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Evaluation of potential biomarkers for the diagnosis and monitoring of Systemic Lupus Erythematosus using the Cytometric Beads Array (CBA)

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ABSTRACT

Background: Systemic Lupus Erythematosus (SLE) is an autoimmune, multisystemic disease. Currently diagnosis depends on complex criteria developed by the American College of Rheumatology. Moreover, the lack of specific biomarkers also challenges the diagnosis.

Methods: Inflammatory biomarkers such as IL-8, IP-10, MIG, MIP-1α and RANTES were measured in serum samples from SLE patients and subjects in control groups (patients with other autoimmune diseases and healthy individuals). Forty-six SLE patients (22 patients with low activity, SLEDAI-2 K ≤ 4, 24 patients with moderate/high activity, SLEDAI-2 K > 4), 42 patients with other autoimmune diseases (OAD group), and 8 healthy volunteers participated in this study.

Results: MIG ($p < .001$) and RANTES ($p < .001$) concentrations in SLE patients and healthy controls, and IP-10 concentrations in SLE patients with different disease activities (low activity, $p < .01$, moderate/high activity, $p < .05$) differed significantly. IL-8 ($p < .001$) and MIP-1α ($p < .001$) concentrations in SLE patients differed from those in patients from the OAD group. IL-8 ($p < .05$), IP-10 ($p < .01$), MIG ($p < .05$), MIP-1α ($p < .001$), and RANTES ($p < .05$) were correlated with SLE activity; their concentrations in SLE patients with low and moderate/high activity differed significantly.

Conclusions: Given the findings of this study, one can envision the possibility of future use of some of these cytokines to assist in the screening of SLE patients, or even in monitoring disease activity.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease of unknown etiology; it is characterized by the loss of immunological tolerance, production of autoantibodies against nuclear and cytoplasmic antigens, and the formation of circulating immune complexes. Besides, SLE is also influenced by genetic and environmental factors [1–4]. The deposition of these immunocomplexes induces inflammatory processes affecting several organs and tissues [5]. SLE is predominant in females, with the ratio of the proportion of affected females to that of affected males being 8–15:1 [6].

SLE is a disease with an unpredictable clinical course, with episodes alternating between periods of activity and remission [7]. In this

Abbreviations: ACR, American College of Rheumatology; AIDS, Acquired Immunodeficiency Syndrome; ANAs, Antinuclear Antibodies; BD, Becton Dickinson; BILAG, British Isles Lupus Assessment Group; CBA, Cytometric Beads Array; ECLAM, European Consensus Lupus Activity Measure; HC-UFMG, Clinics Hospital of the Federal University of Minas Gerais; HIV, Human Immunodeficiency Virus; IL-1, 6, 8, Interleukins 1, 6, and 8; IP-10, Interferon gamma-induced protein 10; LA, Low Activity; MFI, Medium Fluorescence Intensity; MHA, Moderate/high Activity; MIG, Monokine induced by gamma interferon; MIP-1α, Macrophage inflammatory protein-1 alpha; NK, Natural Killer; OAD, Other Autoimmune Diseases; RANTES, Regulated on Activation, Normal T Cell Expressed and Secreted Chemokine; SLAM-R, Systemic Lupus Activity Measure Revised; SLE, Systemic Lupus Erythematosus; SLEDAI, Systemic Lupus Erythematosus Diseases Activity Index; SLEDAI-2K, Systemic Lupus Erythematosus Diseases Activity Index 2000; SLICC, Systemic Lupus International Collaborating Clinics; TNF- α, Tumor Necrosis Factor alpha

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context, the possibility of measuring SLE activity is very important not only for clinical research, but also for deciding the therapeutic scheme to be followed [8,9]. To this end, several indexes have been proposed to evaluate the SLE activity, such as SLEDAI (Systemic Lupus Erythematosus Disease Activity Index), BILAG (British Isles Lupus Assessment Group), ECLAM (European Consensus Lupus Activity Measure), and SLAM-R (Systemic Lupus Activity Measure Revised) [10].

SLEDAI-2 K is a modified version of SLEDAI, established after its revision in 2002; it consists of the same scoring principle, allowing a longer evaluation window (30 days) to measure the disease activity based on important manifestations of SLE, such as proteinuria, arthritis, rashes, and mucosal ulcers [11].

The diagnosis of SLE is extremely complicated and challenging. Due to its innumerable and variable symptoms, SLE can be confused with infectious or inflammatory diseases and other autoimmune diseases that present constitutional (arthralgia, fever, fatigue) and specific symptoms (vasculitis, joint pain, arthritis), in addition to rheumatoid arthritis [12,13].

The current SLE diagnosis is based on classification criteria proposed by the American College of Rheumatology (ACR) in 1971, which were revised in 1982 and 1997 [14]; they are associated with the SLICC (Systemic Lupus International Collaborating Clinics) criteria proposed by Petri et al. [15].

The search for new biomarkers that can help monitor the individual response to therapy is of great importance for the management of SLE patients, since the currently used markers, such as antinuclear antibodies (ANAs), have variable sensitivity and specificity. Several potential biomarkers have been associated with the susceptibility the pathogenesis of SLE. Among them are products of complement activation, autoantibodies and cytokines, which can play an important role in disease activity and SLE pathogenesis [16–18].

Cytokines play an important immunemodulating role in the inflammatory process, acting on local or systemic inflammatory responses, cell proliferation, tissue repair, and taking part in both acute and chronic inflammation through complex interactions. In some cases, they show contradictory actions, due to their pro-inflammatory and/or anti-inflammatory activities [19,20].

Studies have shown a strong association between dysregulation of cytokine production and SLE pathophysiology, since cytokines play a critical role in the differentiation, maturation, and activation of immune cells and loss of immunogenic tolerance. They also participate in the local inflammatory processes that lead to the characteristic tissue lesions of the disease [21,22].

Among the various cytokines associated with SLE are IL-8 (interleukin 8), IP-10 (interferon gamma-induced protein 10), MIG (monokine induced by gamma interferon), MIP-1α (macrophage inflammatory protein – 1 alpha), and the regulated upon activation, normal T cell expressed and secreted chemokine (RANTES), which are involved in the disease activity and tissue damage especially in the progression to nephropathies [23,24].

As reviewed by Adhya et al. [23], cytokines evaluation in isolation or in combination, together with existing markers, may help earlier identification of flare, in monitoring response to therapy and providing information regarding organ involvement, which may be important for making decision. Also according to the same authors, only those cytokines capable of discriminating better between inactive and active SLE are likely to be successful candidates.

2. Patients and methods

2.1. Participants

Patients were recruited between August 2016 and July 2018 at the Rheumatology Outpatient Clinic of the Medical School Hospital of Federal University of Minas Gerais (HC-UFMG), Belo Horizonte, Minas Gerais, Brazil. Ethical approval was granted by the Research Ethics Committee of the Federal University of Minas Gerais and by the teaching and research management of the HC-UFMG. Informed consent has been sought from all participants. Relevant clinical details and laboratory data were obtained from the medical records. SLE group comprised women diagnosed with SLE according to the American College of Rheumatology (ACR), who were under clinical follow-up and undergoing (or not undergoing) treatment, and were not affected by another autoimmune condition. The SLEDAI-2 K score was calculated by the physician at the time of the medical appointment. Patients with other systemic autoimmune diseases (OAD), diagnosed according to the criteria for each disease, were also recruited at the HC-UFMG rheumatology department.

The SLE group (n = 46) comprised of patients with low activity SLE (LA) – SLEDAI-2 K ≤ 4 (n = 22), patients with moderate/high activity SLE (MHA) – SLEDAI-2 K > 4 (n = 24), patients with other systemic autoimmune diseases (OAD) such as rheumatoid arthritis (n = 11), scleroderma (n = 10), ankylosing spondylitis (n = 10), and primary Sjögren's syndrome (n = 11) comprised the OAD group. Healthy female volunteers from the HC-UFMG and Faculty of Pharmacy of Federal University of Minas Gerais, matched for age, were included in the Control group (n = 8).

2.2. Inclusion and exclusion criteria

The control group consisted of healthy women with no family history of autoimmune diseases, selected through a standard questionnaire. SLE patients with other immunosuppressive diseases and associated conditions, including HIV / AIDS were excluded in addition to pregnant women.

2.3. Sample collection

A 10 ml venous blood sample was randomly collected from each participant into vacutainer tubes (Becton-Dickinson) without anticoagulant. Samples were then rapidly centrifuged for 10 min at 3000 × g in a non-refrigerated centrifuge for obtaining serum. Then, 500 µl aliquots were stored at −80 °C until use.

2.4. Quantification of inflammatory biomarkers by flow cytometry - CBA

For samples and reagent preparation, the manufacturer's guidelines for flow cytometry / CBA technique were strictly followed. Biomarkers were quantified in serum samples from patients and healthy volunteers using the CBA-Flex soluble protein kit (Human soluble protein Master Buffer kit: IL-8, IP-10, MCP-1, MIG, MIP-1α, RANTES, Becton Dickinson Biosciences Pharmingen). The methodology used was adapted from the original protocol proposed by the manufacturer. Standard curves were made for all biomarkers to calibrate the equipment for the determination of the analyte concentrations in each sample. The concentrations of the standards and samples were estimated through mean fluorescence intensity (MFI).

2.5. Data acquisition and analysis

Plate reading was performed using the FACVerse ™ BD flow cytometry equipment (BD Biosciences) which acquired 300 events per well. The FCAParray ™ software (ver. 3.0) provided by BD Biosciences was used for the analysis of the results, which were expressed in pg/ml.

2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism ver. 5.01. The comparison of the mean ages of the subjects in each group was performed by the Tukey test. The non-parametric variables were analyzed by means of the Mann-Whitney test. In addition, the Spearman Rho correlation test was performed to analyze the correlation between

Table 1
Characteristics of the study participants.

Characteristics	SLE	LA	MHA	OAD	CONTROL
Number of patients	46	22	24	44	8
Age (± SD) - years	39.64 (9.61)	36.55 (9.51)	42.29 (11.09)	51.93 (13.48)	42.50 (11.50)
Disease time (± SD) - years	10.81 (7.34)	8.64 (7.04)	12.80 (7.18)	11.93 (9.15)	
SLEDAI-2 K (± SD)	5.02 (4.63)	1.32 (1.52)	8.42 (3.82)		

Manifestations in the diagnosis of SLE	Total (%)		
Malar erythema	23 (50%)	11 (50%)	12 (50%)
Discoid lesions	12 (22%)	5 (23%)	7 (29%)
Photosensitivity	21 (46%)	9 (41%)	12 (50%)
Oral ulcers	15 (33%)	7 (32%)	8 (33%)
Arthritis	28 (61%)	13 (59%)	15 (63%)
Serositis	15 (33%)	8 (36%)	7 (29%)
Glomerulonephritis	32 (70%)	14 (64%)	18 (75%)
Neurological disorders	7 (15%)	4 (18%)	3 (13%)
Hematological findings	35 (76%)	17 (77%)	18 (75%)
Immunological findings	32 (70%)	16 (73%)	16 (67%)
Antinuclear antibodies (ANA-Hep2)	42 (91%)	20 (91%)	22 (92%)

SLE - Systemic Lupus Erythematosus; LA - Systemic Lupus Erythematosus of low activity; MHA - Systemic Lupus Erythematosus of moderate to high activity; OAD - Other autoimmune diseases. SLEDAI-2 K - Disease Activity Index of Systemic Lupus Erythematosus 2000. Sm - Smith. VDRL - Venereal Disease Research Laboratory. "ANA" - Antinuclear factor.

the patients' parameters and their demographic and laboratory data. The level of statistical significance was $p < .05$.

3. Results

3.1. Baseline characteristics

The population of this study consisted of 96 female participants, of which 46 belonged to the SLE group, with an average age of 39 ± 10 y. This group was divided into the LA (patients with low activity) group (SLEDAI-2 K less than or equal to 4) comprising 22 participants with an average age of 37 ± 10 y; and the MHA (patients with moderate/high activity) group (SLEDAI-2 K > 4), comprising 24 participants with an average age of 42 ± 11 y (Table 1). The mean disease time (y) for SLE patients was 11 ± 7. When the SLE activity was considered, the means of disease time (y) were 9 ± 7 for the LA group and 130 ± 7 for the MHA group. The mean disease time for the patients in the OAD group was 12 ± 9 y (Table 1) and the mean age of the patients in this group was 52 ± 13 y. The subjects in the control group had an average age of 42 ± 11 y.

The most frequent clinical manifestations among SLE patients at the time of diagnosis were the presence of antinuclear antibodies (ANA-Hep2), presented by 91% of the participants, followed by hematological disorders (76%), nephritis (70%), and immunological changes (70%) (Table 1).

3.2. Quantification of inflammatory biomarkers

The dosages of the inflammatory biomarkers in the patients' serum samples were quantified through CBA analysis. The results are expressed in pg/ml.

3.2.1. Interleukin-8 (IL-8)

Serum concentrations of IL-8 between control SLE patients and other autoimmune diseases groups showed no significant differences (Fig. 1A).

However, significant differences were observed between OAD and LA groups ($p < .001$), OAD and MHA groups ($p < .05$), and between the groups of SLE patients with low activity and moderate/high activity ($p < .05$). When all the patients with lupus were clubbed into a single group, a significant difference was observed between this group and the OAD group ($p < .001$) (Fig. 2A). IL-8 concentrations were higher in the OAD group, with a median (interquartile range) of 35.80 pg/ml (5.50–137.40) and lower in patients with SLE, 1.17 pg/ml (0.00–20.50), especially in patients with low disease activity, 0.00 pg/ml (0.00–8.09).

3.2.2. Interferon gamma-induced protein-10 (IP-10)

Serum IP-10 concentrations showed significant differences between healthy individuals and SLE patients with different concentrations of disease activity: control group compared to the MHA group ($p < .05$), and control group compared to the LA group ($p < .01$). There was a significant difference between the LA and MHA groups ($p < .01$), as well as between LA and OAD groups ($p < .01$). However, it was not observed difference when the OAD group was compared to the MHA group (Fig. 1B). When all SLE patients were grouped, no statistically significant difference was observed among the groups (Fig. 2B). Higher concentrations of IP-10 were observed in patients with SLE, with a median value (interquartile range) of 578.00 pg/ml (0.00–1.236,00); the highest values were found in the group of patients with low activity of the disease: 988.00 pg/ml (602.00–1.470.00). The dosages were lower in the group of patients with moderate to high activity, with a median value (interquartile range) of 0.00 pg/ml (0.00–431.00).

3.2.3. Monokine induced by gamma interferon (MIG)

MIG cytokine concentrations were the most promising among all the biomarkers studied here, as they showed significant differences between almost all the groups. MIG concentrations in the control group differed significantly from those in the MHA ($p < .001$) and LA ($p < .01$) groups, as well as from those in the OAD group ($p < .001$). Significant differences between SLE patients with moderate to high activity and low activity ($p < .05$), and between patients with autoimmune diseases and low activity SLE patients ($p < .01$) were observed. However, no significant difference was observed when OAD and MHA groups were compared (Fig. 1C). Instead, there was a significant difference between control group and all patients with SLE ($p < .001$), as well as between control and OAD group ($p < .001$). However, no difference was found when SLE and OAD groups were compared (Fig. 2C). The concentrations were the highest in the OAD group, with a median value (interquartile range) of 1302 pg/ml (537–2022), and lower in the healthy controls, 217 pg/ml (167–258).

3.2.4. Macrophage inflammatory protein-1 alpha (MIP-1α)

Significant differences were observed in MIP-α serum

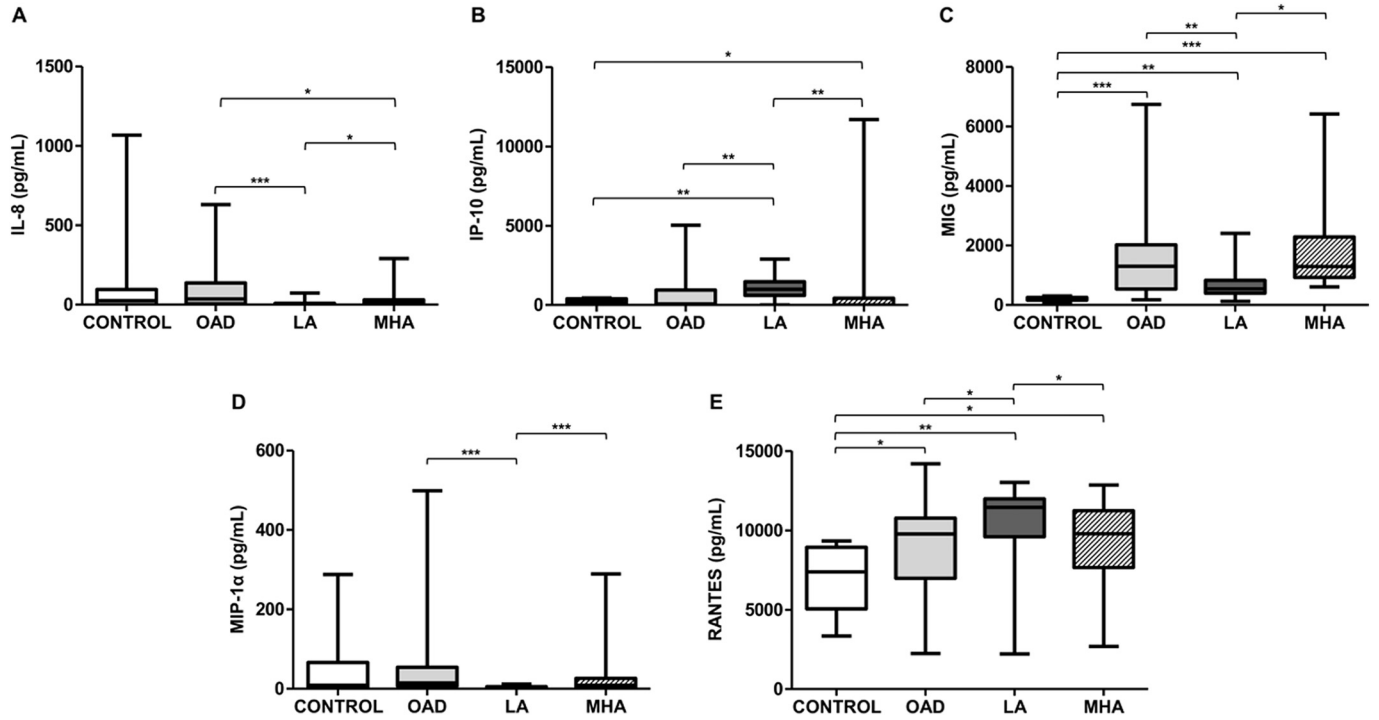

Fig. 1. Serum concentrations of inflammatory markers in the CONTROL, OAD, LA, and MHA groups. A) IL-8; B) IP-10; C) MIG; D) MIP-1α; E) RANTES. * $p < .05$; ** $p < .01$; *** $p < .001$. Mann-Whitney test (significant value: $p < .05$).

concentrations between MHA and LA groups ($p < .001$), and those in the LA and OAD groups ($p < .001$), as shown in Fig. 1D. Statistical analysis also showed a significant difference for MIP-α concentrations between OAD and SLE ($p < .001$). No significant differences were found when the results for SLE patients and healthy individuals (Fig. 2D) were compared. MIP-1α chemokine concentrations were higher in the OAD group, with a median (interquartile range) of 14.90 pg/ml (6.70–54.30) and lower in patients with SLE, 4.84 pg/ml (2.90–10.36), especially in patients with low disease activity, 2.94 pg/ml (1.97–5.23).

3.2.5. Regulated on activation, normal T cell expressed and secreted chemokine (RANTES)

Evaluation of the RANTES concentrations showed promising results,

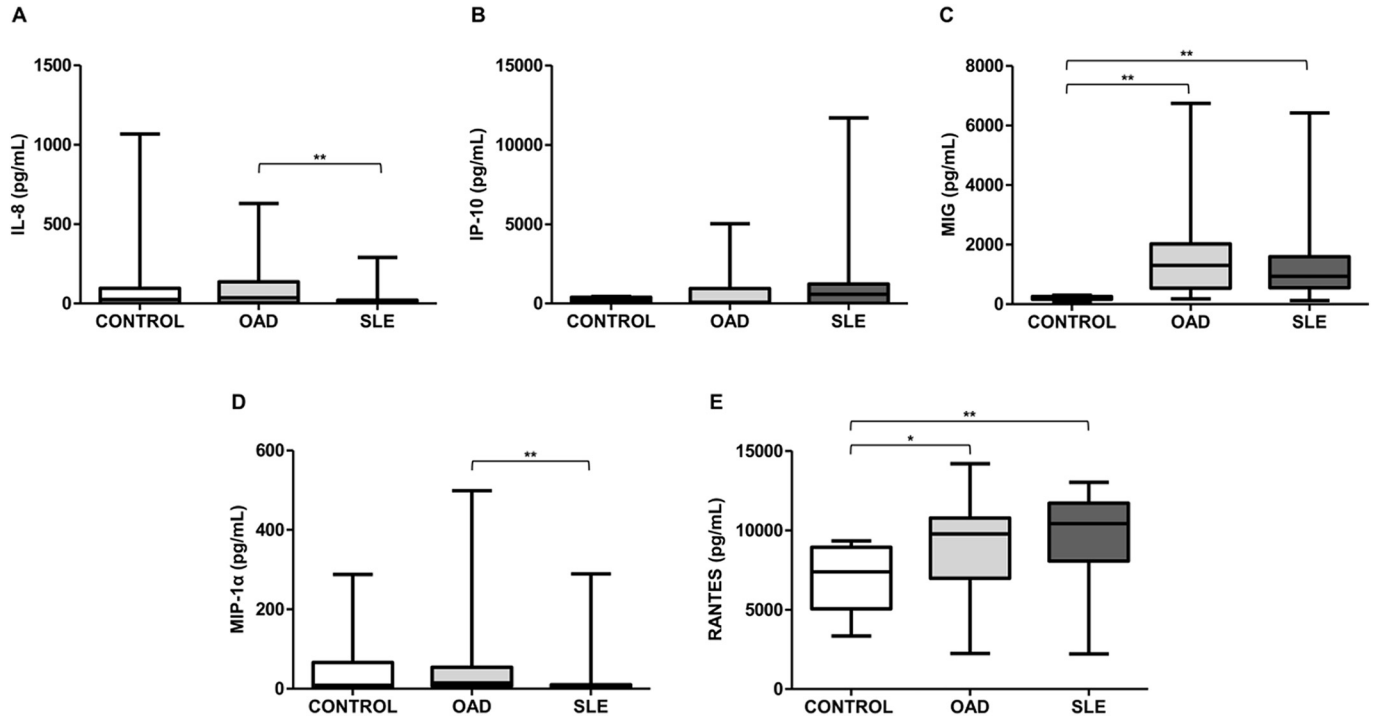

Fig. 2. Serum concentrations of inflammatory markers in the CONTROL, OAD, and SLE groups. A) IL-8; B) IP-10; C) MIG; D) MIP-1α; E) RANTES. * $p < .05$; ** $p < .001$. Mann-Whitney test (significant value: $p < .05$).

similar to MIG results, showing significant differences between almost all the groups analyzed. Thus, a statistically significant difference for RANTES concentrations was observed between control group, MHA ($p < .05$) and LA ($p < .01$) groups, and when the results of the LA group were compared to those of the OAD ($p < .05$). This analysis also revealed significant difference between groups of patients with different concentrations of disease activity (LA and MHA, $p < .05$). A significant difference between the control and OAD groups ($p < .05$) was also observed (Fig. 1E), as well as between healthy individuals and all patients with SLE ($p < .001$) (Fig. 2E). SLE group had the highest concentrations of RANTES, with a median value (interquartile range) of 10,417 pg/ml (8057-11,712), especially in patients with low disease activity, with a median of 9590 pg/ml (9590-11,982). The lowest concentrations were observed in healthy controls, with a median of 7378 pg/ml (5061-8931).

3.3. Cytokine concentrations and disease activity

The analysis between SLEDAI-2 K scores of patients with SLE and IL-8 ($r = 0.601$, $p = .000$), MIG ($r = 0.609$, $p = .000$), and MIP-1α ($r = 0.601$, $p = .000$) biomarkers presented a positive and significant correlation. These findings could indicate that the higher the disease severity, the higher the concentration of these biomarkers in the patients' serum (Fig. 3A, 3C and 3D). In contrast, the biomarkers IP-10 ($r = -0.448$, $p = .002$) and RANTES ($r = -0.445$; $p = .002$) had a significant negative correlation with the SLEDAI-2 K scores in patients with SLE, showing that the concentrations of both these biomarkers are inversely proportional to the disease severity (Fig. 3B and E).

4. Discussion

SLE is an autoimmune chronic inflammatory disease characterized by the production of autoantibodies which interact with nuclear autoantigens, producing immunocomplexes responsible for the release of mediators, influx of inflammatory cells, and a type III hypersensitivity. In addition, SLE presents a heterogeneous clinical and laboratory aspect showing various signs and symptoms that compromise its diagnosis and treatment [25].

Current methods for the diagnosis of SLE are based on complex criteria associated with clinical and laboratory findings, since there is no specific test to define it, as well as because such findings may also be presented by other systemic autoimmune disorders. Besides, patients may also present a lower number of manifestations resulting in a lower sensitivity and specificity of the criteria currently employed for SLE diagnosis [26].

The search for strategies that could aid the development of new methods for diagnosing SLE is necessary due to the increasing number of people affected by the disease. These methods, according to Ahearn et al. [27], should be more specific, faster, and less invasive than the tests currently employed.

In SLE, different potential biomarkers have been identified [28]. However, because just a few number of these molecules satisfy the validation criteria, there is a lack of reliable biomarkers to recognize the disease, predict and monitor response to therapy [29].

In this context, this study aimed to evaluate potential inflammatory biomarkers, preferably those that could be correlated with the stage of disease activity, which may aid the diagnosis of SLE, as well as its differentiation from other systemic autoimmune diseases.

Due to the predominance of SLE in females, 46 women aged ≥18 y were recruited. The age average of the groups was 40 ± 10 y among SLE patients, being 37 ± 10 y among patients with low activity (SLEDAI-2 K ≤ 4), 42 ± 11 y among patients with moderate to high activity (SLEDAI-2 K > 4), and 51 ± 13 y for the OAD group (42 patients). Some evolutionary characteristics of have driven many researchers to study this disease in groups of patients with similar to that presented in this study [30].

Studies have suggested a strong association between the dysregulation of cytokine production and the SLE pathophysiology, since cytokines play a critical role in the differentiation, maturation and activation of immune cells, contributing to the loss of immunological tolerance, and also participating in local inflammatory processes which are responsible for the disease lesions [21,22].

IL-8 is an important mediator of the inflammatory process and is involved in the chemotactic action for leukocytes acting mainly on the recruitment of neutrophils to the inflammatory site [31]. IL-8 is one of the major mediators of the inflammatory response, leading to neutrophil recruitment in the pulmonary interstitium airspace. It can also activate a wide range of neutrophil functions, including degranulation and respiratory burst causing injury and intertitial fibrosis [32]. Studies have indicated that in SLE patients with pulmonary (fibrosis) and renal (nephritis) involvement, IL-8 concentrations are elevated and may be correlated with disease activity [32–34]. In our study, serum concentrations of IL-8 did not present a statistical difference between the groups comprising patients with SLE and healthy controls. However, when serum IL-8 concentrations in SLE patients were compared to those in the OAD group, a significant difference was observed even when SLE group was stratified at different concentrations of disease activity defined by the SLEDAI-2 K score (low and moderate/high activity), suggesting that this biomarker could help in distinguishing SLE from other systemic autoimmune diseases. Studies have shown a positive correlation between IL-8 concentrations and SLE activity in patients with renal [35] and pulmonary involvement [32]. Similarly, our data showed that IL-8 concentrations were higher in SLE patients with high disease activity.

IP-10 is a CXC chemokine secreted by peripheral blood mononuclear cells, fibroblasts, and endothelial cells [36]. Its biological functions include the stimulation of monocytes, natural killer (NK) cell and T cell migration, regulation of T cell and bone marrow progenitor cell maturation, modulation of adhesion molecule expansion and inhibition of angiogenesis [37]. IP-10 concentrations increase in inflammatory diseases, such as autoimmune disorders; besides, it is an important indicator of the severity of various diseases [38,39]. Recently, elevated serum IP-10 concentrations were found in several studies involving small numbers of SLE patients [40–44]; however, its correlation with disease activity was variable. These previous evidences support the involvement of IP-10 in the immunopathogenesis of SLE. Our findings, as well as those from other studies [45–47], validate that IP-10 serum concentrations are increased in patients with different stage of disease activity, compared to control group.

In humans, MIP-1α, which is now officially named CCL3, is a major factor produced by macrophages. It is crucial for immune responses to infection and inflammation [48]. The involvement of the chemokine MIP-1α in the response to acute and chronic infections has been proven [49,50]. It also induces the synthesis and release of other pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6 and TNF-α from fibroblasts and macrophages [48]. Some studies have associated this cytokine with other inflammatory conditions including the development of cutaneous, hematological, and renal manifestations in SLE [41,51]. However, when evaluating MIP-1α concentrations by the CBA technique, our findings did not show a significant difference between patients with SLE and healthy individuals. On the other hand, our results were consistent with those studies by Vilá et al. [51] and Kaneko et al. [52], who quantified this chemokine in Puerto Rican and Japanese populations, respectively, by ELISA technique. These authors also concluded that even in different populations, serum concentrations of MIP-1α between patients and healthy controls tend to show no significant variations. In the present study, a positive correlation was observed between MIP-1α serum concentrations and SLEDAI-2 K, in view of the fact that a significant difference was observed when its concentrations in patients with low and moderate/high disease activity were compared. Studies conducted by Bauer et al. [41] have indicated the participation of MIP-1α in the systemic inflammation associated

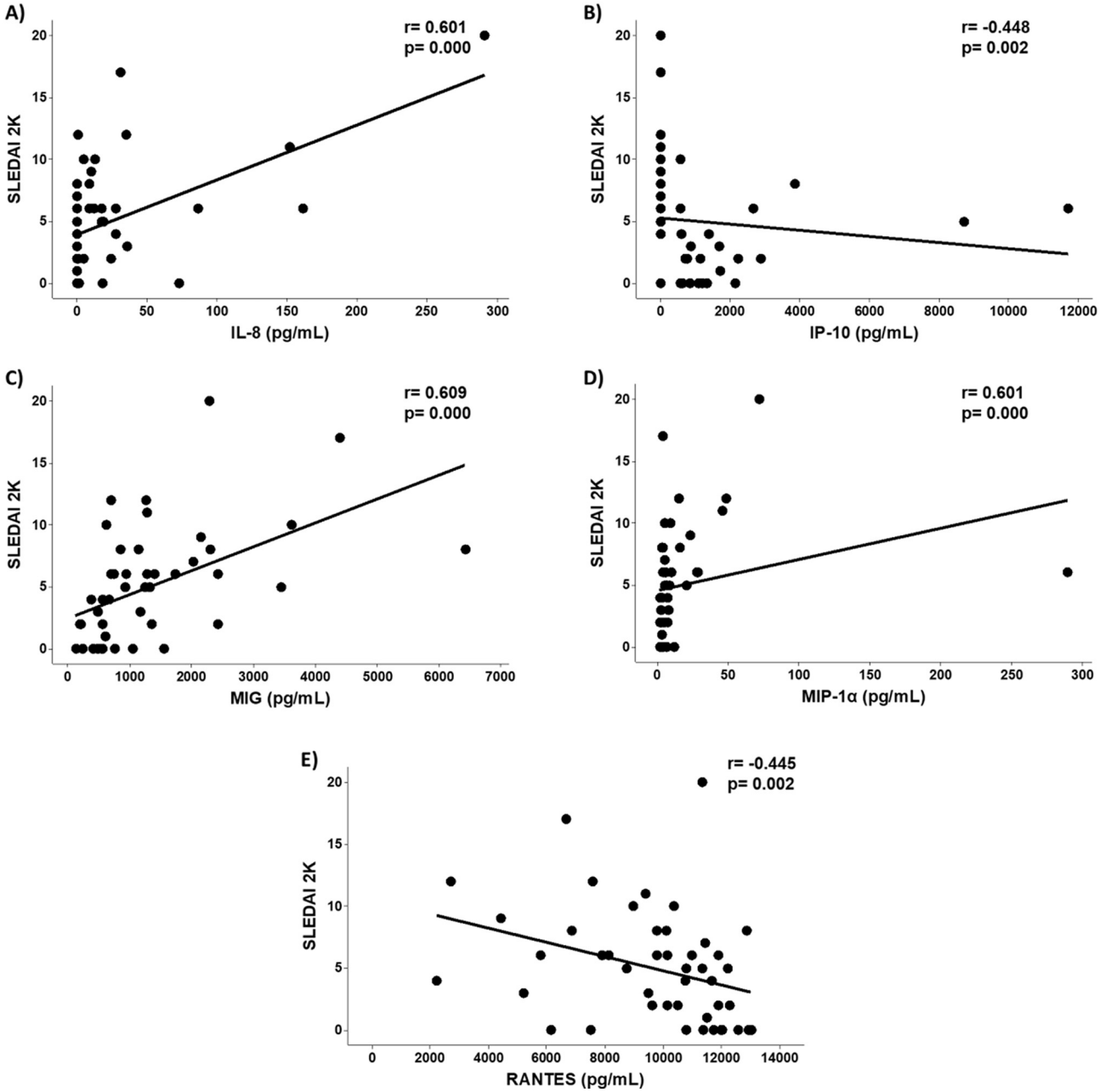

Fig. 3. Scatter plot showing the correlations between the SLEDAI-2 K scores in patients with SLE and the serum concentrations of IL-8 (A), IP-10 (B), MIG (C), MIP-1α (D), and RANTES (E). Data analyzed by the Spearman Rho correlation test (significant value: $p < .05$).

with SLE. Thus, the positive correlation observed between concentrations of MIP-1α and the SLEDAI-2 K score seems to be quite coherent with this result, since its concentrations rise according to the increase in the SLEDAI-2 K scores presented by the patients. These findings are also consistent with the data observed by Ferreira et al. [53], when they evaluated the concentrations of this chemokine in SLE patients under treatment. Similarly, Petrackova et al. [54] correlated the lesions in the organs of SLE patients with the serum protein pattern, and observed an increase in the MIP-1α concentrations, which was proportional to the observed injury index.

The chemokines MIG and RANTES are widely associated with inflammatory processes in response to pathogens and other clinical conditions, for example, systemic autoimmune diseases. They act on cell recruitment, maintenance of inflammation and, in the case of MIG, playing a role in tissue repair and the inhibition of angiogenesis [19,55–58]. In a study with SLE patients, a significant increase in the concentrations of RANTES [59,60] and MIG [43,61] was observed, compared to healthy individuals. In addition, in other studies, these cytokines have been shown to correlate with disease activity [41,53]. Particularly, increased MIG concentrations have been found and correlated with the disease activity. According to Ruffilli [62], there is a growing evidence that MIG plays a role in the pathogenesis of several manifestations of cutaneous SLE.

Our study, similar to those observed in the literature, showed higher concentrations of MIG and RANTES in SLE patients when compared to controls. Significant differences with regards to their concentrations were found, even when the results for the subjects in the control group were compared to those for patients with low and moderate/high disease activity. This suggests that these chemokines could be participating actively in the inflammatory state of these patients. When results of the SLE group were compared with those of the OAD group, no significant difference was observed. The first report on the involvement

of MIG in SLE was published in 2001 by Flier et al. [63] using biopsy samples. They showed that MIG is expressed by infiltrating CD_4^+ and CD_8^+ T cells, suggesting that they play a significant role in the recruitment of T cells in SLE inflammatory lesions [64]. According to Ferreira et al. [53], our results for the MIG concentrations showed a positive correlation with SLEDAI-2 K scores, whereby the lowest concentrations were obtained from patients with a disease activity less than or equal to 4, while the highest concentrations were observed in patients with a disease activity > 4. Similar findings were also observed by Bauer et al. [41], who have confirmed an important role for this chemokine as a mediator of the inflammatory process, being associated mainly with cutaneous, cardiac, and renal manifestations [41,43,53].

The role of RANTES in SLE might be restricted to induce leukocyte infiltration and its activation in the pathogenesis of this disease, which is contrary to previously thought role in SLE; this would have significant implications for the design of novel therapeutic strategies [65]. Regarding the concentrations of RANTES, our findings corroborated the data reported by Kaneko et al. [52], who observed a negative correlation of the RANTES concentrations with SLE activity. Although there are only a few studies that have simultaneously evaluated the serum concentrations of these chemokines in systemic autoimmune disorders, thereby limiting the validity of our findings, we can infer that MIG and RANTES presented promising results for monitoring SLE activity.

Finally, we emphasize the importance of this search for new biomarkers that can assist in the early diagnosis and monitoring of SLE, as this is a highly debilitating disease and can cause death. The present study is the first to report the use of the CBA technique for the simultaneous quantification of all these biomarkers in SLE patients.

5. Conclusions

The analysis of the data obtained in this study support the potential application of the biomarkers for screening and monitoring of SLE. Concentrations of MIG and RANTES ($p < .001$) in patients with SLE and healthy controls differed significantly. The concentrations of IL-8 ($p < .001$) and MIP-1α ($p < .001$) in SLE patients differed significantly compared to those in patients with other autoimmune diseases. The IL-8 ($p < .05$), IP-10 ($p < .01$), MIG ($p < .05$), MIP-1α ($p < .001$), and RANTES ($p < .05$) concentrations were correlated with SLE activity; their concentrations in SLE patients with low disease activity and moderate/high disease activity showed statistically significant differences. IP-10, MIG, and RANTES concentrations also showed significant differences when their concentrations in patients with low and moderate/high disease activity and controls were compared. Given the findings of this study, one can envision the possibility of future use of some of these cytokines to assist in the screening of SLE patients, or even in monitoring disease activity. However, further studies are warranted to validate the potential of these cytokines as additional markers of SLE.

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