Cystatin C, Renal Function, and Atherosclerosis in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** We examined the hypothesis that cystatin C, a novel marker of renal function, is elevated in rheumatoid arthritis (RA) and is associated with inflammation and coronary atherosclerosis.

Methods. We measured serum cystatin C, creatinine, tumor necrosis factor-α and interleukin 6 concentrations, coronary artery calcium score (CACS), and Modified Diet in Renal Disease estimated glomerular filtration rate in 167 patients with RA and 91 controls.

Results. Cystatin C was higher in RA patients [median (IQR) 1.16 (0.99–1.35) mg/l] than controls [1.01 (0.90–1.19) mg/l; $p < 0.001$] and correlated positively with erythrocyte sedimentation rate ($p < 0.001$), C-reactive protein ($p = 0.01$), 28-joint Disease Activity Score ($p = 0.006$), and Framingham risk score (FRS; $p = 0.02$). Cystatin C was correlated with CACS ($p < 0.001$) in RA, but this was not significant after adjustment for age, race, sex, and FRS ($p = 0.44$).

Conclusion. Cystatin C concentrations are higher in RA than controls and may reflect inflammation and undetected subclinical renal dysfunction. Cystatin C provides information regarding the risk of atherosclerosis in RA, but this is not independent of the information provided by conventional cardiovascular risk factors. (J Rheumatol First Release Aug 15 2011; doi:10.3899/jrheum.110168)

Key Indexing Terms:
CYSTATIN RHEUMATOID ARTHRITIS RENAL FUNCTION ATHEROSCLEROSIS

Cystatin C is a novel marker of renal function. Unlike creatinine, it is not substantially affected by muscle mass or diet[1] and thus is a more accurate measure of glomerular filtration rate (GFR). Cystatin C concentrations also predict cardiovascular events[2,3]. Rheumatoid arthritis (RA) is frequently associated with chronic inflammation, subclinical renal impairment[4], and increased cardiovascular risk[5]. We examined the hypotheses that cystatin C concentrations (1) are higher in patients with RA than in controls, independent of renal function; (2) are associated with measures of inflammation in RA; and (3) are associated with coronary atherosclerosis.

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MATERIALS AND METHODS

As described, we studied 167 patients with RA and 91 controls frequency matched for age, sex, and race[5,6]. Our study was approved by the Institutional Review Board of Vanderbilt University Hospital and all subjects gave written informed consent. Clinical and demographic variables were measured: Agatston coronary artery calcium score (CACS), Framingham risk score (FRS), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukin 6 (IL-6), as described[5,6]. Cystatin C concentrations in serum were measured by ELISA. The Modification of Diet in Renal Disease (MDRD) formula was used to estimate GFR (eGFR)[7].

Statistical analysis. Descriptive statistics are presented as the mean ± SD or as the median with interquartile range (IQR), as appropriate. Concentrations of cystatin C, creatinine, and MDRD-eGFR were compared in patients with RA and controls using Wilcoxon signed-rank tests and multivariable linear regression with adjustment for age, race, and sex. In RA, correlation between cystatin C and measures of renal function, traditional cardiovascular risk factors, inflammation, other clinical variables, and CACS was examined using Spearman's rank correlation coefficient. The association between clinical and laboratory variables and renal function measures (cystatin C, creatinine, and MDRD-eGFR) was assessed using separate multivariable linear regression adjusting for age, race, and sex.

The effect of the renal function measures on the CACS was examined by applying a proportional odds logistic regression model with adjustment for age, race, sex, and FRS. Age was allowed to have nonlinear terms because it has the strongest predictive value for atherosclerosis. Concentrations of cystatin C, creatinine, homocysteine, CRP, TNF-α, and IL-6 were natural logarithm-transformed to improve normality. Statistical

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analyses were performed using R version 2.10.0 (http://www.r-project.org), and 2-sided p values < 0.05 were considered statistically significant.

RESULTS

Characteristics of patients with RA (n = 167) and controls (n = 91) are shown in Table 1. Cystatin C was significantly higher in patients with RA (p < 0.001); however, creatinine and MDRD-eGFR did not differ significantly. Cystatin C remained significantly higher in RA after adjustment for age, race, and sex (p < 0.001), and after additional adjustment for MDRD-eGFR (p < 0.001).

In RA, cystatin C was positively correlated with creatinine (p < 0.001) and negatively correlated with MDRD-eGFR (p < 0.001). In controls, cystatin C was negatively correlated with MDRD-eGFR (p = 0.01) but not with creatinine (p = 0.14). In RA, serum creatinine was significantly higher in men than women (p < 0.001); however, cystatin C (p = 0.12) and MDRD-eGFR (p = 0.39) were not (Table 2). Cystatin C, creatinine, and MDRD-eGFR were not significantly associated with smoking, diabetes mellitus, or current use of corticosteroids, nonsteroidal antiinflammatory drugs, and cyclooxygenase-2 inhibitors after adjustment for age, race, and sex. However, methotrexate (MTX) use was associated with lower cystatin C concentrations (p < 0.001; Table 2). Anti-TNF therapy did not affect cystatin C concentrations.

Table 1. Baseline characteristics of control subjects and patients with rheumatoid arthritis (RA). Variables are shown as median (interquartile range) unless otherwise indicated.

Variables	Controls, n = 91	RA, n = 167	p*
Age, yrs, mean ± SD	53.3 ± 11.6	54.2 ± 11.8	0.46
% Women	63	69	0.31
% White	85	89	0.36
BMI, kg/m^2, mean ± SD	28.5 ± 5.9	29.2 ± 6.7	0.46
Current smokers, %	9	24	0.003
Hypertension, %	38	53	0.03
Diabetes, %	4	11	0.06
Cardiovascular disease, %	10	14	0.37
Systolic blood pressure, mm Hg	129 (115–137)	133 (118–146)	0.07
Total cholesterol, mg/dl	195 (170–216)	184 (157–211)	0.09
CRP, mg/l	0.6 (0.2–1.5)	4.0 (1.2–10.5)	< 0.001
TNF-α, pg/ml	3.3 (2.4–4.7)	5.5 (2.8–11.0)	< 0.001
IL-6, pg/ml	4.2 (1.2–17.9)	13.8 (4.4–43.0)	< 0.001
Homocysteine, μmol/l	8.2 (7.2–9.7)	10.2 (8.1–11.9)	< 0.001
Cystatin C, mg/l	1.01 (0.90–1.19)	1.16 (0.99–1.35)	< 0.001
Creatinine, mg/dl	0.8 (0.7–0.9)	0.8 (0.7–0.9)	0.62
MDRD-eGFR, ml/min/1.73 m^2	90.6 (81.7–100.1)	92.8 (78.4–109.5)	0.53
Coronary calcium score, Agatston units	0.0 (0–19.2)	2.7 (0–150.4)	0.02

* Wilcoxon signed-rank test was used for comparing continuous variables, and categorical variables were compared using the chi-square test. BMI: body mass index; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; IL: interleukin; MDRD-eGFR: Modified Diet in Renal Disease formula estimated glomerular filtration rate.

Cystatin C and MDRD-eGFR were correlated significantly with the FRS, but after adjustment for age, race, and sex, only cystatin C remained significant (p = 0.02). After statistical adjustment for age, race, and sex, cystatin C was significantly associated with erythrocyte sedimentation rate (p < 0.001), CRP (p = 0.01), and 28-joint Disease Activity Score (p = 0.006), but not with IL-6 (p = 0.32) or TNF-α (p = 0.11; Table 3). MDRD-eGFR and serum creatinine were not significantly associated with measures of inflammation (Table 3).

Cystatin C (p < 0.001), creatinine (p = 0.01), and MDRD-eGFR (p = 0.03) all correlated significantly with CACS in univariate analyses, but not after adjustment for age, race, sex, and FRS (Table 3).

DISCUSSION

Cystatin C is superior to creatinine in providing an accurate measure of renal function[1]. However, cystatin C also predicts cardiovascular risk[2,3,8], perhaps through association with inflammation[9,10,11], as well as renal impairment[1].

The concentrations of cystatin C in RA [median (IQR) 1.16 (0.99–1.35) mg/l] and controls [1.01 (0.90–1.19) mg/l] were similar to those found in other populations without known renal impairment. For example, in blood donors the reference range for cystatin C concentrations was 0.53–0.92 mg/l in those < 50 years of age and 0.58–1.02 mg/l in those > 50 years of age[12], and in the Framingham Offspring Study (n = 3214, mean age 61 yrs) the mean cystatin C concentration was 0.93 ± 0.18 mg/l[13]. Cystatin C was significantly higher in patients with RA than controls, even after adjustment for age, race, sex, and a creatinine-based measure of GFR. Therefore, it seems likely that inflammation rather than subclinical renal dysfunction is the major explanation for the increased concentrations of cystatin C in RA. MTX, but not other drugs commonly used to treat RA, was associated with lower cystatin C concentrations. Because serum creatinine did not differ significantly in patients using MTX, the lower cystatin C concentrations in MTX users are unlikely to be explained by the preferential prescription of MTX to patients with better renal function. MTX possibly affects the inflammatory pathways involved in the production or elimination of cystatin C.

In the Framingham Offspring Study, cystatin C was associated with several cardiovascular risk factors even in the absence of chronic kidney disease[13]. Our study confirmed the association between cardiovascular risk, measured as FRS, and cystatin C in RA. Generally, cystatin C has been associated with CACS in univariate but not multivariable analyses that adjusted for cardiovascular risk factors[14,15]. This is similar to our findings in RA.

Cystatin C concentrations are higher in patients with RA even after adjustment for renal function. Cystatin C provides

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Table 2. Measures of renal function according to categorical clinical characteristics in patients with rheumatoid arthritis. Cystatin C, creatinine, and MDRD-eGFR values are represented as median (interquartile range).

Variable	N	Cystatin C, mg/l	p	Adjusted p*	Creatinine, mg/dl	p	Adjusted p*	MDRD-eGFR, ml/min/1.73 m^2	p	Adjusted p*
Sex										
Men	52	1.20 (1.07–1.37)	0.12	—	0.9 (0.8–1.0)	< 0.001	—	94.2 (82.6–105.6)	0.39	—
Women	115	1.11 (0.95–1.35)			0.7 (0.6–0.8)			90.7 (77.5–110.9)		
Current smoking										
Yes	40	1.22 (1.05–1.34)	0.35	0.10	0.8 (0.6–0.83)	0.11	0.13	100.7 (83.6–113.8)	0.04	0.16
No	127	1.14 (0.97–1.35)			0.8 (0.7–0.9)			91.5 (76.8–106.5)		
Hypertension										
Yes	88	1.25 (1.05–1.49)	< 0.001	0.10	0.8 (0.7–1.0)	0.07	0.10	88.8 (69.7–105.4)	0.002	0.10
No	79	1.07 (0.91–1.26)			0.8 (0.65–0.9)			97.1 (85.2–111.8)		
MTX										
Yes	119	1.11 (0.97–1.30)	< 0.001	< 0.001	0.8 (0.6–0.9)	0.007	0.10	94.5 (79.0–110.7)	0.17	0.11
No	48	1.31 (1.08–1.75)			0.8 (0.7–1.0)			89.5 (72.6–103.9)		
Corticosteroid										
Yes	90	1.12 (0.99–1.35)	0.97	0.86	0.8 (0.63–0.9)	0.39	0.08	97.3 (81.0–111.1)	0.05	0.05
No	77	1.16 (1.01–1.35)			0.8 (0.7–0.9)			89.6 (75.2–105.2)		
NSAID/Cox-2 inhibitor										
Yes	102	1.11 (0.98–1.34)	0.12	0.11	0.8 (0.7–0.9)	0.34	0.44	92.7 (79.3–106.8)	0.59	0.47
No	65	1.25 (1.03–1.40)			0.8 (0.7–0.9)			92.8 (78.0–112.0)		

* p value adjusted for age, race, and sex with multivariable linear regression. MDRD-eGFR: Modified Diet in Renal Disease formula estimated glomerular filtration rate; NSAID: nonsteroidal antiinflammatory drugs; COX: cyclooxygenase; MTX: methotrexate.

Table 3. Relationship between measures of renal function and cardiovascular risk factors, inflammation, and coronary calcium score in patients with rheumatoid arthritis.

Factor	Cystatin C			Creatinine			MDRD-eGFR		
	Rho†	p*	Adjusted p**	Rho†	p*	Adjusted p**	Rho†	p*	Adjusted p**
Age	0.442	< 0.001	—	0.182	0.02	—	–0.329	< 0.001	—
Body mass index	–0.096	0.22	0.36	–0.134	0.09	0.58	0.120	0.12	0.56
Systolic blood pressure	0.198	0.01	0.89	0.095	0.22	0.30	–0.154	0.05	0.32
Diastolic blood pressure	0.116	0.14	0.66	0.113	0.15	0.80	–0.019	0.81	0.95
HDL cholesterol	–0.149	0.06	0.02	–0.072	0.36	0.87	–0.032	0.68	0.95
LDL cholesterol	–0.042	0.59	0.36	–0.136	0.08	0.12	0.112	0.15	0.20
Homocysteine	0.493	< 0.001	< 0.001	0.355	< 0.001	0.004	–0.238	0.002	0.02
Framingham score	0.438	< 0.001	0.02	0.038	0.63	0.63	–0.237	0.002	0.57
Disease duration	0.149	0.06	0.83	–0.082	0.29	0.06	–0.014	0.86	0.09
DAS28 Index	0.193	0.01	0.006	–0.074	0.35	0.40	0.014	0.85	0.37
ESR	0.256	0.001	< 0.001	–0.021	0.79	0.88	–0.035	0.65	0.50
CRP	0.200	0.01	0.01	–0.085	0.27	0.46	0.045	0.57	0.43
TNF-α	0.198	0.01	0.11	0.021	0.79	0.31	–0.023	0.77	0.25
IL-6	0.104	0.19	0.32	–0.057	0.47	0.41	0.037	0.64	0.41
Coronary calcium score	0.323	< 0.001	0.44	0.202	0.01	0.47	–0.172	0.03	0.78

† Univariate Spearman correlation coefficient (n = 167). * Spearman's correlation test. ** Multivariable linear regression was used for adjustment of age, race, and sex; except for coronary calcium score, which used proportional odds logistic regression with additional adjustment for Framingham risk score. MDRD-eGFR: Modified Diet in Renal Disease formula estimated glomerular filtration rate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; DAS28: 28-joint Disease Activity Score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6 interleukin 6.

information regarding the risk of atherosclerosis in RA, but this is not independent of the information provided by conventional cardiovascular risk factors.

REFERENCES

1. Chew JS, Saleem M, Florkowski CM, George PM. Cystatin C — a paradigm of evidence based laboratory medicine. Clin Biochem Rev 2008;29:47-56.
2. Shlipak MG, Sarnak MJ, Katz R, Fried LF, Seliger SL, Newman AB, et al. Cystatin C and risk of death and cardiovascular events among elderly persons. N Engl J Med 2005;352:2049-60.
3. Jernberg T, Lindahl B, James S, Larsson A, Hansson L, Wallentin L. Cystatin C: a novel predictor of outcome in suspected or confirmed non-ST-elevation acute coronary syndrome. Circulation 2004;110:2342-8.
4. Boers M. Renal disorder in rheumatoid arthritis. Semin Arthritis Rheum 1990;20:57-68.

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5. Chung CP, Oeser A, Raggi P, Gebretsadik T, Shintani AK, Sokka T, et al. Increased coronary-artery atherosclerosis in rheumatoid arthritis: Relationship to disease duration and cardiovascular risk factors. Arthritis Rheum 2005;52:3045-53.
6. Chung CP, Oeser A, Solus JF, Avalos I, Gebretsadik T, Shintani A, et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. Atherosclerosis 2008;196:756-63.
7. Botev R, Mallie JP, Couchoud C, Schuck O, Fauvel JP, Wetzels JF, et al. Estimating glomerular filtration rate: Cockcroft-Gault and modification of diet in renal disease formulas compared to renal inulin clearance. Clin J Am Soc Nephrol 2009;4:899-906.
8. Ix JH, Shlipak MG, Chertow GM, Whooley MA. Association of cystatin C with mortality, cardiovascular events, and incident of heart failure among persons with coronary heart disease: data from the Heart and Soul Study. Circulation 2007;115:1173-9.
9. Keller CR, Odden MC, Fried LF, Newman AB, Angleman S, Green CA, et al. Kidney function and markers of inflammation in elderly persons without chronic kidney disease: The health, aging, and body composition study. Kidney Int 2007;71:239-44.
10. Keller C, Katz R, Cushman M, Fried LF, Shlipak M. Association of kidney function with inflammatory and procoagulant markers in a diverse cohort: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis (MESA). BMC Nephrol 2008;9:9.
11. Knight EL, Verhave JC, Spiegelman D, Lillege HL, Zeeuw DD, Curhan GC, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int 2004;65:1416-21.
12. Finney H, Newman DJ, Price CP. Adult reference ranges for serum cystatin C, creatinine and predicted creatinine. Ann Clin Biochem 2000;37:49-59.
13. Parikh NI, Hwang SJ, Yang Q, Larson MG, Guo CY, Robins SJ, et al. Clinical correlates and heritability of cystatin C (from the Framingham Offspring Study). Am J Cardiol 2008;102:1194-8.
14. Parikh NI, Hwang SJ, Larson MG, Hoffmann U, Levy D, Meigs JB, et al. Indexes of kidney function and coronary artery and abdominal aortic calcium (from the Framingham Offspring Study). Am J Cardiol 2008;102:400-3.
15. Ix JH, Katz R, Kestenbaum B, Fried LF, Kramer H, Stehman-Breen C, et al. Association of mild to moderate kidney dysfunction and coronary calcification. J Am Soc Nephrol 2008;19:579-85.

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