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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 associated with inflammation and coronary atherosclerosis.

Methods—We measured serum cystatin C, creatinine, TNF- α and IL-6 concentrations, coronary artery calcium score (CACS), and Modified Diet in Renal Disease estimated GFR (MDRD-eGFR) in 167 patients with RA and 91 controls.

Results—Cystatin C was higher in RA (median [IQR]: 1.16 [0.99–1.35] mg/L) than controls (1.01 [0.90–1.19] mg/L), ($P < 0.001$) and positively correlated with ESR ($P < 0.001$), CRP ($P = 0.01$), DAS28 ($P = 0.006$), 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 ($P = 0.44$).

Conclusions—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 linked to conventional cardiovascular risk factors.

Key Indexing Terms

cystatin; rheumatoid arthritis; renal function; atherosclerosis

Introduction

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).

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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 are: 1) higher in patients with RA than in controls, independent of renal function; 2) associated with measures of inflammation in RA; and 3) associated with coronary atherosclerosis.

Materials and Methods

As described in detail previously, we studied 167 patients with RA and 91 controls frequency matched for age, sex, and race(5,6). The study was approved by the Institutional Review Board of Vanderbilt University Hospital and all subjects gave written informed consent. Clinical and demographic variables, Agatston coronary artery calcium score (CACS), Framingham risk score (FRS) and laboratory tests including C-reactive protein (CRP), tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were measured as described previously (5,6). Cystatin C concentrations in serum were measured by ELISA (Biovendor). 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 $\pm$ SD or as the median with interquartile range (median [IQR]) as appropriate. Concentrations of cystatin C, creatinine and MDRD-eGFR were compared in RA patients and controls using Wilcoxon's rank sum 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 non linear 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 analyses were performed using R version 2.10.0 (<http://www.r-project.org>) and 2-sided P values less than 0.05 were considered statistical 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 (Table 1). 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, NSAIDs and COX2-inhibitors after adjustment for age, race and sex. However,

methotrexate use was associated with lower cystatin C concentrations ($P<0.001$) (Table 2). Anti-TNF therapy did not affect cystatin C concentrations.

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 ESR ($P<0.001$), CRP ($P=0.01$) and DAS28 ($P=0.006$), 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 inflammation (9–11), 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 very 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 years) 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. Methotrexate, 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 methotrexate, the lower cystatin C concentrations in methotrexate users are unlikely to be explained by the preferential prescription of methotrexate to patients with better renal function. Methotrexate possibly affects the inflammatory pathways that involve 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.

In summary, cystatin C concentrations are higher in patients with RA even after adjustment for renal function. 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.

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References

1. Chew JS, Saleem M, Florkowski CM, Goerge PM. Cystatin C-a paradigm of evidence based laboratory medicine. *Clin Biochem Rev.* 2008; 29:47–56. [PubMed: 18787643]
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(20):2049–60. [PubMed: 15901858]
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; 110:2342–48. [PubMed: 15477399]
4. Boers M. Renal disorder in rheumatoid arthritis. *Semin Arthritis Rheum.* 1990; 20:57–68. [PubMed: 2218554]
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. [PubMed: 16200609]
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. [PubMed: 17266963]
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. [PubMed: 19406960]
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(2):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. [PubMed: 17183246]
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). *Nephrol.* 2008; 9:9.
11. Knight EL, Verhave JC, Spiegelman D, Lillegge 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(4):1416–21. [PubMed: 15086483]
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. [PubMed: 10672373]
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. [PubMed: 18940290]
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. [PubMed: 18235089]

Table 1

Baseline Characteristics of Control Subjects and Patients with Rheumatoid Arthritis

Variables	Controls (n=91)	RA (n=167)	P value *
Age, years, mean±SD	53.3±11.6	54.2±11.8	0.46
Sex,% female	63	69	0.31
Race, % 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, mmHg	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, umoles/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

Variables are shown as median (interquartile range) unless otherwise indicated.

* Wilcoxon's rank sum 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-6=Interleukin-6; MDRD-eGFR= The MDRD (Modified Diet in Renal Disease) formula estimated GFR.

Table 2
Measures of Renal Function According to Categorical Clinical Characteristics in Patients with Rheumatoid Arthritis

Variable	N	Cystatin C (mg/L)	P value	Adjusted P value *	Creatinine (mg/dl)	P value	Adjusted P value *	MDRD-eGFR (ml/min/1.73 m ²)	P value	Adjusted P value *
Gender										
Male	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	-
Female	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)		
Methotrexate										
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)		
NSAIDS/COX2-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)		

Cystatin C, Creatinine and MDRD values are represented as median (IQR)

MDRD-eGFR= The MDRD (Modified Diet in Renal Disease) formula estimated GFR

NSAIDS= Non-steroidal anti-inflammatory drugs

* Adjust P value for age, race and sex with multivariable linear regression.

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# (ρ)	P value ⁺	Adjusted P value [*]	Rho# (ρ)	P value ⁺	Adjusted P value [*]	Rho# (ρ)	P value ⁺	Adjusted P value [*]
Age	0.442	<0.001	-	0.182	0.02	-	-0.329	<0.001	-
BMI	-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 that used proportional odds logistic regression with additional adjustment for Framingham risk score.

MDRD-eGFR= The MDRD (Modified Diet in Renal Disease) formula estimated GFR

BMI=Body Mass Index; HDL=High Density Lipoprotein; LDL=Low Density Lipoprotein; DAS28=Disease Activity Score 28-joint assessment; ESR=Erythrocyte Sedimentation Rate; CRP= C-Reactive Protein; TNF-α=Tumor Necrosis Factor-α; IL-6=Interleukin-6