# Cystatin C: A Promising Marker of Renal Function in Patients with Systemic Lupus Erythematosus?

## Cistatina C: Um Marcador de Função Renal Promissor em Doentes com Lúpus Eritematoso Sistémico?

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### ABSTRACT

**Purpose:** Cystatin C has a higher correlation with glomerular filtration rate and a more significant clinical prognosis than creatinine. We sought to determine whether it is a marker of renal function different from creatinine (cystatin C potentially superior to creatinine), in patients with systemic lupus erythematosus.

**Material and Methods:** 37 patients with systemic lupus erythematosus were evaluated. Serum cystatin C was determined by nephelometry and creatinine by modified Jaffe method. We compared five formulas: Chronic Kidney Disease – Epidemiology Collaboration cystiatin; Chronic Kidney Disease – Epidemiology Collaboration creatinine-cystatin; Cockcroft-Gault; Modification of Diet in Renal Disease and Chronic Kidney Disease – Epidemiology Collaboration creatinine, using the latter as a reference. We analyzed the influence of clinical and laboratory factors in cystatin C variation, using multivariate linear regression.

**Results:** Cystatin C was singly elevated in ten participants, versus none isolated creatinine elevation, and this difference was significant ($p$ = 0.002). There was a difference between the estimated glomerular filtration rate by Chronic Kidney Disease – Epidemiology Collaboration cystatin and by Chronic Kidney Disease – Epidemiology Collaboration creatinine (-6.0541 mL/min/1.73 m$^2$, $p$ = 0.07), more pronounced for lower glomerular filtration rate. Consequently, Chronic Kidney Disease – Epidemiology Collaboration cystatin reclassified 4 patients as having chronic kidney disease *de novo* and 1 patient as not having chronic kidney disease ($p$ = 0.375). Cystatin C was only significantly influenced by age ($p$ < 0.001).

**Discussion:** Several reports showed cystatin C as a better marker to define chronic kidney disease, allowing more accurate classification and risk stratification, compared with creatinine. In this study, Cystatin C revealed as a promisor marker of renal function in patient with lupus, mainly in patients with lower glomerular filtration rates. The correlation between age and cystatin C seems to be a confounding factor, as glomerular filtration rate physiologically declines with ageing.

**Conclusion:** Cystatin C was potentially superior to creatinine and in this study and cystatin C seems to detect changes in glomerular filtration rate earlier than creatinine and may be a better screening method for chronic kidney disease in systemic lupus erythematosus.


**Keywords:** Biological Markers; Cystatin C; Lupus Erythematosus, Renal Insufficiency; Systemic.


### RESUMO

**Introdução:** A cistatina C possui uma correlação superior com a taxa de filtrado glomerular e um prognóstico clínico mais significativo do que a creatinina. Procurou-se averiguar se constitui um marcador de função renal diferente da creatinina (cistatina C potencialmente superior à creatinina), em doentes com lúpus eritematoso sistémico.

**Material e Métodos:** Foram avaliados 37 doentes com lúpus eritematoso sistémico, sem evidência de nefrite lúpica activa. Determinou-se a cistatina C sérica por nefelometria e a creatinina pelo método de Jaffe modificado. Compararam-se cinco fórmulas: Chronic Kidney Disease – Epidemiology Collaboration cystatin; Chronic Kidney Disease – Epidemiology Collaboration creatinine-cystatin; Cockcroft-Gault, Modification of Diet in Renal Disease e Chronic Kidney Disease – Epidemiology creatinine, utilizando-se esta última como referência. Analisou-se a influência de factores clínicos e laboratoriais na variação da cistatina C, por regressão linear multivariada.

**Resultados:** A cistatina C encontrava-se isoladamente elevada em dez participantes, ao invés de nenhuma elevação isolada da creatinina, sendo esta diferença significativa ($p$ = 0,002). Verificou-se uma diferença entre a taxa de filtrado glomerular estimada pela Chronic Kidney Disease – Epidemiology Collaboration cystatin e pela Chronic Kidney Disease – Epidemiology Collaboration creatinine (-6,0541 mL/min/1,73 m$^2$, $p$ = 0,07), mais acentuada para taxas de filtração glomerular mais baixas. Assim, a fórmula Chronic Kidney Disease – Epidemiology Collaboration cystatin reclassificou 4 doentes como tendo doença renal crónica de novo e um doente como não tendo doença renal crónica ($p$ = 0,375). A cistatina C foi influenciada significativamente apenas pela idade ($p$ < 0,001).

**Discussão:** Vários estudos demonstraram que a cistatina C melhora a definição de doença renal crónica, permitindo uma classificação e uma estratificação do risco mais exactas, comparativamente à creatinina. A cistatina C revelou-se, neste estudo, um marcador de função renal promissor nos doentes com lupus, principalmente para taxas de filtrado glomerular mais baixas. A correlação da cistatina C com a idade para ser um factor confundente, na medida em que existe um declínio fisiológico da taxa de filtração glomerular com o envelhecimento.

**Conclusão:** A cistatina C foi potencialmente superior à creatinina e nesta amostra a cistatina C pareceu detectar mais precocemente do que a creatinina alterações na taxa de filtrado glomerular, podendo ser um melhor método de rastreio de doença renal crónica no lúpus eritematoso sistémico.


**Palavras-chave:** Cistatina C; Insuficiência Renal; Lúpus Eritematoso Sistémico; Marcadores Biológicos.

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## INTRODUCTION

Chronic kidney disease (CKD) is an emerging major health issue worldwide, related to its increasing incidence and prevalence over the last few decades and to its clinical and economic impact as an independent risk factor for cardiovascular disease and acute kidney lesion.[1]

Therefore, early diagnosis is crucial to slow down the progression of disease and its complications. According to the Kidney Disease: Improving Global Outcomes (KDIGO), CKD may be defined as the presence of structural and renal function abnormalities extending for more than three months, with health implications.[2] A period of functional decrease is therefore observed and the glomerular filtration rate (GFR) is the best indicator of global renal function.

Diagnosis and classification of CKD largely depends on the use of renal function biomarkers as CKD is often asymptomatic until the later stages. This is an area of intense research and discussion within the scientific world.[3,4]

GFR may be measured through the clearance of a marker of exogenous filtration, urinary inulin clearance measured during a continuous intravenous infusion becoming the gold standard. Other possible exogenous markers include itothalamate, iohexol or ethylenedieminetetraacetic acid (EDTA).[5] GFR measurement is expensive, invasive, inconvenient and not routinely available.

As such, GFR is generally estimated (eGFR) from the serum concentration of endogenous markers. The ideal biomarker would be produced at a constant rate and exclusively eliminated by glomerular filtration. In such conditions, its serum concentration would reflect the GFR.

Creatinine, the classic GFR marker, is an amino acid derivative from muscle creatine non-enzyme degradation. It is a good marker of renal function as it does not bind to proteins, is not metabolised by kidney and is freely filtered in the glomerulus.[3]

Nevertheless, it has several recognized limitations, as its serum concentration does not exclusively depend on GFR and is largely influenced by other physiological factors; the creatinine production rate is proportional to the muscle mass. Its excretion does not exclusively depend on the glomerular filtration, with some proximal tubular secretion (10-20%), as well as some extra-renal elimination produced by bacterial intestinal flora. The latter mechanisms become more important with a declining renal function, allowing for the serum creatinine concentration to be maintained within a normal range until a very significant reduction in GFR is reached (around 60 mL/min/1.73 $m^2$) – the so-called creatinine blind range.[4,6]

Overcoming inter-individual variability in creatinine production has been attempted through the development of equations improving GFR calculation, involving different anthropometric factors, from which the Cockroft-Gault equation, the Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CDK-EPI) study equations are the most frequently used.[3,6,7]

According to the KDIGO (2012), the CKD-EPI 2009 equation (CKD-$EPI_{creat}$) is the recommended equation for GFR calculation as it has shown to be the most exact and accurate, as well as with less biased when compared to MDRD's equation, especially for higher GFRs.[2,8]

Cystatin C has been studied as an alternative GFR marker and is a non-glycosylated low molecular weight protein from the cysteine proteinase inhibitors family, produced at a constant rate by every nucleated cell, freely filtered in the glomerulus and finally reabsorbed and metabolised in the proximal tubule. As there is no significant tubular secretion or extra-renal elimination, its peripheral concentration depends on glomerular filtration rate, which makes it a promising marker of renal function. Its production has been shown to be more uniform, as it does not depend on the muscle mass or on diet. Therefore, cystatin C serum levels have consistently shown a higher correlation with the GFR than creatinine.[9,10]

Cystatin C, like creatinine, should be used to assess kidney function from equations that estimate GFR and the CKD-EPI 2012 equation (CKD-$EPI_{cist}$) is recommended by the KDIGO.

Other GFR-independent factors may change cystatin C concentration but have not considered for these equations. In fact, the production of this biomarker seems to be increased in situations of increased cellular turnover like hyperthyroidism or malignant diseases and, despite non-consensual literature and further studies being required to demonstrate causality, it has been hypothesized that it may be influenced by inflammatory states, diabetes, obesity, smoking or the use of steroid therapy.[6,11]

KDIGO's most recent recommendations on CKD (2012) include several suggestions related to cystatin C.

Patients with systemic lupus erythematous (SLE) should be considered. The kidney lesion is an important factor of morbidity and mortality in these patients and at least 60% of them will have some degree of kidney involvement during SLE progression.[12] Nephritis is asymptomatic in most patients and therefore the renal function assessment becomes even more relevant in the early clinical identification, risk stratification and therapy adjustment in this population.[13]

The 2009 European Recommendations on lupus nephritis suggested an assessment of renal function in patients with SLE through serum creatinine levels or the different GFR estimation equations, except in cases with eGFR above 60 mL/min/1.73 $m^2$, in which the creatinine clearance obtained from a 24-hour urine collection is the recommended method.[14,15] However, current global recommendations of renal function assessment rather suggest the use of GFR estimating equations rather than the creatinine clearance which is an inconvenient and time-consuming method with high rates of inadequate collections and a tendency to overestimate GFR.

The formula that best applies to estimating renal function in SLE patients has not yet been determined.

Our study aimed (1) to determine whether cystatin C may be used as an alternative marker of renal function, potentially better than creatinine, in patients with SLE, (2) to compare bias, accuracy and precision of cystatin C-based GFR equations ($CKD\text{-}EPI_{cist}$ and $CKD\text{-}EPI_{creat\text{-}cist}$) to other creatinine-based methods used in clinical practice, using the Cockroft-Gault, MDRD and $CKD\text{-}EPI_{creat}$ equations, (3) to determine whether the CKD classification (eGFR < 60 mL/min/1.73 $m^2$) of patients is different from the other methods and from the reference method and finally (4) to determine the presence of any extra-renal factors influencing cystatin C serum levels.

## MATERIAL AND METHODS

This was a cross-sectional, prospective and observational study, including patients with SLE attending the Autoimmune Disease outpatients from the Medicine I Department from the *Centro Hospitalar Lisboa Norte - Hospital de Santa Maria* and selected from 30 July to 14 August 2014.

The single criteria for inclusion in the study was the presence of diagnosed SLE according to the American College of Rheumatology 1997 criteria.[16] The exclusion criteria included: below 18 years of age, the presence of any kidney disease with other aetiology except lupus nephritis, including diabetes and increased cell turnover situations, namely pregnancy and neoplasm-related.

This study was approved by the Ethics Committee of the *Centro Hospitalar Lisboa Norte, EPE*, according to the Helsinki Declaration.

### Assessed variables

Different parameters were assessed from all the patients included in the study. Serum cystatin C level was determined by nephelometry (reference values provided by the producer: 0.5 – 0.96 mg/dL) and creatinine by the modified Jaffe's method (reference values provided by the producer: 0.7 – 1.3 mg/dL). GFR was estimated from these two values for each patient. The formula for estimated GFR that was considered as reference was the $CKD\text{-}EPI_{creat}$, which is currently the recommended preferred equation for GFR calculation, according to the recent KDIGO recommendations.[2]

In order to assess a possible influence on serum cystatin C levels, the following clinical and laboratory data were also obtained: patient's gender, ethnicity, age, body weight, height, body surface (calculated by the DuBois formula),[17] smoking habit, thyroid function (based on the values of thyroid stimulating hormone and thyroxine), C-reactive protein (CPR) and steroid therapy dose at the time when renal function biomarkers were collected. This last variable was converted to the equivalent dose of prednisolone.

### Statistical analysis

The statistical analysis used SPSS® (Statistical Package for the Social Sciences, version 21) software.

The descriptive statistics of categorical variables are presented as frequencies and continuous variables as mean and standard deviation.

A paired design was used in a 2 x 2 table where each patient was classified according to the absolute values of cystatin C and/or creatinine (according to laboratory reference values) and the McNemar test was used to assess the similarities and differences found, with $\alpha < 0.05$ considered as statistically significant.

A graphic comparison between each of the alternative (eGFR) and reference equations (rGFR - $CKD\text{-}EPI_{creat}$) was established by plotting the differences found between the equations (eGFR – rGFR) and eGFR, together with a smoothed regression line.

Bias for each alternative equation was defined as the average difference (eGFR – rGFR), precision as the interquartile range (IQR) of differences and accuracy as the percentage of eGFR values that differed less than 30% from rGFR (P30).

Due to the lack of a method that could be defined as gold standard, the analysis of specificity and sensitivity of each alternative equation was not carried out, although concordance between each alternative equation and the reference equation in the CKD classification (eGFR < 60 mL/min/1.73 $m^2$) was established, through the McNemar's test and $\alpha < 0.05$ was defined as statistically significant.

Finally, the different factors that could influence cystatin C serum concentration were also analysed through linear regression methods, with univariate analysis and multivariate adjustment and $\alpha < 0.05$ as statistically significant.

## RESULTS

### Characteristics of our group of patients

In total, 37 patients were included in our study, all of these meeting the previously established inclusion and exclusion criteria.

The characteristics of our group of patients are shown in Table 1. Patients were predominantly female (94.6%) and from Caucasian ethnicity (94.6%), aged on average 45.08 (± 13.6) years, treated with an average steroid daily dose of 8.31 (± 7.841) mg.

### Assessment of kidney function markers

Frequencies of creatinine and cystatin C values ranged as normal or high for each patient, according to the described reference values are shown in Table 2. A single elevation in cystatin C was found in ten patients whilst no single elevation in creatinine was found. The McNemar's test showed a statistically significant difference ($p = 0.002$) between both methods, in favour of an elevation of cystatin C levels in a larger number of patients.

When comparing the average eGFR values obtained

Table 1 - Characteristics of our group of patients. Continuous variables are presented by the mean (μ) ± standard deviation (δ) and categorical variables by the absolute number and percentage.

| Characteristic | Absolute value (%) (n = 37) | μ ± δ | Variation |
|---|---|---|---|
| **Female** | 35 (94.6%) | -------- | -------- |
| **Ethnicity** | | | |
| **Caucasian** | 35 (94.6%) | -------- | -------- |
| **Black** | 2 (5.4%) | -------- | -------- |
| **Smoking habit** | 6 (16.2%) | -------- | -------- |
| **Thyroid dysfunction** | 2 (5.4%) | -------- | -------- |
| **Age (years)** | -------- | 45.08 ± 13.63 | 23 – 83 |
| **Body weight (Kg)** | -------- | 69.22 ± 16.39 | 50 – 115 |
| **Height (m)** | -------- | 1.65 ± 0.07 | 1.53 – 1.85 |
| **Body surface area ($m^2$)** | -------- | 1.73 ± 0.20 | 1.5 – 2.4 |
| **Steroid therapy (mg/day)** | -------- | 8.31 ± 7.84 | 0 – 30 |
| **CPR (mg/dL)** | -------- | 0.44 ± 0.60 | 0.04 – 2.61 |

by the different formulae with those obtained through the reference method, we found that those were lower, except the ones obtained with the Cockroft-Gault formula (Table 3).

Despite the GFR measurement not having been obtained with a gold standard method, the bias, precision and accuracy (Table 4 and 5) for each equation were calculated by comparison with rGFR. A statistically significant difference was found ($p < 0.05$) between the rGFR and the eGFR based on the Cockroft-Gault and MDRD formulae, with a +9.9162 ($p = 0.009$) and -4.9216 ($p < 0.001$) bias, respectively and suggesting that the first overestimates and the second underestimates the GFR. Despite a statistically non-significant difference, the CKD-$EPI_{cist}$ formula also presents a clear tendency to calculate lower eGFR values than the rGFR (-6.0541 mL/min/1.73 $m^2$, $p = 0.07$). The MDRD equation showed the best correlation with the standard method and was the more accurate (P30 = 100) and precise (IQR = 9.50).

Table 2 - Frequency of normal or elevated creatinine and cystatin C levels

| | | Creatinine | |
|---|---|---|---|
| | | **Normal** | **Elevated** |
| **Cystatin C** | Normal | 25 | 0 |
| | Elevated | 10 | 2 |

Table 3 - GFR estimated according to the different formulae

| Formula | Mean (μ) | Standard Deviation (δ) |
|---|---|---|
| **CKD – $EPI_{Creat}$ (rGFR)** | 99.59 | 23.37 |
| **CKD – $EPI_{Cist}$** | 93.54 | 29.96 |
| **CKD – EPICreat-cist** | 97.19 | 27.54 |
| **MDRD** | 94.67 | 26.38 |
| **Cockroft-Gault** | 109.51 | 31.63 |

Table 4 - Bias of the different formulae for estimated GFR

| Formula | μ | δ | 95% Confidence interval | *p - value* |
|---|---|---|---|---|
| **CKD – $EPI_{cist}$** | -6.0541 | 19.7230 | [-12.6300, 0.5219] | 0.070 |
| **CKD – $EPI_{creat-cist}$** | -2.4054 | 12.1117 | [-6.4436, 1.6328] | 0.235 |
| **MDRD** | -4.9216 | 8.4265 | [-7.7311, -2.1121] | 0.001 |
| **Cockroft-Gault** | 9.9162 | 21.8579 | [2.6284, 17.2040] | 0.009 |

Bias was calculated as the mean difference eGFR - rGFR (μ); δ - standard deviation

Table 5 - Precision and accuracy of different formulae for estimated GFR

| Formula | IQR | P30 (%) |
|---|---|---|
| **CKD - EPI$_{cist}$** | 28.50 | 81.1 |
| **CKD - EPI$_{creat-cist}$** | 17.50 | 97.3 |
| **MDRD** | 9.50 | 100.0 |
| **Cockroft-Gault** | 32.85 | 81.1 |

However, as the CKD-EPI$_{creat}$ equation is not the gold standard method, the determination of bias (Table 4), precision and accuracy measurements (Table 5) has no value in terms of the best method definition, despite allowing for the inference as to whether there is any difference in renal function with the different equations.

Precision was calculated as the interquartile range (IQR) and accuracy as the percentage of eGFR values that differed less than 30% from the rGFR (P30).

The fact that the differences found between the eGFR obtained by the CKD-EPI$_{cist}$ method and the reference method were more obvious for lower GFR values (close to the 60 mL/min/1.73 m$^2$ threshold) and faded for higher values should be mentioned, a result obtained upon the analysis of the differences between the rGFR and the eGFR, in turn obtained by each equation through the whole range of eGFR values (Fig. 1). The remaining formulae seem to maintain a uniform and less significant difference through the renal function spectrum. As this was a small group of patients, we were not able to calculate 95% confidence intervals through the whole eGFR spectrum and therefore to reach a conclusion as to whether the difference was statistically significant for each eGFR value.

When we compared the CKD classification (defined in our study as GFR < 60 mL/min/1.73 m$^2$) obtained with the different formulae with the one obtained with the reference method, we found that, despite statistically non-significant ($p$ = 0.375), there is in fact a difference between CKD classification according to the CKD-EPI$_{cist}$ formula and to the CKD-EPI$_{creat}$ and the equation based on cystatin C has reclassified four patients as with CKD *de novo* and one patient as not having CKD. As regards the other formulae, we did not find any relevant difference in classification (Table 6).

**Other variables that may have influenced serum cystatin C level**

Gender, ethnicity and thyroid function were not analysed

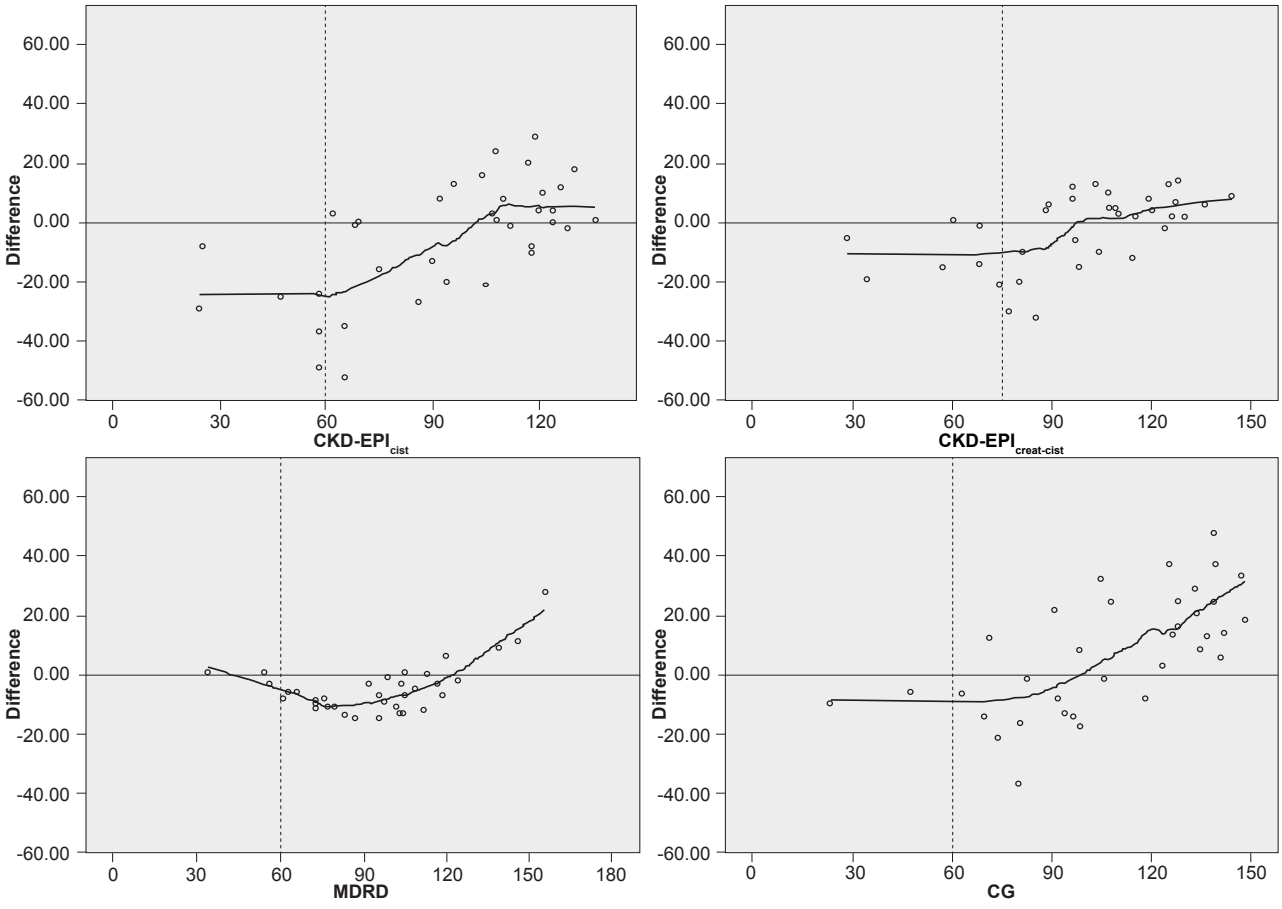


Figure 1 – Correlation between eGFR and the difference (rGFR – eGFR)

Table 6 - Presence or absence of CKD (GFR < 60 mL/min/1.73 m²) according to the different equations

| | | CKD - $EPI_{creat}$ | | | *p* – value |
|---|---|---|---|---|---|
| | | GFR < 60 | GFR ≥ 60 | Total | |
| **CKD - $EPI_{cist}$** | eGFR < 60 | 2 | 4 | 6 | *p* = 0.375 |
| | eGFR ≥ 60 | 1 | 30 | 31 | |
| **CKD - $EPI_{creat-cist}$** | eGFR < 60 | 3 | 1 | 4 | *p* = 1 |
| | eGFR ≥ 60 | 0 | 33 | 33 | |
| **MDRD** | eGFR < 60 | 3 | 0 | 3 | *p* = 1 |
| | eGFR ≥ 60 | 0 | 34 | 34 | |
| **Cockroft-Gault** | eGFR < 60 | 2 | 0 | 2 | *p* = 1 |
| | eGFR ≥ 60 | 1 | 34 | 35 | |

Table 7 - Univariate and multivariate regression analysis

| Characteristic | Univariate | | Multivariate | | |
|---|---|---|---|---|---|
| | *r* | *p - value* | β | SE | *p - value* |
| **Age** (years) | 0.745 | < 0.001 | 0.019 | 0.004 | < 0.001 |
| **Body surface area** ($m^2$) | 0.190 | 0.911 | -0.171 | 0.246 | 0.492 |
| **Smoking habit** | 0.104 | 0.541 | 0.031 | 0.130 | 0.813 |
| **Steroid therapy** (mg/dia) | 0.416 | 0.010 | 0.007 | 0.006 | 0.272 |
| **CPR** (mg/dL) | 0.126 | 0.457 | 0.056 | 0.76 | 0.464 |

when assessing other extra-renal factors that could have affected cystatin C concentration, due to the very small number of patients that were male, black or had thyroid dysfunction. Their inclusion would have prevented a reliable statistical analysis.

Univariate linear regression analysis (Table 7) showed that factors 'age' and 'steroid therapy' are significantly correlated ($p < 0.05$) and correlate positively with cystatin C. However, the regression analysis adjusted to the multiple variables, from the five analysed, 'age' was the only that had a statistically significant influence on cystatin C variation.

## DISCUSSION

The 2012 CKD-EPI study showed that the equation combining both markers (CKD-$EPI_{creat-cist}$) is the most precise and accurate method to estimate GFR, having correctly reclassified above or below the GFR limit of 60 mL/min/1.73 $m^2$ 19% of the patients with borderline GFR (45-74 mL/min/1.73 $m^2$) calculated with the CKD-$EPI_{creat}$ equation and these results were statistically significant.[7,18,19] Nevertheless, there was a similar bias on the three equations.

Several studies have showed that the $GFR_{cist}$ improves CKD definition, allowing for a more accurate classification and risk stratification compared to the $GFR_{creat}$. Based on these evidences, the KDIGO includes in their more recent recommendations for CKD (2012) several suggestions related to cystatin C, namely the measurement of cystatin C in adult patients with $GFR_{creat}$ 45-59 mL/min/1.73 $m^2$ and with no other markers of kidney lesion, when the confirmation of CKD is needed. Use of cystatin C has also been suggested as a confirmation test in specific clinical situations in which creatinine would be predictably less accurate or could be influenced by factors independent from the GFR. The fact that these suggestions are still not recommendations regards questions of clinical application, such as cost or lower availability.[2]

Cystatin C is therefore only used to improve CKD diagnostic specificity. However, the $GFR_{cist}$ allows for the identification of patients with CKD non-identified by the $GFR_{creat}$ and therefore should be used as a screening test to improve CKD diagnostic sensitivity. Its economic feasibility would depend on factors such as the cost of the test, the frequency of positive results and the usefulness of CKD's early detection, such as implications for treatment, as well as creatinine GFR overestimate.[6] Therefore, in order to have a beneficial and cost-efficient screening with cystatin C, its application would have to be specific, limited for instance to increased CKD risk populations, diabetic nephropathy

being the most studied case where this new marker has shown superiority in most patients.[20]

We consider SLE patients as a target population for this new test. The kidney lesion is an important factor of morbidity and mortality in these patients. The formula that best applies to patients with SLE for estimating renal function has still not been found. Two studies[15,21] compared Cockroft-Gault to MDRD equations and have found a better result with the latter, having nevertheless used creatinine clearance as gold standard and with a high percentage of inadequate 24-hour urine collections. Another study[22] compared several creatinine-based equations with the CKD-EPI$_{creat}$ formula as reference and the MDRD equation obtained the best results. However, as SLE is a disease with a very specific population, predominantly female, with reduced muscle mass, involving different ethnicities, using steroid therapy and other specific immunosuppressive drugs and with a very variable range of renal function, it is questionable whether the recommended formulae for general population would be those that would better adapt to these patient's characteristics.[13] Cystatin C, for the abovementioned evidences, may become a promising marker of renal function in this specific population, which so far has been scarcely studied.[13,23]

The major concern regarding the use of this marker in patients with SLE is the fact that a consensus is still to be reached regarding the influence in these patients that different relevant factors would have on its serum concentration. In fact, some studies found there is no correlation between the values of cystatin C and the use of steroids[23,24] or the level of activity of the disease.[23] However, Chew et al.[25] found that patients with SLE had higher basal levels of cystatin C than the control group ($p < 0.0001$), which did not occur with other markers of renal function and Lertnawapan et al.[26] found a positive and significant correlation between cystatin C and several markers of inflammation and activity of the disease (CPR, ESR, TNF-A, IL-6 and SLICC). Similar results were found in studies with other rheumatological diseases like rheumatoid arthritis.[26] It is possible that this association is showing the immune modulator role of cystatin C while acting as an endogenous inhibitor of the cystein-protheases, like cathepsin or elastase, as well as inhibitor of chemotaxis of polymorphonuclear cells, of oxygen radical liberation or phagocytosis. In addition, it may be difficult to exclude that inflammation may on its own lead to subtle declines in renal function, eventually only detected by cystatin C, as this is clearly more accurate than creatinine in the identification of slight changes in GFR, which would acknowledge cystatin C as a better marker of the renal function in patients with SLE in whom the degree of involvement is extremely variable.

Based on these data, Martinez-Martinez[13] aimed to assess which creatinine-base equation would be the best in patients with SLE. In the first stage of his study, different equations were compared (iothalamate clearance as the gold standard) and the equation developed by Steven et al.[27] showed the best performance, combining creatinine and cystatin C. However, his group of patients only included 14 Mexican patients specifically selected due to low-activity SLE, low-dose steroid therapy, not having hypothyroidism and being non-smokers, in order to ensure that these factors would not interfere with cystatin C levels. In a second stage, 55 participants were included and the study aimed to determine which was the best creatinine-based equation, compared to the equation by Steven[27] and the CKD-EPI$_{creat}$ was the most accurate and with the lowest bias.

The results obtained in our study showed differences between the assessment of renal function based on the markers cystatin C and creatinine in patients with SLE. In fact, a statistically significant difference was found between the number of patients with high serum cystatin C and creatinine levels, more often in favour of the single elevation of cystatin C levels ($p = 0.002$). In line with this, despite a statistically non-significant difference, the CKD-EPI$_{cist}$ equation showed a clear tendency to estimate lower GFR than with the CKD-EPI$_{creat}$ equation (-6.0541 mL/min/1.73 m$^2$, $p = 0.07$).

Due to the absence of a real gold standard method for GFR measurement, we are not able to determine which marker is the closest to reality. Our study only allows us to reach the conclusion that depending on the method, different results are obtained. However, it is well known that creatinine estimates relatively imprecise values for GFR, due to its determinants being independent from glomerular filtration. This fact may be particularly relevant in patients with SLE, due to a tendency for lower muscle mass, both regarding the strong female predominance, the chronic disease itself and the use of steroid therapy, leading to a lower production of creatinine, which may explain for a possible GFR overestimation by the creatinine-based formulae.

The fact that the difference between both methods is more evident for lower GFRs (close to the 60 mL/min/1.73 m$^2$ limit) should also be mentioned as GFR$_{cist}$ allowed for the clinical relevant reclassification of four patients with *de novo* CKD and one patient as not having CKD. However, this difference did not show statistical significance ($p = 0.375$). These were in line with previous results considering cystatin C as a more sensitive marker of slight GFR changes, when compared to creatinine,[15,25] allowing for an earlier detection of CKD and therefore corresponding to a better screening method, especially in patients with GFR not so accurately estimated with creatinine,[2] as it seems to be the case with patients with SLE.

In addition, the correlation between certain factors in patients with SLE, such as the use of steroids or inflammation, and the variation in serum cystatin C levels, is not absolutely clear. In our study, age and steroid therapy factors were significantly ($p < 0.05$) and positively related to cystatin C in univariate analysis; nevertheless, only

the age showed a statistically significant correlation to cystatin C in multivariable-adjusted analysis. Also, with a GFR physiological decline with ageing, this seems to be a confounding factor and therefore, in our study, cystatin C did not seem significantly influenced by any factor beyond renal function. Furthermore, the doses of steroids used by our group of patients were relatively low (8.31 ± 7.8410, range - 0 – 30 mg) and given the small dimension of the sample, further correlation studies are needed to definitely exclude the influence these and other factors may have on the levels of cystatin C, leading to a possible overestimation of the values of $GFR_{cist}$.

### Main strength and limitations of the study

The main limitation to our study regards the absence of a gold standard method for GFR measurement with which we could compare the remaining equations that were assessed. This is due to technical complexity as well as financial costs of the procedures involved. This issue is reduced by the fact that the CKD-$EPI_{creat}$ equation, used as reference, has shown a high concordance with the GFR assessed through exogenous filtration markers.[8] Further studies are needed, using a gold standard measurement method of renal function in order to validate the preliminary results obtained by this study.

In addition, this was a small group of patients and with scarce ethnic (94.6% Caucasian) and gender diversity (94.6% female). However, these factors are in line with SLE's epidemiological specific characteristics, with a female prevalence (9:1 female: male ratio) and in line with the Portuguese population, of Caucasian majority.

In addition, when compared to a similar study by Martinez-Martinez,[13] our study offers the advantage of including a higher number of participants and a more random population (compared to stage 1 of that study). In fact, from the 37 patients initially assessed, all were included in the study according to the established inclusion and exclusion criteria. Therefore, this study may have a good external validity, representative for daily clinical practice and may apply to general population of Portuguese patients with SLE.

## CONCLUSION

Further research on more accurate methods of determination of renal function and risk stratification is crucial, especially in susceptible patients, such as in patients with SLE. The results of our study support the hypothesis that serum cystatin C is a different marker of renal lesion from serum creatinine and may be a more sensible marker of slight changes in GFR and potentially better than creatinine as a screening test for CKD, both regarding an earlier diagnosis and a higher predictive value. This has important clinical implications particularly in patients with SLE, whose renal complications are one of the major factors of morbidity and mortality. However, based in this study, we are not able to conclude that serum cystatin C is superior to serum creatinine, as GFR was not assessed through the clearance of an exogenous filtration marker (considered the gold standard). In addition, cystatin C is an expensive marker compared to creatinine and not largely available in clinical practice, which may limit its applicability in clinical practice in Portugal.

## HUMAN AND ANIMAL PROTECTION

The authors declare that the followed procedures were according to the regulations established by the responsible body of the Ethics and Clinical Research Committee and according to the Helsinki Declaration of the World Medical Association.

## DATA CONFIDENTIALITY

The authors declare that they have followed the protocols of their work centre on the publication of patient data.

## CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest in writing this manuscript.

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