

# Serum cystatin C is independently associated with renal impairment and high sensitivity C-reactive protein in systemic lupus erythematosus

C. Chew<sup>1</sup>, P.W. Pemberton<sup>2</sup>, A. Al-M. Husain<sup>1,3</sup>, S. Haque<sup>3,4</sup>, I.N. Bruce<sup>1,3</sup>

<sup>1</sup>The Kellgren Centre for Rheumatology, and <sup>2</sup>Department of Clinical Biochemistry, Central Manchester Foundation Trust, Manchester; <sup>3</sup>Arthritis Research UK Epidemiology Unit, School of Translational Medicine, Manchester Academic Health Science Centre, The University of Manchester; <sup>4</sup>Department of Rheumatology, East Lancashire Hospitals Trust, Blackburn, United Kingdom.

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## Abstract

### Objectives

In systemic lupus erythematosus (SLE) patients, glomerular filtration rate (GFR) is usually estimated using the modified Cockcroft-Gault (mCG) and Modification of Diet in Renal Disease (MDRD) equations. We aimed to study cystatin C (sCysC) in SLE to assess its agreement with standard renal indices and investigate factors affecting sCysC in SLE.

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### Methods

SLE patients ( $\geq 4$  ACR criteria) and healthy women from Greater Manchester were recruited and clinical assessments were undertaken. sCysC was measured using R&D Systems' ELISA. Agreement between renal measures was assessed using Deming plots and factors associated with sCysC in SLE were examined by multiple linear regression analyses.

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### Results

178 patients and 68 controls had median (IQR) ages of 53 (46–61) and 50 (39–60) years, respectively. In an age-adjusted analysis, SLE patients had higher sCysC (1.16 [0.98–1.36] vs. 0.950 [0.73–1.13] mg/l;  $p < 0.0001$ ) and within SLE those with a history of lupus nephritis had higher sCysC (1.31 [1.10–1.66] vs. 1.11 [0.95–1.29] mg/l;  $p < 0.005$ ). sCysC correlated positively with serum creatinine, and inversely to renal measures ( $r = -0.530$ ;  $p < 0.0001$  [mCG], and  $r = -0.620$ ;  $p < 0.0001$  [MDRD]). There was closer agreement between the two eGFR measures than between either eGFR measures and sCysC.

In addition to age and serum creatinine, a multivariate analysis ( $\beta$ ,  $p$ ) found that high-sensitivity C-reactive protein (hs-CRP) (0.03, 0.026) was also independently associated with sCysC in SLE.

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### Conclusion

In SLE, sCysC may be influenced by low grade inflammation as well as by renal dysfunction. Therefore, sCysC should not supplant current assessment of renal dysfunction in SLE.

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### Key words

lupus, renal impairment, cystatin C

Christine Chew, MBChB, BSc  
Philip W. Pemberton, MSc  
Awal Al-M. Husain, MSc, MBChB  
Sahena Haque, MRCP, PhD, MBChB  
Ian N. Bruce, MD, FRCP

Please address correspondence  
and reprint requests to:

Ian N. Bruce,  
Arthritis Research UK,  
Epidemiology Unit,  
School of Translational Medicine,  
Stopford Building,  
The University of Manchester,  
Oxford Road,  
Manchester M13 9PT, United Kingdom.  
E-mail: ian.bruce@manchester.ac.uk

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## Introduction

Systemic lupus erythematosus (SLE) is a multisystem inflammatory disease commoner in females with increased morbidity and mortality from rheumatic fever (RF) and cardiovascular disease (CVD) complications (1). In the general population, chronic kidney disease (CKD) is associated with CVD and atherosclerosis (2). Traditionally, renal function has been estimated using serum creatinine or chromium-51 EDTA (3). The mCG index increased the accuracy of creatinine clearance (CrCl) (4), but is known to overestimate glomerular filtration rate (GFR). Three multiple regression models improving eGFR (5) were developed, followed by an 'adjusted-MDRD' based on Cr, age, sex and race (6). The National Kidney Foundation (NKF) recommended the modified Cockcroft-Gault (mCG) and Modification of Diet in Renal Disease (aMDRD) equations for accurate GFRs (7).

CysC has been proposed as a stable marker of renal impairment. CysC, a cysteine proteinase inhibitor, is produced by all nucleated cells at a relatively constant rate (8). Studies have shown that sCysC is influenced by age, BMI, sex, smoking and inflammation (9). CysC is more sensitive than creatinine at detecting minor reductions in GFR, and is potentially valuable in diagnosing CKD and predicting CVD (10).

In SLE, little is known about CysC with relation to inflammation, disease activity and validated renal investigations. This study compared CysC in patients and matched controls, observed the agreement between CysC with creatinine-based indices and examined factors associated with increased CysC in SLE patients.

## Patients and methods

### Study groups

The study was approved by the Northwest Multi-Centre Research Ethics Committee and written informed consent was obtained from subjects.

Women with SLE (>18 years old) were recruited from lupus clinics in Greater Manchester (2006–2009) fulfilling  $\geq 4$  of the updated ACR criteria or, if they had 3 criteria, with no alternative diag-

nosis (11). We also recruited 68 age-matched healthy women. Pregnant and lactating mothers, and patients with infections <1 month before the study were excluded as were patients who had adjustments to their therapy in the past 22 months. SLE disease activity and cumulative damage were assessed using the SLEDAI-2K (12) and SDI indices (13), respectively. Patients with a current or past history of persistent proteinuria (>500 mg/day), unexplained haematuria, CKD, nephrotic syndrome or any grade of biopsy-proven lupus nephritis were noted. Current nephritis was defined using the SLEDAI domains (12). A history of diabetes mellitus, age, BMI, smoking and menopause were also recorded.

### Specimen processing

Following a 48-hour fast, subjects had lipid profiles, inflammatory markers, biochemistry, haematology and autoantibodies measured by standard automated techniques. After centrifugation (1500g for 15min), serum aliquots were stored (-80°C).

### CysC measurement

SCysC was measured using a DuoSet developmental ELISA (R&D Systems, Minneapolis, USA). The working range up was 2ng/mL with a minimum detection limit of 0.025ng/mL. The intra-assay and inter-assay coefficients of variation were 6.5% and 12.7%, respectively. SCysC was stable for  $\geq 4$  freeze/thaw cycles. High-sensitivity C-reactive protein (hs-CRP) was assayed by an in-house sandwich ELISA using antibodies, standards and controls (Dakocytomation, Glostrup, Denmark).

### Statistical analysis

Values are shown as median (IQR) or the number of affected individuals (percentage of the total). The Mann-Whitney or Fisher's Exact Probability test was used to calculate significance between groups. Correlation between sCysC and risk factors was assessed by the Spearman Rank test. eGFR and CrCl were calculated using the MDRD and CrCl formulae, respectively. The Deming fit allowed comparison of three renal tests. Risk factors associ-

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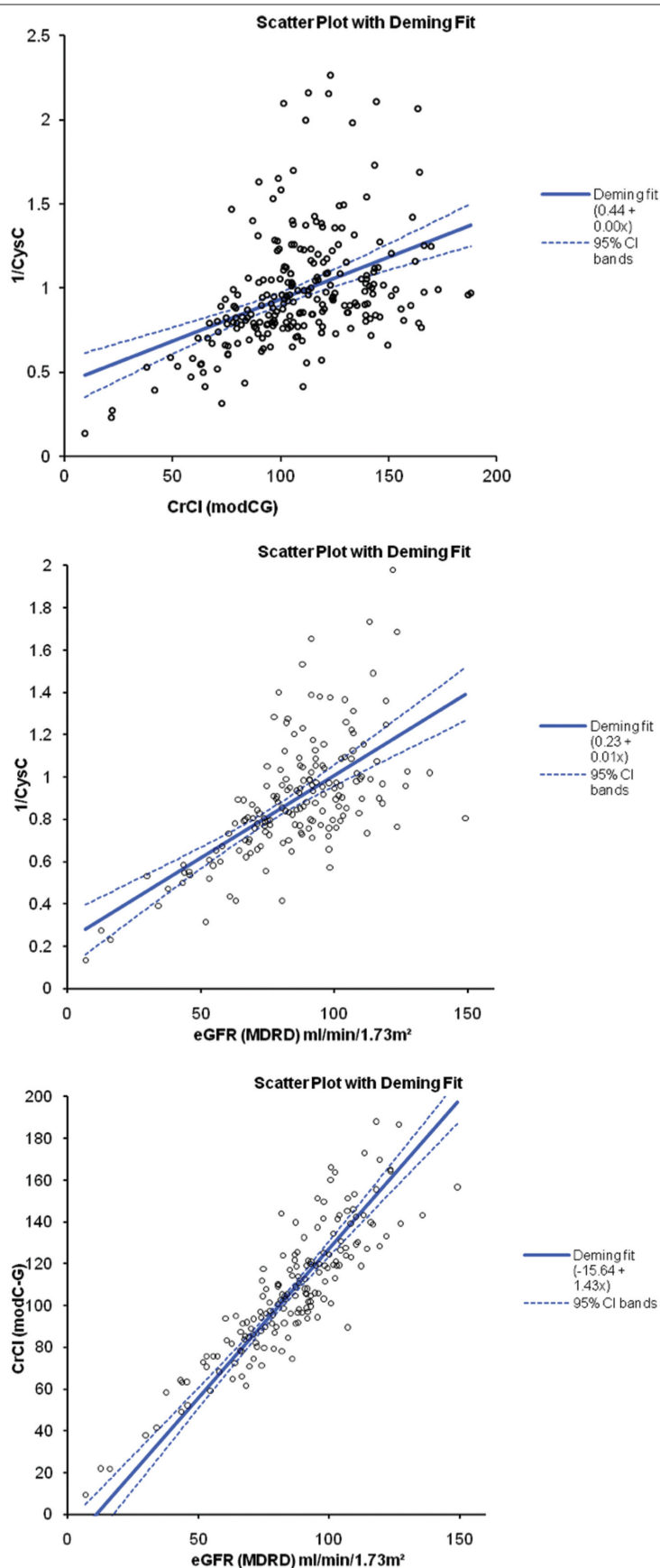
ated with CysC were analysed using a univariate regression analysis. Variables with  $p < 0.2$  were included in stepwise regression models. Analyses were performed using Stata 10.1 (2009) and the 'Analyse It Method Evaluation' programme (Microsoft Excel 2003).

## Results

178 SLE patients and 68 controls had median (IQR) ages 53 (46–61) and 50 (39–60) years, respectively ( $p = 0.0437$ ). In the SLE cohort, 36 (22%) and 15 (8.4%) had a history of nephritis or nephrotic syndrome, respectively. In an age-adjusted analysis, serum creatinine and both renal function indices showed no overall difference in both groups. SCysC, however, was significantly increased in SLE patients compared to controls (1.16 [0.98–1.36] vs. 0.95 [0.73–1.13] mg/l;  $p < 0.0001$ ).

Whilst serum creatinine was not significantly increased in patients with a history of lupus nephritis, sCysC was significantly higher in this subset (1.31 [1.10–1.66] vs. 1.11 [0.95–1.29] mg/l;  $p < 0.005$ ). In patients, serum creatinine correlated positively with sCysC ( $r = 0.622$ ,  $p < 0.0001$ ) and there was a significant negative correlation between CysC and renal function tests: CrCl ( $r = -0.530$ ,  $p < 0.0001$ ) and eGFR ( $r = -0.620$ ,  $p < 0.0001$ ). The correlation between eGFR calculations was also high ( $r = 0.859$ ,  $p < 0.001$ ). Deming fits assessing agreement showed more scattered data with broader lines of 95% confidence levels for 1/CysC vs. MDRD and 1/CysC vs. mCG than mCG vs. MDRD (Fig. 1).

In the univariate regression, sCysC in controls was associated with age, BMI, SBP, LDL, triglycerides, Cr and eGFR. In patients, sCysC was associated with age, LDL, triglycerides, total cholesterol, hs-CRP, Cr, eGFR, history of nephritis, SLEDAI-2K score and SDI. In a forward stepwise multiple regression, age and BMI were significant in controls. In SLE, age, hs-CRP and SDI were significant. In SLE, age, hs-CRP and SDI were significantly associated with sCysC. When serum creatinine was added to the SLE model ( $\beta$ , 95%CI,  $p$ -value), creatinine (0.01, 0.010–0.012,  $< 0.0001$ ) and hs-CRP (0.01, 0.009–



**Fig. 1.** Deming fit plots comparing CysC vs. CrCl, CysC vs. MDRD and mCG vs. MDRD in SLE patients.

**Table I.** Forward stepwise regression of predictors of sCysC in SLE patients and controls.

Risk factor	SLE patients			Controls		
	$\beta$ -coefficient	95%CI	p-value	$\beta$ -coefficient	95%CI	p-value
Age	0.008	-0.0001–0.016	0.050	0.007	0.002–0.01	0.003
BMI	-0.011	-0.0028–0.005	0.178	0.01	0.0002–0.02	0.045
hs-CRP	0.03	0.002–0.04	0.026	0.001	-0.014–0.016	0.870
SLEDAI	0.0029	-0.022–0.0285	0.823	–	–	–
*SLICC damage index (SDI)	0.17	0.1–0.2	<0.0001	–	–	–

\*Adding serum creatinine to the model displaced SDI with hs-CRP remaining significant and age becoming of borderline significance ( $p=0.045$ ).

**Table II.** Age-adjusted demographic data, cardiovascular risk factors, incidence of renal disease and markers of renal function and injury in SLE patients and controls.

Risk factor	SLE patients	Controls	p-value
Age	53 (46–61)	50 (39–60)	0.0437
BMI	26.5 (22.9–30.8)	25.6 (23.5–28.5)	NS
Waist/hip	0.88 (0.84–0.93)	0.87 (0.82–0.92)	NS
SBP	127 (115–142)	121 (110–135)	0.045
DBP	71 (64–76)	68 (60–74)	0.028
Total cholesterol	4.64 (4.06–5.27)	4.99 (4.45–5.93)	0.0097
HDL-cholesterol	1.65 (1.41–2.00)	1.64 (1.40–1.88)	NS
Triglycerides	1.07 (0.81–1.49)	0.85 (0.7–1.26)	NS
LDL-cholesterol	2.3 (1.8–2.9)	2.8 (2.2–3.2)	0.0001
Current smoking	16/173 (9.2%)	9/68 (13.2%)	NS
Glucose	4.5 (4.2–5.1)	4.7 (4.5–4.9)	NS
Diabetes	10/76 (5.7%)	1/65 (1.5%)	NS
Menopause	102/178 (57.3%)	29/68 (42.6%)	0.0458
Hypertension	82/177 (46.3%)	16/66 (24.2%)	0.002
10-year CVD risk	3.8 (2.0–6.7)	4.8 (1.2–8.5)	NS
Family history of IHD	50/178 (28.1%)	12/68 (17.6%)	NS
hs-CRP	2.01 (0.77–5.39)	1.91 (0.43–3.93)	NS
Nephritis (current)	5/170 (2.9%)	0/65 (0%)	NS
Nephritis (past)	31/166 (18.7%)	0/63 (0%)	<0.0001
Nephrotic syndrome (current)	2/160 (1.3%)	0/65 (0%)	NS
Nephrotic syndrome (past)	13/163 (8%)	0/65 (0%)	0.022
Creatinine	66 (59–76)	66 (62–71)	NS
eGFR (MDRD)	87.4 (73.4–100.7)	88.3 (82.0–98.0)	NS
CrCl (mCG)	105 (88–124)	111 (100–132)	NS
CysC	1.16 (0.98–1.36)	0.95 (0.73–1.13)	<0.0001

Results are expressed either as median (interquartile range) or as the number of affected individuals (percentage of the total). Data were analysed using the Mann-Whitney U-test (continuous variables) or Fisher's Exact Probability test (dichotomous variables). NS: not significant. Units were expressed as follows: age as years; SBP and DBP as mmHg; total cholesterol, HDL-cholesterol, triglycerides, LDL-cholesterol, glucose and creatinine as mmol/l; 10 year cardiac risk as %; hs-CRP and CysC as mg/l.

0.03, <0.0001) remained significant and age retained its previous trend (0.039, -0.002–0.04, 0.06) (Table I).

## Discussion

In SLE, monitoring renal function is pivotal in long-term management. In practice, creatinine-based tests are used. However, serum creatinine is also influenced by muscle mass, vigorous exercise and diet. Additionally, serum creatinine undergoes glomerular filtration and tubular secretion, resulting in

CrCl overestimation. CysC has been suggested as a more accurate measure of renal function (10). A meta-analysis (49 studies, 4,492 patients) showed better GFR prediction and earlier detection of renal failure with CysC than creatinine as indicated by AUC (0.926 vs. 0.837) (10). We found that sCysC and creatinine correlated strongly, however, only sCysC was increased in SLE patients compared to controls and in SLE, CysC was raised in nephritis patients.

The mCG and aMDRD correlate and agree strongly because creatinine is utilised in both formulae. The NKF however recommends a predefined limit of agreement ( $\pm 10\%$ ) between tests used (7). In our study, CysC showed good correlation but poor agreement with eGFR measures. Our data suggest that inflammatory mechanisms, as reflected by hs-CRP and SLEDAI-2K, also affects CysC in patients, and in our multivariate analysis hs-CRP was retained in the final model. This accords with a previous study in SLE (14). Therefore, CysC may reflect other factors in SLE, and both eGFR measures may be more sensitive to changes in renal function than CysC. Conversely, sCysC may be more sensitive to early renal dysfunction, but this hypothesis needs further study in a larger prospective population. The range of SLEDAI-2K scores were limited in this outpatient population and studying patients with very active disease would also be informative. These limitations may also explain why hs-CRP, rather than SLEDAI, was retained in the final model.

The cross-sectional nature of our study prevented trend comparisons of renal investigations against changes in disease activity over time. We also lacked a 'gold standard' such as inulin clearance or  $^{51}\text{Cr}$ -EDTA. However, our aim was to compare several laboratory assessments in a routine clinical setting. Therefore, relative agreement between renal investigations and variables influencing CysC remains valid. Furthermore, objective measures have practical and ethical limitations. Inulin, whilst freely filtered, not protein-bound, reabsorbed, or secreted, requires intravenous administration. Similarly,  $^{51}\text{Cr}$ -EDTA involves a radioisotope and is potentially limited in its usefulness in young females. Nevertheless, a prospective evaluation of circulating measures against a 'gold standard' would help to evaluate the relative contribution of inflammation and renal function to CysC measurement in SLE and determine which measure best predicts future decline in renal function in SLE.

In conclusion, sCysC is increased in SLE patients, particularly in nephritis. Both eGFR measures have good agree-



ment with each other but less agreement with CysC. Additionally, sCysC is also influenced by levels of hs-CRP. At present, SCysC should not supplant current methods of assessing renal dysfunction in SLE patients.

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