Cystatin C and the Risk for Cardiovascular Events in Patients With Asymptomatic Carotid Atherosclerosis

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Background and Purpose—Renal dysfunction is a risk factor for cardiovascular events in patients with atherosclerosis. Unlike serum creatinine or estimated glomerular filtration rate, cystatin C reflects renal dysfunction independent of factors such as sex, weight, and race. We investigated whether baseline serum levels of cystatin C predict major cardiovascular events in patients with asymptomatic carotid atherosclerosis and compared the predictive value of cystatin C to these established markers of renal function.

Methods—We prospectively studied 1004 of 1286 consecutive patients with carotid ultrasound scanning. Patients were followed for the occurrence of major cardiovascular events, a composite of myocardial infarction, percutaneous coronary intervention, coronary bypass graft, stroke, and death.

Results—During a median of 3 years of follow-up, we recorded 346 major cardiovascular events in 311 patients. The risk for a first major cardiovascular event increased significantly with increasing quintiles of cystatin C; hazard ratios ranged from 1.18 to 1.94 for the highest versus the lowest quintile (P<0.001 for trend). Creatinine levels showed no significant association with major cardiovascular events, and for glomerular filtration rate, only the lowest quintile was moderately associated with adverse cardiovascular outcome.

Conclusions—Cystatin C was significantly and gradually associated with future cardiovascular events in patients with carotid atherosclerosis. In contrast, neither serum creatinine nor estimated glomerular filtration rate were significant predictors of adverse cardiovascular outcomes. (Stroke. 2010;41:674-679.)

Key Words: carotid atherosclerosis ■ renal dysfunction ■ cystatin C ■ major adverse cardiovascular events

Renal dysfunction is associated with cardiovascular morbidity and mortality, and the risk for major adverse cardiovascular events (MACE) increases continuously with a decline in kidney function.1,2 Previously, it has been demonstrated that even mild to moderate elevation of serum creatinine indicate an increased risk for cardiovascular events.3 Several large, community-based cohort studies observed an independent, graded association between a reduced estimated glomerular filtration rate (GFR) and the risk of MACE.4 However, serum creatinine and estimated GFR levels are influenced by several variables, such as sex, age, race, lean muscle mass, and weight, and, particularly, minor and moderate declines in renal function are only inaccurately displayed by these markers.5 Therefore, by measurement of serum creatinine or glomerular filtration, minor renal dysfunction remains undetected in a considerable number of patients who are already at a higher risk for cardiovascular events.

Cystatin C (CysC) is a serum marker of renal function, a protein with a low molecular weight of ≈13.3 kDa, freely filtered by the renal glomerulus and then metabolized by the proximal tubule.6 CysC serum concentrations seem to be independent of individual factors. Thus, CysC is thought to be a more sensitive parameter for renal function assessment compared with serum creatinine or estimated GFR.7 In this context, CysC was also identified as a stronger predictor for death and cardiovascular events in patients with symptomatic coronary heart disease and diabetes mellitus.8–10 We hypothesized that CysC is associated with future MACE in neurologically asymptomatic patients with carotid atherosclerosis indicated by presence of carotid plaques or carotid stenosis, and that CysC more accurately predicts adverse outcomes compared with serum creatinine levels or estimated GFR.

Methods

Study Design
Between March 2002 and March 2003, 1286 consecutive patients without recent transient ischemic attack or stroke were prospectively enrolled in the Inflammation and Carotid Artery Risk for Athero-
sclerosis Study. The study was approved by the local ethics committee, and all patients gave their written informed consent.

Inclusion and Exclusion Criteria
Inclusion and exclusion criteria have been reported previously. Briefly, patients with prevalent atherosclerotic carotid artery disease, as identified in the ultrasound laboratory of a university hospital, who were clinically asymptomatic at the time of enrollment were eligible. Patients with a cardiovascular event (myocardial infarction, stroke, coronary revascularization, or peripheral vascular surgery) during the preceding 6 months were excluded. The rationale to exclude patients with recent cardiovascular events was to avoid the potential impact of prerenal factors such as low-output heart failure, sepsis, or multigorgan failure or complications of interventions (renal dysfunction attributable to contrast administration) on CysC levels, which may rather reflect the severity of the acute situation than the level of chronic renal dysfunction.

Study End Point
Occurrence of a first cardiovascular event, a composite of myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, stroke, or death during the follow-up period were defined as the primary clinical study end point. As a secondary objective, we investigated the association between renal function and the occurrence of a triple-end point of myocardial infarction, stroke, and death.

Clinical Data
After enrollment of a patient, the medical history and the clinical results from physical examination were recorded. This included age, sex, height, weight, blood pressure, arterial hypertension, smoking status, diabetes mellitus, hyperlipidemia, family history of atherosclerotic disease, history of coronary artery disease, history of peripheral artery disease, history of cerebrovascular disease, and current medication. All demographics and vital parameters were checked on completeness and accuracy by 2 independent observers.

Laboratory Analysis
All standard laboratory analyses were determined according to local standard procedures. Serum CysC was measured from frozen samples (−80°C) collected at the baseline study visit with the use of a BNII nephelometer (Dade Behring, Inc) with a particle-enhanced immunonephelometric assay (N Latex Cystatin C; Dade Behring, Inc). The assay range is 0.195 to 7.330 mg/L; the reference range for young healthy persons is 0.53 to 0.95 mg/L. The intra-assay coefficient of variation ranges from 2.3% to 3.1%. Serum creatinine was determined by the Jaffe method on a Roche/Hitachi MODULAR (Roche Diagnostics, Inc). The assay range is 0.195 to 7.330 mg/L; the reference range for young healthy persons is 0.53 to 0.95 mg/L. The intra-assay coefficient of variation ranges from 2.0% to 2.8%, and the interassay coefficient of variation ranges from 2.3% to 3.1%. Serum creatinine was determined by the Jaffe method on a Roche/Hitachi MODULAR (Roche Diagnostics). For estimated GFR calculation, the Modification of Diet in Renal Disease equation was used. Briefly, GFR=186×(serum creatinine ^1.154)×(age ^0.203)×(0.742 if female)×(1.21 if black).

Surveillance Protocol
Patients were scheduled for follow-up visits in 6- to 9-month intervals after the initial presentation for clinical re-examination. In addition, a follow-up questionnaire was sent to each patient after 3 years, re-evaluating the occurrence of cardiovascular events. Information from the follow-up questionnaire was validated by reviewing the original hospital discharge reports of corresponding readmissions because of cardiovascular events. A total of 743 of 1004 (75%) questionnaires were returned. If the follow-up questionnaire was not returned, personal telephone contact to the patients, their relatives, or to the treating physicians was established. Additional information was obtained by reviewing the hospital discharge reports of any other readmission during the follow-up period. The performance of percutaneous coronary interventions or coronary bypass graft was validated by review of the original procedure protocols. Outcome was assessed by 2 independent observers who were blinded with respect to patients’ baseline clinical and laboratory data.

Definitions
Myocardial infarction and stroke were defined according to published guidelines. For stroke, cranial computed tomography or MRI was used for confirmation of the diagnosis. Definitions of traditional cardiovascular risk factors are given previously.

Statistical Methods
Continuous data are presented as the median and the interquartile range (range from the 25th to the 75th percentile), or the total range. Discrete data are given as counts and percentages. We used χ² tests, Mann–Whitney U tests, exact tests, and Spearman correlation coefficients for univariate analyses, as appropriate. Time-dependent variables were analyzed using the Kaplan–Meier method and compared by the log rank test. For this purpose, levels of CysC, serum creatinine, and estimated GFR were categorized in quintiles. Multi-variable Cox proportional hazards models were applied to assess the association between CysC, serum creatinine, and estimated GFR and the occurrence of a first cardiovascular event. Results of the Cox models are presented as the hazards ratio and 95% CIs. We assessed the overall model fit using Cox–Snell residuals. Further, we tested the proportional hazard assumption for all covariates using Schoenfeld residuals (overall test) and the scaled Schoenfeld residuals (variable-by-variable testing). According to the tests, the proportional hazards assumption was not violated. In addition, we analyzed the association between CysC, serum creatinine, and estimated GFR and cardiovascular events in predefined strata according to patients’ cardiovascular risk profile at baseline. A 2-sided P<0.05 was considered statistically significant. Calculations were performed with Stata (release 8.0; Stata) and SPSS for Windows (version 15.0; SPSS Inc).

Results
Patient Characteristics and Follow-Up
In total, 1364 patients were screened for the study. Of these, 78 subjects did not meet eligible criteria because of recent cardiovascular events. A sensitivity analysis including these 78 patients with recent cardiovascular events showed virtually identical effect sizes as the final analysis of the study population (data not shown).

A total of 1286 patients met eligible criteria and were enrolled in the study. From 61 patients, no frozen samples for determination of CysC were available, and another 221 patients (17%) were lost to clinical follow-up, leaving 1004 patients for the final analysis. Of these, 668 patients (63%) were male, and the median age was 69 years (interquartile range, 61 to 76). Demographic data and baseline characteristics of 1004 patients are given in Table 1. There were no significant differences of baseline clinical characteristics (age, sex, frequency of atherothrombotic risk factors, and cardiovascular comorbidities) of the 282 patients who had to be excluded compared with the study population of 1004 patients (data not shown).

Of 1004 patients included in the final analysis, the association of CysC at baseline, other measures of renal function, and cardiovascular risk factors and comorbidities is presented in Table 2. As expected, hypertension, diabetes, HbA1c, creatinine, and estimated GFR were significantly associated with CysC levels.

Follow-Up for Cardiovascular Events
During a median of 3 years of clinical follow-up (interquartile range, 2.5 to 3.5 years), we recorded 359 cardiovascular events in 271 (27%) patients, including 38 myocardial infarc-
tions (3.8%), 75 percutaneous coronary interventions (7.5%), 43 coronary bypass grafts (4.3%), 52 strokes (5.2%), and 151 deaths (15%) of which 120 (79.5%) were attributable to vascular causes. Cumulative event-free survival rates at 1, 2, and 3 years were 89% (95% CI, 0.88 to 0.92), 82% (95% CI, 0.80 to 0.85), and 73% (95% CI, 0.69 to 0.77).

**Renal Function and MACE**

CysC levels (in quintiles) were significantly associated with the occurrence of a first cardiovascular event by univariate analysis (log rank \( P < 0.001 \)). To account for potential confounding effects, we calculated the risk for MACE by multivariable Cox proportional hazards analysis adjusting for age (years), sex (male/female), body mass index (kg/m²), smoking (in categories), hypertension (yes/no), low-density lipoprotein cholesterol level (mg/dL), glycohemoglobin A1 level (%), history of myocardial infarction (yes/no), peripheral artery disease (yes/no), history of stroke (yes/no), baseline degree of carotid stenosis (in categories), and statin treatment (yes/no; Figure 1). Adjusted hazard ratios for a first MACE for increasing quintiles of CysC were 1.18 (95% CI, 0.77 to 1.8), 1.36 (95% CI, 0.89 to 2.08), 1.65 (95% CI, 1.11 to 2.47), and 1.94 (95% CI, 1.31 to 2.88), compared with the lowest quintile, respectively (Figure 2; \( P < 0.001 \) for trend), indicating a gradual, significant, and independent association between CysC and MACE. Reanalyzing the association between CysC and MACE, including only vascular death, showed similar results as for all-cause mortality, and the analyses on individual disease end points revealed that the association between CysC and MACE is mainly driven by stroke and vascular death (data not shown).

Serum creatinine levels were significantly associated with MACE by univariate analysis (log rank; \( P < 0.001 \)). However, by multivariable analysis adjusting for the same variables as listed above, increasing quintiles of serum creatinine were not significantly associated with the risk for a first MACE; adjusted hazard ratios were 0.86 (95% CI, 0.58 to 1.27), 0.67 (95% CI, 0.46 to 1.09), 1.14 (95% CI, 0.77 to 1.69), and 1.31 (95% CI, 0.89 to 1.91) compared with the lowest quintile, respectively (Figure 2; \( P = 0.17 \) for trend).

Estimated GFR was significantly inversely associated with MACE by univariate analysis (log rank \( P < 0.001 \)). Adjusted hazard ratios for a first cardiovascular event for decreasing quintiles of estimated GFR were 0.97 (95% CI, 0.66 to 1.42), 1.17 (95% CI, 0.83 to 1.63), 0.86 (95% CI, 0.58 to 1.27), and 0.67 (95% CI, 0.46 to 1.09), respectively (Figure 2), indicating that only patients in the lowest quintile of GFR had a significantly increased risk for MACE (\( P = 0.31 \) for trend).

### Table 1. Baseline Characteristics of 1004 Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Subjects</td>
<td>1004</td>
</tr>
<tr>
<td>Age, y</td>
<td>69 (61 to 76)</td>
</tr>
<tr>
<td>Male sex, no. (%)</td>
<td>632 (62.9)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.1 (24.0 to 28.7)</td>
</tr>
<tr>
<td>Diabetes mellitus, no. (%)</td>
<td>226 (22.5)</td>
</tr>
<tr>
<td>Hypertension, no. (%)</td>
<td>689 (68.6)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>1 to 10 cigarettes daily 99 (9.9)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL*</td>
<td>205 (177 to 236)</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol, mg/dL*</td>
<td>50 (42 to 60)</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol, mg/dL*</td>
<td>119 (94 to 146)</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.9 (5.6 to 6.5)</td>
</tr>
<tr>
<td>CysC, mg/L</td>
<td>0.69 (0.54 to 0.89)</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>1.06 (0.93 to 1.23)</td>
</tr>
<tr>
<td>Estimated GFR, mL/min/1.73 m²</td>
<td>66 (55 to 76)</td>
</tr>
<tr>
<td>History of myocardial infarction, no. (%)</td>
<td>245 (24.4)</td>
</tr>
<tr>
<td>History of stroke, no. (%)</td>
<td>168 (16.7)</td>
</tr>
<tr>
<td>Peripheral artery disease, no. (%)</td>
<td>433 (43.1)</td>
</tr>
<tr>
<td>Family history of atherosclerosis</td>
<td>542 (54)</td>
</tr>
</tbody>
</table>

Continuous data are presented as the median and the interquartile range. Discrete data are given as counts and percentages.

* Multiply by 0.0259 to convert variables to mmol/L.

### Table 2. Correlation Between CysC and Standard Vascular Risk Factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>CysC (mg/dL)</th>
<th>( r^2 )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperlipidemia, yes/no</td>
<td>0.68 (0.55 to 0.89)/0.70 (0.54 to 0.88)</td>
<td>—</td>
<td>0.66</td>
</tr>
<tr>
<td>Diabetes, yes/no</td>
<td>0.74 (0.59 to 0.98)/0.67 (0.53 to 0.86)</td>
<td>—</td>
<td>0.002</td>
</tr>
<tr>
<td>Hypertension, yes/no</td>
<td>0.71 (0.56 to 0.93)/0.66 (0.52 to 0.81)</td>
<td>—</td>
<td>0.001</td>
</tr>
<tr>
<td>Smoking, yes/no</td>
<td>0.69 (0.56 to 0.86)/0.69 (0.53 to 0.89)</td>
<td>—</td>
<td>0.25</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol</td>
<td>—</td>
<td>0.002</td>
<td>0.15</td>
</tr>
<tr>
<td>HbA1c</td>
<td>—</td>
<td>0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine</td>
<td>—</td>
<td>0.59</td>
<td>0.001</td>
</tr>
<tr>
<td>Estimated GFR</td>
<td>—</td>
<td>0.35</td>
<td>0.001</td>
</tr>
<tr>
<td>Peripheral artery disease, yes/no</td>
<td>0.71 (0.57 to 0.93)/0.67 (0.52 to 0.83)</td>
<td>—</td>
<td>0.22</td>
</tr>
<tr>
<td>Coronary artery disease, yes/no</td>
<td>0.71 (0.55 to 0.93)/0.67 (0.53 to 0.83)</td>
<td>—</td>
<td>0.67</td>
</tr>
<tr>
<td>Degree of carotid stenosis</td>
<td>—</td>
<td>0.002</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Continuous data are presented as the median and the interquartile range.
Discussion

Renal dysfunction is a risk factor for future cardiovascular events among patients with carotid atherosclerosis. CysC serum levels showed a gradual and independent association with MACE and seem to be a potent predictor for adverse cardiovascular outcome. In contrast, serum creatinine was not significantly associated with cardiovascular end points, and estimated GFR predicted adverse outcomes only in the lowest quintile, thus underestimating the cardiovascular risk, particularly in patients with mild to modest renal dysfunction.

Serum creatinine levels within the normal range do not necessarily reflect normal renal function. The Modification of Diet in Renal Disease equation tries to adjust for creatinine confounding variables, but it is still a creatinine-dependent method for renal function assessment. In fact, the sensitivity of serum creatinine and estimated GFR for the detection of mild decrements in renal function is rather low. Because even moderate renal dysfunction impacts patients’ cardiovascular prognosis, this delays the identification of patients at high risk for cardiovascular events and death. In patients with carotid atherosclerosis, we found CysC to be a more potent and more sensitive indicator of cardiovascular risk, superior to serum creatinine and estimated GFR.

Our results are consistent with data from previous studies, which also described renal dysfunction as a risk factor for cardiovascular events and death. Shlipak et al found an association between CysC and the risk for death and cardiovascular events among an elderly study population. Similar findings were described by Ix et al among a predominately male population with coronary heart disease. However, our study is the first to demonstrate that CysC is a strong predictor for MACE among a cohort of patients with carotid atherosclerosis without signs of transient ischemic attack or stroke. Patients from our study had generally lower CysC serum levels compared with patients from other trials. This might be explained by the demographics of our study population. Our patients were younger, and females were almost equally represented.

Risk stratification is a key issue in the treatment of patients with atherosclerotic disease. Patients with carotid atherosclerosis clearly benefit from an early identification for increased risk of future cardiovascular events. Early onset of renal
impairment indicates an increased risk for MACE among patients with cardiovascular disease. The mechanisms for increased cardiovascular disease risk in patients with renal dysfunction are not completely understood so far, but it seems to be a multifactorial process. In addition to the accumulation of traditional risk factors in patients with chronic renal disease, there are other specific factors that are thought to be related to renal dysfunction. This includes higher levels of inflammation and oxidative stress and additionally hypoalbuminemia, anemia, and dysregulation of the calcium and phosphorus metabolism. Although numerous studies suggest that CysC is a potent parameter for renal function assessment, serum creatinine is still the most commonly used laboratory marker in renal diagnostic. This might be explained by the lower costs of creatinine measurement compared with CysC. Further, establishing a new parameter for routine renal function assessment certainly remains a challenge. Based on current data, we conclude that CysC measurement should be considered for renal function assessment as well as for risk stratification in patients with carotid atherosclerosis. However, discussing the prognostic value of renal dysfunction in the context of risk stratification of patients with carotid atherosclerosis, other nonlaboratory investigations provide important information, particularly with respect to stroke risk. It has been demonstrated recently that the presence of microemboli on transcranial Doppler monitoring strongly correlate with future cerebrovascular events in patients with asymptomatic carotid stenosis. Microemboli signals indicate a substantially higher incremental risk for stroke compared with elevation of renal parameters such as CysC. Nevertheless, CysC, in contrast to transcranial Doppler measurements, also indicates an increased risk for cardiac adverse events and mortality and thus reflects a broad spectrum of cardiovascular and cerebrovascular risk stratification.

Limitations
We are aware of several limitations of our study. First, selection of patients was hospital based, displayed by a rather high prevalence of atherosclerotic comorbidities, diabetes, and arterial hypertension, compared with a community-based population. Further, our sample size was too small to observe meaningful numbers of renal end points (dialysis or kidney transplantation). Therefore, it remains unclear in these patients whether CysC levels also predict adverse renal outcomes.

Conclusion
CysC was significantly and gradually associated with the occurrence of future cardiovascular events in patients with asymptomatic carotid atherosclerosis. In contrast, neither serum creatinine nor estimated GFR were significant predictors of adverse cardiovascular outcomes.

Acknowledgments
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Disclosures
None.

References


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