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Is there Correlations among SLEDAI, Pro-Inflammatory Biomarkers and Urine NGAL in SLE?

Bianchini MS, Madureira P, Volpe CMO and Nogueira-Machado JA*

Núcleo de Pós-Graduação e Pesquisa Santa Casa de Belo Horizonte Ensino e Pesquisa, Minas Gerais, Brazil

***Corresponding author:** Nogueira-Machado JA, Núcleo De Pós-Graduação E Pesquisa, Santa Casa De Belo Horizonte Ensino E Pesquisa, Domingos Vieira 590, Santa Efigênia, 30150-240, Belo Horizonte, MG, Brazil, Fax: +55 31 32388838, Tel: +55 31 32388838, E-mail: nogueira.machado@pq.cnpq.br; aunog@santacasabh.org.br

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Abstract

Objective: Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease. Several indexes can measure SLE activity, and Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) is one of them. The aim was to associate SLEDAI with inflammatory biomarkers present in serum (C3, C4, CH50, C-reactive protein, and creatinine), proteinuria (urine 24h) and urine NGAL in SLE patients.

Methods: This study included 43 SLE patients who were arranged in two groups according to the value of SLEDAI. One group, SLEDAI 0-4 (n=25), included SLE patients with low activity, and the other group, SLEDAI >4 (n=18), included SLE patients form moderate to high activity. Level of urine NGAL (uNGAL) was determined by ELISA, and the data were analyzed using GraphPad Prism 5.

Results: Levels of serum creatinine and 24h urinary protein were significantly higher in the group SLEDAI >4 compared to the group SLEDAI 0-4. Values of uNGAL were similar between the groups. Correlation studies of the SLEDAI versus C3 and CH50 indicated moderate negative correlation (r = -0.43 and r = -0.57, respectively), and SLEDAI versus C4 high negative correlation (r = -0.73) for SLEDAI 0-4 group. In the SLEDAI >4 group, a moderate positive correlation was found between the SLEDAI and uNGAL (r = 0.50). 24h urinary protein was highly positively correlated with SLEDAI renal (r = 0.72) and uNGAL (r = 0.71) in the SLE patients with lupus nephritis.

Conclusion: Urine NGAL is a potential predictor biomarker of renal involvement in patients with SLE. It could be used in conjunction with SLEDAI for evaluating SLE activity.

Keywords: Systemic Lupus Erythematosus; Lupus Nephritis; SLEDAI; NGAL

Introduction

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammatory disease. Clinical and laboratory characteristics may be diverse, and evolution is usually chronic, with periods of exacerbation and remission [1]. Survival has increased over the years mostly due to its earlier diagnosis [2]. Anti-nuclear antibody (ANA) and autoantibody for double-stranded DNA (anti-dsDNA) are the most common autoantibodies found in patients with disease activity mainly in renal manifestation [2,3]. The presence of ANA has a sensitivity of approximately 90% and specificity of 70%, while anti-dsDNA antibody, although less sensitive, is more specific than ANA and more associated with disease activity [4]. Its diagnosis is based on laboratory tests and clinical manifestations ranging from classic cutaneous manifestations to renal, pulmonary, hematological and central nervous system alterations. All of them encompassed by the American College of Rheumatology (ACR) of 1997, in which the presence of at least four criteria, a total of 11 parameters, in following or serial form establishes the diagnosis of SLE [5,6]. After diagnosis, it is necessary to define the degree of disease activity to help the therapeutic decisions. The use of indexes is routine in clinical practice to measure the improvement or the exacerbation of SLE. Since 1980 several studies have been carried out to find a valid and sensitive assessment tool to measure SLE activity. Several indexes are used as The Index of Lupus Assessment of the British Isles (BILAG), European Lupus Activity Measurement Consensus (ECLAM), Systemic Lupus Activity Measurement (SLAM), Lupus Activity Index (LAI) and the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). All of them are grounds for criticism and SLEDAI is one of the most used indexes. It measures the SLE activity within the last ten days [7]. SLEDAI measure

the disease activity, and it can range from 0 to 105 points. However, in SLEDAI scores above five is suggested the initiating therapy [8]. In the present study, we used SLEDAI, an index composed by several clinical and serological parameters, such as proteinuria, urine sediments, creatinine clearance, complement levels (C3, C4, and CH50), and anti-dsDNA. The summation of different parameters leads to total SLEDAI score that supports the therapeutic decision. Few pro-inflammatory parameters are used to define the SLEDAI score. A new biomarker with predictive appeal could be used in conjunction with SLEDAI. In this context, the role of NGAL (Neutrophil gelatinase-associated lipocalin) as a possible future biomarker of renal injury, in SLE is suggested [9,10]. NGAL monomeric or dimeric forms are the predominantly synthesized molecules in the renal tubules which can be quantified in the urine. NGAL had prognostic value for clinical outcomes, such as the need for dialysis and mortality [11,12]. According to Schmidt-Ott, NGAL fulfilled the criteria of a promising renal biomarker and maybe stepped away from clinical practice [13]. The number of clinical and laboratory parameters used in SLEDAI is high but compromises its objectivity. In the present study, the SLEDAI score was calculated as proposed by Bombardier, *et al*. and the urine NGAL was quantified separately [14]. We aimed to associate the SLEDAI score with inflammatory biomarkers present in the serum (C3, C4, CH50 and C-reactive protein) or the urine (proteinuria and NGAL) in SLE patients.

Methods

Ethical approval

The Ethical Committee from Santa Casa Hospital of Belo Horizonte – Brazil approved this study, and the informed consent was obtained from all participants included in the study.

Study population

Patients diagnosed with SLE were selected at Santa Casa Hospital -Belo Horizonte, Minas Gerais, Brazil, - Rheumatology Service under the responsibility of Dr. Paulo Madureira and Dr. Marília Simões Bianchini, at Traumatology and Orthopedics Center (Governador Valadares, Minas Gerais, Brazil). Patients aged between 18 and 65 years and ANA > 80 were included in this study. Patients were grouped according to the SLEDAI score, SLEDAI 0-4 and SLEDAI >4 constitute the groups for study [8]. Characteristics of the study population and laboratory data as levels of C3, C4, CH50 and C-reactive protein (CRP) of these subjects were evaluated (Table 2). Subjects presenting other autoimmune diseases other than SLE, cancer, pregnant women, diabetics, smokers, chronic renal failure, and acute renal failure without SLE were excluded from the study.

Score	Description
8	Psychosis
8	Organic brain syndrome
8	Visual disturbance
8	Cranial nerve disorder
8	Lupus headache
8	Cerebro vascular accident (excluding atherosclerosis)
8	Vasculitis
4	Arthritis
4	Myositis
4	Heme-granular or red blood cells casts in urine
4	Hematuria (excluding stone, infection or other cause)
4	Proteinuria
4	Pyuria (excluding infection)
2	New onset or recurrence of inflammatory rash
2	Alopecia
2	Mucosal ulcers
2	Preurisy
2	Pericarditis
2	Low Complement
2	Increased DNA binding
1	Fever (excluding infection cause)
1	Thrombocytopenia
1	Leukopenia (excluding drug causes)

Table 1: Score to define SLEDAI
Adapted from Bombardier *et al*. [14].

Blood and Serologic parameters

Leukocytes counting were performed in a cytometer. Complement components, C3 and C4, C reactive protein (CRP) and urine protein, were quantified by the turbidimetric assay. Complement activity (CH50) was evaluated by ELISA, antibodies anti-DNA and anti-nuclear factor (Hep2 cells) were determinate by indirect immunofluorescence.

SLEDAI

SLEDAI was calculated according to Bombardier, *et al.* [14] (Table 1). The following activity categories have been defined on the basis of SLEDAI scores: no activity (SLEDAI = 0), mild activity (SLEDAI = 1–5), moderate activity (SLEDAI = 6–10), high activity (SLEDAI = 11–19), very high activity (SLEDAI = 20). Some authors use the scores to define inactivity, low activity, moderate, high and very high activity and others consider as active disease index greater or equal than 4 and severe disease index greater or equal than 12 [15,16].

Renal SLEDAI ranges from 0 to 16, and its calculation is performed through the following tests: Proteinuria 24h >0.5g/day, hematuria (cells > 5 red blood cells/field in the urine excluding other causes of hematuria), pyuria (> 5 leukocytes/field, excluding infection) and haematic or hyaline cylinders [17].

Urine collection

Peripheral venous blood collection was performed using vacutainer tubes, containing or not EDTA. Approximately 30 ml of urine was collected from each volunteer. After centrifugation (200g for 15 min, at room temperature), the urine was stored at -80 ˚C until further analysis.

Quantification of NGAL

The NGAL levels in urine were determinate by ELISA method (LEGEND MAX™ Human NGAL ELISA Biolegend® - catalog number 443408) according to the manufacturer's guidelines.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the normal distribution of the continuous variables; values were expressed as mean ± standard error or median (minimum-maximum) as appropriate. Comparisons between groups were performed using unpaired Student t-test or Mann-Whitney test. Within-group correlations were performed using Spearman's r calculation; interpretations were based on the study Mukaka [18]. In order to evaluate the association between the variables, we used the Spearman correlation test. The correlation coefficient (r) can be assessed qualitatively as follows: $0.00 < r < 0.25$, small or no correlation; $0.25 \leq r < 0.50$, weak correlation; $0.50 \leq r < 0.75$, moderate correlation; $0.90 \leq r < 1.00$, strong correlation. All analyses were considered significant at P values < 0.05 using GraphPad Prism 5 (GraphPad Software, Inc).

Results

Table 2 shown characteristic of the two groups of SLE patients. All subjects were female patients. A significant difference ($p<0.05$) regarding SLEDAI value and levels of C3 were found between SLEDAI 0-4 and SLEDAI>4.

Parameters	SLEDAI		p
	0-4	>4	
SLE patients (N)	25	18	-
Lupus Nephritis (N)	3	11	-
Disease duration (months)b	60 (24 – 396)	48 (12 – 160)	ns
Age (years)[a]	41.3 ± 2.4	34.8 ± 2.6	ns
SLEDAI[b]	0 (0 – 4)	8 (5 – 22)	<0.05
C3 (mg/dL)[a]	103.6 ± 5.7	81 ± 10.3	<0.05
C4 (mg/dL)[b]	21 (8 – 60)	10.5 (7 – 65)	ns
CH50 (U/CAE)[b]	109 (50 – 171)	83 (39 – 213)	ns
CRP (mg/L)[b]	0.8 (0 – 12)	3 (0 – 81)	ns

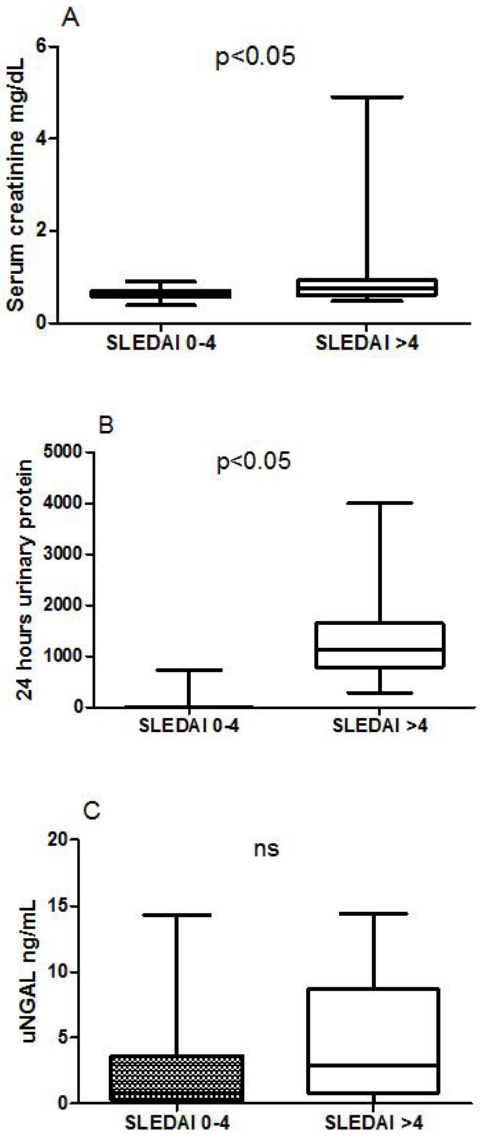
Table 2: Characteristics of the studied SLE patients
[a]values expressed as mean ± standard error; [b]values expressed median (minimum – maximum). CRP: C-reative protein; ns = non-significant; U/CAE: Units/Complement Activity Enzyme Immunoassay.

Figure 1 shows significant differences between SLE patients grouped according to disease activity in serum creatinine and 24h urinary protein, but not to uNGAL. Values, expressed in median (minimum-maximum), to serum creatinine were 0.6 (0.4 – 0.9) and 0.8 (0.5 – 4.9), to 24h urine protein were 0 (0 – 730) and 1127 (286 – 4000) and to uNGAL were 0.8 (0.0 – 14.3) and 2.9 (0.0 – 14.4) for SLEDAI 0-4 and SLEDAI >4, respectively.

In the SLEDAI 0-4 group, a negative correlation was found between the SLEDAI and C3 (r = -0.43, moderate negative correlation), C4 (r = -0.73, high negative correlation) and CH50 (r = -0.57, moderate negative correlation) (Table 3). In the SLEDAI >4 group, a positive moderate correlation was found between the SLEDAI and uNGAL (r = 0.50) (Table 3). Figure 2 shows high positive correlation between 24h urinary protein and SLEDAI renal (r = 0.72) and uNGAL (r = 0.71).

Parameters	Spearman correlation coefficient (r)	
	SLEDAI 0-4 N=25	SLEDAI >4 N=18
C3	-0.43* (low)	-0.22(negligible)
C4	-0.73*(high)	-0.04(negligible)
CH50	-0.57*(moderate)	-0.11(negligible)
CRP	-0.09(negligible)	0.34(low)
uNGAL	0.27(low)	0.50*(moderate)

Table 3: Spearman Correlation coefficient for SLEDAI and biomarkers in SLE patients grouped *p<0.05; CRP: C-reative protein; uNGAL: urine NGAL. The interpretation of the value of r was as proposed by Mukaka [18].



SLEDAI 0-4, N=25 and SLEDAI>4, N=18
ns = non-significant

Figure 1: Levels of serum cretianine (Panel A), 24h urinary protein (Panel B) and urine NGAL (uNGAL, Panel C) in SLE patients grouped according to disease activity.
Values expressed median (minimum - maximum), significant differences between the groups were determined using Mann-Whitney test.

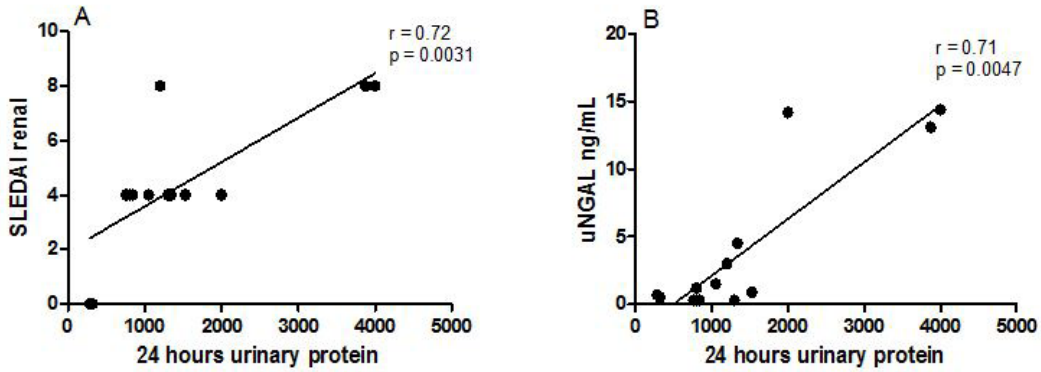


Figure 2: Spearman Correlation coefficient for 24 hours urinary protein and SLEDAI renal (Panel A) and urine NGAL (uNGAL, Panel B) in SLE patients with Lupus Nephritis (N=14)

Discussion

The proposition of one or more biomarkers for using in conjunction with SLEDAI to define the SLE activity is attractive. Our results demonstrated that SLEDAI leads to low correlation with the pro-inflammatory parameters used for its calculation. The conjunction of uNGAL and renal SLEDAI could improve the reliability of the index and help the physician in the definition of the therapeutic approach. SLEDAI is not able to detect the worsening or the improvement of an already diagnosed parameter. The improvement in mental function (8 points) or the worsening of arthritis (4 points) will not change the final SLEDAI score. In both examples, the total SLEDAI score will always equal 12. Thus, it is not a reliable index for determining the severity or activity of SLE. SLE is an inflammatory disease. Will there be a correlation between the level of inflammatory biomarkers and the total SLEDAI score used for determining the SLE activity? Recent studies propose new pro-inflammatory biomarkers and some specifically for renal injury assessment. Could not they be included or used for evaluation of patients with SLE? They could be a new tool for the clinician in association with SLEDAI.

Correlations between SLEDAI and pro-inflammatory biomarkers showed negative correlation for C3, C4, and CH50 in the SLEDAI 0-4 group (inactive and mild SLE activity) but not for the group SLEDAI >4 (moderate and high SLE activity) (Table 3). Tseng *et al*., in a study. Hinze, *et al*. reported that NGAL was present in patients' urine three months before the onset of LN. Suzuki, *et al*., Pitashny, *et al*.and Hammad, *et al*. reported no association between renal injury and plasma levels of NGAL [17,19,21-23]. It can be explained since plasma NGAL is predominant in the NGAL-MMP9-bound form, which will not appear in the urine. Considering only the population of patients with Lupus Nephritis, our results demonstrate high positive correlations between renal SLEDAI and uNGAL levels and proteinuria (24h urinary protein) (Figure 2). Similar results were reported by Elewa, *et al*., Yang, *et al*., Sharifiporu, *et al*. and Satirapoj, *et al*. [24,27]. However, Pitashny, *et al*., Alharazy, *et al*. and Brunner, *et al*. found no correlation between proteinuria (24h urinary protein) and urinary NGAL [17,20,24,27,28].

Conclusion

SLEDAI is an index for the measure of SLE activity that showed low or variable correlation with the pro-inflammatory mediators present in this pathology. Our results suggest the evaluation of SLEDAI in conjunction with urine NGAL in patients without diagnosed renal complication could be a useful predictive biomarker. The possibility of the inclusion of the urine NGAL measurement in the SLEDAI calculation is attractive, but it will demand hard and further studies.

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