Journal of Translational Autoimmunity 5 (2022) 100158

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

journal homepage: www.sciencedirect.com/journal/journal-of-translational-autoimmunity

Longitudinal comparison of IL-6, IL-10, and IL-12 cytokine profiles in adult and childhood-onset systemic lupus erythematosus

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A B S T R A C T

Objective: To compare the levels of Th1 (IL-12) and Th2 (IL-6 and IL10) cytokines over a two-year period among systemic lupus erythematosus patients with childhood-onset (cSLE), adult-onset (sSLE), and healthy controls, and correlate with their clinical, laboratory, and treatment manifestations.

Methods: The study included 63 patients with cSLE [57 (90%) women; mean age 19.7 ± 4.3 years (range = 10–29); mean disease duration 7.3 ± 4.2 years (range 2–15)], 67 patients with aSLE [65 (97%) women; mean age of 39.9 ± 11.8 years (range 21–68); disease duration 7.7 ± 3.1 years (range 4–16)], and 40 healthy controls [36 (90%) women; mean age of 29.6 ± 10 years (range 12–49)]. cSLE and aSLE patients were paired by disease duration. Clinical and laboratory manifestations, disease activity (SLEDAI), cumulative damage (SDI), and current drug exposures were evaluated. Symptoms of anxiety and depression were evaluated by the Beck inventory (BAI and BDI, respectively). Th1 (IL-12) and Th2 (IL-6 and IL-10) cytokines were measured by the ELISA test. Data were collected at four different time points (TI, TII, TIII, and TIV) and compared by non-parametric tests.

Results: IL-6 levels were significantly higher in aSLE patients compared to healthy controls at times I, II, and III (TI $p = 0.013$, TII $p = 0.015$, TIII $p = 0.004$, and TIV $p = 0.634$). However, no difference was observed between cSLE patients and healthy controls (TI $p = 0.223$, TII $p = 0.613$, TIII $p = 0.341$, and TIV $p = 0.977$). In addition, no difference was observed between aSLE and cSLE patients (TI $p = 0.377$, TII $p = 0.123$, TIII $p = 0.105$, and TIV $p = 0.591$). The levels of IL-12 were significantly higher in cSLE patients compared to healthy controls at all time points (TI $p = 0.04$, TII $p < 0.001$, TIII $p = 0.015$, and TIV $p = 0.021$). aSLE patients showed significantly elevated levels when compared to healthy controls at time III and IV (TI $p = 0.752$, TII $p = 0.827$, TIII $p = 0.011$*, and TIV $p < 0.001$*). cSLE patients showed significantly higher levels than aSLE patients at times I and II (TI $p = 0.07$*, TII $p < 0.001$*, TIII $p = 0.998$, and TIV $p = 0.140$). In aSLE patients, IL-6 was associated with headache ($p = 0.006$), arthritis ($p = 0.044$), and nephritis ($p = 0.012$); IL-10 was associated with nephritis ($p = 0.043$), hypocomplementemia ($p = 0.001$), and disease activity ($p = 0.001$); in these patients, IL-12 was associated with alopecia ($p = 0.025$) and leukopenia ($p = 0.044$). In cSLE patients, IL-6 was associated with arthritis ($p = 0.022$) and malar rash ($p = 0.012$).

Conclusion: aSLE and cSLE patients with long disease duration present similar levels of cytokines, despite differences in clinical activity patterns over time.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by periods of activity and remission affecting mainly women of reproductive age [1,2]. The deregulation of the immune system through exacerbation of the production of pathogenic autoantibodies contributes to a wide range of clinical manifestations including cutaneous lesions, arthritis, and damage to the renal and nervous system among others [3].

Approximately 15–20% of SLE patients have disease-onset before the age of 18, which characterizes the childhood-onset SLE (cSLE) [4,5]. The male/female ratio in cSLE is 4:3 when the disease starts during the first decade of life and 4:1 during the second decade of life compared to a ratio of 9: 1 in adult-onset SLE (sSLE) [6].

The age at disease onset has an impact on the course of the disease [7,8]. cSLE has greater disease severity and morbidty in manifestations,

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https://doi.org/10.1016/j.jtauto.2022.100158
Received 16 May 2022; Accepted 20 May 2022
Available online 26 May 2022

such as nephritis and central nervous system (CNS) dysfunctions [9–12]. Studies have shown that patients with cSLE have a more active disease not only at the disease-onset, but also throughout its course compared to aSLE patients [13,14].

Regardless of the difference in disease profile, there are evidence that support a pathogenic role of cytokines in SLE [15]. Interleukin-12 (IL-12) is among the most important in the Th1 group and is associated with cellular immunity while IL-6 and IL-10 are cytokines belonging to the Th2 group and are associated with the induction of humoral immunity and production of B lymphocytes [16]. Due to differences in disease severity and outcome we aimed to compare the levels of Th1 (IL-12) and Th2 (IL-6 and IL10) cytokines over a two-year period between CSLE and ASLE patients and healthy controls, and evaluated their correlation with clinical, laboratory, and treatment manifestations.

2. Patients and methods

2.1. Subjects

All patients in the study presented 4 or more SLE classification criteria reviewed by the American College of Rheumatology (ACR) [17]. Those with a diagnosis before 18 years of age were considered cSLE patients [4]. cSLE and aSLE patients were recruited consecutively at the pediatric rheumatology outpatient clinic and the rheumatology outpatient clinic of the General Hospital at the State University of Campinas. cSLE and aSLE patients were paired by disease duration. Healthy controls paired by sex to patients and no apparent infections at the day of blood collection were included in the study for the comparison of cytokine levels. This study was approved by the ethics committee of our institution and all participants and/or guardians signed the voluntary participation consent form.

2.2. Data collection

All patients (cSLE and aSLE) and individuals in the control group had 4 blood samples collected over a period of 2 years (with a minimum interval of 4 months between collections). Clinical, laboratory, and patient data were also collected on the day of blood collection, including the completion of questionnaires about anxiety and depression symptoms. All data were separated and tabulated according to collection times (TI, TII, TIII, and TIV). Data such as age and time of disease were collected in the last blood collection time (TIV).

2.3. Clinical characteristics

The medical histories and clinical and serological characteristics of all patients were assessed at study entry according to the ACR [18]. One of the characteristics included in the protocol of this study was the age at disease onset, defined as the age at which patients fulfilled 4 or more out of the 1.982 criteria for SLE classification [18].

All information on clinical and laboratory manifestations was obtained on the day of blood collection. Nephritis was diagnosed based on proteinuria greater than 0.5 g/L, changes in urinary sediments, and/or histological findings. Nephrotic syndrome was defined as proteinuria above 3.0 g/day. Hematologic alterations were attributed to lupus only in the absence of bone marrow suppression (leukopenia $<$4000 cells/mm^3; thrombocytopenia $<$100.000 cells/mm^3; and hemolytic anemia). We also analyzed the presence of erythema malar, discoid lesions, subacute cutaneous lesions, cutaneous vasculitis, photosensitivity, oral ulcers, arthritis, and serositis. Neurological and psychiatric involvement was defined according to the ACR [19]. The treatments used at the time of blood collection, such as prednisone, chloroquine, and azathioprine was also taken into consideration.

2.4. Laboratory characteristics

Antinuclear antibodies (ANA) were determined by immunofluorescence using HEp-2 cells as the substrate, and considered positive if greater than 1:40. The anti-double stranded DNA antibodies (dsDNA) were determined by indirect immunofluorescence using Crithidia as the substrate and considered positive if greater than 1:10. Extractable nuclear antigen precipitating antibodies (ENAs), including Anti-Ro (SSA), Anti-La (SSB), and Anti-Sm were detected by ELISA and considered positive if greater than 1:40. The rheumatoid factor (RF) was detected by nephelometry and considered positive if higher than 10 UI/mL. The anticardiolipin antibodies (aCL) of the IgG and IgM isotopes were measured by the ELISA method [20]. The active lupus anticoagulant (LA) was detected by platelet-free plasma coagulation assays performed by double centrifugation as recommended by the Scientific and Standardization Committee of the International Society of Thrombosis and Homeostasis [21]. These measurements were performed twice over a 12-week interval.

2.5. Disease activity and damage

Disease activity was measured by the "Systemic Lupus Erythematosus Disease Activity Index" (SLEDAI) with a variation from 0 to 105 [22]. Cases with SLEDAI $\geq$3 were considered with active disease [23]. Active nephritis was diagnosed based on SLEDAI renal manifestations (proteinuria greater than 0.5 g/L, abnormal urinary sediment, and low complement levels). SLEDAI was evaluated on the day of each blood collection; SLEDAI measurements were obtained by time points (TI, TII, TIII, and TIV). The cumulative damage associated with SLE was determined using the "Systemic Lupus International Collaborative Clinics/ACR" (SLICC)/ACR Damage Index (SDI) [24], whose SDI scores range from 0 to 47. Cumulative damage was considered when the score was $\geq$1 [24]. Because these are cumulative damages, one single evaluation was performed at the end of the 4 collection time points.

2.6. Evaluation of symptoms of anxiety and depression

All patients completed the Beck Depression Inventory (BDI) [25] and Beck Anxiety Inventory (BAI) [26] on the day of each blood collection. The Child Depression Inventory (CDI) was applied for patients under 16 years old. These scales that evaluate symptoms of depression and anxiety, respectively, consist of 21 items each and describe common symptoms of depression/anxiety. Patients were asked to rate, using a 4-point scale ranging from 0 to 3, how much they were bothered by each symptom in the past month. Items were summed to obtain a total score that ranges from 0 to 63. The cut-off used for BDI were: 0–13, without depression; 14–19, with mild depression; 20–28, with moderate depression; and 29–63, with severe depression. The cut-off used for BAI were: 0–7, with no anxiety/minimal level of anxiety; 8–15, with mild anxiety; 16–25, with moderate anxiety; and 26–63, with severe anxiety. The limit used for CDI was 17.

2.7. Determination of the serum levels of cytokines

Four blood samples were collected from each participant. These samples were centrifuged at 3000 rpm for 15 min after being allowed to coagulate for 30 min at room temperature. Sera were separated from the red blood cell clot after centrifugation and maintained in aliquots at 80 °C until assayed. No sample was collected during episodes of infection requiring hospitalization in order to avoid the detection of cytokines at elevated levels due to secondary causes [31]. Samples were analyzed in duplicate to ensure reproducibility of results. Commercially available kits from R & D Systems (London, UK) were used for the measurement of IL-6, IL-10, and IL-12 by the enzyme-linked immunosorbent assay (ELISA) and according to the manufacturer's instructions. The minimum detectable dose (MDD) was 0.039 pg/mL for IL-6, 3.9

pg/mL for IL-10, and 0.5 pg/mL for IL-12.

2.8. Statistical analysis

All statistical analyses were performed using the SYSTAT software version 12.0. The results were presented as the mean and standard deviation (SD). We used the Shapiro-Wilks test to verify test normality. Because this was a non-normal distribution, the Kruskal-Wallis test was used to compare cytokine levels in the three groups (CSLE, ASLE, and controls) at each evaluated time point (TI, TII, TIII, and TIV). An analysis of variance was used when a significant difference was observed between the results from time points; the Tukey's test was used to compare all groups and determine statistical differences between them. The Spearman correlation was used to correlate variables (levels of cytokines and SLEDAI, SDI, BAI, and BDI). The Chi-square test or Fisher's exact test were used to compare clinical and laboratory manifestations between the two groups of patients (cSLE vs. aSLE). The Mann-Whitney *U* test was used to evaluate associations between cytokine levels and categorical variables (clinical and laboratory manifestations), and performed on combined data from the four time points. The value of $p < 0.05$ was considered statistically significant in all analyses.

3. Results

3.1. Demographic data

The study included 63 patients with cSLE [57 (90%) women at the mean age of 19.7 ± 4.3 years (in the 10–29 range) and mean disease time of 7.3 ± 4.2 years (in the 2–15 range)], 67 patients with aSLE [65 (97%) women at the mean age of 39.9 ± 11.8 years (in the 21–68 range) and mean disease time of 7.7 ± 3.1 years (in the 4–16 range)], and 40 healthy controls [36 (90%) were women at the mean age of 29.6 ± 10 years (in the 12–49 range)]. cSLE and aSLE patients were paired by disease duration.

3.2. Comparison of clinical and laboratory manifestations

Clinical and laboratory manifestations were compared in all 4 collection time points (Table 1). The presence of headaches was significantly more frequent in cSLE patients than in aSLE patients ($p = 0.01$) in time III, however, no other significant differences were observed in relation to clinical and laboratory manifestations in any of the time points (Table 1).

3.3. Comparison of disease activity and cumulative damage

At baseline, 23 cSLE patients (36%) presented disease activity [mean score of 3.61 ± 4.87] compared to 33 aSLE patients (49%) [mean of 3.46 ± 4.36] (Table 2). Disease activity decreased significantly in aSLE patients throughout the study ($p = 0.05$) while remaining stable in cSLE patients ($p = 0.50$).

At the end of the study, 32 cSLE patients (51%) and 43 aSLE patients (64%) showed SLICC/SDI scores ≥1 ($p = 0.02$).

3.4. Comparison of symptoms of anxiety and depression

Anxiety symptoms were significantly more frequent in aSLE patients compared to cSLE patients at all time points (TI $p = 0.001$*, TII $p = 0.004$*, TIII $p < 0.001$*, and TIV $p < 0.001$*). Symptoms of depression

Table 2
Comparison between scores for anxiety and depression symptoms and disease activity in cSLE and aSLE patients.

	Anxiety symptoms			Depression symptoms		
	cSLE N63 (%) Average + SD	**aSLE N67 (%) Average + SD**	**p[a]**	**cSLE N63 (%) Average + SD**	**aSLE N67 (%) Average + SD**	**p[a]**
Time points						
TI	25 (40%) 9.4 ± 10.8	35 (52%) 16 ± 12.6	0.001*	9 (14%) 6.6 ± 7.5	22 (32%) 13 ± 8.3	<0.001*
TII	20 (32%) 8.4 ± 10.1	32 (48%) 17.6 ± 17.1	0.004*	10 (16%) 8.1 ± 7.3	20 (30%) 13.2 ± 7.7	0.001*
TIII	14 (22%) 6.3 ± 6.2	32 (48%) 19.2 ± 14.4	<0.001*	11 (17%) 8.6 ± 7.7	19 (28%) 12.8 ± 9.8	0.019*
TIV	22 (35%) 6.2 ± 6.7	26 (39%) 19.8 ± 16.1	<0.001*	4 (6%) 5.6 ± 5.6	17 (25%) 14.2 ± 10.1	<0.001*
p[b]	0.52	0.13		0.29	0.62	

*p ≤ 0.05 is considered statistically significant.
P[a] Results from the Mann-Whitney test.
P[b] Results from the Friedman test.

Table 1
Comparison of clinical and laboratory manifestations in cSLE and aSLE patients at four time points.

	TI			TII			TIII			TIV		
	cSLE N63 (%)	aSLE N67 (%)	p	cSLE N63 (%)	aSLE N67 (%)	p	cSLE N63 (%)	aSLE N67 (%)	p	cSLE N63 (%)	aSLE N67 (%)	p
Clinical Manifestations												
Alopecia	8 (13%)	6 (9%)	0.67	2 (3%)	4 (6%)	0.69	–	4 (6%)	0.12	1 (2%)	2 (3%)	0.75
Arthritis	2 (3%)	2 (3%)	0.88	–	4 (6%)	0.12	1 (2%)	1 (1%)	1.00	–	2 (3%)	0.50
Headache	4 (6%)	4 (6%)	1.00	1 (2%)	4 (6%)	0.38	5 (8%)	–	0.01*	1 (2%)	5 (8%)	0.39
Nephritis	3 (5%)	5 (7%)	0.71	3 (5%)	2 (3%)	0.87	2 (3%)	2 (3%)	1.00	1 (1%)	2 (3%)	0.87
Malar Rash	8 (13%)	7 (10%)	0.56	4 (6%)	2 (3%)	0.40	3 (5%)	5 (7%)	0.73	2 (3%)	5 (8%)	0.69
Oral Ulcers	0	1 (1%)	0.59	0	0	–	0	2 (3%)	0.50	0	0	–
Vasculitis	1 (2%)	0	0.46	1 (2%)	0	0.44	0	0	–	0	0	–
Laboratory Manifestations												
Anti DNA	4 (6%)	4 (6%)	1.00	3 (5%)	1 (1%)	0.32	3 (5%)	4 (6%)	0.98	3 (5%)	3 (4%)	0.69
Hematuria	5 (8%)	9 (13%)	0.39	2 (3%)	8 (12%)	0.18	1 (1%)	5 (7%)	0.23	1 (1%)	3 (4%)	0.63
Hipocomplementemia	14 (22%)	16 (24%)	0.97	12 (19%)	16 (24%)	0.87	12 (19%)	15 (22%)	0.88	13 (21%)	11 (16%)	0.17
Leukocyturia	9 (14%)	10 (15%)	0.92	4 (6%)	11 (16%)	0.17	11 (17%)	10 (15%)	0.35	9 (14%)	10 (15%)	0.61
Thrombocytopenia	–	3 (4%)	0.24	1 (1%)	2 (3%)	0.70	2 (3%)	1 (1%)	0.57	2 (3%)	1 (1%)	0.57
Proteinuria	5 (8%)	9 (13%)	0.39	4 (6%)	4 (6%)	0.73	4 (6%)	4 (6%)	0.72	1 (1%)	4 (6%)	0.39

*p ≤ 0.05 is considered statistically significant.

were also significantly higher in aSLE patients compared to cSLE at all time points (TI $p < 0.001$*, TII $p = 0.001$*, TIII $p = 0.019$*, and TIV $p < 0.001$*) (Table 3).

3.5. Treatment

At the study start, 57 cSLE patients (90%) vs. 65 aSLE patients (97%) were taking medication. In relation to prednisone, 54 (86%) cSLE patients [mean dosage of 29.4 ± 20.5 mg] used this medication compared to 62 (92%) aSLE patients [mean dosage of 19.8 ± 17.6 mg] ($p = 0.04$). The use of chloroquine was observed in 36 (57%) cSLE patients [mean dosage of 267 ± 156 mg] vs. 42 (63%) aSLE patients [mean dosage of 352.3 ± 180.2 mg] ($p = 0.01$). The use of azathioprine was observed in 23 (36%) cSLE patients [mean dosage of 137.2 ± 82.5 mg] compared to 23 (34%) aSLE patients [mean dosage of 129.3 ± 66.3 mg] ($p = 0.86$) (Table 4).

cSLE patients used significantly higher doses of prednisone compared to aSLE patients at all time points (TI $p = 0.048$*, TII $p = 0.012$*, TIII $p = 0.007$*, and TIV $p = 0.030$*). The prednisone dosages decreased significantly over the study time in cSLE patients ($p < 0.001$*) and aSLE patients ($p < 0.001$*). The chloroquine dosages significantly increased in cSLE patients ($p = 0.003$*) and was maintained in aSLE patients ($p = 0.518$) over the study time. The azathioprine dosages did not change significantly over the study time in cSLE ($p = 0.245$) and aSLE patients ($p = 0.476$).

3.6. Comparison of cytokine levels

The IL-6 levels were significantly higher in aSLE patients compared to healthy controls at the I, II, and III time points (TI $p = 0.013$*, TII $p = 0.015$*, TIII $p = 0.004$*, and TIV $p = 0.634$). However, no significant differences were observed between aSLE vs. cSLE patients at any of the time points (TI $p = 0.377$, TII $p = 0.123$, TIII $p = 0.105$, and TIV $p = 0.591$). Moreover, no statistical difference was observed between cSLE and controls at any of the time points (TI $p = 0.223$, TII $p = 0.613$, TIII $p = 0.341$, and TIV $p = 0.977$) (Fig. 1).

No statistical difference was observed at the levels of IL-10 in any of the groups over the study time (Fig. 2).

Levels of IL-12 were significantly increased in cSLE vs. controls at all time points (TI $p = 0.04$*, TII $p < 0.001$*, TIII $p = 0.015$*, and TIV $p = 0.021$*). The comparison between aSLE and controls showed IL-12 levels significantly higher in aSLE at times III and IV (TI $p = 0.752$, TII $p = 0.827$, TIII $p = 0.011$*, and TIV $p < 0.001$*). Levels of IL-12 were significantly increased in cSLE compared to aSLE at times I and II (TI $p = 0.07$*, TII $p < 0.001$*, TIII $p = 0.998$, and TIV $p = 0.140$) (Fig. 3).

3.7. Associations between cytokines, clinical and laboratory manifestations, and treatment

IL-6 levels were associated with nephritis ($p = 0.012$), headache ($p = 0.006$), and arthritis ($p = 0.044$) in aSLE patients; and associated with arthritis ($p = 0.022$) and malar rash ($p = 0.012$) in cSLE patients. IL-10 levels were associated with nephritis ($p = 0.043$), hypocomplementemia ($p = 0.001$), and disease activity ($p = 0.001$) in aSLE. No associations were observed between levels of IL-10 and manifestations in cSLE patients. IL-12 was associated with alopecia ($p = 0.025$) and leukopenia ($p = 0.044$) in aSLE patients. No associations were observed between levels of IL-12 and manifestations in cSLE patients. No associations were observed between detected levels of cytokines and medications used by participants (Table 4).

4. Discussion

This study compared the profiles of Th1 and Th2 cytokines in patients with aSLE and cSLE with prolonged disease time. No significant differences were observed in serum cytokine patterns between the studied groups, despite differences in disease activity pattern over time. However, we observed that cytokines are associated with disease activities in certain organs or systems, which varied between groups. Levels of IL-6 were significantly increased in aSLE patients when compared to healthy controls and were associated with nephritis, headache, and arthritis; these levels were associated with arthritis and malar rash in cSLE patients. Previous studies have demonstrated an increase in IL-6 in patients with cSLE and aSLE compared to controls [27–30,48]. In this study, the increase of IL-6 in patients with aSLE was longitudinally evaluated and confirmed in the four time points when compared to healthy controls. However, an increase was not observed in cSLE patients compared controls. Our results corroborate the association of IL-6 with nephritis reported in some studies [30–33]. In addition, an anatomopathological study demonstrated increased IL-6 expression along glomeruli and tubules in the kidneys of patients with lupus nephritis, suggesting that IL-6 may be a biomarker for lupus nephritis [34].

Our study observed an association of IL-6 with headaches in aSLE patients. There are no studies demonstrating the association of IL-6 with headaches in SLE patients or patients with primary headaches. However, some studies have demonstrated an increase in IL-6 serum levels in aSLE patients with neuropsychiatric manifestations [35,36].

IL-12 levels were significantly higher in cSLE patients at all time points when compared to healthy controls. Although there are no studies showing increased levels of IL-12 specifically in cSLE patients, some studies have shown that IL-12 was significantly increased in aSLE patients compared to controls [37–39]. We observed an increase in IL-12 in aSLE patients in relation to healthy controls only at times III and IV;

Table 3
Comparison between treatments used by cSLE and aSLE patients.

	Prednisone			Chloroquine			Azathioprine		
Time points	cSLE N = 63 (%) Average + SD	aSLE N = 67 (%) Average + SD	p^a value	cSLE N = 63 (%) Average + SD	aSLE N = 67 (%) Average + SD	p^avalue	cSLE N = 63 (%) Average + SD	aSLE N = 67 (%) Average + SD	p^a value
TI	54 (86%) 29.4 ± 20.5	62 (92%) 19.8 ± 17.6	0.048*	36 (57%) 267 ± 155	42 (63%) 352 ± 180	0.010*	23 (36%) 137 ± 82	23 (34%) 129 ± 66	0.864
TII	58 (92%) 26.5 ± 19.5	60 (89%) 18.2 ± 16.4	0.012*	45 (71%) 291 ± 149	43 (64%) 350 ± 178	0.429	25 (40%) 120 ± 68	24 (36%) 122 ± 63	0.597
TIII	58 (92%) 21.4 ± 15.3	55 (82%) 15.5 ± 12.2	0.007*	42 (67%) 283 ± 149	39 (52%) 329 ± 170	0.718	27 (43%) 125 ± 68	24 (36%) 132 ± 69	0.414
TIV	58 (92%) 18.8 ± 14.7	53 (79%) 14.7 ± 11.7	0.030*	39 (62%) 308 ± 159	41 (61%) 318 ± 165	0.879	28 (44%) 126 ± 71	24 (36%) 121 ± 65	0.209
p^b value	<0.001*	<0.001*		0.003*	0.518		0.245	0.476	

*$p \leq 0.05$ is considered statistically significant.
P^a Results from the Mann-Whitney test.
P^b Results from the Friedman test.

Table 4
Clinical and laboratory manifestations associated with cytokines IL-6, IL-10, and IL-12 in aSLE and cSLE patients.

Associated manifestations	IL-6		IL-10		IL-12	
	cSLE	aSLE	cSLE	aSLE	cSLE	aSLE
Alopecia	0.728	0.906	0.543	0.268	0.521	0.025*
Arthritis	0.022*	0.044*	0.831	0.830	0.550	0.068
Disease activity	0.159	0.110	0.829	0.001*	0.921	0.695
Headache	0.941	0.006*	0.065	0.468	0.817	0.727
Hipocomplementemia	0.398	0.475	0.707	0.001*	0.830	0.801
Leukopenia	0.866	0.358	0.596	0.481	0.607	0.044*
Nephritis	0.979	0.043*	0.405	0.043*	0.120	0.897
Malar Rash	0.012*	0.694	0.480	0.932	0.650	0.117

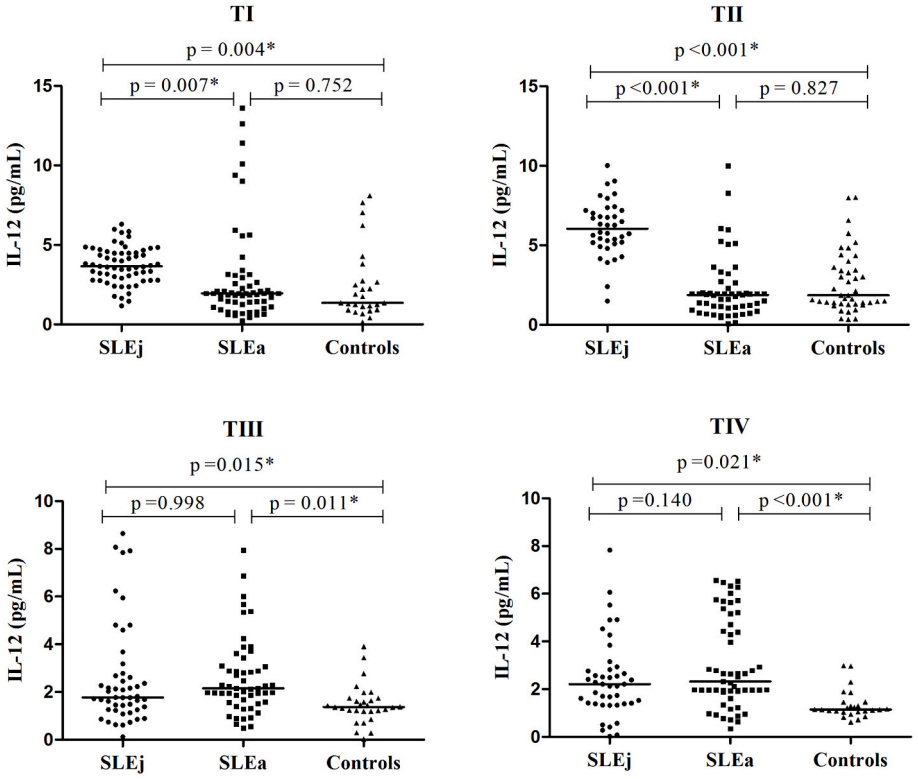

Fig. 1. Comparison of levels of IL-6 between cSLE, aSLE, and controls at the four time points evaluated. *p ≤ 0.05 is considered statistically significant.

moreover, IL-12 was associated with alopecia and leucopenia. These associations have not been reported in the literature.

The levels of IL-10 were not increased in aSLE or SLE patients, however, we observed an association of IL-10 with nephritis, hypocomplementemia, and disease activity in aSLE patients. Some studies have previously demonstrated an association of IL-10 with disease activity [40–42]. One study evaluated IL-10 levels in a group of SLE patients with active disease and observed a direct correlation with the SLEDAI score [42].

One characteristic of SLE patients is the abnormal increase in T cell response caused by an imbalance in the production of cytokines [16]. B-cell hyperactivity and immune complex formation are driven by Th2 lymphocytes; however, Th1 has also been similarly related suggesting that the Th1/Th2 axis imbalance plays a key role in the inflammatory response and the pathogenesis of SLE [15,16,43]. Our study is the first to longitudinally describe the pattern of Th1/Th2 cytokines in aSLE and cSLE. Some studies have demonstrated differences in profiles of clinical, laboratory, and disease activity in these two groups of patients, however, the involvement of cytokine profiles has not been demonstrated [7, 9–12,44–46].

In our study, we observed a significant improvement in disease activity over time in aSLE. However this difference in disease activity pattern could not be explained by the examined cytokine levels. The longitudinal SLEDAI was not performed, which could better evaluate activity over time. Some studies report more active disease in cSLE [9, 47] while another reports a higher frequency of disease activity in aSLE patients [14].

In our study, aSLE patients presented a significantly higher frequency of cumulative damage than cSLE patients; however, some studies have observed more cumulative damage in cSLE patients (9.48). A recent study found that the number of patients without cumulative damage during the study period was significantly higher in cSLE patients compared to other patients (60% cSLE vs. 22% aSLE, $p < 0.001$); a significant higher number of aSLE patients presented cumulative damage when this score was 1–3 (26% cSLE vs. 62% aSLE, $p < 0.001$); and similar cumulative damage was observed between the two groups when the score was ≥4 (14% cSLE vs. 16% aSLE, $p = 0.304$) [10] corroborating the findings in our study. Most of the previous studies did not evaluate the frequency of cumulative damages but rather the average score; hence, the findings are contradictory in the literature. Two studies comparing patients found that cSLE had significantly more cumulative damage than aSLE (9.48). However, two other studies did not observe a

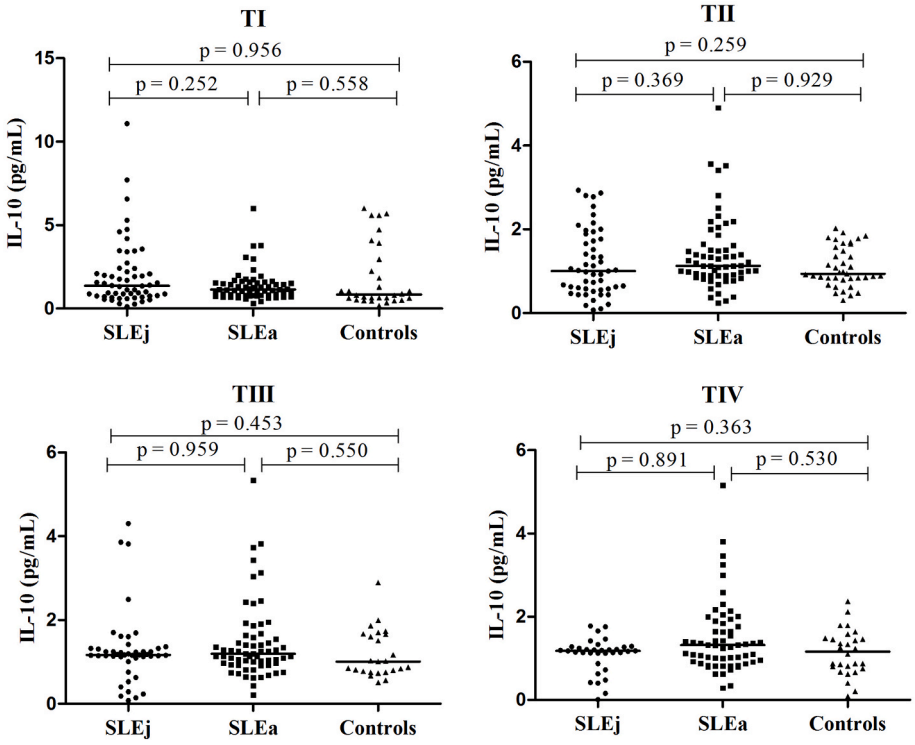

Fig. 2. Comparison of levels of IL-10 between cSLE, aSLE, and controls in the four time points evaluated. *p ≤ 0.05 is considered statistically significant.

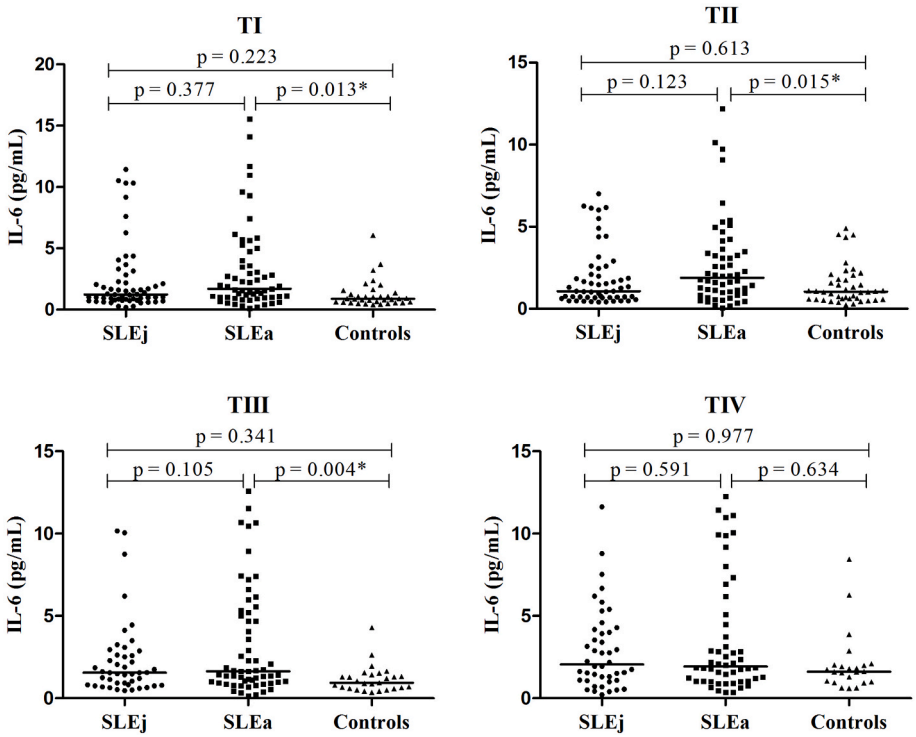

Fig. 3. Comparison of levels of IL-12 between cSLE, aSLE, and controls in the four time points evaluated. *p ≤ 0.05 is considered statistically significant.

statistical difference between aSLE and cSLE patients (49–50). In our study, we did not observe a statistical difference in the mean cumulative damage score between the studied groups (p = 0.102).

Among the evaluated clinical and laboratory manifestations, headaches was found more frequently in cSLE than in aSLE patients. Some studies comparing aSLE and cSLE found a higher frequency of neuropsychiatric symptoms in cSLE patients, particularly psychosis, headache, and seizure (10–12.44).

In our study, cSLE patients received significantly higher doses of prednisone at all time points. Most studies compare the frequency of medication use [9,10,12,14]; some studies observed that cSLE patients make use of prednisone at higher frequency than aSLE patients (9.14),

others did not observe a significant difference in the use of prednisone between aSLE and cSLE patients [46,47].

The limitations of our study include the lack of cumulative SLEDAI and absence of patients with the short-term disease. It is discussed in the literature that the profile of cytokines in SLE patients can vary with the duration of disease [15].

In summary, aSLE and cSLE patients with long disease duration present similar levels of cytokines, despite differences in disease activity pattern over time.

Consent for publication

All authors have reviewed the final version and agree with its submission and publication.

Funding

Simone Appenzeller received grants from Fundação Apoio À Pesquisa Estado São Paulo-Brasil (FAPESP 08/02917–0, 12/21071–0), Conselho Nacional Pesquisa Desenvolvimento-Brasil CNPq 306,723/2019–0), CAPES 001.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Simone Appenzeller reports financial support was provided by São Paulo Research Foundation. Karina de Oliveira Pelicari reports financial support was provided by São Paulo Research Foundation. Simone Appenzeller reports financial support was provided by National Council for Scientific and Technological Development. Simone Appenzeller reports financial support was provided by Coordination of Higher Education Personnel Improvement.

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