Os níveis séricos de cistatina C sofrem influência da dose de corticoide em pacientes com nefrite lúpica?

Are serum cystatin C levels influenced by steroid doses in lupus nephritis patients?

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**Resumo**

Introdução: A cistatina C é considerada como um teste promissor para avaliar a taxa de filtração glomerular, pois apresenta características de um marcador endógeno ideal, sendo similar ou até superior à creatinina sérica, segundo alguns estudos. No entanto, é possível que alguns fatores (como corticoterapia) influenciem os níveis séricos da cistatina C, independente da taxa de filtração glomerular. Procurou-se investigar se diferentes doses de glicocorticoides afetariam os níveis do marcador em pacientes com nefrite lúpica.

Métodos: Foram avaliados 42 pacientes com nefrite lúpica, submetidos a 109 coletas de sangue diferentes; a idade média deles era de 37,7 ± 13,1 anos, e 88% eram do sexo feminino; a taxa de filtração glomerular estimada média era de 61,9 ± 20,0 mL/min. Os pacientes foram divididos, de acordo com a dose de corticoide, em dois grupos: A – altas (pulsoterapia com metilprednisolona e prednisone > 0,5 mg/kg/d, n = 14) versus B – baixas doses (prednisone ≤ 0,5 mg/kg/d, n = 28). Os níveis de creatinina sérica foram usados como parâmetros de comparação em relação à função renal. A cistatina C foi determinada por metodologia desenvolvida in-house, usando citometria de fluxo na plataforma Luminex.

Resultados: Considerando esses dois grupos, os níveis de cistatina C foram diferentes apenas nas amostras da segunda consulta (p = 0,106). Mas, quando considerados os níveis de creatinina sérica nos mesmos grupos, foi observada uma diferença marginalmente significante entre eles (p=0,070), sugerindo que a diferença nos níveis de cistatina C entre os grupos foi causada por suas respectivas taxas de filtração glomerular. Não houve diferença entre os que receberam, ou não, pulsoterapia.

Conclusões: Embora alguns

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**Abstract**

Introduction: Cystatin C is considered a promising test to evaluate glomerular filtration rate, since it has characteristics of an ideal endogenous marker, being similar or even superior to serum creatinine according to some studies. However, it is possible that some factors (as corticotherapy) could have an influence on serum cystatin C levels regardless of the glomerular filtration rate. The aim of this study was to investigate if different doses of glucocorticoid could have an influence on serum cystatin C levels in lupus nephritis patients.

Methods: We evaluated 42 patients with lupus nephritis that performed 109 different blood collections; their mean age was 37.7 ± 13.1 years old, and 88% were female; the mean estimated glomerular filtration rate was of 61.9 ± 20.0 mL/min. Patients were divided according to their glucocorticoid dose in two groups: A – high (pulse therapy with methylprednisolone and prednisone > 0.5 mg/kg/d, n = 14) versus B – low doses (prednisone ≤ 0.5 mg/kg/d, n = 28). Serum creatinine levels were used as parameters for renal function comparison. Cystatin C was determined by an in-house methodology, using Luminex system flow citometry.

Results: Considering these groups, cystatin C levels were different only in the second visit (p = 0.106). But, when the serum creatinine levels were considered in the same groups, a marginally significant difference among them (p = 0.070) was observed, which suggested that the difference in cystatin C levels between the groups was caused by their respective glomerular filtration rate. There was not any difference between those groups that received or did not receive pulse therapy.

Conclusion:
estudos tenham mostrado que os glicocorticoides podem influenciar os níveis de cistatina C, não foi observada tal interferência nesta população de pacientes com nefrite lúpica submetidos à corticoterapia.

Keywords: Glicocorticoides. Cistatina C. Creatinina. Taxa de filtração glomerular. Nefrite lúpica.

INTRODUCTION

There are many markers and methods to estimate renal function, and the gold standard used to determine glomerular filtration rate (GFR) is the measure of the clearances of exogenous substances, such as inulin, iohexol, Cr¹-ErLDTA, Tc⁹⁰-DTPA and I¹²⁵-iothalamate. On the other hand, urea and creatinine serum (sCreat) levels are endogenous markers of renal function frequently used, but they present several limitations. The inexistence of an ideal index of renal function, which is easily applicable in daily practice, imposes the search for new substances.

Serum cystatin C (sCysC) has been proposed as a promising marker of the GFR and it is seen as equivalent or even superior to sCreat. However, it is not clear if other factors beyond renal function have an influence on sCysC levels. Initially, it was accepted there were not in vivo factors that could have interference on its levels. Nevertheless, since its clinical application was initiated, conditions that could interfere on its serum concentration beyond renal function were reported, as corticosteroid therapy, thyroid dysfunction, chronic liver disease, malignancies, organ transplantation, and many others. In addition, some authors consider the possibility that sCysC values could be increased in the event of male gender, increasing age, weight, and height. Moreover, its levels could be influenced by smoking, similarly to C-reactive protein, as a marker of inflammation and cardiovascular mortality.

In fact, the superiority of sCysC over sCreat in the evaluation of GFR was demonstrated in some circumstances, but it is still questionable whether nonrenal factors could interfere on its levels, as the use of glucocorticoids.

The aim of this study is to evaluate whether corticosteroids have an influence on sCysC levels. In order to test this hypothesis, we evaluated patients with lupus nephritis using different doses of PO and IV glucocorticoids.

Although some previous studies have shown that glucocorticoid has an influence on serum cystatin C levels, we have not observed such interference in the lupus nephritis patients submitted to corticotherapy.

Keywords: Glucocorticoids. Cystatin C. Creatinine. Glomerular filtration rate. Lupus nephritis.

SUBJECTS AND METHODS

Forty-two patients with systemic lupus erythematosus (SLE), which diagnosis was established by the presence of at least four criteria of angiotensin receptor antagonists (ARA), were enrolled, and 109 blood samples were collected. These patients were followed in the Glomerulopathy Section (Division of Nephrology) of Universidade Federal de São Paulo (UNIFESP). All of them had renal involvement during the course of SLE. Patients with a previous renal biopsy were classified according to the World Health Organization (WHO) Lupus Nephritis Classification. The study was approved by the Ethics Committee, and patients were included in the study after signing their informed consent.

All patients included in this study had already used corticosteroids during the course of SLE, and in this study doses were defined according to their clinical status and presence of lupus activity evidence, which was evaluated by the application of SLEDAI-2K criteria. Patients were evaluated during approximately five months (range from three to ten months). Renal function was assessed in three different visits in order to have more information about the association between sCysC and corticosteroid use. At each visit, clinical history, physical examination, sCysC and routine laboratory exams were performed, as well as the determination of the laboratory items necessary to calculate the SLEDAI-2K.

The exclusion criteria corresponded to concurrent lymphoproliferative or autoimmune diseases (as rheumatoid arthritis, ankylosing spondylitis, and Crohn disease), chronic infectious diseases (as AIDS), active infections (as tuberculosis and viral hepatitis), pre-dialysis chronic kidney disease, renal transplantation, malignancies, and nonlupus related glomerulopathies. Many therapies were used to control SLE and lupus nephritis manifestations, including: corticosteroids, azathioprine, cyclosporine, cyclophosphamide, and antimalarials. Statins, angiotensin converting enzyme inhibitors (ACEi) and ARA II were also frequently administered. In addition,
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no patient used nonsteroidal anti-inflammatory drugs (NSAID) during the follow-up period, in order to reduce nephrotoxicity risk.

Patients were divided into two groups, according to the current corticosteroid doses: high (pulse therapy with methylprednisolone and prednisone > 0.5 mg/kg/day) versus low doses (prednisone ≤ 0.5 mg/kg/day). The classification was based on the highest corticosteroid dose they received daily; the pulse therapy group was defined when this type of treatment was used regardless of the oral prednisone dose, since pulse therapy had been administered within the last three or four weeks before sample collection, exceptionally six weeks before (when, for any reason, the drugs infusion was postponed but the cycles of IV pulse therapy were not interrupted). sCystatin C was determined by an in-house developed assay, using an automated microsphere-based flow cytometric methodology (Luminex, Austin, TX). Briefly, the technique was as follows: captured monoclonal antibody A3P3 was covalently coupled to microspheres (beads) according to the manufacturer’s instructions. For the assay, 50 μL (containing 3,000 beads) were incubated with 50 μL of serum (samples and standards) diluted 1:20 in PBS-BSA, 1.0% in individual wells of a 96-well filter-bottom microtiter plate (Millipore – MABV 1210). After one hour of incubation at room temperature with agitation, the beads were washed with PBS-BSA at 0.5%, and biotinylated anti-Cystatin C (polyclonal antibody – DAKO) was added. This step was followed by incubation at room temperature for one hour and a new cycle of washings, and streptavidin-phycocerythrin (Molecular Probes USA) were added and incubated for 30 minutes. After washings, 100 μL of PBS-BSA at 1.0% were added and the plate was analyzed in Luminex. Results were expressed in mg/L, using as reference a standard curve with calibrators from the Cystatin C kit - PET by DAKO.

This in-house assay was validated in linearity range and precision. The results obtained using this noncommercial assay were comparable to those of the commercial assays, using the kits N Latex Cystatin C, Dade Behring (r² = 0.884) and Cystatin C PETKit, Dako (r² = 0.814). We have also determined GFR in healthy voluntary individuals using iohexol clearance and the sCystatin C in-house assay, and a good correlation coefficient was obtained between them (r² = 0.821). As the Cystatin C methodology was developed in-house, normal reference values were also determined by the authors of this article. There was not a difference between the means when compared gender (Student’s t-test, p = 0.844). The range of Cystatin C corresponded to 0.40 - 0.91 mg/L, and the reference interval of normality (mean ± two standard deviations – SD) was 0.38 - 0.86 mg/L, similar to that observed in other studies.

STATISTICAL ANALYSIS

Continuous variables were presented as mean ± SD. For comparison among samples means, Student’s t-test, ANOVA or Kruskal-Wallis tests (followed by Tukey multiple comparison test) were performed. The association among renal function markers was evaluated by Pearson’s correlation test. It was considered significant p ≤ 0.05. The software Sigma Stat 2.0 was used for all analyses.

RESULTS

Mean age of the patients was 37.1 ± 13.1 years old (18 – 78); 37 (88%) of them were females; 67%, Caucasians; 19%, mulattos; and 14%, Afro-descendents. Mean time of SLE diagnosis was 9.0 ± 6.5 years (0.1 - 26.0). Mean e-GFR was 61.9 ± 20.0 mL/min. The mean interval between sample collections was 5.4 ± 1.9

Table 1

<table>
<thead>
<tr>
<th>Characteristics (n = 42)</th>
<th>Range**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.2 ± 13.1* [18 - 78]</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>5/37</td>
</tr>
<tr>
<td>Race (Caucasian/ mullato/ Afro-Brazilian)</td>
<td>28/8/6</td>
</tr>
<tr>
<td>Hypertension</td>
<td>26</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>1</td>
</tr>
<tr>
<td>Renal biopsy (class III/IV/V/VI)</td>
<td>03/18/11/01</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.20 ± 0.62 * [0.70 – 4.30]</td>
</tr>
<tr>
<td>Serum Cystatin C (mg/L)</td>
<td>1.26 ± 0.78 * [0.45 – 4.86]</td>
</tr>
<tr>
<td>e-GFR (MDRD - mL/min/1.73m²)</td>
<td>61.9 ± 20.0 * [11.4 – 101.0]</td>
</tr>
</tbody>
</table>

* Mean ± SD; ** if applicable.
months for the three visits (Table 1). Patients that presented higher SLEDAI-2K score received (or had a tendency to receive) a higher dose of corticosteroid, when the four groups of doses (data not shown) were considered. Mean sCreat corresponded to $1.20 \pm 0.62$ mg/dL and sCysC to $1.26 \pm 0.78$ mg/L. The correlation coefficients were: sCysC versus sCreat, $r = 0.900$, sCreat versus e-GFR (MDRD), $r = 0.949$, 1/sCreat versus sCysC and e-GFR (MDRD), $r = 0.716$.

When Groups A and B were analyzed, there was a statistically significant difference in the second visit, $p = 0.035$ (Table 2 and Figure 1). When sCreat levels were considered in order to evaluate eventual interference of renal function on these findings, it was still observed a tendency of a significant difference ($p = 0.070$). Therefore, it is possible that the alteration observed in sCysC levels was due to GFR deficit, instead of the use of medication.

When patients that have received pulse therapy with glucocorticoids versus those who have not were evaluated, regardless of the oral dose of prednisone, there was a tendency to observe an association between sCysC and glucocorticoid dose only in the first visit ($p = 0.052$), which was not seen in the following ones (second visit, $p = 0.116$; third visit, $p = 0.522$), as seen in Figure 2.

**Discussion**

Cystatin C is being considered as a potential candidate to replace serum creatinine in renal function evaluation, because it seems to be less affected by muscle mass. However, recent reports have shown substantial variability in the relationship between GFR and sCysC among the populations evaluated, suggesting that there may be differences in generation, tubular reabsorption, or extra-renal elimination. Stevens et al. found a stronger association of sCreat rather than sCysC with surrogates of muscle mass, including age, sex, race, and urine creatinine. This reflects smaller contribution of muscle mass to generation of CysC than creatinine.

**Table 2**

<table>
<thead>
<tr>
<th>Visits</th>
<th>Serum levels</th>
<th>Prednisone $\leq$ 0.5 mg/kg/day</th>
<th>Prednisone $&gt; 0.5$ mg/kg/day and pulse therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n = 28)</td>
<td>(n = 14)</td>
</tr>
<tr>
<td>1st visit</td>
<td>Creat</td>
<td>$1.09 \pm 0.59^*$</td>
<td>$0.97^{**}$</td>
</tr>
<tr>
<td></td>
<td>CysC</td>
<td>$1.04 \pm 0.62^*$</td>
<td>$0.82^{**}$</td>
</tr>
<tr>
<td>2nd visit</td>
<td>Creat</td>
<td>$1.20 \pm 0.72^*$</td>
<td>$1.10^{**}$</td>
</tr>
<tr>
<td></td>
<td>CysC</td>
<td>$1.24 \pm 0.89^*$</td>
<td>$1.00^{**}$</td>
</tr>
<tr>
<td>3rd visit</td>
<td>Creat</td>
<td>$1.28 \pm 0.72^*$</td>
<td>$1.01^{**}$</td>
</tr>
<tr>
<td></td>
<td>CysC</td>
<td>$1.35 \pm 0.88^*$</td>
<td>$1.00^{**}$</td>
</tr>
</tbody>
</table>

* Mean $\pm$ SD, ** Median.

**Figure 1.** Comparison between the groups that received higher (pulse therapy and $> 0.5$ mg/kg/day) and lower doses ($\leq 0.5$ mg/kg/day) of corticosteroids.

**Figure 2.** Comparison between patients that received or did not pulse therapy (independent of the oral prednisone dose).
It is possible that GFR estimates, based on sCysC, are more accurate than estimates based on sCreat in patients with variation in creatinine generation due to diet or clinical conditions, which affect muscle mass, including chronic use of glucocorticoid.

In this study, we were not able to demonstrate any relation between corticosteroids and CysC levels in patients with lupus nephritis. In fact, initially, a difference between two groups was observed (patients that received ≤ 0.5 mg/kg versus pulse therapy in the first visit), but as renal function was evaluated based on sCreat levels at the same moment, a tendency to elevation of these values was documented, suggesting that increased levels of sCysC could be due to GFR decline. This was also observed when compared groups that received low and high doses of corticosteroid, and there was not a difference between those that used or did not pulse therapy. These data are in accordance with studies involving children with nephrotic syndrome, who received high doses of glucocorticoids, in which authors have not observed a correlation between sCysC levels and glucocorticoid use.22

On the other hand, Cimerman et al. observed an increase in CysC levels in patients with asthma, who had utilized methylprednisolone and oral glucocorticoid in comparison with those that had not used these drugs or received cyclosporine.12 Similarly, it was shown in patients using methylprednisolone due to severe subarachnoidal hemorrhage that sCysC levels were higher after seven days of treatment than at the time of admission in the hospital.37

Risch et al. showed that kidney transplant recipients on low-dose prednisone therapy had greater sCysC levels compared with those on steroid-free immunosuppressive therapy, but it was reported that the groups were not perfectly matched.10

Pöge et al. performed a paired analysis in which matching was based on GFR, sex, age, and weight in 20 kidney transplant recipients. They showed that patients receiving doses of prednisone higher than 10 mg/day had more increased levels of sCysC than those using 5 mg/day.18 In a cross-sectional community based study, Wasen et al. found that glucocorticoid therapy was associated with higher sCysC levels, but they did not mention the dose of steroids.38 On the contrary, White et al. have not found such association.39

Rule et al. have shown that kidney transplant recipients had higher sCysC levels than patients with chronic kidney disease. This study used iothalamate clearance as gold standard.16 Le Bricon et al. have observed that sCysC has underestimated GFR in 14%, three months after transplantation.40 Manetti et al. showed in Graves ophthalmopathy patients treated with high doses of methylprednisolone a significant increase of sCysC after 24 and 48 h of medication use, but the levels of sCreat have also increased after 48 hours, therefore, it was supposed that such increase could be due to the GFR change.11 Discrepancies in these studies might be caused by lack of adjustment for kidney function using an accepted reference standard.

As previously mentioned, several reports have shown an association between steroid use and sCysC concentration. The absence of sCysC elevation associated with corticotherapy in our population of lupus nephritis patients could be explained, among other reasons, by their previous and concurrent use of glucocorticoids. We could speculate that the gene that codifies CysC was already activated by the induction of the promoter, determined by the previous use of the drug, and, consequently, there would not be current activation changes, neither alteration of sCysC levels. Bjarnadottir et al. have proposed this mechanism in their in vitro study, with HeLa cells. They have demonstrated that after 48 hours of dexamethasone use, a dose-dependent increase in sCysC levels occurred and this was attributed to the induction of the promoter mediated by glucocorticoids, increasing the transcription of the CysC gene.11 Such gene activation could be transitory and occur only during the use of such drugs.

It is noteworthy that the present study has some limitations, and the most important are: the small population and lack of a “gold standard” method to determine the GFR of this specific patient sample. It is also necessary to mention that thyroid function abnormalities are known to interfere in sCysC levels, which increase in hyperthyroidism and decrease in hypothyroidism.8,17 Multifactorial thyroid disorders are frequent in SLE patients, in which higher prevalence of hypothyroidism as well as of thyroid autoantibodies detection is widely described.41,42 However, we did not have cases of thyroid disorders at the onset of this study, since we did not evaluate periodically the thyroid function of these patients.

This study adds evidence against an interference of steroids on sCysC levels, but further randomized studies are certainly necessary to evaluate this possible association to avoid misinterpretation of this GFR marker, in patients under treatment with glucocorticoids.
REFERENCES


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