Cytokine imbalance in patients with systemic sclerosis and resilience: the key role of interleukin-6

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ABSTRACT

Objective. *Resilience, the ability to respond positively to adverse events, may be influenced by long-term stressors and autoimmune/inflammatory conditions such as systemic sclerosis (SSc). Since the immune system plays a role in the development of resilience, we aimed to evaluate the relationship between a panel of cytokines and resilience in patients with SSc.*

Methods. *Thirty-five consecutive women with established SSc were involved in this exploratory study. Clinical characteristics, including severity of symptoms and resilience, a panel of 15 serum cytokines and 17 autoantibodies were assessed simultaneously. Multivariate methods were used to analyse the data.*

Results. *Interleukin-6 (IL-6) levels were associated with severity of symptoms (β=1.8395, p=0.04), and low resilience scores (β= -0.581120, p=0.02). Furthermore, resilience was not associated with clinical manifestations nor polyautoimmunity. Cytokine levels did not significantly differ between groups based on regular physical activity.*

Conclusion. *The results highlight the importance of IL-6 as a key mediator in the altered cytokine network of SSc.*

Introduction

Systemic sclerosis (SSc) is an autoimmune disease (AD), characterised by fibrosis of the skin, internal organs, and vascular obliteration phenomena (1). SSc patients typically show burdensome symptoms of psychological distress determined by disfiguration, pain, fatigue, and marked impairment for daily life occupations (1, 2), thus displaying the high burden of disease on quality of life (3, 4). It has been described that symptoms of depression in SSc are common (5-7) and nearly 65% of SSc patients develop major depressive disorder (MDD) (8, 9). Pain and the physical domains have a critical role in quality of life and development of depression in SSc (10, 11).

Resilience, defined as the ability to bounce back or recover previous levels of functioning after a stressful event (12), was recently evaluated in a group of patients with SSc (13). Results showed that a history of physical activity (*i.e.* regular exercise) and socioeconomic status influenced it (13). However, the role of biological factors associated with the development of resilience in SSc have not been studied yet.

It has been proposed that the imbalance between T-helper 1 (Th1) and T-helper 2 (Th2) profiles may elicit a dysregulation of serotonin and glutamate seesaw, which lead to changes in behaviour and stress response, including an impaired capacity to respond adequately to stress (14–16). In fact, in some patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) an increase in IL-4 and IFN-γ after stressful events has been shown (17). Since the effect of cytokines is a plausible mechanism to explain the changes on behaviour in patients with SSc, we aimed to investigate the relationship between a panel of Th1, Th2, and Th17 cytokines and resilience in women with SSc.

Methods

Study population

An exploratory cross-sectional analytical study was conducted on 35 women previously reported with established SSc (13). The subjects had been followed at the Center for Autoimmune Diseases Research (CREA) in Bogotá, Colombia. All the patients fulfilled the 2013 American College of Rheumatology/European League Against

Rheumatism (EULAR) classification criteria for SSc (18). Polyautoimmunity (PolyA) was considered in those patients fulfilling criteria for more than one autoimmune condition (19). In this case, patients with RA, SLE and Sjögren's syndrome (SS) fulfilled the 1987 American College of Rheumatology (ACR) classification criteria for RA (20), the 1997 ACR criteria for SLE (21), and the revised American-European Consensus Group for SS, respectively (22). For autoimmune thyroid disease (AITD), patients with autoimmune hypothyroidism (AH) were classify as follows: 1) confirmed AH (*i.e.* thyroid dysfunction, TSH >4.1 mIU/L or levothyroxine treatment, and the presence of anti-TPO or anti-Tg autoantibodies), 2) euthyroid patients with positive anti-TPO or anti-Tg, 3) non-autoimmune hypothyroidism (thyroid dysfunction and absence of TPO-Ab/TgAb) (23).

This study was done in compliance with Act 008430/1993 of the Ministry of Health of the Republic of Colombia, which classified it as minimal-risk research. The institutional review board of the Universidad del Rosario approved the study design.

Data collection

As previously reported, resilience was measured by the Spanish validated version of the Brief Resilience Scale (BRS) (24). Scoring was assigned according to the author's guidelines (low BRS: 1-2.99; b Normal BRS: 3-4.3; C high BRS: 4.31-6) (12). Severity of symptoms was measured by the scleroderma skin patient report outcome questionnaire (SSPRO, 1 to 108) (13, 25). The patients' socio-demographic and cumulative clinical and laboratory data were obtained by interview, standardised report form, physical examination and chart review. Data regarding age, age at onset, duration of disease, socioeconomic status (*i.e.* low, middle and high), physical activity (*i.e.* more than 30 minutes of exercise at least 3 times per week), occupation and educational level were previously analysed and reported (13). All data were collected in an electronic and secure database as described elsewhere (13).

Laboratory measurements

The sera of patients was collected in fasting state and spite of the treatment status (*i.e.* 60% of patients were on therapy at the moment of the study). Concentration of 15 cytokines (IL-2, IL-10, IL-6, IL-8, IL-9, IL-13, IL-12/23p40, G-CSF, IFNγ IFNα IL-4, IL-1β, TNF-α IL-5, IL-17A) in serum samples from patients was assessed by Cytometric Bead Array (CBA, Becton Dickinson Biosciences, San Diego, CA, USA). The test was done according to the manufacturer's protocols. Concentration of the cytokines was calculated using the FCAP Array™ Software (BD Bioscience) as reported elsewhere (26). In addition, 17 autoantibodies were evaluated by immunoblot assay (Nucleosomes, Histones, dsDNA, Ro60, Ro52, SSB/La, U1 RNP, smD1, PCNA, P0, ACApB, Scl70, AMA M2, Jo-1, PM-Scl, Mi-2, Ku) (IMTEC ANA-LIA Maxx from Human diagnostics) according to the manufacturer's protocols. The anti-RNA polymerase III positivity status was obtained from clinical records.

Statistical analyses

In the univariate analysis, categorical variables were analysed by frequencies, and quantitative continuous variables were expressed as mean and standard deviation (SD) as well as being in the median and interquartile range (IQR). To assess associations between outcomes of interest and other variables, the Kruskall-Wallis, Mann-Whitney U-test, and the unpaired *t*-test were used. Correlations between continuous variables were assessed by the Spearman's correlation coefficient. The joint effect of cytokines and therapy on BRS and SSPRO scores was evaluated by means of linear regression with an interaction term. A significance level of 0.05 was set for the study. Data were analysed using R v. 3.3.2.

Results

Cohort

The general characteristics of patients with SSc are shown in Table I. Almost all patients showed limited subphenotype (94.20%), and more than the half of patients (66%) reported a history of regular physical activity (*i.e.* at least 30 minutes of exercise three times per week) at the time of the study (13). PolyA was found in 40% of patients with AITD, SLE and RA as the most common. Data about treatment for 30 patients was registered. Of these 18 (60%) were on medication as follows: inmmunosupressors 4 (13.3%), corticosteroids 7 (23.3%), antimalarials 5 (16.7%), disease-modifying anti-rheumatic drugs 10 (33.3%), and biological drugs 1 (3.3%).

Table I. General characteristics and resilience of women with systemic sclerosis.

Variable	Frequency
Sociodemographic data	
Age (IQR)	58 (51.50-62.50)
Age at onset disease (IQR)	48 (37.0-53.50)
Duration of disease (IQR)	7 (4.0-13.0)
Years of education (IQR)	11 (9.0-16.0)
Socioeconomic status (%)	
Low	8 (24.20)
Middle	15 (45.50)
High	10 (30.30)
Occupation (%)	
Employed	20 (57.0)
Unemployed	15 (43.0)
Polyautoimmunity (%)	14 (40.0)
SLE	3 (8.60)
RA	3 (8.60)
AITD	6 (17.14)
SS	1 (2.90)
AH	1 (2.90)
PBC	1 (2.90)
APS	2 (5.71)
Subphenotype	
Diffuse	2 (5.70)
Limited	33 (94.20)
Symptoms severity	
SSPRO (IQR)	52 (30.50-64.0)
Resilience assessment	
Total BRS (IQR)	3.33 (2.80-3.70)
Low (%)[a]	10 (28.60)
Moderate (%)[b]	21 (60.0)
High (%)[c]	4 (11.40)

IQR: interquartile range; SSPRO: scleroderma skin patient report outcome (SSPRO) questionnaire; BRS: Brief Resilience Scale Total Score (*i.e.* Total sum by the total number of questions answers); [a]low BRS: 1-2.99; [b]moderate BRS: 3-4.3; [c]high BRS: 4.31-6. SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; AITD: autoimmune thyroid disease; SS: Sjögren's syndrome; AH: autoimmune hepatitis; PBC: primary biliary cholangitis; APS: antiphospholipid syndrome.

Clinical manifestations and resilience

Clinical manifestations are shown in Table II. The most common features

Table II. Clinical manifestations of patients with systemic sclerosis and the association with resilience.

Clinical manifestation	Frequency (%)	BRS[a]	SSPRO[a]
Skin thickening MCP joints	13 (37.14)	0.2172	0.8912
Skin thickening	26 (74.29)	0.4152	0.2989
Puffy fingers	7 (20.0)	0.1472	0.6798
Sclerodactyly	22 (62.86)	0.1072	0.7198
Digital tip ulcers	6 (17.14)	0.3228	0.0336*
Pitting scars	5 (14.29)	0.1423	0.0851
Telangiectasia	29 (82.86)	0.5980	0.3692
Abnormal capillaroscopy	8 (22.86)	0.2781	0.3355
PAH	11 (31.43)	0.8305	0.3645
Interstitial lung disease	4 (11.43)	0.3623	0.9173
Raynaud's phenomenon	34 (97.14)	0.1494	0.1372
Scleroderma related antibodies	23 (65.7)	0.0619	0.4138
Anticentromere antibodies	22 (62.90)	0.0898	0.7455
Anti SCL-70 antibodies	1 (2.90)	-	-
Anti-RNA polymerase III	0 (0.00)	-	-

[a]Analyses were done with Mann-Whitney U-test to find associations between organic involvement, BRS and SSPRO scores. *Statistical significant. BRS: Brief Resilience Scale; SSPRO: scleroderma skin patient report outcome questionnaire; MCP: metacarpophalangeal joints; PAH: pulmonary artery hypertension.

Table III. Cytokines in patients with systemic sclerosis.

Cytokines	All patients[a]	Limited subphenotype[a]	*p*-value[b]
IL-1β	5.80 (11.90)	6.17 (12.19)	0.8996
IL-2	1.18 (3.57)	1.26 (3.67)	0.9277
IL-4	2.50 (5.20)	2.66 (5.35)	0.9009
IL-5	1.00 (2.22)	1.01 (2.89)	0.9872
IL-6	4.84 (7.10)	4.65 (7.09)	0.9125
IL-8	13.20 (7.6)	12.25 (6.36)	0.5792
IL-9	1.02 (3.12)	0.96 (3.17)	0.9376
IL-10	2.40 (4.60)	2.41 (4.78)	0.9930
IL12_23p40	46.00 (76.40)	47.34 (78.63)	0.9434
IL-13	0.84 (3.00)	0.88 (3.11)	0.9571
IL-17A	34.70 (72.30)	36.81 (73.98)	0.9057
TNFα	10.10 (20.60)	10.62 (21.10)	0.9184
G-CSF	6.72 (12.30)	6.67 (12.64)	0.9869
IFNα	14.80 (26.50)	15.70 (27.07)	0.8903
IFNγ	0.66 (1.40)	0.61 (1.41)	0.8838

[a]Mean in pg/mL (Standar deviation), [b]unpaired t-test was used to compare cytokines levels between all patients and those with only limited subphenotype. IL: interleukin; G-CSF: granulocyte colony-stimulating factor; IFN: interferon; TNF: tumour necrosis factor.

were Raynaud's phenomenon, telangiectasia and skin thickening. Although digital tip ulcers were associated with SSPRO (p=0.0336), BRS scores were not associated with any of the clinical manifestations reported (Table II). PolyA was not associated with resilience (p=0.5886) nor with severity of symptoms (p=0.9462). Furthermore, since only two patients showed a diffuse subphenotype, comparison between limited and diffuse subphenotypes regarding resilience and severity of symptoms was precluded.

Cytokines, severity of symptoms, and resilience

Cytokine results are shown in Table III. In the bivariate analysis, there was not a specific pattern of cytokines associated with resilience. Highest levels of pro-inflammatory (Th1 and Th17) and Th2 cytokines were observed in patients with low resilience scores as compared to those with normal scores (Fig. 1 and 2). Furthermore, resilience was negatively correlated with IL-5, IL-8, IL-10, and IL-13 levels (Table IV), and these associations persisted after exclusion of patients with diffuse subphenotype (Table IV). Interestingly, in this bivariate analysis, a positive correlation was observed between IL-6 levels and severity of symptoms (r_s=0.39, p=0.0201). Although exercise was previously associated with better resilience (13), cytokine levels did not significantly differ between groups based on regular physical activity (data not shown).

After the bivariate exploration for treatment (Suppl. Table I), a linear regression analysis with an interaction term (*i.e.* therapy) was made in order to find those cytokines, which could have been affected by the treatment. IL-6 was associated with severity of symptoms (β=1.8395, p=0.0435) regardless of treatment (Fig. 3A), and with low BRS scores (β=0.581120, p=0.0291) in those patients under therapy and regardless of severity of symptoms (Fig. 3B). Associations with other cytokines were not found.

Autoantibodies, severity of symptoms, and resilience

Multiple autoantibodies have been associated with different subphenotypes in patients with SSc. In this line, autoantibodies evaluated did not influence resilience nor severity of symptoms (Suppl. Table II).

Discussion

This study reports the imbalance of cytokines in patients with SSc and resilience. Levels of IL-6 were associated with low resilience scores and a worse symptomatology, in patients with predominantly limited subphenotype. Furthermore, PolyA and autoantibodies were not associated with resilience nor severity of symptoms. Although a history of exercise was previously associated with resilience (13), cytokine levels did not significantly differ between groups based on regular physical activity.

Since ADs, including SSc, are characterised by a pro-inflammatory state driven by cytokines, changes in behaviour and response to stress are expected (27). Data regarding cytokine profiles and changes in behaviour are controversial. Some reports have shown the role of these molecules in psychiatric illnesses such as MDD. In fact, both

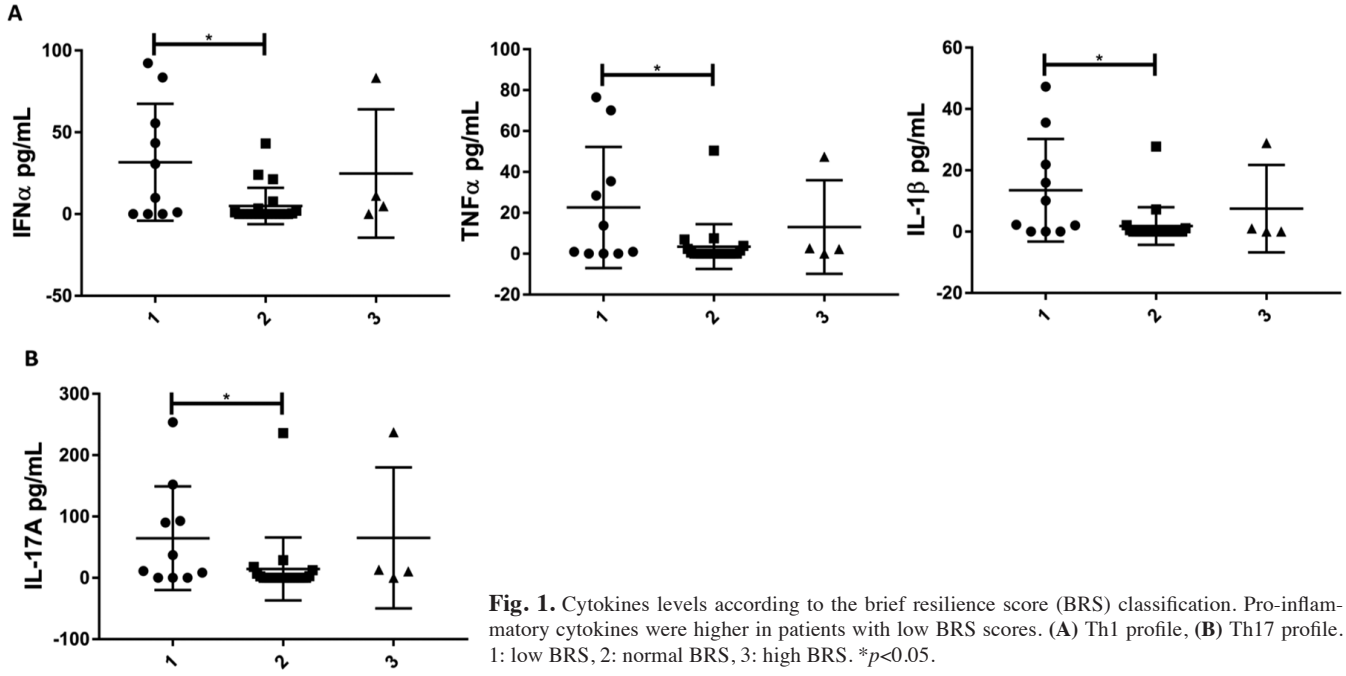

Fig. 1. Cytokines levels according to the brief resilience score (BRS) classification. Pro-inflammatory cytokines were higher in patients with low BRS scores. **(A)** Th1 profile, **(B)** Th17 profile. 1: low BRS, 2: normal BRS, 3: high BRS. *p<0.05.

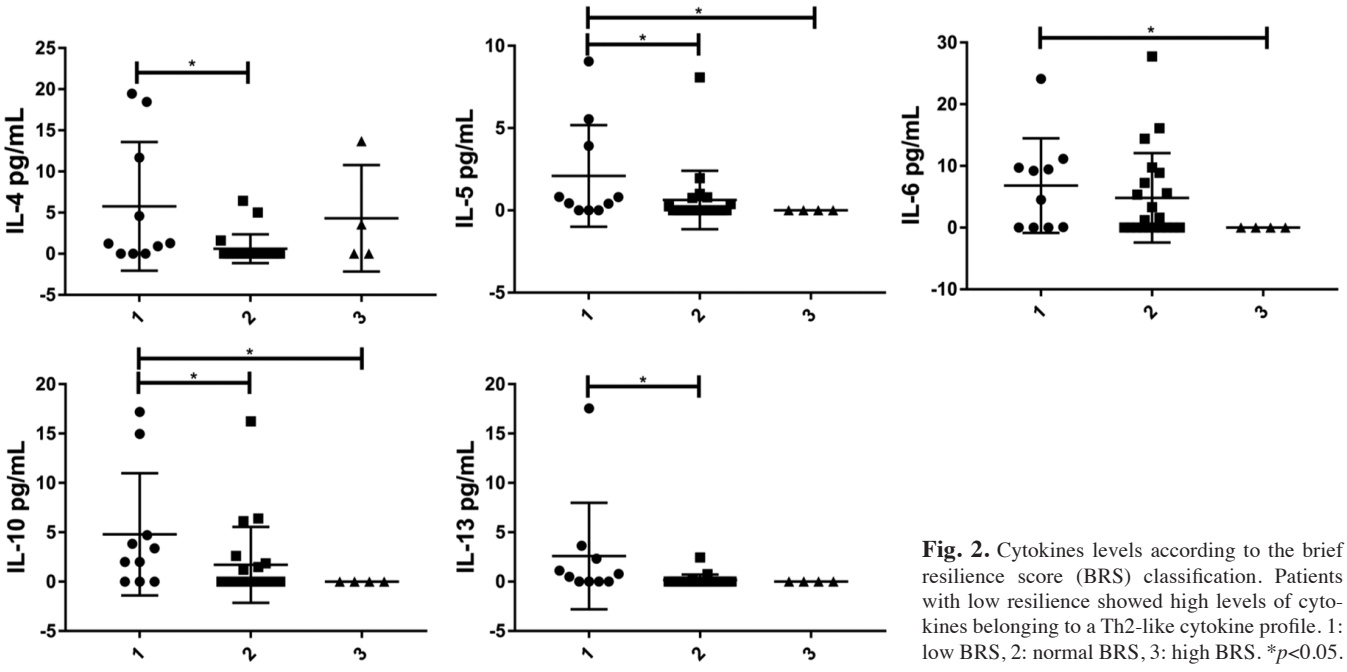

Fig. 2. Cytokines levels according to the brief resilience score (BRS) classification. Patients with low resilience showed high levels of cytokines belonging to a Th2-like cytokine profile. 1: low BRS, 2: normal BRS, 3: high BRS. *p<0.05.

proinflammatory (*i.e.* Th1 and Th17) and regulatory (*i.e.* Th2) cytokines have been associated with changes in behaviour (15, 28). However, the heterogeneity of cytokine results across the studies has hindered finding a specific profile (28, 29).

The cytokine hypothesis of depression states that several neuroendocrine processes are disturbed due to peripheral inflammatory cytokines (15). These molecules communicate with the central nervous system (CNS) through either neural pathways, vascular mechanisms or infiltration across circumventricular organs. In this sense, a neuroimmune communication process embracing the vagus nerve, and cytokine stimulating receptors in endothelial cells promoting inflammation in the CNS, has been proposed (30). This induces an imbalance between Th1 and Th2 profiles which might elicit a dysregulation of serotonin and glutamate leading to changes in behaviour and stress response (15) (Fig. 4).

In the early stages of SSc, a predominance of Th1 and Th17 has been defined, whereas in later stages, when skin fibrosis occurs, a Th2 profile prevails (31). IL-6 and IL-13 have been associated with skin fibrosis (32, 33), IL-5 and IL-17 are associated with interstitial lung disease (34, 35), and reduced levels of IL-10 by the innate immune system after *in vitro* stimulation

Table IV. Correlations between cytokines and resilience (BRS) in patients with systemic sclerosis.

Cytokines	All patients		Limited subphenotype	
	r_s	*p*-value	r_s	*p*-value
IL-1β	-0.18	0.2916	-0.20	0.2699
IL-2	0.15	0.3991	0.15	0.4181
IL-4	-0.22	0.1953	-2.3	0.1912
IL-5	-0.45	0.0072*	-0.45	0.0090*
IL-6	-0.30	0.0773	-0.30	0.0899
IL-8	-0.38	0.0250*	-0.36	0.0379*
IL-9	-0.02	0.9290	-0.02	0.9037
IL-10	-0.41	0.0153*	-0.39	0.0243*
IL-12/IL-23p40	-0.15	0.3791	-0.14	0.4330
IL-13	-0.50	0.002*	-0.50	0.0032*
IL17A	-0.06	0.7276	-0.07	0.6917
TNF-α	-0.08	0.6524	-0.10	0.5902
G-CSF	-0.25	0.1494	-0.22	0.2093
IFN-α	-0.11	0.5368	-0.12	0.4955
IFN-γ	-0.07	0.6722	-0.06	0.7524

*Statistical significant. IL: interleukin; G-CSF: granulocyte colony-stimulating factor; IFN: interferon; TNF: tumour necrosis factor.

is characteristic of SSc (36). These evidences indicate an unsuccessful regulatory process by the immune system and a complex interaction among cytokines in SSc. Although a specific cytokine profile has not been associated with changes in behaviour, the role of IL-5, IL-6, IL-10, and IL-13 has been previously reported, thus supporting their possible role in behavioural illnesses (29, 37). This is in line with our results since these cytokines were associated with low BRS scores in SSc patients.

Noteworthy, the multivariate analysis revealed that IL-6 was associated with low BRS scores in presence of therapy. The IL-6 is considered a potent inflammatory mediator of the immune system and it has been associated with MDD development (38). Maes *et al*. (39) provided evidence of increased levels of IL-6 in patients with treatment resistant depression (TRD). Twin studies have suggested that the association of IL-6 with depression is strongly genetically influenced (40). Thus, IL-6 blockade could be a therapeutic option for depression (41).

Some antidepressant therapies have shown to decreased levels of IL-6 and it was correlated with clinical improvement (42). On the other hand, some patients with TRD treated with either imipramine and venlafaxine, or in combination of 5-hidroxitriptamine (5-HT) and fluoxetine, exhibited increased levels of IL-6 (43). This is in line with our results, since those patients under treatment for SSc, showed a paradoxical effect of IL-6 on resilience. It has been proposed that the activation of the 5-HT4 and 5-HT7 receptors augmented the release of IL-1β, IL-6, IL-12p40 and IL-8/CXCL8 driven by LPS (44), suggesting that under treatment some patients may exhibit a paradoxical effect on cytokines. However, the role of immunomodulatory treatments on this mechanism has not been fully evaluated, and only a study found than those patients using methotrexate and leflunomide reported lower scores on suicidal ideation (45). Altogether, data indicate that IL-6 is a central cytokine on behaviour, and plays a key role on resilience in patients with SSc.

In addition, the role of IL-6 in skin fibrosis has been previously proposed, and some authors have found an association between its levels and the activ-

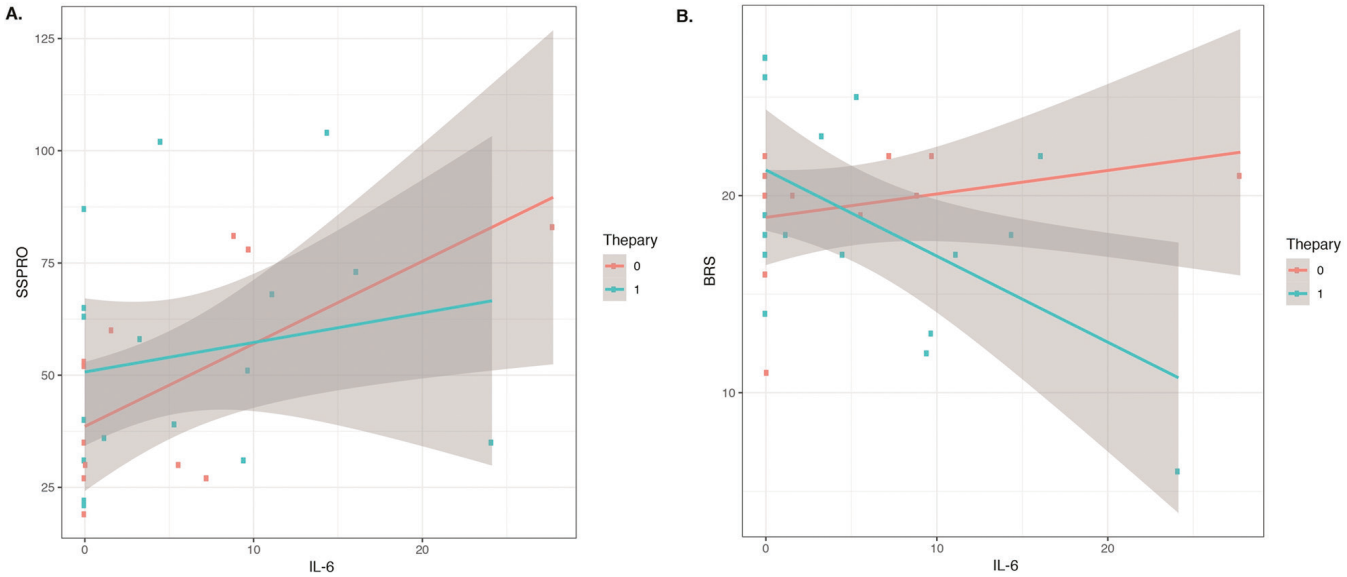

Fig. 3. A. joint effect of IL-6 and therapy on SSPRO scores, **B.** joint effect of IL-6 and therapy on BRS. Statistical analysis by means of linear regression with an interaction term. Therapy: includes any combination of treatment with immunosupressors, DMARDs, corticoids or antimalarials. SSPRO: scleroderma skin patient report outcome questionnaire, BRS: Brief Resilience Scale.

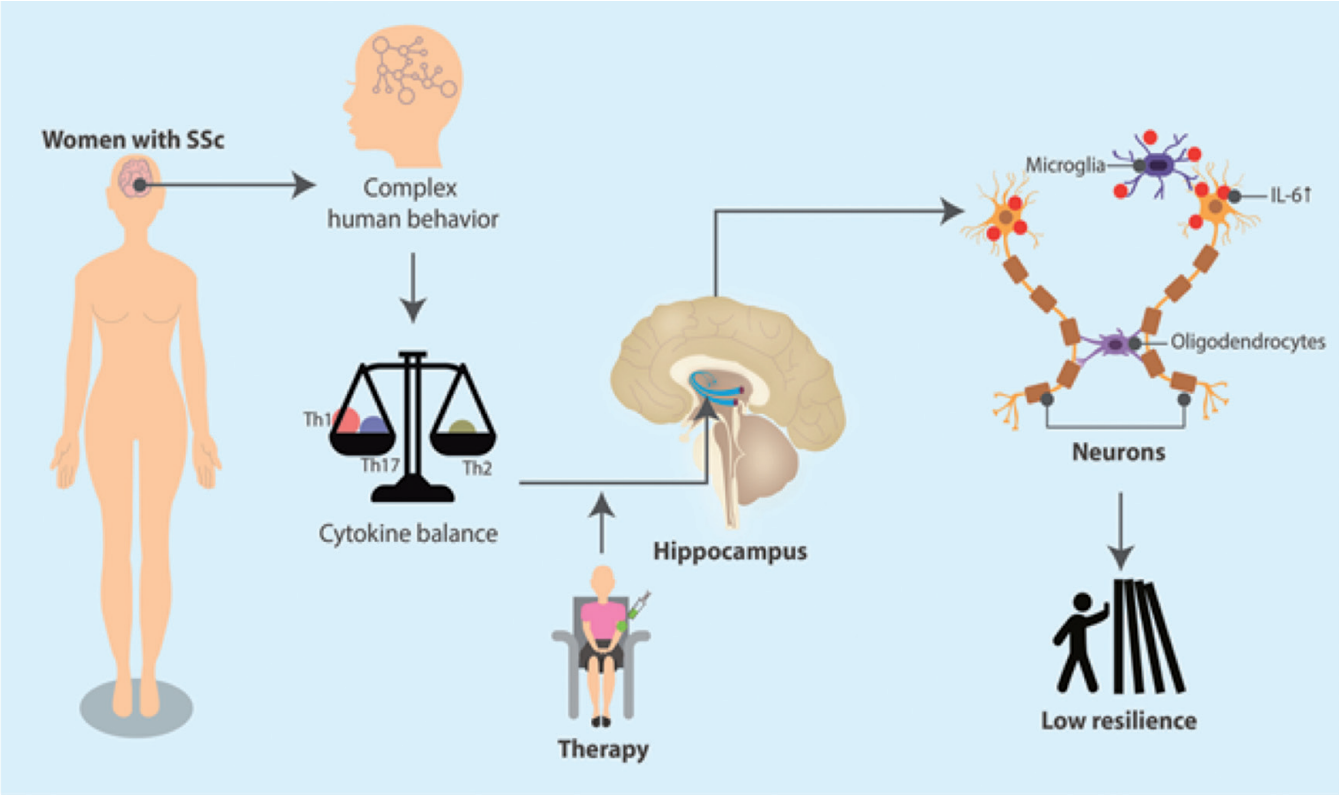

Fig 4. Cytokine imbalance in women with SSc. Immunomodulation may increase concentration of serotonin in the central nervous system, which, in turn, may activates microglia to produce pro-inflammatory cytokines in the hippocampus (*i.e.* IL-6). This imbalance impairs the ability to positively respond to acute stress, reducing resilience (see text for details).

ity of disease (46), especially in those patients with diffuse subphenotype (47, 48). Phase 2 trials (faSScinate clinical trial) with interleukin 6 receptor-α inhibitor (Tocilizumab) showed an improvement of the modified Rodnand skin score as well as benefits in predicted forced vital capacity after 96 weeks of treatment (49).

Patients included in the faSScinate trial demonstrated improvement in quality of life and fatigue (49), both of which variables have been previously associated with resilience in chronic diseases (50, 51). In the current study, IL-6 was also associated with severity of symptoms in patients with limited SSc. Adding further evidence as a key factor on both SSc subphenotypes (*i.e.* limited and diffuse). Physical activity has been associated with better resilience scores, low rates of anxiety, depression, fatigue, and a greater ability to participate in social roles and activities in SSc (13, 52). It has been suggested that physical activity modulates the production of inflammatory mediators (53), and regular physical activity can lead to physiological 'stress training' and thus improve the psychological and physical response to stress (54). Furthermore, it has been found that physical therapy in SSc significantly reduced pain, disability scores, and improved hand motility (55).

Although IL-6 has been associated with proinflammatory functions, exercise has been found to induce an increase in serum levels of IL-6 (56). This, in turn, acting as a myokine, induces the production of IL-10, a strong immunomodulatory cytokine (57). In the current study, cytokine levels did not significantly differ among groups based on a history of regular physical activity. Although an increase in systemic inflammation and oxidative stress secondary to physical activity in SSc has been reported (58), cumulative evidence suggests that physical activity is safe and tolerable, including in those patients with pulmonary involvement (53). Thus, physical activity should be promoted in patients with SSc since may improve resilience (13), and may not induce a deleterious effect secondary to the increase of inflammatory cytokines.

PolyA is frequent in patients with SSc (59). PolyA has been associated with an earlier onset of disease, lung fibrosis and heart involvement, with particular distinctive pattern of autoantibodies (60). In our study, patients with PolyA did not show lower resilience or worse severity of symptoms, and other autoantibodies were no associated with these outcomes.

The possible shortcomings of our study must be acknowledged. The main objective of this exploratory cross-sectional analytical study was to describe the role of cytokines on resilience and severity of symptoms. The small sample size and the fact that about all patients exhibited limited subphenotype may hinder the analysis and extrapolation of the results obtained. Nevertheless, such a possibility would be unlikely given the highly significant results seen as well as their consistent direction and magnitude within the different analyses. Spite of limitations of our study, the results are encouraging and deserve to be validated prospectively.

Conclusion

Our results highlight the importance of IL-6 as a crucial factor in severity of symptoms and resilience, indicating the relevance of this cytokine as a new biomarker of activity of disease and as a target treatment in SSc. Further studies are warranted to clarify the role of this cytokine role on behaviour, and the potential mechanisms by which they may disturb neurotransmitters in SSc.

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