

## Cystatin C and Cardiovascular Risk

Nevio Taglieri,<sup>1</sup> Wolfgang Koenig,<sup>2</sup> and Juan Carlos Kaski<sup>1\*</sup>

**BACKGROUND:** Patients with chronic kidney disease (CKD) are at high risk for developing cardiovascular disease (CVD) and cardiovascular events. Cystatin C, a protease inhibitor synthesized in all nucleated cells, has been proposed as a replacement for serum creatinine for the assessment of renal function, particularly to detect small reductions in glomerular filtration rate.

**CONTENT:** This report presents a review of the role of cystatin C as a predictor of cardiovascular risk.

**SUMMARY:** Patients with higher circulating cystatin C concentrations appear to have an increased cardiovascular risk profile, i.e., they are older and have a higher prevalence of systemic hypertension, dyslipidemia, documented CVD, increased body mass index, and increased concentrations of C-reactive protein. Prospective studies have shown, in various clinical scenarios, that patients with increased cystatin C are at a higher risk of developing both CVD and CKD. Importantly, cystatin C appears to be a useful marker for identifying individuals at a higher risk for cardiovascular events among patients belonging to a relatively low-risk category as assessed by both creatinine and estimated glomerular filtration rate values. Of interest, elastolytic proteases and their inhibitors, in particular cystatin C, have been shown to be directly involved in the atherosclerotic process. Increased concentrations of cystatin C appear to be indicative of preclinical kidney disease associated with adverse outcomes. Clinical studies involving direct glomerular filtration rate measurements are required to ascertain both the true role of this promising marker in renal disease and whether atherogenic factors like inflammation can account for increases in cystatin C concentrations, thus explaining its predictive value in CVD.

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Chronic kidney disease (CKD)<sup>3</sup> is an important public health problem worldwide, with an estimated prevalence of 13% in the Western world (1, 2). CKD patients are known to be at an increased risk of developing cardiovascular disease (CVD) and cardiovascular events (2–6).

In clinical practice, serum creatinine is the marker most commonly used to assess renal function (RF). However, creatinine appears to be a rather unreliable marker of RF because creatinine serum concentrations are affected by tubular secretion, age, sex, muscle mass, physical activity, and diet, and therefore creatinine does not have a direct relationship with the glomerular filtration rate (GFR) (7). The Cockcroft-Gault (8) and the Modification of Diet in Renal Disease (MDRD) (9) equations, both based on serum creatinine, are being used increasingly because they overcome, at least in part, some of the limitations of creatinine measurements. Both equations are currently recommended for the estimation of GFR, which is an established method for detection and classification of CKD in clinical practice (2). However, these creatinine-based equations, which have been validated in patients with CKD, have several limitations, particularly among CKD patients with comorbid conditions, elderly individuals, obese patients, and patients with only mild impairment of RF (10–12).

In recent years, cystatin C has been proposed as a more reliable marker of RF than serum creatinine, in particular for the detection of small reductions in GFR (13–15). Cystatin C is a 13-kDa protein and a member of a family of competitive inhibitors of lysosomal cysteine protease synthesized at a constant rate in all nucleated cells (16). Owing to its free filtration in the glomerulus, complete reabsorption and catabolism in the proximal tubule, and lack of tubular secretion, plasma cystatin C concentration is thought to depend almost completely on GFR. Recent studies, however, have shown that plasma cystatin C concentration is influenced by factors such as age (17, 18), body mass index (BMI) (17, 19), sex (17, 18), smoking status (17),

<sup>1</sup> Cardiovascular Biology Research Centre, Division of Cardiac and Vascular Sciences, St George's University of London, London, UK; <sup>2</sup> Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, Ulm, Germany.

\* Address correspondence to this author at: Division of Cardiac and Vascular Sciences, St George's, University of London, Cranmer Terrace, London SW17 0RE, UK. Fax +44-208-725-3328; e-mail jkaski@sgul.ac.uk.

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<sup>3</sup> Nonstandard abbreviations: CKD, chronic kidney disease; CVD, cardiovascular disease; RF, renal function; GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated GFR; MI, myocardial infarction; CHS, Cardiovascular Health Study; HR, hazard ratio; HF, heart failure; CrCl, creatinine clearance; NSTEMI, non-ST-elevation acute coronary syndrome.

and high concentrations of C-reactive protein (CRP) (17, 18). Recent studies (20, 21) have demonstrated that for the estimation of GFR in patients with CKD, a cystatin C–based equation performed better if variables such as age, sex, race, and BMI were included. Therefore, although cystatin C is a promising marker of RF, further investigation is needed to elucidate its role in both the classification of CKD and the management of patients with CKD (22).

In recent years cystatin C has emerged as a potential marker for cardiovascular risk. Here we present a review of the role of cystatin C as a predictor of cardiovascular events.

### Cystatin C and Established Cardiovascular Risk Factors

Parikh et al. evaluated the association between cystatin C and conventional cardiovascular risk factors in 3241 predominantly white participants (mean age 61 years; 53% women) in the Framingham Offspring prospective cohort study (23, 24). In this patient cohort, RF was assessed by means of the MDRD equation. CKD, defined as an estimated GFR (eGFR)  $<60 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ , (2) was present in 8.6% of individuals and CVD in 13.1%. Participants were subdivided according to the 95th percentile of cystatin C concentration (1.07 mg/L). The authors showed that high concentrations of cystatin C were independently associated with cardiovascular risk factors such as age, female sex, BMI, low HDL cholesterol, and smoking, even in individuals without CKD or microalbuminuria. A cross-sectional analysis of data on individuals with CKD in the study disclosed a similar risk profile but, interestingly, those with high cystatin C concentrations and no CKD had a higher prevalence of obesity and hypertension compared to individuals with CKD and low cystatin C.

These findings confirmed previous results from the Third National Health and Nutrition Examination Survey. In a cross-sectional study Muntner et al. enrolled 4991 adults age  $\geq 20$  years from National Health and Nutrition Examination Survey participants without CKD, i.e., eGFR  $<60 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ , or micro- or macrohematuria (25). Participants were subdivided according to cystatin C quartiles. After age standardization, the prevalence of risk factors such as cigarette smoking, hypertension, and low HDL cholesterol was higher in individuals with higher cystatin C concentrations. Moreover, the prevalence of CVD, myocardial infarction (MI), angina pectoris, and stroke increased with increasing concentrations of cystatin C. After adjustment for age, sex, race, and principal cardiovascular risk factors, cystatin C was still independently associated with CVD. These re-

sults suggested an association between cystatin C and cardiovascular risk factors but gave little insight as to the mechanisms responsible for the association. An important limitation of these studies was their retrospective design.

The association between cystatin C and incident hypertension has been prospectively studied in a cohort of individuals from the Multi-Ethnic Study of Atherosclerosis (26). Kestenbaum et al. evaluated whether early kidney dysfunction, measured by serum cystatin C or urinary albumin excretion, was a risk factor for new-onset hypertension (27). They assessed 2767 individuals (mean age 58 years) without prevalent hypertension, CVD, or eGFR  $<60 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ . The whole population was subdivided into quartiles, and the study showed that individuals with higher concentrations of cystatin C were older and had a worse clinical risk profile. During a median follow-up of 3.1 years, 19.7% of individuals developed hypertension which, after multivariable adjustment for the most important clinical factors, was found to be associated with cystatin C concentrations, older age, African-American ethnicity, diabetes, and higher baseline systolic blood pressure (27). After adjustment for established risk factors associated with hypertension, it was established that for each 15 nmol/L (0.2 mg/L) increase in cystatin C concentration there was a 15% greater incidence of hypertension ( $P = 0.017$ ). The association between cystatin C and incident hypertension was similar when patients with eGFR  $\geq 90 \text{ mL/min per } 1.73 \text{ m}^2$  were considered. Thus, this clinically important study, which included a large number of individuals belonging to different ethnic groups, identified an independent association between cystatin C and hypertension, after correction for relevant confounders.

### Cystatin C and Prediction of Cardiovascular Outcome

Reported data regarding cystatin C and prediction of cardiovascular outcome are summarized in Table 1.

#### FINDINGS IN ELDERLY PATIENTS

The prognostic role of cystatin C in elderly persons (age  $\geq 65$  years) was thoroughly evaluated by Shlipak et al. in the Cardiovascular Health Study (CHS) (28, 29). The study comprised 5201 individuals, recruited between 1989 and 1990, and an additional 687 African-American persons recruited in 1992–1993. For 4637 ambulatory elderly persons, frozen samples had been collected during their 1992–1993 follow-up visit, and information was available on cystatin C and creatinine concentrations. Follow-up was carried out by means of an annual clinical examination and interim telephone calls at 6 months, and continued until June 2001 (me-

Table 1. Cystatin C and prediction of cardiovascular events.

Study: reference (year published)	Patients, N	Cystatin C assays <sup>a</sup>	Characteristics of recruited participants	Renal function mL · min <sup>-1</sup> · (1.73 m <sup>2</sup> ) <sup>-1</sup>	Variables associated with cystatin C <sup>b</sup>	Study endpoint	Main findings <sup>c</sup>
Elderly patients							
Shlipak et al. (29) (2005)	4637	CV 7.7%	Individuals age >65 years	Mean eGFR = 61	Creatinine ( $r = 0.79$ ; $P < 0.001$ ) eGFR ( $r = -0.63$ ; $P < 0.001$ )	Cardiovascular death, death from all causes, MI, stroke	HR (95% CI) for the 5th quintile of cystatin C ( $\geq 1.29$ mg/L) vs 2 lowest quintiles  Cardiovascular death 2.27 (1.73–2.97)
							MI 1.48 (1.08–2.02) Stroke 1.47 (1.09–1.96)
Samak et al. (30) (2005)	4384	Intraassay CV 2.0%–2.8%, interassay CV 2.3%– 3.1%	Individuals age >65 years, without previous HF	Mean eGFR = 72	Not reported	Incident HF	HR (95% CI) for the 3rd– 5th vs 1st quintile of cystatin C, respectively below:  HR = 1.44 (1.07–1.94) HR = 1.58 (1.18–2.12) HR = 2.16 (1.61–2.91)
Shlipak et al. (31) (2005)	279	Intraassay CV 2.0%–2.8%; interassay CV 2.3%– 3.1%	Individuals age >65 years, with HF	Mean eGFR = 61	Not reported	Death from all causes	HR (95% CI) for the 4th quintile vs the lowest:  HR = 2.15 (1.30–3.54)
O'Hare et al. (32) (2005)	4025	Intraassay CV 2.0%–2.8%, interassay CV 2.3%– 3.1%	Individuals age >65 years, with peripheral artery disease	Mean eGFR = 72	Not reported	Peripheral artery disease procedure (bypass, angioplasty, amputation)	HR (95% CI) for the 5th quintile vs the lowest:  HR = 2.5 (1.2–5.1)
Patients with CHD <sup>d</sup>							
Ix et al. (34) (2007)	990	Assay range 0.195–7.330 mg/L; intraassay CV 2.0%–2.8%; interassay CV 2.3%–3.1%	Patients with stable CHD	Mean eGFR = 77	Not reported	All-cause mortality, cardiovascular events, incident HF	HR (95% CI) for the highest quartile vs lowest:  Death (3.6; 1.8–7.0) Cardiovascular events (2.0; 1.0–3.8) Incident HF (2.6; 1.0–6.9) <i>Continued on page 1935</i>

Table 1. Cystatin C and prediction of cardiovascular events. (Continued from page 1934)

Study: reference (year published)	Patients, N	Cystatin C assays <sup>a</sup>	Characteristics of recruited participants	Renal function mL · min <sup>-1</sup> · (1.73 m <sup>2</sup> ) <sup>-1</sup>	Variables associated with cystatin C <sup>b</sup>	Study endpoint	Main findings <sup>c</sup>
Koenig et al. (36) (2004)	1033	Interassay CV 3.8%	Patients with stable CHD	5.6% of patients with CrCl <60 mL/min	Creatinine ( <i>r</i> = 0.58; <i>P</i> < 0.0001), CrCl ( <i>r</i> = -0.48; <i>P</i> < 0.0001), CRP ( <i>r</i> = 0.16; <i>P</i> < 0.0001)	Combined outcome of fatal and nonfatal cardiovascular events	HR (95% CI) for the highest quintile vs lowest:  HR = 2.27 (1.05–4.91)
Jernberg et al. (37) (2004)	726	Total analytical imprecision for cystatin C was 4.8% at 0.56 mg/L and 3.7% at 2.85 mg/L	Patients with suspected or confirmed NSTE- ACS	Cystatin C derived eGFR = 77	Creatinine ( <i>r</i> = 0.61; <i>P</i> < 0.001), cTnT ( <i>r</i> = 0.25; <i>P</i> < 0.001), CRP ( <i>r</i> = 0.31; <i>P</i> < 0.001), NTproBNP ( <i>r</i> = -0.59; <i>P</i> < 0.001)	Death (follow-up 40 months)	Death HR (95% CI) for 3rd vs 1st quartile: 3.2 (1.2–8.5)
Windhausen et al. (38) (2009)	1128	Total CV 2% at 1.03 mg/L and 2% at 1.43 mg/L	Patients with NSTE- ACS and increased cTnT	Mean eGFR = 85	Not reported	Death within 4 years, MI within 3 years	Death HR (95% CI) for 3rd vs 1st tertile: 2.04 (1.02–4.10)  MI HR (95% CI) for 3rd vs 1st tertile 1.95; (1.05–3.63)
Patients with established CKD							
Menon et al. (41) (2007)	825	Interassay imprecision for the assay, 5.6%	Nondiabetic adult patients with stage 3–4 CKD	Mean GFR = 45	Creatinine ( <i>r</i> = 0.80; <i>P</i> < 0.001)  GFR ( <i>r</i> = 0.85; <i>P</i> < 0.001)	All-cause mortality, CVD mortality, kidney failure, composite outcome of kidney failure and all-cause mortality	HR (per SD); 95% CI: All-cause mortality  HR = 1.41, 1.18–1.67  Cardiovascular mortality  HR = 1.64 (1.28–2.08)  <i>Continued on page 1936</i>

Table 1. Cystatin C and prediction of cardiovascular events. (Continued from page 1935)

Study: reference (year published)	Patients, N	Cystatin C assays <sup>a</sup>	Characteristics of recruited participants	Renal function mL · min <sup>-1</sup> · (1.73 m <sup>2</sup> ) <sup>-1</sup>	Variables associated with cystatin C <sup>b</sup>	Study endpoint	Main findings <sup>c</sup>
Patients without clinical CKD							
Kestenbaum et al. (27) (2008)	2767	CV 7.7%	Individuals without: hypertension cardiovascular disease, clinically apparent kidney disease, microalbuminuria	Mean eGFR = 84	Not reported	Incident hypertension, all cause death, CV death, MI, heart failure, stroke	For each 15-nmol/L increase in cystatin C there was a significant 15% greater incidence of hypertension ( <i>P</i> = 0.017)
Shlipak et al. (33) (2006)	4663	Assay range = 0.195– 7.330 mg/L Reference ranges for the young = 0.53–0.92 mg/L for persons >50 years = 0.58–1.02 mg/L	Community-based elderly population (>65 years), 78% without clinical CKD	Mean eGFR = 83	Patients with CKD: creatinine ( <i>r</i> = 0.81; <i>P</i> < 0.001), eGFR ( <i>r</i> = –0.75; <i>P</i> < 0.001) Patients without CKD: creatinine ( <i>r</i> = 0.38; <i>P</i> < 0.001), eGFR ( <i>r</i> = –0.46; <i>P</i> < 0.001)	HR (per SD); 95% CI <sup>e</sup> : Death (1.33; 1.25–1.40) Cardiovascular death (1.42; 1.30–1.54) Noncardiovascular death (1.26; 1.17–0.36) Heart failure (1.28; 1.17– 1.40) Stroke (1.22; 1.08–1.38) MI (1.20 CI; 1.06–1.36)	
Keller et al. (39) <sup>f</sup> (2009)	1827	CVs <1.8% in concentration 0.87–4.63 mg/L	Patients with coronary artery disease	Mean serum creatinine concentration 0.94 mg/dL	Creatinine ( <i>r</i> = 0.32; <i>P</i> < 0.001), eGFR ( <i>r</i> = –0.33; <i>P</i> < 0.001), age ( <i>r</i> = 0.37; <i>P</i> < 0.001), BMI ( <i>r</i> = 0.15; <i>P</i> < 0.001), NTproBNP ( <i>r</i> = –0.21; <i>P</i> < 0.001), CRP ( <i>r</i> = 0.15; <i>P</i> < 0.001)	Cardiovascular death	HR <sup>a</sup> = 1.94, 95% CI 1.59–2.37; HR <sup>b</sup> = 3.87, 95% CI 2.33–6.62
Studies comparing cystatin C to established cardiovascular risk factors							

Continued on page 1937

Table 1. Cystatin C and prediction of cardiovascular events. (Continued from page 1936)							
Study: reference (year published)	Patients, N	Cystatin C assays <sup>a</sup>	Characteristics of recruited participants	Renal function mL · min <sup>-1</sup> · (1.73 m <sup>2</sup> ) <sup>-1</sup>	Variables associated with cystatin C <sup>b</sup>	Study endpoint	Main findings <sup>c</sup>
Zethelius et al. (42) (2008)	1135 (of whom 661 had no cardiovascular disease)	Not reported	Elderly men (mean age 71 years, range 69.4–73.6 years)	Not reported	Not reported	Comparison of c-statistics between a model of risk stratification including biomarkers (cystatin C, NTproBNP, troponin I, and CRP) and a model without these markers	Cardiovascular death (all patients): Model with 4 biomarkers, c-statistic 0.766 vs 0.664, <i>P</i> < 0.001  Model with cystatin C, c-statistic 0.691 vs 0.664, <i>P</i> = 0.07  Cardiovascular death (patients without CV disease)  Model with 4 biomarkers, c-statistic 0.748 vs 0.688, <i>P</i> < 0.03  Model with cystatin C, c-statistic 0.70 vs 0.688, <i>P</i> = 0.45

<sup>a</sup> Cystatin C was measured in all studies by means of particle-enhanced immunonephelometric assay (N Latex Cystatin, Dade Behring) with a nephelometer (BNII, Dade Behring).

<sup>b</sup> Only the relation expressed by correlation tests is reported here.

<sup>c</sup> Only multivariable adjusted HR are reported.

<sup>d</sup> CHD, coronary heart disease; NTproBNP, N-terminal probrain natriuretic peptide; cTnT, cardiac troponin I.

<sup>e</sup> Main findings reported relate only to patients without clinical CKD.

<sup>f</sup> In the present study the authors did not find a linear association between cystatin C and clinical outcomes, and a threshold effect was proposed for the first time. This observation must be confirmed in future studies because this study differs from others in the literature in that cystatin C levels were not normally distributed. The reason for this finding is not known.

<sup>g</sup> Logarithmically transformed standard cystatin C values.

<sup>h</sup> Upper quartile vs quartile 1–quartile 3 (pooled together).



dian follow-up 7.4 years). The whole population was subdivided for each marker of RF, initially into quintiles and subsequently into 7 categories, as the fifth quintile was further subdivided in thirds. The study revealed that with regard to all-cause mortality and death from CVD, the adjusted hazard ratio (HR) increased from the lowest to the highest concentrations of cystatin C. Moreover, the seventh category was independently associated with the risk of MI and the sixth and seventh with the risk of stroke.

With respect to conventional markers of RF and mortality, very high concentrations of creatinine and low eGFR were independently associated with all-cause mortality (category 7 and 6 and 7, respectively), whereas only category 7, i.e., abnormal eGFR, was associated with death from CVD. Importantly, within each quintile of creatinine, increasing concentrations of cystatin C were associated with increased mortality. Although the results of this large study are of interest, the question remains as to whether the post hoc statistical assessment of individuals in the highest quintile could have introduced some bias in the analysis, thus affecting the final conclusions of the study.

Individuals in CHS without previous heart failure (HF) at baseline, and those who did not develop HF before the year 1992–1993 visit, were recruited for the evaluation of cystatin C as a risk factor for the development of HF (30). Overall, 4384 patients were enrolled and followed up for a median of 8.3 years. The association of RF and risk for incident HF was assessed using 3 measures of renal function, cystatin C, creatinine concentrations, and eGFR, as assessed by means of the MDRD equation. Mean age was 75 years; 41% of participants were male and 17% were of African-American origin. The study showed that individuals with the highest cystatin C concentrations had the worst cardiovascular risk profile. During follow-up, 763 persons developed HF (annual incidence 2.5%), and the incidence of HF increased linearly through the 5 cystatin C quintiles. In contrast, quintiles of creatinine and eGFR showed a flat or J-shaped relationship with incident HF in the lowest 4 quintiles. After multivariable adjustment, patients in the third-to-fifth cystatin C quintile showed an increased incidence of HF. However, only patients in the highest quintile of eGFR showed an independent association between this marker and incident HF. Thus these findings suggest that high cystatin C concentration predicts increased risk for HF more accurately than conventional markers of RF, although the reason for this difference remains unclear. In a sub-study (31) of CHS involving 279 patients with HF at baseline (as assessed during the year 1992–1993 visit), the highest concentrations of cystatin C and the lowest eGFR were independently associated with an increased risk of mortality (HR = 2.15, 95% CI 1.30–3.54; HR =

1.62, 95% CI = 1.01–2.59, respectively). This was, however, a small substudy and therefore these results require confirmation in larger prospective studies. Results from the same group of investigators (32) showed that cystatin C concentrations were predictors of incident peripheral arterial disease in the elderly, whereas creatinine and eGFR were not, thus confirming findings in other studies of a superior predictive value of cystatin C, compared to other markers of RF, in the cardiovascular setting.

## FINDINGS IN ELDERLY PATIENTS WITHOUT CLINICAL CKD

To assess the prognostic role of cystatin C in patients without clinical CKD, the CHS investigators prospectively evaluated the association between cystatin C concentrations and the risk of cardiovascular and renal outcomes in elderly individuals with  $\text{eGFR} \geq 60 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$  (33), followed up for a median of 9.3 years. Study endpoints were: all-cause death, cardiovascular death, heart failure, MI, and stroke. Of 4663 individuals included, 1004 (22%) had CKD and 3659 (78%) had no CKD. Among those with  $\text{eGFR} \geq 60 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$ , cystatin C concentrations showed a strong association with each outcome whereas creatinine concentration was a weaker predictor of cardiovascular death compared to cystatin C (HR for creatinine = 1.17 95% CI 1.03–1.32 vs HR for cystatin C = 1.42 95% CI 1.39–1.54). In individuals without CKD ( $n = 2508$ ) those with cystatin C values  $\geq 1 \text{ mg/L}$  were also at a higher risk to develop incident CKD compared with patients with lower cystatin C concentrations, during a 4-year follow-up. This finding was remarkable because patients who developed CKD had a significantly higher risk of death, cardiovascular death, and heart failure. As the authors stated, these findings suggested that increased concentrations of cystatin C identify a state of preclinical kidney disease, highly prevalent in the elderly population (39%), associated with adverse outcomes. Findings in this large study further suggest a superiority of cystatin C, compared to other markers of RF, as a marker of both kidney dysfunction and cardiovascular risk.

## FINDINGS IN PATIENTS WITH CORONARY HEART DISEASE

Ix et al. (34) evaluated whether serum cystatin C concentrations were associated with all-cause mortality, cardiovascular events, and incident HF in outpatient clinic patients included in the prospective “Heart and Soul” study (35). Patients in the study ( $n = 990$ ) had a history of MI, angiographic coronary stenosis  $\geq 50\%$  in at least 1 epicardial vessel, stress-induced myocardial ischemia, or a history of coronary revascularization. GFR was estimated by means of the MDRD equation. Patients had a mean age of 67 years, 82% were male, and 61% were white. Mean cystatin C concentration

was 1.20 (0.56) mg/dL and mean eGFR 77 (23) mL/min per 1.73 m<sup>2</sup>. With subdivision by quartiles, high concentrations of cystatin C were found to be associated with older age and higher CRP concentrations. During a median follow-up of 37 months, 132 patients died and 101 had nonfatal cardiovascular events. Among 816 patients without a prior history of HF, 57 were hospitalized for HF. Compared to patients in the first quartile, patients in the highest cystatin C quartile (cystatin C concentration  $\geq 1.3$  mg/L) had a higher annual rate of events; on multivariable assessment these patients showed a >3-fold mortality hazard, 2-fold cardiovascular events hazard, and >2-fold incident HF hazard. Importantly, the risk association with higher cystatin C concentrations did not differ among patients with or without renal dysfunction or microalbuminuria. This was a medium size study including well-characterized, nonhospitalized patients with CAD. There was, however, a small representation of women in the study population, and the nonwhite population comprised approximately 40% of the whole cohort. Cystatin C in this study was not compared to other markers of RF.

Koenig et al. evaluated the risk of secondary cardiovascular events, according to cystatin C and other markers of renal function, in 1033 patients (age 30–70 years) admitted to a hospital rehabilitation program 3 months after an acute coronary event or coronary artery revascularization (36). The endpoint of the study (median follow-up, 33.5 months) was the combined outcome of fatal and nonfatal cardiovascular events. Creatinine clearance (CrCl) was estimated by use of the Cockcroft-Gault formula.

Patients were subdivided in quintiles according to cystatin C concentrations. High concentrations of cystatin C were associated with older age, history of diabetes, extension of coronary heart disease, CRP concentrations, and angiotensin-converting enzyme inhibitor and diuretic therapy. As in previous studies, study patients were subdivided by quintiles, but in this study no cutoff points were identified. After adjustment for several clinical confounders, patients in the upper cystatin C quintile showed a >2-fold risk for cardiovascular events. Adjustment by CrCl did not modify these findings. Thus, the authors suggested that cystatin C could be more than simply a marker of renal dysfunction. However, owing to the lack of direct measurements of GFR (the gold standard for renal function evaluation) in the Koenig study, the authors' conclusions may require confirmation in studies that assess RF directly. Moreover, from a statistical standpoint, a significant correlation was reported between cystatin C and CrCl in both the study by Koenig et al. (36) and by Shlipak et al. (29), thus making it difficult to rule out the effect of colinearity in these studies.

#### FINDINGS IN ACUTE CORONARY SYNDROME PATIENTS

Few studies have evaluated the prognostic role of cystatin C specifically in acute patients. Jernberg et al. (37) enrolled 726 patients admitted to the coronary unit at Uppsala University Hospital between March 1997 and February 1998 with symptoms suggestive of non-ST-elevation acute coronary syndrome (NSTEMI-ACS). Endpoints were: death during a median follow-up of 40 months (range, 35–47 months) and recurrence of MI after a follow-up of 6 months. Kidney function was evaluated by means of cystatin C, creatinine, and CrCl (Cockcroft-Gault formula). Patients with high cystatin C concentrations were older and had worse baseline clinical characteristics. In patients with confirmed NSTEMI-ACS (n = 380) and in those with other diagnoses, the mortality rate increased with increasing cystatin C concentrations. After multivariable adjustment, patients in the highest cystatin C quartile (cystatin C concentration  $\geq 1.25$  mg/L), showed increased mortality (HR = 4.28 95% CI 1.64–11.2) but not increased MI rates. Compared with other markers of renal function, cystatin C had better discrimination power than CrCl or creatinine (c-statistic = 0.79, 0.72, and 0.66, respectively). Once again, this time in patients with ACS, a superior predictive value was observed for cystatin C compared to other markers of RF.

Recently, these observations have been confirmed and expanded by investigators in the ICTUS (Invasive vs Conservative Treatment in Unstable Coronary Syndromes) trial, in which Windhausen et al. found that in patients with NSTEMI-ACS and increased cardiac troponin T concentrations, high plasma cystatin C concentrations are associated with a higher risk of death and spontaneous MI (38).

#### FINDINGS IN PATIENTS WITH CORONARY HEART DISEASE BUT WITHOUT CLINICAL CKD

Keller et al. (39) recently evaluated the prognostic role of cystatin C in 1827 patients with stable coronary artery disease or ACS and normal or mildly impaired eGFR, who were taking part in the Atherogene study (40). Patients were subdivided according to cystatin C quartiles and followed up for a median of 3.7 years with reference to all-cause mortality and mortality from CVD. Those in the upper cystatin C quartile had a higher risk of cardiovascular mortality compared to patients in the lower quartiles (HR = 3.87; 95% CI 2.33–6.42,  $P < 0.001$ ). This association, however, became non-statistically significant after adjustment for clinical risk factors, CRP, and N-terminal probrain natriuretic peptide (HR = 1.86, 95% CI 0.9–3.81,  $P = 0.09$ ). Logarithmically transformed cystatin C was significantly associated with cardiovascular death (HR = 1.94, 95% CI = 1.59–2.37,  $P < 0.001$ ). The results of this study show disparities with previous studies re-



garding the independent role of cystatin C as a marker of cardiovascular risk, because the univariate association became nonsignificant after appropriate adjustments in multivariable analyses.

## FINDINGS IN PATIENTS WITH ESTABLISHED CKD

The prognostic role of cystatin C in patients with CKD was evaluated by Menon et al. (41) in a pivotal study in which cystatin C was compared with GFR measured by iothalamate clearance. The study population comprised 825 nondiabetic adults (age 18–70 years) with stage 3–4 CKD (mean age was 52 years, 85% of the patients were white and 61% were men). The study showed that 1/cystatin C strongly correlated with directly measured GFR ( $r = 0.85$ ;  $P < 0.001$ ).

Patients were followed for a median of 10 years and multivariable analysis revealed that 1-SD decreases in 1/creatinine, GFR, and 1/cystatin C were associated with adverse outcome. In particular, increased cystatin C concentrations showed the highest risk for both all-cause mortality and CVD mortality (HR = 1.41, 95% CI 1.18–1.67) compared to creatinine (HR = 1.27, 95% CI 1.06–1.49) and measured GFR (HR = 1.27, 95% CI 1.08–1.49). These results, similar to data from previous studies, suggest that cystatin C may be a stronger predictor of risk than measured GFR. The very high correlation found between 1/cystatin C and iothalamate clearance in this study suggests that at least in patients with CKD, kidney dysfunction is the most likely explanation for the prognostic value of cystatin C. However, the overlapping 95% CI in the study did not allow firm conclusions to be drawn in this regard.

## CARDIOVASCULAR RISK STRATIFICATION: CYSTATIN C VS ESTABLISHED RISK FACTORS

The evidence reported so far appears to suggest that cystatin C concentrations provide independent and incremental prognostic information in addition to that of traditional markers of RF. However, the potential additional contribution of cystatin C to a comprehensive clinical model of cardiovascular risk stratification has not been extensively investigated. Recently Zethelius et al. (42) assessed whether a combination of biomarkers, including cystatin C, N-terminal pro-brain natriuretic peptide, troponin I, and CRP, improved patient risk stratification compared to established cardiovascular risk factors. These investigators enrolled 1135 (661 free from cardiovascular disease) elderly men (mean age 71 years, range 69.4–73.6 years) from the Uppsala Longitudinal Study of Adult Men. These individuals were followed for a median of 10 years with reference to all-cause mortality and cardiovascular death. The main finding of the study was that the discrimination power for cardiovascular death increased

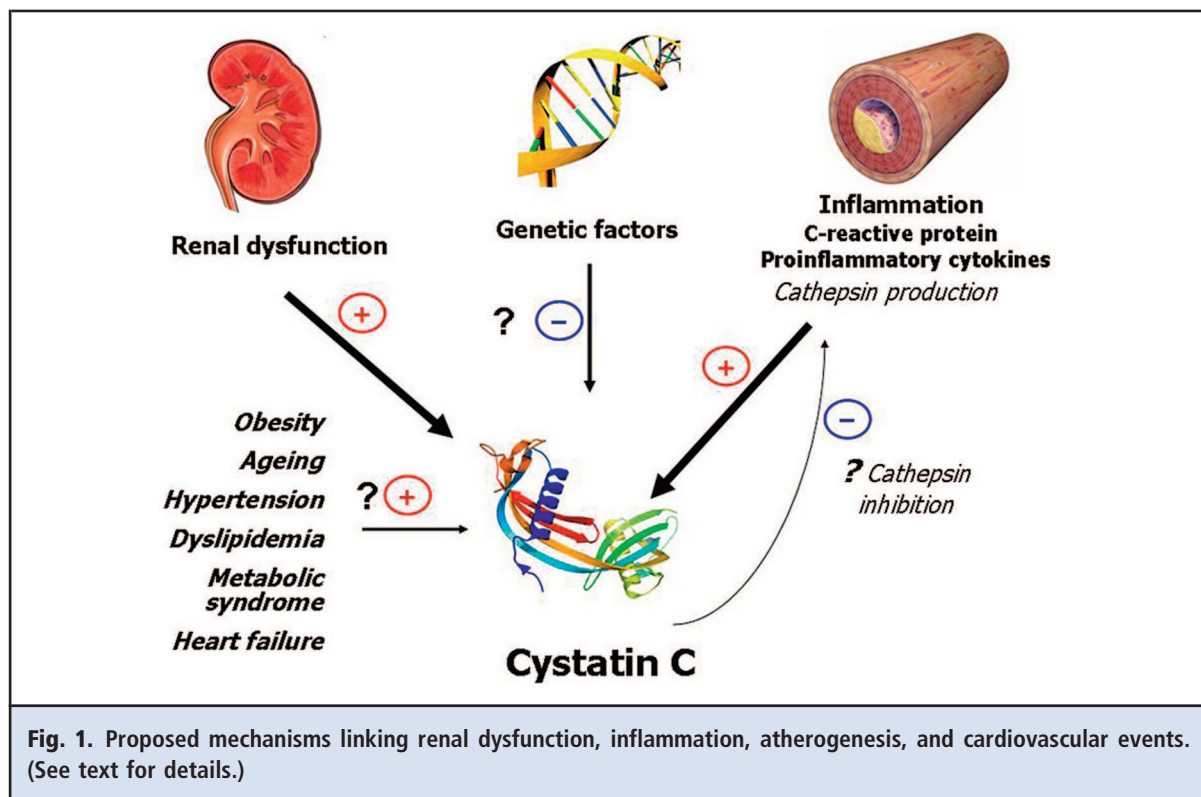
significantly when the 4 biomarkers mentioned above were added into a model that included established cardiovascular risk factors. The c statistic in the whole patient group, with and without the inclusion of established biomarkers, showed an area under the ROC curve of 0.766 vs 0.664, respectively; ( $P < 0.001$ ). The areas under the ROC curve for the group without cardiovascular disease at baseline were 0.748 and 0.688, respectively ( $P = 0.03$ ) and for established risk factors with and without cystatin C in the model, 0.691 vs 0.664 ( $P = 0.07$ ). This study, albeit of interest, had an important limitation, that the cohort comprised only white men in a limited age range and the study design did not allow testing the impact of age, race, and sex on the biomarkers assessed (18, 20).

Patients with CKD are known to be at an increased risk of cardiovascular events (2–6), and cystatin C, a novel marker of RF, has been proposed to be a more sensitive and accurate marker of kidney dysfunction than plasma creatinine concentrations in the clinical arena, especially for the estimation of small reductions in GFR (13–15). So far, however, and possibly because of its still poorly understood pathophysiological role, the use of this marker in the management of patients with CKD has been limited in clinical practice.

## Cystatin C Improves Cardiovascular Risk Stratification

Cystatin C appears to be a marker of cardiovascular risk, and high concentrations of circulating cystatin C have been shown to be consistently and strongly associated with cardiovascular outcomes in different clinical scenarios. Moreover, Cystatin C seems to offer more complete prognostic information than other markers of renal function. Most importantly, cystatin C appears to be useful for identifying individuals at a higher risk for cardiovascular events among patients classified as belonging to a low-risk category according to both creatinine and estimated GFR values (Table 1), but the reasons for the incremental prognostic information afforded by cystatin C are still speculative.

Increased cystatin C concentrations identify early GFR abnormalities (13), and it has been suggested that this marker is highly sensitive to “preclinical” kidney dysfunction (33), which may be associated with adverse clinical outcomes. Cystatin C may thus help, in the early phases of CKD, to identify individuals who are at increased risk both for further impairment of CKD and for the development of CVD, and who may benefit from more “aggressive” preventative measures, such as lowering blood pressure to stringent targets and enrollment into strict monitoring pro-



grams. To date, however, no study has evaluated therapeutic strategy based on risk stratification using cystatin C values.

Most published studies have focused on the predictive role of cystatin C compared to traditional markers of RF, using a similar approach, i.e., the assessment of adjusted odds ratios or HRs, as appropriate. Only 1 study (35), involving well-characterized patients with a meticulous follow-up, used analysis of C-statistics for comparison of different biomarkers. The results of these studies, however, are rather similar, indicating an independent contribution of cystatin C for both RF and CVD risk prediction.

#### Cystatin C: Just a Marker of RF or a Marker of Inflammation and Atherogenesis?

A plausible link between increased cystatin C concentrations and impaired cardiovascular outcome, as reported in most of the studies so far, is renal dysfunction. However, none of the published studies so far has specifically addressed the issue of whether cystatin C is a predictor of cardiovascular risk even in patients with completely normal RF. An important common limitation of many of the studies mentioned in this review is that they have not measured GFR directly. Specific, well-designed trials are required to answer the impor-

tant question of whether pathogenetic mechanisms other than renal dysfunction could account for a high cystatin C concentration and explain predictive value for future cardiovascular risk.

Inflammation, associated with atherogenic changes, may be one mechanism associated with cystatin C and cardiovascular risk, and high cystatin C concentrations have been found to be associated with high concentrations of CRP (17, 43). Although it is possible that this association could just be the result of the presence of renal dysfunction (43–46), it has been suggested that high cystatin C concentrations are directly related to both inflammation and atherosclerosis (47) (Fig. 1). There is evidence that both elastolytic cysteine proteases and their inhibitors, an important one being cystatin C, are involved in the pathogenesis of atherosclerosis. Studies have suggested that rather than the circulating levels, the imbalance between proteases and inhibitors determines their net effects on the cardiovascular system (48–51). Inflammatory cytokines associated with atherosclerosis stimulate the production of lysosomal cathepsins, and increased plasma concentrations of cystatin C, a cathepsin inhibitor, may reflect, at least in part, an attempt to counterbalance a potentially damaging increased elastolytic activity. Studies have demonstrated that human cathepsins are expressed in endothelial cells, smooth muscle cells, and macro-

phages, and that they are involved in the progression, the composition, and the rupture of atherosclerotic plaques (48, 52–54). This response is likely to involve the interaction of mechanisms determined genetically (55, 56) (Fig. 1). High concentrations of cystatin C have been also associated with a hypermetabolic status (57, 58). Given the various possible mechanisms responsible for changes in cystatin C concentrations, it is conceivable that, depending on the clinical setting considered, increased cystatin C concentrations may variably reflect renal dysfunction, the effects of heart failure on RF as a result of hypertension and/or fluid retention (59–61), or coronary artery disease associated with inflammation and atherosclerosis (36). Further research is required to gain insight into the true significance of increased cystatin C concentrations in these various clinical settings.

In conclusion, increased cystatin C is emerging as a marker of both CKD and cardiovascular risk. If confirmed, the role of cystatin C as a sensitive early marker of RF will have clinical importance. Conceivably, a reliable method for early diagnosis of renal dysfunction will lead to more accurate and efficient patient management and the development of strategies for cardiovascular risk stratification and prevention. Large, well-

designed prospective studies in patients without renal dysfunction are needed to completely elucidate the link between high concentrations of circulating cystatin C and the risk of cardiovascular events.

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