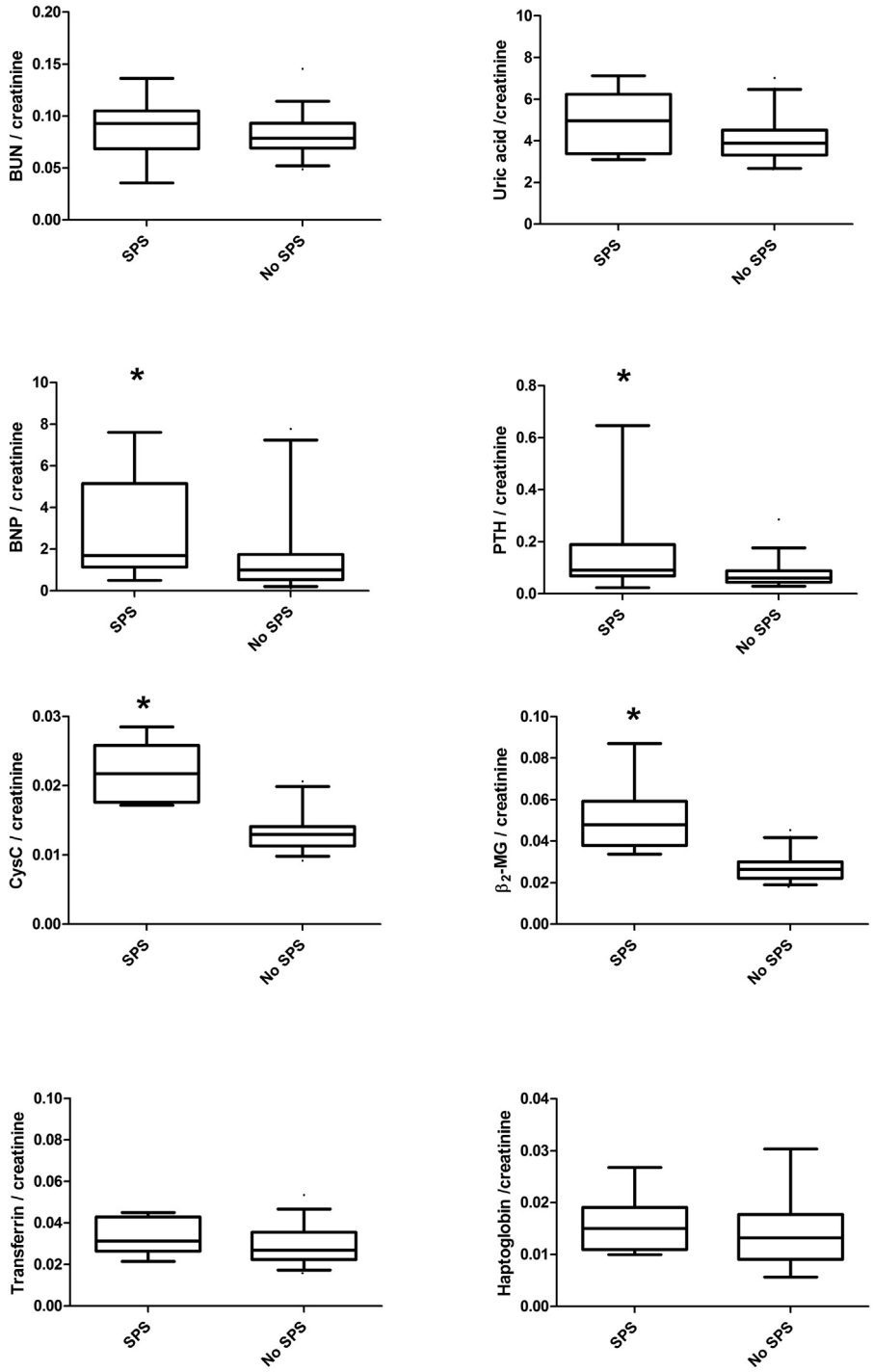

Fig 2. Ratios of different molecules to creatinine in participants with and without shrunken pore syndrome. The ratios were not different between molecules with lower and higher molecular weights. Significant differences are marked by asterisks. β_2-MG, β_2-microglobulin; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CysC, cystatin C; PTH, parathyroid hormone; SPS, shrunken pore syndrome. An asterisk indicates a significant difference ($P < 0.05$).

<60%; n = 4, $P = 0.01$ at 70%; n = 22, $P < 0.001$ at 80%).

The survival curves of participants with and without shrunken pore syndrome according to the different definitions are given in Fig 3. It can be observed that survival differences become larger when the cutoff for $eGFR_{Cys}/eGFR_{Crea}$ for the definition of shrunken pore syndrome is decreased. It also becomes apparent that

Table IV. Molecule-to-creatinine ratio (μmol/L) in 10 participants with shrunken pore syndrome (as obtained with CKD-EPI equations and a cutoff of <60% for definition of the syndrome) and 40 controls matched for age, sex, and eGFR

Parameter	Molecular weight	Participants with SPS	Participants without SPS	*P* value
Blood urea nitrogen (mmol/L)	60 Da	0.0927 (0.0704–0.105)	0.0785 (0.0699–0.0927)	0.11
Uric acid (μmol/L)	168 Da	4.969 (3.411–5.976)	3.894 (3.331–4.512)	0.08
BNP (pg/mL)	3.5 kDa	1.678 (1.222–5.127)	1.0 (0.567–1.741)	0.03
PTH (pmol/L)	9.4 kDa	0.0918 (0.0721–0.164)	0.061 (0.0457–0.0876)	0.03
β_2-microglobulin (mg/L)	11.7 kDa	0.048 (0.0381–0.0571)	0.0264 (0.0225–0.030)	<0.001
Cystatin C (mg/L)	13.3 kDa	0.0217 (0.0176–0.0258)	0.0130 (0.0114–0.0140)	<0.001
RBP (mg/L)	20.6 kDa	0.0687 (0.0623–0.0715)	0.0539 (0.0456–0.0676)	0.03
TSH (U/L)	28 kDa	0.0298 (0.0154–0.0413)	0.0138 (0.0118–0.0287)	0.04
α_1-acid glycoprotein (g/L)	40 kDa	0.0097 (0.0091–0.0111)	0.0085 (0.0068–0.0107)	0.04
Lipase (U/L)	47 kDa	0.601 (0.358–0.746)	0.357 (0.274–0.553)	0.04
Prealbumin (g/L)	55 kDa	0.0033 (0.0029–0.0034)	0.0026 (0.0021–0.0030)	0.02
Pancreatic amylase (U/L)	55.4 kDa	0.461 (0.382–0.580)	0.349 (0.203–0.481)	0.049
Albumin (g/L)	66.5 kDa	0.543 (0.519–0.655)	0.453 (0.366–0.519)	0.002
Transferrin (g/L)	78 kDa	0.0312 (0.0264–0.0427)	0.0259 (0.0224–0.0249)	0.13
Haptoglobin (g/L)	>100 kDa	0.0150 (0.0111–0.195)	0.0132 (0.0092–0.176)	0.39

Abbreviations: BNP, brain natriuretic peptide; *eGFR,* estimated glomerular filtration rate; *IQR,* interquartile range; *PTH,* parathyroid hormone; *RBP,* retinol-binding protein; *SPS,* shrunken pore syndrome; *TSH,* thyroid-stimulating hormone.
The ratios are given for different molecules with increasing molecular weight and are displayed as median and IQR showing the 25th and 75th percentiles.

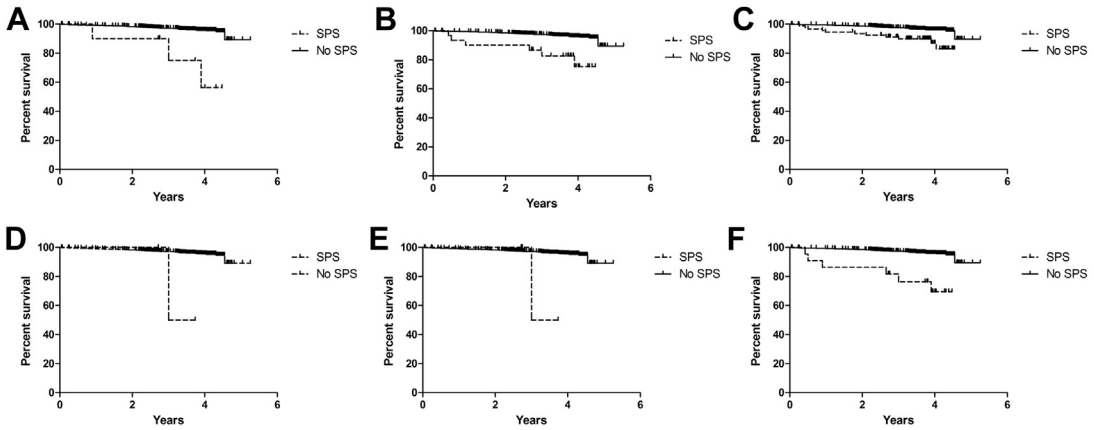

Fig 3. Survival curves are given for differences in survival among participants with and without SPS at different cutoffs of 60% (**A** and **C**), 70% (**B** and **E**), and 80% (**C** and **F**). $eGFR_{CysC}$ and $eGFR_{Crea}$ were estimated with CKD-EPI equations (**A**–**C**) and with CAPA and LM_{rev} equations (**D**–**F**). In all comparisons, the survival of participants with SPS is significantly worse than that of participants without SPS. Ticks represent censored cases. LM_{rev}, Lund-Malmö revised equation; SPS, shrunken pore syndrome.

shrunken pore syndrome is associated with survival differences at a cutoff of 80%, independent of the methods used for the estimation of GFR.

DISCUSSION

This study evaluated the reference intervals of the serum cystatin C/creatinine ratio in seniors aged 60 years and older. This ratio increased with advancing age and was higher in women than in men. Furthermore, the ratio of these 2 kidney function markers predicted overall mortality and morbidity after nearly 4 years of follow-up, even after adjustment of the regression models for kidney function and other covariates. Finally, in participants with shrunken pore syndrome, molecules with a molecular weight between 3.5 and 66.5 kDa showed relative retention similar to that of cystatin C. The proteins displaying higher ratios to creatinine belong to the functional families of hormones, transport proteins, enzymes, kidney function markers, and acute phase reactants.

The higher cysC-crea ratio among females and older participants was expected because serum creatinine is lower in females and older populations.[2,14] In a model adjusted for the measured GFR, Stevens et al[14] found the serum cystatin C concentration to be lower in women and to decrease with age, although to a lesser extent than creatinine. Therefore, the age-related increase in the cysC-crea ratio may partly be explained by an unequal decrease with age in the levels of markers.

However, other nonrenal factors exert a differential influence on cystatin C and creatinine.[14] The cysC-crea ratio increases with lower creatinine values and/or higher cystatin C levels. Lower creatinine levels occur in the presence of lower body weight, higher white blood cell count, and lower protein intake.[4,14] Higher cystatin C levels have been observed in the presence of diabetes, higher body weight, higher systolic blood pressure, and increased markers of inflammation—all known cardiovascular risk factors.[14]

The differential behavior of creatinine and cystatin C may be due to the influence of not only nonrenal factors but also factors that affect the quality of filtration of different substances in the glomerulus. Grubb et al[1,35] described the decrease in glomerular filtration quality as a pathophysiological phenomenon occurring because of the shrinking of the glomerular pores with subsequent unimpeded filtration of creatinine and the retention of 3 different low–molecular weight proteins in serum. This phenomenon suggests morphologic changes in the kidneys in these states at the glomerular filtration barrier. The structure of the glomerular filtration barrier is complex, and there is no generally accepted 3-D model.[1] Consequently, these morphologic changes—in contrast to biochemical changes—in shrunken pore syndrome have not yet been demonstrated by pathoanatomic methods.

It has been hypothesized that glomerular pores shrink in many renal diseases.[1,35] This changes the composition of glomerular filtrate, but measured GFR does not decrease. Proteins with a molecular weight up to approximately 20 kDa are excreted primarily by glomerular transport.[36] Owing to the pore shrinkage, the larger molecules can no longer traverse glomerular pores and exit the bloodstream. Therefore, their serum levels start to increase. A small reduction in pore size is identified by increased serum concentrations of these larger molecules. As the pores shrink further, smaller molecules are also retained and start to accumulate in the blood. Because cystatin C (13.3 kDa) is >100 times larger than creatinine (113 Da), it is retained at a much lower degree of shrinkage and can therefore indicate kidney disease at an earlier stage.

The ratio of serum cystatin C to creatinine has not yet been extensively researched. There are sporadic reports on glomerular filtration quality in different collectives.[1,35,37,38] To our knowledge, reference intervals have only been reported for pediatric patients.[37] The present study is the first to provide reference intervals for the cysC-crea ratio in seniors. In general, both the lower and upper limits of the reference intervals increased with age and were higher in the female study participants. The values observed in the study by Grubb et al[1] are within the range observed in our study. The median values for patients with shrunken pore syndrome reported by Grubb et al[1] and for the 10 participants with shrunken pore syndrome in the present study are above the upper limit of the reference intervals for all investigated age classes. We also provided reference intervals for $eGFR_{CysC}/eGFR_{Crea}$, which in both our study and another study performed in patients undergoing elective coronary artery bypass grafting has been demonstrated as an independent predictor of mortality.[39]

The prevalence of shrunken pore syndrome, as described by Grubb et al,[1] is low in the present cohort of healthy elderly people. However, patients with shrunken pore syndrome in our study cohort of healthy seniors had a significantly higher prevalence of morbidity and mortality. Further indication that shrunken pore syndrome might be a risk factor for morbidity can be obtained from the fact that shrunken pore syndrome occurs significantly more frequently in hospitalized patients (8.2%) than in healthy seniors (0.2%; $P < 0.0001$; χ^2 test).[1]

Our analyses have also shown that the prevalence of shrunken pore syndrome depends on the equations used for the calculation of $eGFR_{CysC}/eGFR_{Crea}$. As demonstrated by others,[39] the CKD-EPI formula results in higher prevalence compared with CAPA and LM_{rev} equations, which were originally used to characterize shrunken pore syndrome.[1] However, both sets of equations can indicate an increased risk for mortality. Interestingly, an increased risk for mortality was also present when cutoffs higher than the originally proposed cutoff of <60% were used for $eGFR_{CysC}/eGFR_{Crea}$, irrespective of the equations used. This observation is in line with the findings of Dashdati et al[39] in patients undergoing elective coronary artery bypass grafting. The magnitude of survival impairment in participants of the present study is also comparable to that observed in patients undergoing elective coronary artery bypass grafting.[39]

Interestingly, molecules with a very low–molecular weight, such as BUN and uric acid, did not show different filtration behaviors in participants with and without shrunken pore syndrome.[1] This result corroborates the findings of Grubb et al,[1] who hypothesized that molecules with a very low–molecular weight, such as creatinine, do not undergo impeded glomerular filtration in patients with shrunken pore syndrome.

We further confirmed that the levels of low–molecular weight proteins such as cystatin C and β_2-microglobulin are higher in individuals with shrunken pore syndrome than those in controls with normal glomerular filtration quality. Our observations indicate that the same mechanisms leading to their relative retention might affect not only the levels of kidney function markers but also of hormones, acute-phase reactants, transport proteins, and enzymes with a molecular weight up to 66.5 kDa. However, it remains unclear whether the increased BNP/creatinine, PTH/creatinine, and TSH/creatinine ratios are due to alterations in glomerular filtration quality or due to endocrinological feedback mechanisms.

In summary, we observed increased protein/creatinine ratios for proteins with a molecular weight of 3.5–66.5 kDa, which encompasses the range of molecular weights filtered by the renal glomeruli. We did not investigate β-trace protein, with a molecular weight between 23–29 kDa, which has also shown increased ratios in patients with shrunken pore syndrome.[1] Altogether, in patients with shrunken pore syndrome, low–molecular weight proteins up to approximately 66.5 kDa (but not very low–molecular weight metabolites such as BUN and uric acid) indicate altered glomerular filtration quality by their relative retention.

It has already been hypothesized that the discrepant behavior of creatinine and other markers of GFR may lead not only to retention of GFR markers but also to increases in other compounds with molecular weights similar to the low–molecular weight markers of kidney function, such as interleukin 6 and a variety of other proteins with signaling function (ie, cytokines, hormones, and growth factors).[1] These retained mediators are thought to lead to increased overall and disease-specific morbidity and mortality. Our study now demonstrates the relative retention of such signaling proteins, that is, BNP and PTH, which both have been shown to lead to higher all-cause mortality in the general population.[40-42] The relative retention of compounds other than kidney function markers offers a pathophysiological link for our observation of increased overall mortality and decreased well-being. Several low–molecular weight markers of GFR (ie, β_2-microglobulin, beta-trace protein, and cystatin C) improve risk prediction compared with creatinine.[43] The cysC-crea ratio may thus be a tool by which to estimate the retention of low–molecular weight proteins (ie, mediators) relative to creatinine that could be easily introduced to automatically generated laboratory reports.[28]

The main strengths of the study are its prospective design, the large number of included participants, complete follow-up, and the use of standardized laboratory results for measuring cystatin C and creatinine. All laboratory analyses were performed on freshly collected material. One limitation is the relatively low incidence of endpoints, which limited the use of covariates in the regression models. Another limitation is that we did not collect urine samples. Consequently, it was not possible to investigate the associations between the cysC-crea ratio and urinary parameters, such as albuminuria. Therefore, albuminuria could not be included as a covariate in the regression model. Finally, we only included Caucasian participants. The results can thus not be extrapolated to populations of different origin. However, we do not believe that these limitations invalidate our findings.

In conclusion, this study is the first to provide reference intervals for the serum cysC-crea ratio for seniors. Several associated factors were also identified. Finally, we identified the cysC-crea ratio as a predictor of mortality and morbidity. This result may be due to the selective retention of signaling molecules in individuals with diminished filtration quality.

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Conflicts of Interest: All authors confirm that they have read the journal's policy on disclosure of potential conflicts of interest. All authors declare that there are no conflicts of interest. Further, all authors have disclosed any financial or personal relationship with organizations that could potentially be perceived as influencing the described research.

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The article has been reviewed by and approved by all named authors.

The SENIORLAB study is registered in the ISRCTN registry (ISRCTN53778569).

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Appendix

Supplementary Table 1. Reference intervals for the CKD-EPI $eGFR_{Cys}/eGFR_{Crea}$ ratio (given as %) stratified according to age and sex

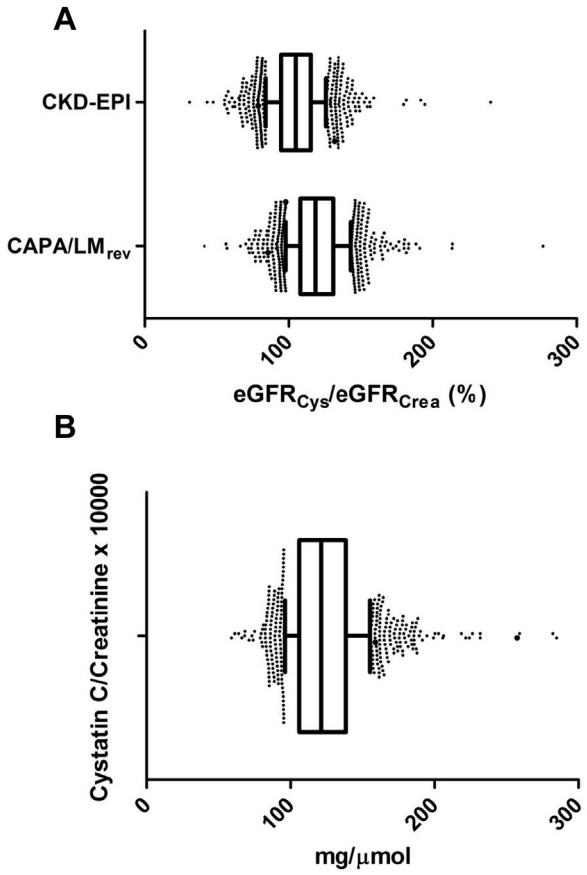
	Females			Males		
Age	**Subjects**	**Lower limit**	**Upper limit**	**Subjects**	**Lower limit**	**Upper limit**
60–64	130	79 (75–83)	137 (133–142)	126	78 (71–85)	147 (140–154)
65–69	173	76 (72–79)	139 (135–143)	154	76 (70–82)	144 (139–150)
70–74	147	71 (59–81)	140 (129–152)	139	79 (76–84)	135 (132–138)
75–79	106	69 (65–74)	134 (129–139)	87	74 (69–79)	142 (136–147)
80–84	66	68 (64–73)	121 (117–125)	61	69 (64–74)	129 (123–135)
≥85	29	72 (65–79)	121 (115–127)	16	63 (52–73)	134 (118–145)

Abbreviation: eGFR, estimated glomerular filtration rate.
Upper and lower limits are provided with 90% confidence intervals in parentheses. Reference intervals overlap substantially. Combined sex-specific reference intervals are 72 (69–75)% to 136 (133–140)% for women (n = 653) and 75 (73–78) to 141 (138–143)% for men (n = 583). A sex- and age-independent reference interval is 74 (71–76)% to 139 (137–141)%.

Supplementary Table II. Reference intervals for the CAPA/LM_{rev} $eGFR_{Cys}/eGFR_{Crea}$ ratio (given as %) stratified according to age and sex

	Females			Males		
Age	**Subjects**	**Lower limit**	**Upper limit**	**Subjects**	**Lower limit**	**Upper limit**
60–64	130	88 (84–92)	157 (151–161)	126	83 (77–89)	156 (150–162)
65–69	173	85 (81–89)	160 (155–164)	154	81 (75–88)	155 (149–162)
70–74	147	82 (69–93)	162 (150–175)	139	89 (85–93)	146 (143–150)
75–79	106	82 (77–88)	159 (152–165)	87	78 (69–88)	162 (153–171)
80–84	66	84 (79–90)	147 (141–152)	61	83 (78–89)	147 (141–154)
≥85	29	91 (81–102)	157 (148–164)	16	78 (66–91)	161 (144–174)

Abbreviations: eGFR, estimated glomerular filtration rate*; LM_{rev},* Lund-Malmö revised equation.
Upper and lower limits are provided with 90% confidence intervals in parentheses. Reference intervals overlap substantially. Combined sex-specific reference intervals are 85 (81–88)% to 158 (154–161)% for women (n = 653) and 83 (80–86)% to 153 (150–156)% for men (n = 583). A sex- and age-independent reference interval is 84 (82–86)% to 156 (153–158)%.



Supplementary Fig 1. (**A**) The distribution of $eGFR_{Cys}/eGFR_{Crea}$ as estimated by CKD-EPI (upper) and CAPA/Lund-Malmö revised ($CAPA/LM_{rev}$; lower) are shown as boxplots with median, interquartile range, 10th and 90th percentile, and outliers. The prevalence of participants is 0.7% (CKD-EPI) and 0.2% ($CAPA/LM_{rev}$) at a ratio of <60%, 2.2% (CKD-EPI) and 0.3% ($CAPA/LM_{rev}$) at a ratio of <70%, and 6.7% (CKD-EPI) and 1.6% ($CAPA/LM_{rev}$) at a ratio <80%. (**B**) Displays the distribution of cystatin C/creatinine (mg/µmol) times a factor of 10,000. eGFR, estimated glomerular filtration rate.