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Plasma Concentrations of Cystatin C in Patients with Coronary Heart Disease and Risk for Secondary Cardiovascular Events: More than Simply a Marker of Glomerular Filtration Rate

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Background: **Renal impairment (RI) is associated with worse prognosis. Recently, cystatin C has been shown to represent a potentially superior marker of the glomerular filtration rate compared with creatinine clearance (CrCl). We evaluated the impact of cystatin C and other markers of RI on prognosis in a large cohort of patients with coronary heart disease (CHD).**

Methods: **Cystatin C, creatinine (Cr), and CrCl were determined at baseline in a cohort of 1033 patients (30–70 years) with CHD. Patients were followed for a mean of 33.5 months, and a combined endpoint [fatal and nonfatal cardiovascular disease (CVD) events] was used as the outcome variable. Cystatin C was measured by immunonephelometry, and CrCl was calculated.**

Results: **During follow-up, 71 patients (6.9%) experienced a secondary CVD event. Neither Cr ($P = 0.63$) nor CrCl ($P = 0.10$) were associated with incidence of CVD events, whereas cystatin C was clearly associated with risk of secondary CVD events ($P < 0.0001$). In multivariate analyses, patients in the top quintile of the cystatin C distribution at baseline had a statistically significantly increased risk of secondary CVD events even after adjustment for classic risk factors, severity of coronary disease, history of diabetes mellitus, treatment with angiotensin-converting enzyme inhibitors, and C-reactive protein (hazard ratio, 2.27; 95% confidence interval, 1.05–4.91) compared with patients in the bottom quintile.**

Conclusions: **These data support the possibly important prognostic value of cystatin C among patients with known CHD and suggest that it may be a useful clinical marker providing complementary information to established risk determinants.**

There is an increasing burden of chronic renal impairment (RI)[3] in the general population *(1)*. In addition, RI is often not recognized and treated adequately *(2–4)*. Individuals with RI have a high risk for end-stage renal disease, a condition requiring dialysis or transplantation to avoid uremia *(5)*. It is also evident that RI is an independent risk factor for cardiovascular disease (CVD), congestive heart failure, and total mortality *(1, 6, 7)*. Furthermore, in numerous studies including patients with manifest CVD or diabetes *(8)* and congestive heart failure *(9)*, a decreased glomerular filtration rate (GFR) has been found to be an independent risk factor for future CVD events and total mortality, even in cases in which RI was mild *(1)*. In addition, percutaneous coronary interventions have a less favorable outcome if conducted in patients with RI *(10)*. Therefore, early identification of patients with RI is of utmost interest because preventive and renoprotective measures are available.

In clinical practice, GFR is assessed by measurement of serum or plasma creatinine (Cr) or by determination of creatinine clearance (CrCl) *(1)*. However, serum Cr is of

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[3] Nonstandard abbreviations: RI, renal impairment; CVD, cardiovascular disease; GFR, glomerular filtration rate; Cr, serum creatinine; CrCl, creatinine clearance; CHD, coronary heart disease; ACS, acute coronary syndrome; MI, myocardial infarction; CRP, C-reactive protein; BMI, body mass index; ACE, angiotensin-converting enzyme; HR, hazard ratio; and CI, confidence interval.

limited value in early detection of RI, and CrCl overestimates true GFR because Cr is not only filtered by the glomeruli but is also secreted by the tubules *(11)*.

Recently, cystatin C, a cysteine protease inhibitor, has been proposed to represent a superior marker for detection of RI *(12, 13)* that seems very sensitive to small changes in GFR. Cystatin C is produced at a constant rate by all nucleated cells, is freely filtered in the glomeruli, and is almost completely reabsorbed and catabolized by the proximal renal tubular cells *(12, 14)*. Its serum concentration is thought to be determined mainly by GFR, its renal plasma clearance is virtually identical to that of ^{51}Cr-labeled EDTA *(13, 15)*, and its production has been claimed to be unaltered in inflammatory conditions *(16)*. Thus, it seems to fulfill many of the criteria of an ideal marker of GFR *(17)*. However, several recent studies *(18, 19)* have identified other factors that influence cystatin C beyond renal function. To date, no prospective study confirming the prognostic significance of cystatin C in patients with coronary heart disease (CHD) has been published *(20)*.

We therefore sought to investigate the diagnostic value of cystatin C for the prediction of future risk of cardiovascular events in a large cohort of patients with manifest CHD. Furthermore, we wanted to compare its prognostic value with that of routine markers of GFR, i.e., Cr and CrCl.

Materials and Methods

STUDY POPULATION

All patients with CHD (International Classification of Diseases, 9th Revision; codes 410–414) 30–70 years of age and participating in an in-hospital rehabilitation program between January 1999 and May 2000 in two cooperating clinics (Schwabenland-Klinik, Isny, and Klinik im Südpark, Bad Nauheim, Germany) were enrolled in the study (initial response, 58%). In Germany, all patients after acute coronary syndrome (ACS) or coronary artery revascularization are offered a comprehensive in-hospital rehabilitation program after discharge from the acute-care hospital. The aim of this 3-week program is the reduction of cardiovascular risk factors, improvement in health-related quality of life, and preservation of the ability to work (the latter only if a person was still at work at the onset of disease; otherwise, the objective was the prevention of nursing care). This in-hospital rehabilitation program usually starts ~3 weeks after the acute event or coronary artery revascularization. In the current study, only patients who were admitted within 3 months after the acute event or coronary artery revascularization were included.

All participants gave written informed consent. The study was approved by the Ethics Committees of the Universities of Ulm and Heidelberg and of the Physicians' Chamber of the States of Baden-Wuerttemberg and Hessen (Germany).

DATA COLLECTION

At the beginning of the in-hospital rehabilitation program, all patients filled out a standardized questionnaire containing sociodemographic information and medical history. In addition, information was taken from the patients' hospital charts. In all patients, active follow-up was conducted 1 and 3 years after discharge from the rehabilitation center. Information was obtained from each patient by use of a mailed standardized questionnaire. Information regarding secondary cardiovascular events and treatment since discharge from the in-hospital rehabilitation clinic was obtained from the primary care physicians via a standardized questionnaire. Physicians were unaware of the specific purpose of the study. If a patient had died during follow-up, the death certificate was obtained from the local Public Health Department and the main cause of death was coded according to the International Classification of Diseases (9th Revision). Secondary cardiovascular events were defined as CVD as the main cause of death (as stated in the death certificate), nonfatal myocardial infarction (MI), or ischemic cerebrovascular event (stroke or transitory ischemic attack).

LABORATORY METHODS

Blood at baseline was drawn at the end of the rehabilitation phase in a fasting state under standardized conditions. For Cr measurements, in one hospital the conventional kinetic Jaffe method (interassay CV, 2.4–5.7%) was used, whereas in the other hospital Cr was measured by an enzymatic *p*-aminophenazone method (interassay CV, 1.2–2.2%) *(21)*. CrCl was calculated according the Cockcroft–Gault formula *(22)*. Cystatin C concentrations in plasma were measured by immunonephelometry on a Behring Nephelometer II (Dade-Behring), and C-reactive protein (CRP) was determined by a high-sensitivity assay on the same device (N Latex CRP mono; Dade Behring). All markers were measured in a blinded fashion. The interassay CV was 3.8% for cystatin C and 5.0% for CRP. Blood lipid measurements and leukocyte counts were done by routine methods in both participating clinics.

STATISTICAL METHODS

We first described the study population with respect to various sociodemographic and medical characteristics. The association of Cr and CrCl (as indicators of RI) and of various cardiovascular risk factors with cystatin C distribution (proportion in top quintile) was quantified by means of a χ^2 test.

We then assessed the relationships of Cr, CrCl, and cystatin C with CVD events during follow-up by the life table method and quantified these values by use of the log-rank test. Cr was categorized into three groups according to Gibson et al. *(23)*: ≤106, >106 to ≤177, and >177 μmol/L. CrCl was also divided into three groups: >90 mL/min (normal renal function); 60 to <90 mL/min (mild RI); and <60 mL/min (moderate or severe RI).

Further stratification was not possible because of the small number of patients with CrCl <60 mL/min.

The Cox proportional hazards model was then used to assess the independent association of cystatin C distribution with the risk of secondary CVD events. In addition to a model adjusted for age and gender, we considered the following potential confounders: body mass index (BMI; kg/m^2), smoking status (never, current, ex-smoker), school education (<10 years, ≥10 years), family status (married, other), history of MI (yes, no), history of diabetes mellitus (yes, no), severity of cardiovascular disease (number of affected vessels at baseline), HDL-cholesterol (mmol/L), and hospital site (Isny or Bad Nauheim).

In another analysis (and to avoid overadjustment), in addition to the main factor cystatin C and the variables age, gender and hospital side, we added all covariates [school education, family status, smoking status, history of diabetes, history of MI, clinical score, HDL-cholesterol, intake of β-blockers, intake of angiotensin-converting enzyme (ACE) inhibitors, intake of diuretics] to the model if they were significant predictors of a secondary event at an α-level of 0.1 or if their inclusion changed the parameter estimates for the main variables (cystatin C) by more than 10% (BMI, HDL-cholesterol, history of diabetes mellitus, treatment with ACE inhibitors). Finally, CRP (and in an additional model, CrCl) were included. All statistical procedures were carried out with the SAS statistical software package.

Results

Overall, 1206 patients with a diagnosis of CHD within the past 3 months were included in the study at baseline during the in-hospital rehabilitation program. Three years of follow-up information was complete for 1033 patients (85.7%). The main characteristics of the study population are shown in Table 1.

Table 1. Baseline sociodemographic, clinical, and laboratory characteristics of the 1033 patients with CHD.

Mean (SD) age, years	59.0 (7.9)
Age groups, n (%)	
30–39	23 (2.3)
40–49	123 (11.9)
50–59	301 (29.1)
60–70	586 (56.7)
Men, n (%)	877 (84.9)
History of MI, n (%)	602 (58.3)
Clinical score (angiographic evaluation), n (%)	
One-vessel disease	269 (26.0)
Two-vessel disease	274 (26.5)
Three-vessel disease	442 (42.8)
Unknown	48 (4.7)
School education <10 year, n (%)	613 (59.3)
Married, n (%)	867 (83.9)
Mean (SD) BMI, kg/m^2	27.1 (3.5)
History of high blood pressure, n (%)	572 (55.4)
History of diabetes, n (%)	176 (17.0)
Mean (SD) HDL-cholesterol, mmol/L	1.0 (0.3)
CRP,[a] mg/L	3.48 (1.23;8.6)
Mean follow-up, months	33.5

[a] Geometric mean (first and third tertile cutpoints).

Of the 1033 patients with a diagnosis of CHD, 58.5% had suffered from MI, and 44.9% of patients (with coronary angiography) had three-vessel disease. The mean age of CHD patients was 59 years. Most of them (56.7%) were between 60 and 70 years of age, and 84.9% were male. Most patients had a BMI between 25 and 30 kg/m^2.

The relationships between Cr, CrCl, and various cardiovascular risk factors and cystatin C are shown in Table 2. As expected, patients with moderate or severe RI as determined by Cr or CrCl were more likely to be in the top quintile of the cystatin distribution than those with mild RI or normal renal function. Furthermore, increasing age, history of diabetes, extension of CHD, increased CRP, and intake of ACE inhibitors or diuretics were all strongly and positively related to cystatin C concentrations, whereas there was a U-shaped association with BMI and a negative association with the intake of beta-blockers.

Seventy-one patients (6.9%) experienced a secondary CVD event after a mean follow-up of 33.5 months. Twenty-one patients (2.0%) died from CVD, 30 patients (2.9%) suffered from a nonfatal MI, and 20 patients (1.9%) had been diagnosed with a stroke or transitory ischemic attack.

The associations between the three indicators of RI and CVD events during follow-up are shown in Table 3. Of patients with a Cr >106 μmol/L, 5.4% experienced an event, compared with 7.0% with a Cr ≤106 μmol/L (P = 0.63). Likewise, 7.0% of patients with a CrCl <60 mL/min and 9.0% of patients with a CrCl 60–90 mL/min had an event, compared with 6.3% of patients with a CrCl >90 mL/min (P = 0.10). In contrast, the probability of a secondary CVD event in the top quintile of the cystatin C distribution was 14.0%, whereas it was 7.7%, 4.3%, 3.9%, and 5.0%, respectively, from the fourth to the first quintile (P <0.0001).

Shown in Table 4 are the results of multivariable analysis to estimate the independent association of cystatin C concentrations at baseline with risk of fatal and nonfatal cardiovascular endpoints during follow-up after adjustment for a variety of potential confounders. Compared with patients in the bottom quintile of the cystatin C distribution at baseline, patients in the top quintile had a hazard ratio (HR) of 2.83 [95% confidence interval (CI), 1.35–5.93] for a CVD event during follow-up after adjustment for age and gender (P = 0.0005 for trend). The HR was slightly attenuated after adjustment for classic risk factors and severity of CHD (HR = 2.27; 95% CI, 1.05–4.88), and it remained unchanged after further adjustment for all factors from Table 2 that contributed significantly (P <0.1) to the model (BMI, HDL-cholesterol, history of diabetes mellitus, treatment with ACE inhibitors) and for CRP (HR = 2.27; 95% CI, 1.05–4.91). Additional adjust-

Table 2. Values for various risk factors and proportion in upper quintile of cystatin C distribution.

Risk factors	n	Proportion in upper quintile of cystatin C distribution, %	*P*
Creatinine,[a] μmol/L			
≤106 (normal)	932	15.2	
>106 to ≤177 (mild RI)	84	60.7	
>177 (severe RI)	8	87.5	<0.0001[b]
CrCl,[a] mL/min			
≥90 (normal)	569	8.1	
60 to <90 (mild RI)	398	28.6	
<60 (moderate and severe RI	57	70.2	<0.0001[b]
Gender			
Female	156	23.1	
Male	877	18.7	0.20
Age, years			
30–39	23	0	
40–49	123	4.1	
50–59	301	11.6	
60–70	586	27.3	<0.0001
School education			
<10 years	613	20.7	
≥10 years	420	17.4	0.18
Family status			
Married	867	18.7	
Other	166	22.9	0.20
BMI, kg/m^2			
<25	295	21.4	
25–30	555	16.2	
>30	182	25.3	0.02
Smoking status			
Never	329	20.4	
Ex-smoker	656	18.6	
Current smoker	48	22.9	0.66
History of diabetes			
Yes	176	33.0	
No	857	16.6	<0.0001
History of MI			
Yes	602	20.3	
No	431	18.1	0.38
Clinical score			
Zero-/One-vessel disease	269	8.6	
Two-vessel disease	274	16.8	
Three-vessel disease	442	27.4	<0.0001
HDL-cholesterol, mmol/L			
<1.03	623	19.7	
≥1.03	410	18.8	0.70
CRP, mg/L			
<10 (first–fourth quintiles)	825	14.8	
≥10 (fifth quintile)	208	37.5	<0.0001
Medications			
Beta-blockers			
Yes	900	17.7	
No	131	31.3	0.0002
ACE inhibitors			
Yes	547	23.0	
No	484	15.3	0.002
Diuretics			
Yes	255	43.5	
No	776	11.5	<0.0001

[a] Available for only 1024 patients.
[b] *P* after stratification for hospital site.

ment for CrCl showed an increase in the HR associated with cystatin C distribution (HR for top quintile = 2.75; 95% CI, 1.21–6.27).

Discussion

This prospective cohort study including 1033 patients 30–70 years of age with newly diagnosed CHD at baseline demonstrates for the first time that increased cystatin C concentrations are strongly and independently associated with future secondary cardiovascular events. Patients in the top quintile of the cystatin C distribution had a more than twofold increased risk for a secondary CVD event compared with those in the bottom quintile, even after controlling for a large variety of potential confounders, including markers of inflammation and traditional markers of impaired renal function. Therefore, cystatin C seems to be a promising and clinically useful marker that provides complementary information to the established risk determinants in patients with CHD. Furthermore, the independency of the association between cystatin C and CVD events from Cr and CrCl strongly suggests that cystatin C may represent more than just a marker of glomerular filtration.

RENAL IMPAIRMENT AND PROGNOSIS

This is the first prospective study to evaluate the risk for future CVD events in patients with CHD as assessed by plasma cystatin C concentrations. Thus, no direct comparison with other studies is possible. In the present study, only cystatin C, but not Cr and CrCl, was associated with adverse events during a 3-year follow-up. This may be related to the fact that we studied patients several weeks after they had survived a MI or had undergone revascularization and thus were at relatively low risk. Mild RI, as determined by a CrCl between 60 and <90 mL/min, was found in 38.9% of patients, whereas in only 5.6% it was <60 mL/min. This is in contrast to a recent study of patients with an ACS based on the same criteria, in whom the prevalence of RI was 63.7% *(23)*. Other authors found prevalences of 41%, using a cutpoint of 70 mL/min *(24)*, or 35%, with a cutpoint of 60 mL/min *(25)*. The higher prevalence of RI (especially severe RI) and thus the wider variation in renal function and the fact that these populations consisted of patients admitted to the hospital with ACS clearly puts them at higher risk and may explain why Cr and CrCl were predictive in these groups but not in ours. However, in the present study, the correlations between cystatin C and Cr and CrCl as common markers of renal function were $r = 0.58$ ($P < 0.0001$) and $r = -0.41$ ($P < 0.0001$), respectively.

CORRELATIONS BETWEEN CYSTATIN C AND OTHER CARDIOVASCULAR RISK FACTORS

Cystatin C was strongly correlated with age, as has been shown for other markers of renal function. However, in the bivariate analysis, it was also correlated with BMI, presence of diabetes mellitus, and extension of CHD.

Table 3. Distributions of Cr, CrCl, and cystatin C and relationships with fatal and nonfatal CVD events during follow-up (life table method).

	n (column %) (Total = 1033)		Fatal and nonfatal CVD event during follow-up, n (row %)	*P*
Creatinine,[a] μmol/L				
≤106	932 (91.0)		65 (7.0)	
>106 to ≤177	84 (8.2)	92 (8.9)	5 (5.4)	0.63
>177	8 (0.7)			
CrCl, mL/min				
>90	569 (55.6)		30 (6.3)	
60 to <90	398 (38.9)		36 (9.0)	
30 to <60	54 (5.3)	57 (5.6)	4 (7.0)	0.10
<30	3 (0.3)			
Cystatin C, mg/L				
≤0.91 (quintile 1)	221 (21.4)		11 (5.0)	
>0.91 to ≤0.99 (quintile 2)	207 (20.0)		8 (3.9)	
>0.99 to ≤1.09 (quintile 3)	210 (20.3)		9 (4.3)	
>1.09 to ≤1.24 (quintile 4)	195 (18.9)		15 (7.7)	
>1.24 (quintile 5)	200 (19.4)		28 (14.0)	<0.0001

[a] Available for only 1024 patients.

Furthermore, although it has been reported that the production of cystatin C may not be influenced by inflammation *(16)*, we found a positive correlation with CRP measured by a high-sensitivity assay ($r = 0.16$; $P < 0.0001$). Recently, Knight et al. *(18)* reported determinants of cystatin C from a large cross-sectional study in The Netherlands. They found that age, male gender, greater weight, greater height, current cigarette smoking, and higher CRP concentrations were independently associated with serum cystatin C concentrations after adjusting for CrCl, which is in line with our findings. Results from the Cardiovascular Health Study *(26)* also indicate that plasma concentrations of a variety of inflammatory and hemostatic proteins were increased in elderly individuals with RI (Cr ≥133 μmol/L in men and ≥115 μmol/L in women), supporting our observation. Therefore, the increased concentrations of CRP and other biomarkers may be the result of increased production and decreased clearance in patients with RI or the combination of both.

associations between cystatin c and cardiovascular drug therapy

More patients on ACE inhibitors or diuretics were in the top quintile of the cystatin C distribution compared with those not on these medications. This may reflect more advanced disease in those on the respective drugs, confounding by indication, or they may represent an effect of these drugs on renal function. However, our bivariate analysis cannot explain the association with beta-blocker medication for which only 18% of patients were in the top quintile of the cystatin C distribution compared with 31% of those not receiving a beta-blocker.

cystatin c is more than purely a marker of glomerular filtration

The fact that cystatin C was still an independent predictor of prognosis after adjustments had been made for CrCl can only partly be explained by the lower sensitivity of CrCl to detect RI. Together with the observation by

Table 4. Association of cystatin C concentrations at baseline with fatal and nonfatal cardiovascular events during follow-up: Results of multivariate analysis.

	HR (95% CI)		
	Adjusted for age and gender	Adjusted for multiple covariates[a]	Adjusted for multiple covariates[b]
Cystatin C, mg/L			
≤0.91 (quintile 1)	1 referent	1 referent	1 referent
>0.91 to ≤0.99 (quintile 2)	0.77 (0.31–1.92)	0.86 (0.34–2.15)	0.81 (0.32–2.02)
>0.99 to ≤1.09 (quintile 3)	0.83 (0.34–2.02)	0.83 (0.34–2.04)	0.75 (0.30–1.82)
>1.09 to ≤1.24 (quintile 4)	1.55 (0.70–3.45)	1.36 (0.60–3.09)	1.35 (0.59–3.06)
>1.24 (quintile 5)	2.83 (1.35–5.93)	2.27 (1.05–4.88)	2.27 (1.05–4.91)
P for trend	0.0005	0.01	0.009

[a] Adjusted for age, gender, BMI, smoking status, duration of school education, family status, history of MI, history of diabetes mellitus, severity of CVD (number of affected vessels at baseline), HDL-cholesterol, and hospital site.

[b] Adjusted for age, gender, hospital site, all factors from Table 2 that contributed significantly ($P < 0.1$) to the model (BMI, HDL-cholesterol, history of diabetes mellitus, treatment with ACE inhibitors), and CRP.

Hillege et al. *(7)* in the large PREVEND study, in which cystatin C was clearly superior to Cr in predicting total mortality, this suggests that the information contained by cystatin C goes beyond purely that of a marker of glomerular filtration. Moreover, adjustment for a variety of other determinants seen in our cohort, which have also been reported by others *(18, 19)*, did not appreciably change the results.

Cystatin C is involved in the human immune defense via inhibition of human polymononuclear cell chemotaxis *(27)*. During vascular injury, there is increased production of inflammatory cytokines, which in turn stimulate the production of elastolytic cysteine proteases. This might be counterbalanced by their most abundant inhibitor, cystatin C *(28)*. By regulating protease activities, protease inhibitors play an important role in tissue remodeling, in particular in the postinfarction period. Thus, it might be speculated that increased plasma concentrations of cystatin C in patients with manifest CVD (most of them post-MI) and a future secondary event represent in part a compensatory mechanism for the increased activity of elastolytic proteases in an attempt to restore the physiologic balance between proteases and their main inhibitor. However, in a recent small prospective study of 133 apparently healthy men, who were compared with 133 age- and smoking-matched controls, plasma concentrations of cystatin C were not associated with future risk of developing symptomatic peripheral arterial disease *(29)*, and in a cross-sectional study, lower plasma concentrations of cystatin C were detected in postinfarction patients compared with controls *(30)*.

STUDY LIMITATIONS

The following limitations of our study should be considered. Although we had a large sample of patients with CHD (>50% with a MI), fatal CVD events were rare in this study population. This is explained by the fact that mortality of MI is highest during the pre- and early in-hospital phases. The acute events leading to diagnosis of CHD or MI had occurred at least 3 weeks before inclusion in this study; therefore, selection of patients with a better prognosis compared with a patient population in the early phase of a newly diagnosed CHD must be assumed. Furthermore, not all patients were willing or able to participate in an in-hospital rehabilitation program. This may be another reason for the underrepresentation of severely ill patients in our study sample. However, this does not explain the positive findings between cystatin C serum concentration and CVD events.

These data are in support of an important prognostic value of cystatin C among patients with known CHD and suggest that cystatin C is a useful clinical marker that provides complementary information to the established risk determinants. These data also suggest that cystatin C may be more than a marker of GFR; thus, further mechanistic studies are warranted to fully elucidate its role in renal function and CVD.

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