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Original Investigation

Effect of induction therapy with mycophenolate or cyclophosphamide on serum BAFF levels in patients with systemic lupus erythematosus

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ABSTRACT

Introduction: Systemic lupus erythematosus (SLE) is a complex autoimmune disease in which B cell hyperactivity plays a key role in its pathophysiology. In this disease, the B cell activating factor (BAFF) is implicated in the loss of immunological tolerance, and high concentrations of this factor have been linked to disease progression. It is unknown how induction therapies with cyclophosphamide (CFM) or mycophenolate (MMF) modulate BAFF levels.
Objective: To determine how induction therapies modulate BAFF concentrations.
Materials and methods: An analytical observational study was performed with 20 patients with SLE from two institutions between 2020–2022, clinical and laboratory information was obtained from medical records. Measurement of serum BAFF levels was performed using an ELISA kit and statistical analyses with GraphPad Prism version 9.
Results: 20 patients with a diagnosis of SLE, 18 with CFM and 2 with MMF, were included, nine patients at baseline and eleven at 3–6 months. The median BAFF in SLE patients was 902.2 pg/mL and 379.7 pg/mL in healthy controls, statistically significant differences ($p = .0003$). BAFF levels were also found to be different among patients treated with antimalarials ($p = .0465$) and an inverse correlation with creatinine values and prednisolone doses was also observed.

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Conclusions: BAFF levels are affected during induction therapy, observing an initial reduction and subsequent recovery at 3–6 months, our results suggest that chloroquine and high doses of prednisolone maintain lower serum BAFF levels.

Palabras clave:
Lupus eritematoso sistémico
Factor activador de células B
Nefritis lúpica
Ciclofosfamida
Micofenolato

Efecto de la terapia de inducción con micofenolato o ciclofosfamida sobre los niveles séricos de BAFF en pacientes con lupus eritematoso sistémico

RESUMEN

Introducción: El lupus eritematoso sistémico (LES) es una enfermedad autoinmune compleja, en cuya fisiopatología destaca la hiperactividad de las células B. En esta enfermedad, el factor activador de células B (BAFF) está relacionado con la pérdida de tolerancia inmunológica; además, concentraciones altas de dicho factor se han asociado con la progresión de la enfermedad. Se desconoce de qué manera las terapias de inducción con ciclofosfamida (CFM) o micofenolato (MMF) modulan los niveles de BAFF.

Objetivo: Determinar de qué manera las terapias de inducción modulan concentraciones séricas de BAFF.

Materiales y métodos: Se realizó un estudio observacional analítico con 20 pacientes con LES de dos instituciones entre los años 2020 y 2022, en tanto que la información clínica y de laboratorio se obtuvo de las historias clínicas. La medición de los niveles séricos de BAFF se llevó a cabo mediante un kit de ELISA y los análisis estadísticos con GraphPad Prism versión 9.

Resultados: Se incluyeron veinte pacientes con diagnóstico de LES, 18 de ellos en tratamiento con CFM y dos con MMF, nueve pacientes en inicio y 11 en tres-seis meses. La mediana de BAFF en pacientes con LES fue 902,2 pg/mL, en tanto que en controles sanos fue de 379,7 pg/mL; las diferencias fueron estadísticamente significativas (P = 0,0003). También se encontraron diferencias en los niveles séricos de BAFF entre los pacientes tratados con antimaláricos (P = 0,0465) y una correlación inversa con los valores de creatinina y dosis de prednisolona.

Conclusiones: Los niveles de BAFF se ven afectados durante la terapia de inducción, se observa una reducción inicial y la recuperación posterior en tres-seis meses. Nuestros resultados sugieren que la cloroquina y dosis altas de prednisolona mantienen más bajos los niveles séricos de BAFF.

Introduction

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease, with variable symptoms, characterized by periods of remission and exacerbation. Multiple genetic, hormonal and environmental factors are involved in the pathological process, as well as infectious agents or drugs that can aggravate or reactivate the entity.[1]

B lymphocytes play an important role in the development of autoimmune diseases, as they contribute to exacerbation through different mechanisms such as the production of autoantibodies, the presentation of autoantigens, and the generation of proinflammatory cytokines[2]; all these events are involved in the development of lupus nephritis (LN), which is why B cells are one of the therapeutic targets.[3]

In the process of maturation of B lymphocytes are involved different molecules of the immune system, which exert a stimulating or suppressive action; these are mainly cytokines, such as the B-cell activating factor (BAFF), which intervenes in maturation, participates in the differentiation, survival and proliferation of B-lymphocytes, mainly, through ligand-receptor interactions with the specific receptor BAFF-R and other molecules such as transmembrane activator, calcium modulator, cyclophylin ligand (TACI) and B-cell maturation antigen (BCMA), which also act as receptors.[4]

The increase in serum levels of the cytokine BAFF has been related to different autoimmune pathologies, including SLE.[5] Its high concentration in the serum of the patients has been associated with a worsening of the disease, since it regulates the survival and maturation of B lymphocytes, and therefore has an effect on the humoral response.[6,7]

Currently, thanks to the fact that the importance of B cells in the pathogenesis of SLE is known, some monoclonal antibodies directed to the control of this cell population, which have demonstrated effectiveness in the management, are used.[8–11] An example of these is rituximab, a chimeric antibody, directed against the CD20 antigen of B cells, which once bound to its target induces cell death through various mechanisms, such as cell- or antibody-mediated cytotoxicity, producing a depletion of these subtypes of cells. Its use in SLE has been explored since 2002 and it has been found that its effect is beneficial even in those subjects with a low response to conventional therapy. However, some patients who receive this treatment have persistently high BAFF levels, which lead to the perpetuation of the inflammatory state in SLE, which is why it has been suggested to add BAFF inhibition to the therapeutic schemes, in order to prevent relapses.[12]

Since 2011, the use of belimumab, an antibody specifically directed against soluble BAFF, has been approved for the treatment of SLE. By having a direct effect on the reduction of the levels of the cytokine, it also promotes the decrease of B cell subpopulations, leading to a continuous improvement in disease activity.[13,14] New evidence suggests that belimumab may be useful in patients with LN by inducing remission or partial response and preventing the progression of damage.[15]

Despite the advances that biological therapy has had in recent years, initial management schemes usually use chemically synthesized immunosuppressants,[16] which are drugs that have an effectiveness comparable to that of monoclonal antibodies,[17] but their use prevails since they are easier to access, involve less costs and, in terms of production, are more feasible.[18,19]

Immunosuppressive therapy in nephritis aims to preserve renal function and prevent progression, and is divided into two phases: induction and maintenance. In the first, high doses of immunosuppressants are administered for six months, while in maintenance the dose is reduced and used for long periods.[20] Mycophenolate (MMF) or cyclophosphamide (CFM), in combination with high-dose corticosteroids, are considered the treatment of choice for LN as it has been demonstrated that they improve long-term renal survival. The therapeutic scheme for the induction phase is established taking into account the class of nephritis, the severity and the characteristics of the patient. For classes III and IV, high doses are the choice, with monthly intravenous pulses of CFM of 0.5 to 1 g/m^2 of body surface area for six months or MMF at doses of 2 to 3 g every day for six months. Subsequently, after an adequate response, it is recommended to use MMF or azathioprine for maintenance therapy.[21,22]

In 2016, Mercado et al. found that BAFF levels in patients with SLE treated with various drugs (among them some immunosuppressants) showed fluctuations in the concentrations of this cytokine over time. Although these variations were not uniform among them, no relationship was established between the BAFF levels and the specific type of therapy they were receiving.[23]

Since BAFF levels are related to disease activity and due to the disparity evidenced between studies seeking to establish these levels in patients with immunosuppressive therapy, specifically with CFM and MMF, the need has arisen to assess the effect of these drugs on the serum concentrations of the molecule and to explore their possible relationship with the clinical manifestations of the subjects. Few studies allow us to visualize their behavior over time and whether their effect is transitory or sustained; in addition, a clear relationship with a specific therapy is not established.[17,24,25] The objective of this work was to determine the serum concentrations of BAFF, at the beginning and during the six months of duration of the induction therapy, in such a way that allows to make comparisons between the behavior of BAFF at different times and with both medications.

Materials and methods

Study participants

An analytical observational study was conducted in Medellín (Colombia), which included 20 patients diagnosed with SLE according to the criteria given by the American College of Rheumatology (ACR), version European League Against Rheumatism (EULAR)/ACR 2019,[26] and the parameters revised by Tan et al. in 1982.[27] The inclusion criteria of the study included being over 18 years of age, having a diagnosis of nephritis confirmed by biopsy and being under an induction scheme with MMF or CFM in the period 2020–2022 at the Hospital San Vicente Fundación (HSVF) or the Health Care Provider (IPS) Artmédica. The clinical and demographic characteristics were obtained through the review of medical records. This study was designed following the Declaration of Helsinki and the Colombian legislation (Ministry of Health 008430 of 1993); it was approved by the Ethics Committee of the Medical Research Institute of the Faculty of Medicine of the University of Antioquia. Any evidence of recent infection was considered an exclusion criterion. Eight healthy donors were included as technical controls and reference for BAFF values (without evidence of autoimmune diseases, infectious diseases, carcinogenic processes or pregnancy status and not matched by sex or age).

Sample collection and processing

All participants provided a blood sample, which was taken following the venipuncture protocol.[28] The blood was collected in two 6 mL tubes without anticoagulant, then it was centrifuged (1,500 g) at room temperature for 10 min, and, subsequently, approximately 3 mL of serum was separated from the supernatant. The amount obtained was stored in vials of 500 μL and at −20 °C until use. The samples were taken at different times of the induction therapy: at the beginning and until two months (T0) and during its duration, which ranges between three and six months (T1).

Enzyme-linked immunosorbent assay

The levels of the cytokine were determined in the serum of patients and controls using the human BAFF detection kit from R&D Systems (Minneapolis, MN, USA),[29] according to the instructions of the manufacturer. Briefly, the samples were kept at room temperature, and then diluted 1:2 with RD6Q calibrator diluent. Subsequently, 100 μL of Assay Diluent RD1-111

Table 1 – Clinical and immunological characteristics.

Clinical characteristics		Immunological characteristics			
Clinical manifestations n = 20	n (%)	Antibodies	n (%)	Pattern n = 19	
Nephritis	20 (100%)	ANAS n = 20	20 (100%)	Homogeneous	10 (52.6%)
Non-erosive arthritis	11 (55%)			Speckled	7 (36.8%)
Oral ulcers	5 (25%)	Anti-DNA n = 20	16 (80%)	Fine granular	1 (5.3%)
Discoid rash	4 (20%)			Nucleolar	1 (5.3%)
Serositis	3 (15%)	Anti-RNP n = 18	9 (50%)	Titers (n = 19)	
Photosensitivity	3 (15%)				
Hematological alterations	15 (75%)	Anti-Ro n = 19	8 (42.1%)	1/160	4 (21.1%)
Lymphopenia (<1,500 in two or more occasions)	11 (55%)	Anti-La n = 18	2 (11.1%)	1/320	2 (10.5%)
Leukopenia (<4,000 in two or more occasions)	1 (5%)	Anti-Sm n = 18	7 (38.9%)	1/640	4 (21.1%)
Thrombocytopenia (<100,000)	1 (5%)	Anticardiolipins IgG n = 15	1 (6.7%)	1/1.280	5 (26.3%)
Hemolytic anemia	7 (35%)	Anticardiolipins IgM n = 14	4 (28.6%)	1/2.560	4 (21.1%)

ANAS: Antinuclear antibodies; IgG: immunoglobulin G; IgM: immunoglobulin GM.

were added per sample in the wells of the human BAFF/B lymphocyte stimulator (Blys) microplate, and 50 μL of each sample to proceed to incubate under agitation during three hours at room temperature. Then, 200 μL of the human conjugate BAFF/BlyS were added to each well, and incubated again for one hour under the same conditions. Finally, 200 μL of the substrate were added, incubated for 30 min without shaking and in darkness, and 50 μL of the stop solution were added. The reading of the results was carried out using the Multiskan™ GO (Thermo Scientific) equipment, at a wavelength of 450 nm and with correction at 540 nm.

Statistical analysis

The clinical characteristics and sociodemographic data were documented using descriptive statistics (measures of central tendency and frequency). The normality of the quantitative variables included in the study was assessed using the Shapiro–Wilk test; the statistical significance between two groups was found by applying the Mann–Whitney test, and multiple comparisons were made using analysis of variance (ANOVA) for normal data and the Kruskal–Wallis test for non-normal data. Finally, the correlations were carried out with Spearman's test; p-values < 0.05 were considered statistically significant. All analyses were performed in the GraphPad Prism software version 9.0.0 (San Diego, California, USA).

Results

Characteristics of the population

In total, 20 patients with a diagnosis of SLE were included, 18 (90%) women and two (10%) men, whose ages ranged between 18 and 68 years, with a mean of 37. The ethnicity was assessed by self-designation, and 100% of the group was classified as mestizo. The frequency of the clinical and immunological characteristics of the subjects are summarized in Table 1, while the treatment schemes are shown in Table 2. The most frequent clinical manifestation was non-erosive arthritis, in 11 individuals (55%). A total of 15 (75%) patients had some hematological alteration, the most common were lymphopenia (55%) and hemolytic anemia (35%). In addition, eight healthy donors were included, with a mean age of 29 years (range 19–59), all women.

Table 2 – Drug schemes.

Drugs n (%)[a]	
Mycophenolate	2 (10)
Cyclophosphamide	18 (90)
Chloroquine	9 (45)
Hydroxychloroquine	8 (40)
Prednisolone	19 (95)

[a] Several patients were using more than one medication.

As for the immunological characteristics, antinuclear antibodies (ANAS) were detected in 100% of the patients, with the homogeneous pattern being the most common, occurring in 10 (52.9%) of the 19 subjects.

Polyautoimmunity was found in three patients: two with hypothyroidism and one with dermatomyositis. In addition, four had antiphospholipid antibodies, but did not meet the criteria for the diagnosis of the syndrome.

When collecting data on renal involvement, it was found that the most frequent LN was class IV (35%), which occurred in seven patients; the frequencies of the LN types are summarized in Fig. 1.

Regarding treatment schemes, 18 patients were on induction therapy with CFM and two with MMF. Of them, nine were at the beginning of therapy, that is, at T0, and 11 at T1. A total of 23 determinations were performed (three subjects had measurements at two times).

95% (19) of the patients received doses of prednisolone and 85% received an antimalarial drug (AMA), chloroquine (CQ) (45%) or hydroxychloroquine (HCQ) (40%); the remaining 15% did not receive any drug.

Serum levels of B-cell activating factor

The determination of the serum level of BAFF in the 23 samples had a median of 902.2 pg/mL, (range 209.7−3,998), compared to that of the healthy controls 379.7 pg/mL, (range 284.1–565.6), with a statistically significant difference (p = 0.0003). In the group of the patients, one obtained a very high value of 3,997.6 pg/mL; The results are shown in Fig. 2. The two patients

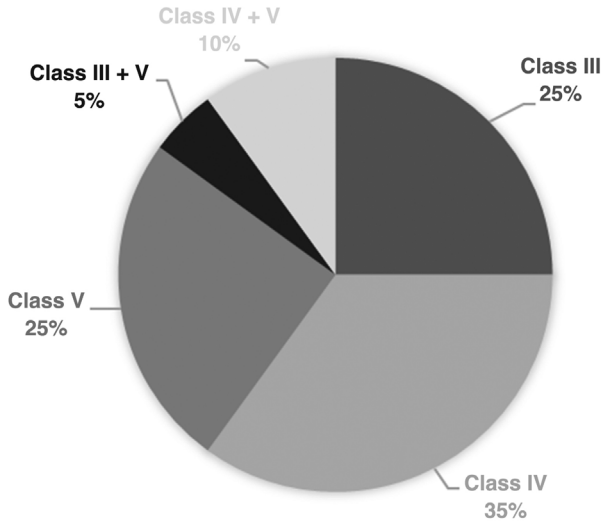

Fig. 1 – Frequency of the different types of lupus nephritis in the 20 patients.

who presented the highest levels of BAFF had increased titers of ANAS (1:2.560), in addition, one of them exhibited polyautoimmunity with concomitance of dermatomyositis, while the other reported a family history of autoimmunity.

Subsequently, BAFF was evaluated at the two times of the induction therapy with MMF or CFM that were defined (T0, T1), and a median of 665.4 pg/mL (range 209.7–1,035.3) was obtained for T0 and of 995.5 pg/mL (range 513.8–3,997.7) for T1; the data are shown in Fig. 3. Statistically significant differences were observed only between controls and T1 (p = 0.0002).

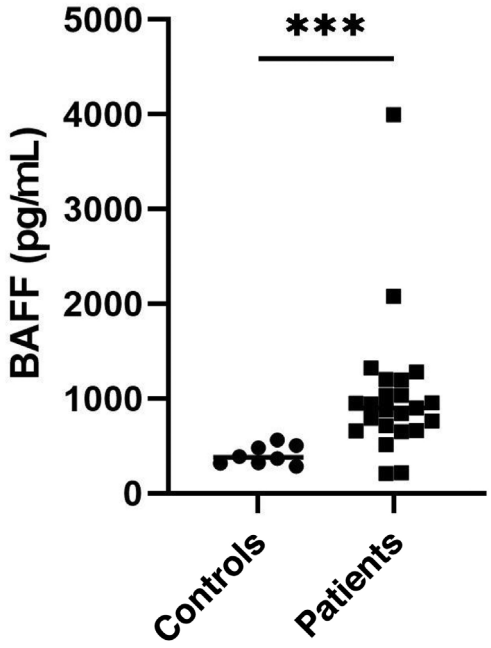

Fig. 2 – Serum BAFF levels in patients with systemic lupus erythematosus and controls.
BAFF: B-cell activating factor.
***p = 0.0003.**

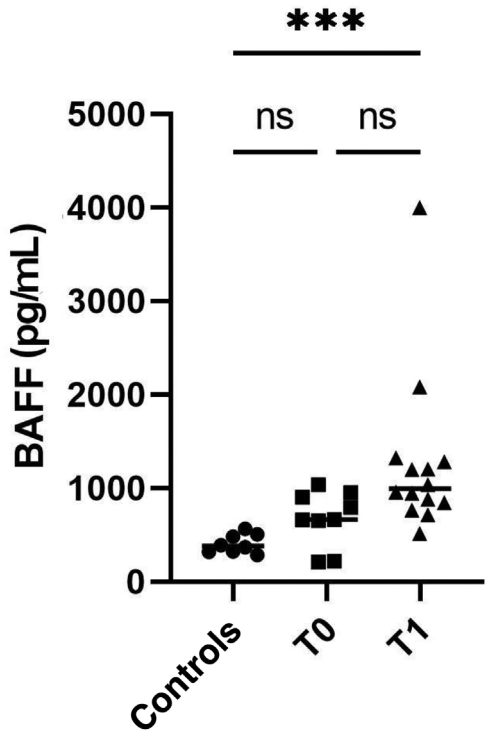

Fig. 3 – Serum BAFF levels in controls, T0 and T1 patients.
BAFF: B-cell activating factor; ns: not significant; T0: time zero (initiation of induction therapy); T1: time one (from two to six months of induction therapy).
***p = 0.0002.**

On the other hand, in three patients was obtained a paired sample between T0 and T1, the results of which are expressed in Fig. 4. In this, contrary to the trend observed in the general group, the subjects show a decrease in serum BAFF levels between the first and second samples. being more notable in the follow-up of patient A, with a decrease of 50.22%, followed by patients B (21.24%) and C (6.08%).

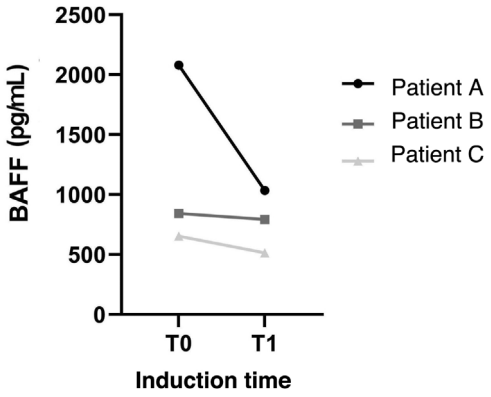

Fig. 4 – Follow-up of serum BAFF levels in three patients.
BAFF: B-cell activating factor.

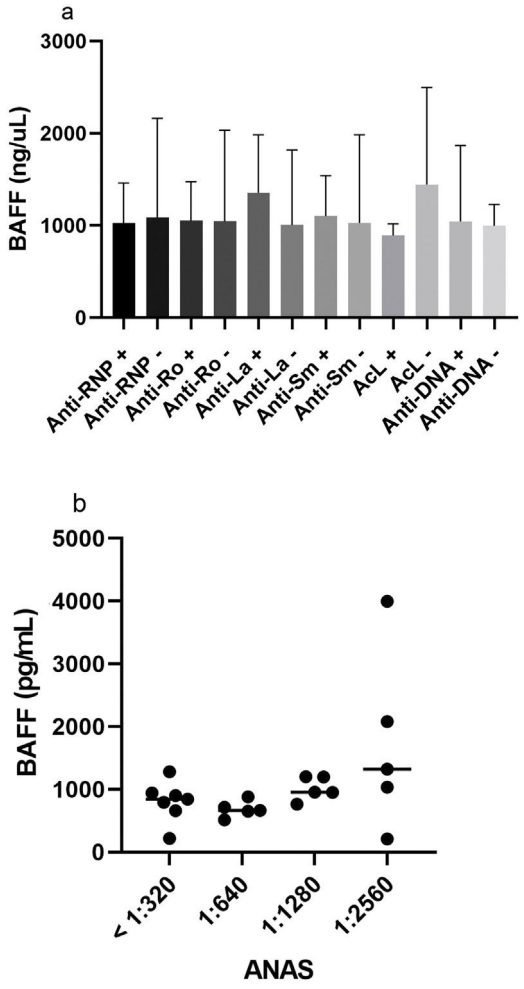

Fig. 5 – Serum BAFF levels and different autoantibodies (a) and ANAS titers (b). ANAS: antinuclear antibodies; BAFF: B-cell activating factor; ns: not significant.

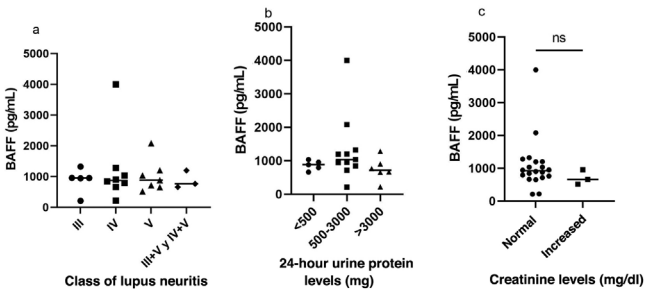

Fig. 6 – Serum BAFF levels and (a) classes of lupus nephritis, (b) 24 h urine protein and (c) serum creatinine. BAFF: B-cell activating factor.

Association between the levels of B-cell activating factor and the immunological characteristics of the patients

The possible association between the serum BAFF levels and each of the characterized antibodies was evaluated; The results are shown in Fig. 5a. The median of patients who had positive anti-DNA antibodies was 879.3 pg/mL (range 209.7–3,788) and of the negative was 995.5 pg/mL (range 715.6–1,280).

With respect to the other specific antibodies, a median of 955.7 pg/mL (range 513.8–2,079) was obtained in the patients with positive anti-RNP and of 872.7 pg/mL (range 209.7–3,998) in the case of negative patients. Only two subjects were positive for anti-La antibodies; the group of negatives had a median BAFF of 872.7 pg/mL (range 209.7–3,998). Anti-Ro patients, positive and negative, had a median of 916.4 pg/mL (range 661.1–2,079) and 923.5 pg/mL (range 209.7–3,998), respectively. The median of individuals positive for anti-Sm antibodies was 995.5 pg/mL (range 665.4–2,079), while the negative had a median of 843.3 pg/mL (range 209.7–3,998). Finally, for the lupus anticoagulant a median BAFF of 843.3 pg/mL (range 792–1,035) was found in those patients positive for the antibody; in the case of the negative, the median was 1,201 pg/mL (range 715.6–3,998). No statistically significant association was found between the BAFF levels and the positivity or negativity of each type of ANAS.

In the titers of ANAS, a mean of 806.2 pg/mL (range 220.4–1,280) was found in the BAFF levels in the case of those patients who presented titers lower than or equal to 1:320. The means for the titers 1:640 and 1:1.280 were 685.3 pg/mL (range 513.8–879.3) and 1,015 pg/mL (range 763.6–1,203), respectively. Despite the highest mean was evidenced in titers of 1:2560 (1,729 pg/mL, range 209.7–3,998), no statistically significant differences were found between each of the antibody titers and serum BAFF levels, as shown in Fig. 5b.

Association between B-cell activating factor levels and characteristics of renal involvement

Possible associations between the serum BAFF levels and the characteristics of renal involvement were assessed (Fig. 6). In relation to nephritis, a median BAFF of 872.7 pg/mL (220.4 pg/mL-3,998 pg/mL) was found for class IV, which was the most frequent. The median for LN class V was 879.3 pg/mL (range 513.8–2,079), for class III it was 953.5 (range 209.7–1,113) and finally, for classes III + V y IV + V it was 763.6 (range 661.1–1,198). No significant difference was found in BAFF levels according to the type of nephritis (Fig. 6a).

With respect to 24 h urine protein, the highest median of BAFF levels (1,035 pg/mL, range 209.7–220.4) was obtained in those patients with proteinuria between 500 and 3,000 mg/24 h, followed by the median of those with values lower than 500 mg/24 h which was 879 pg/mL (range 661.1–1,035). For protein levels higher than 3,000 mg/24 h, the median was 714.5 pg/mL (range 220.4–1,280). In Fig. 6b is shown that no statistically significant differences were found between the different degrees of proteinuria and the BAFF levels.

Regarding serum creatinine, normal values were considered to be between 0.7 and 1.3 mg/dL for men and between 0.6 and 1.1 mg/dL for women. The majority of patients presented normal values for this paraclinical test. The median BAFF level for the group of people with normal creatinine was 923.5 pg/mL (range 209.7–3,998), for those with increased levels it was 661.1 pg/mL (range 513.8–955.7). These data are

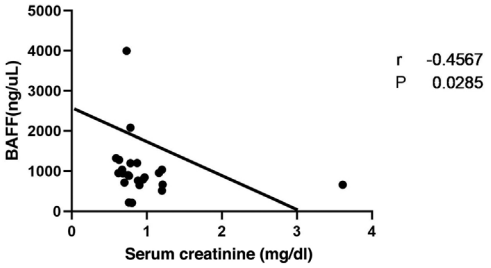

Fig. 7 – Correlation of serum BAFF levels and serum creatinine.
BAFF: B-cell activating factor.

shown in Fig. 6c. There was no statistically significant difference between the two groups.

We established correlations between the 24 h urine protein and creatinine levels and the BAFF concentrations. Only for serum creatinine values, as shown in Fig. 7, an r of -0.456 (p = 0.0285) was obtained, which indicates a negative correlation between these parameters.

Association of the serum levels of B-cell activating factor and the complement

The patients were classified into groups according to the results of complement levels as low (<88 mg/dL for C3 and <15 mg/dL for C4) or normal (88–200 mg/dL for C3 and 15–45 mg/dL for C4). In those with normal concentrations of C3, the median BAFF was 917.5 pg/mL (range 209.7–3,998) with little difference from what was observed in patients with low C3 with levels of 902.2 pg/mL (range 220.4–2,079). On the other hand, the median BAFF in those with normal levels of C4 was 879.3 pg/mL (range 209.7–3,998) and 944.7 pg/mL (range 220.4–2,079) for individuals with decreased C4 levels. No statistically significant difference was found between cytokine concentrations and complement consumption, and we did not find any correlation between the data. The possible association between the levels of BAFF and those of the complement fractions C3 and C4 is shown in Fig. 8.

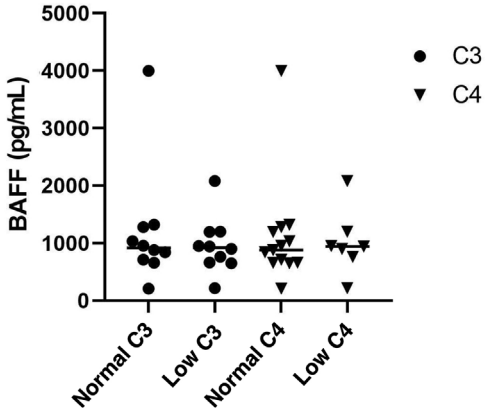

Fig. 8 – BAFF and C3 and C4 levels.
BAFF: B-cell activating factor.

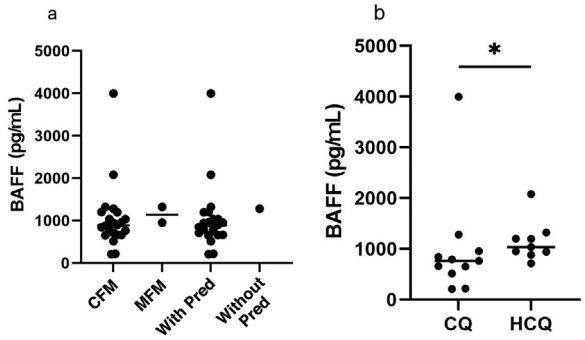

Fig. 9 – Serum BAFF levels and treatment schemes: (a) CFM, MMF, with prednisolone and without prednisolone; (b) chloroquine, hydroxychloroquine.
BAFF: B-cell activating factor; CFM: cyclophosphamide; MMF: mycophenolate.
***p = 0.0465.**

Association between the levels of B-cell activating factor and the treatment schemes

The serum BAFF levels were explored with the different immunosuppressive treatment schemes. The data in Fig. 9a show that the group of patients with CFM had a median of 879.3 pg/mL, (range 209.7–3,998). The two treated with MMF had BAFF levels of 953.475 and 1,322.270 pg/mL, respectively. When comparing the results of the BAFF values between both therapies, no statistically significant difference was found.

The data from the 22 samples of patients who were receiving doses of prednisolone are shown in Fig. 9a, the BAFF levels for this group presented a median of 890.7 pg/mL (range 209.7–3,998). When comparing the doses of pred-

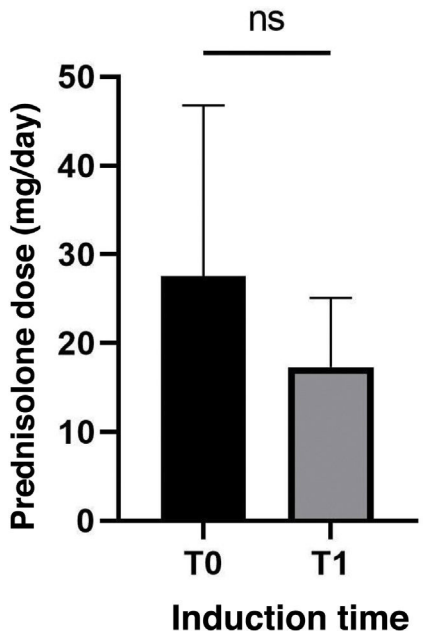

Fig. 10 – Comparison of prednisolone doses between T0 and T1.
ns: not significant.

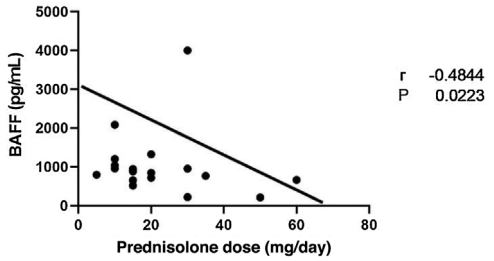

Fig. 11 – Correlation of serum BAFF levels and prednisolone dose.
BAFF: B-cell activating factor.

nisolone between the two evaluated times (T0, T1), a median of 25 mg/day (range 5–60) was obtained for T0 and of 15 mg/day (range 10–35) for T1, which is shown in Fig. 10. In addition, the correlation between the doses of prednisolone and the BAFF levels is found in Fig. 11, with an r = −0.4844, which indicates a negative correlation between them (p = 0.0223).

Finally, when looking for the association between the BAFF levels obtained and the treatment with CQ and HCQ, a median of 763.6 pg/mL (range 209.7−3,998) was observed in the patients treated with CQ, compared to 1,035 pg/mL (range 715.6−2,079) for the subjects who received HCQ. (range 209.7−3,998) in the patients treated with CQ, with respect to one of 1,035 pg/mL (range 715.6−2,079) for the subjects who received HCQ. A statistically significant difference was obtained (p = 0.0465); the data are shown in Fig. 9b.

Discussion

Elevated serum BAFF levels have been previously described in relation to the development of autoimmune diseases such as primary Sjögren's syndrome and rheumatoid arthritis,[30–33] in which the cytokine contributes to the pathogenesis of the entity. Similarly, several studies specifically establish the association between high BAFF concentrations and SLE activity,[34–36] which coincides with what was observed in our study, in which we found a link between the levels of the patients studied and the diagnosis of SLE, since these values were significantly higher in these cases, compared to healthy controls.

When evaluating the levels of the cytokine and the immunosuppression schemes, we found no difference between the patients treated with MMF and those managed with CFM. However, it is important to take into account the limitations of the sample, since only two subjects were under a treatment regimen with MMF. In 2015, Fassbinder et al. analyzed the effect of MMF and CFM on some cellular and serological characteristics of patients with SLE and found that plasmablast and plasma cell counts were significantly lower in those treated with MMF compared to those who received CFM, but the latter had lower total and naive B cell counts; however, serum BAFF levels were not measured in this study.[37] Vincent et al., for their part, found that serum BAFF was much lower in people who received HCQ, while it was significantly higher in those who received immunosuppressants, including CFM and MMF, but the effects of each drug were not discriminated separately.[38] Similarly, Hernandez et al. found higher BAFF levels in individuals treated with MMF than in those who were not receiving any type of immunosuppressive treatment, nevertheless, subjects with severe LN were excluded of this study.[39]

In our study, it was possible to evidence that higher serum levels of BAFF were obtained during the induction scheme compared to controls, without discriminating the immunosuppressive therapy. During T0, the concentration of BAFF was considerably higher than in the controls, but the most significant difference is seen with T1, as the determinations with the highest values are concentrated there. Similar to our results, in a study conducted in 2010, serum levels of BAFF were measured in mice, and it was found that these levels were significantly elevated after the administration of CFM.[40] Similarly, Phatak et al. found a significant increase after six months of induction therapy with CFM in patients with LN.[41] Parodis et al., on the other hand, established that in subjects with membranous LN there was an increase in serum levels of the cytokine when using CFM, while in those treated with MMF no changes were observed. In the case of patients with proliferative nephritis, no significant change was observed after treatment.[42]

Only serum BAFF levels were analyzed in this study, however, the state of B cell depletion is unknown, even so, the results are consistent with previous findings in the literature, in which there is an increase in the BAFF cytokine after a treatment that induces B lymphocyte depletion, as is the case of CFM, which was the scheme in which most of the patients were, and that was also administered intravenously, which entails a sustained depletion of cells accompanied by a reactive production of the cytokine.[42]

Although the general group of patients with SLE shows a clear increase in the serum levels of BAFF, in the three paired follow-ups carried out, a tendency to decrease these over time stands out, therefore, taking into account the heterogeneity in the behavior of BAFF levels, as described by Mercado et al. in their study, where such variety is demonstrated in the follow-up of 32 subjects, the relevance of carrying out a study with paired follow-ups is pointed out.[23]

AMA drugs such as CQ and HCQ are used for the treatment of chronic inflammatory diseases. In SLE, their use dates back to the 1950s, as they contribute to the reduction of the symptoms of the disease and have few side effects. Previous evidence has found that, in other conditions, such as primary Sjögren's syndrome and rheumatoid arthritis, the BAFF levels decreased significantly after starting therapy with HCQ.[43] In a study conducted in 2019 specifically in SLE patients, it was revealed that those who received AMA had a BAFF level lower than those who were not under this type of regimen, however, the effect of each of the AMAs was not defined separately.[39] Lambers et al. observed that BAFF levels in SLE were significantly lower after the treatment with HCQ.[44] Regarding CQ, its use is restricted because it produces more adverse reactions compared to HCQ, so the available evidence is limited.[45] In a work focused on assessing the direct effect of CQ on B cells, Ma et al. found that it potently suppressed the proliferation of B lymphocytes, however, the effect on the BAFF levels was not evaluated.[46] One of the most striking findings of our study was that the concentrations of the cytokine in patients receiving

CQ were significantly lower than in those on HCQ therapy; this suggests the need for further research comparing the effect of the two AMAs that could provide new evidence useful to define better therapeutic schemes.

As described by Yu et al. in their review, taking into account the diversity of factors involved in the pathogenesis of LN, proinflammatory processes and tissue damage to the kidney stand out, so the therapeutic choice is usually corticosteroids combined with immunosuppressants, with the aim of preserving renal function.[22] As for the effect of steroid therapy on BAFF levels, specifically with prednisolone, Hernández et al. highlight that the daily dose of this drug (on average 10.8 ± 8.7 mg/day) was not found to affect serum BAFF levels; they emphasize that the measurement was performed at a single moment and make it clear that the patients were receiving standard treatment for SLE at the time when the sample was taken.[39] As described by Carter et al., the follow-up was carried out for 18 months, associating high levels of BAFF with relapses in various clinical manifestations, including nephritis, but they state that there is no association between the reduction of the doses of corticosteroids and BAFF. It is worth clarifying the variety of characteristics of the patients included in this study.[47]

Due to the disparity of the data found, according to what was found in our study, it is suggested that prednisolone could be affecting serum BAFF levels, since at high doses (median 30 mg/day) low values are found, compared to T1, where the doses of corticosteroids are lower, which establishes an inversely proportional correlation.

With respect to BAFF levels and other patient characteristics, previous evidence has evaluated the association between the former and the presence of autoantibodies. For the first time in 2001, Cheema et al. found a significant correlation between the presence of anti-DNA in serum and high concentrations of the cytokine in North American subjects.[48] In later years, the following researchers obtained similar results: Mercado et al. established a moderate correlation with anti-DNA titers[23] in Mexican patients, while Ju et al. found that the Chinese population with high anti-DNA titers had a higher expression of BAFF messenger ribonucleic acid (mRNA) levels.[49] McCarthy et al. further found a positive correlation between serum BAFF levels and anti-Sm antibodies and antiphospholipid antibodies in Irish subjects.[50] In contrast, Elbirt et al. did not see a correlation with anti-DNA titers.[51] In the same way, in 2022 Sari et al. did not find a link between BAFF values and positivity for anti-DNA or anti-Sm.[52] In our study we also did not find a significant association between the presence of autoantibodies and the levels of the cytokine, however, these differences between studies may be due to the various methods used to detect both anti-DNA antibodies and BAFF concentrations and the diversity of the origin of the populations.[30,51]

With respect to renal involvement, in our study there was no significant association between the BAFF levels and the different types of nephritis. Similar to our results, in 2012, Eilertsen et al. did not observe a relationship between the findings of the renal biopsy and the serum values of BAFF, nor its gene expression.[53] Evidence from later years confirms that there is no correlation between the concentrations of the cytokine and renal histopathology, nor with the activity or chronicity indices.[41] As for proteinuria, we did not find a correlation with BAFF levels either, which coincides with the results of some studies carried out in previous years.[52] Several authors did not establish a correlation between BAFF and creatinine, while in our study we found a negative correlation between creatinine values and serum BAFF levels. The mechanism by which this could be happening is unknown, since it has not been previously described in the literature.

Similar to what was found by Eilertsen, we did not find any significant association between BAFF and the levels of complement fractions C3 and C4.[53] In contrast, Treamtrakanpon et al. found a significant correlation between serum complement and BAFF concentrations.[54] Likewise, Zollars et al. observed a positive correlation between elevated expression of the *BAFF* gene and C3 and C4 hypocomplementemia.[55]

Limitations

They are mainly derived from the small sample size, especially of patients on therapy with MMF (n = 2), which highlights the need for additional research to evaluate the effect of this immunosuppressant, and thus establish more accurate comparisons. Due to the foregoing, it was necessary to limit the analysis to two study times, where T1 covers a very wide range (two to six months), which restricts a more in-depth and detailed analysis of the behavior of serum BAFF levels during and at the end of induction therapy. Likewise, by only having three patients with paired follow-ups, it is not possible to know with certainty the trend of BAFF over time, which reduces the possibility of horizontal analysis.

Conclusions

The serum BAFF levels are affected during the induction therapy, with an initial decrease and subsequent recovery observed in T1, change that appears to be more influenced by the steroid doses that were higher at T0 and reduced at T1, and not by the CFM dose that remains stable during the induction therapy. In the case of MMF, our results are inconclusive due to the small sample size, which highlights the need to conduct larger studies that allow us to better analyze the behavior of the cytokine with this treatment. In addition, we suggest the development of research that includes paired follow-ups over time in larger cohorts of patients, as this could allow a better understanding of the behavior of the cytokine under different therapeutic schemes.

One of the most interesting results of our study shows that administration of CQ and high doses of prednisolone maintain low serum BAFF levels. Determining whether these are reflected in the activity of the disease could be very useful to define therapeutic schemes that lead to a better prognosis.

Ethical considerations

This study was designed following the Declaration of Helsinki and the Colombian legislation (Ministry of Health 008430 of 1993). It was approved by the ethics committee of the Institute of Medical Research of the Faculty of Medicine of the Univer-

sity of Antioquia. All patients and controls were asked for their informed consent to participate in the study.

Authors' contribution

Alejandra Betancur Herrera: Participation in the writing of the project, patient recruitment, sample processing, analysis of results, writing of the article.

Juan José Mariño Restrepo: Participation in the writing of the project, patient recruitment, sample processing, analysis of results, writing of the article.

Tulio Lopera Restrepo: Participation in the writing of the project, patient recruitment, sample processing, analysis of results, writing of the article.

Laura Robledo Montoya: Participation in patient recruitment.

Adriana Lucía Vanegas García: Participation in the administrative and logistical coordination for patient recruitment.

Carlos Horacio Muñoz Vahos: Participation in the administrative and logistical coordination for patient recruitment.

Juan Camilo Díaz Coronado: Participation in the administrative and logistical coordination for patient recruitment.

Ricardo Antonio Pineda Tamayo: Participation in the administrative and logistical coordination for patient recruitment.

Gloria Vásquez: participation in generation of the idea, obtention of resources, writing the project, patient recruitment, processing of samples, analysis of results, writing of the article.

Juan Felipe Soto Restrepo: Participation in patient recruitment.

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Declaration of competing interest

The authors declare that they do not have any conflict of interest.

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