A R T I C L E

Serum cytokines to predict systemic lupus erythematosus clinical and serological activity

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Abstract

We aimed to explore the role of interleukin (IL)-6, interferon-gamma (IFNγ), IL-10, and tumor necrosis factor (TNF) as predictors of systemic lupus erythematosus (SLE) clinical and serological activity, and their correlation with the treatment received. We performed a retrospective analysis of 77 patients with SLE according to the 2012 Systemic Lupus International Collaborative Clinics (SLICC) criteria. The outcomes were serological activity (SA), active disease (AD), complete remission (CR), the low-disease activity state (LDAS), and immunosuppressive treatment. SA was present in 17.1%, AD in 17.3%, CR in 13%, and LDAS in 64.9% of patients. IL-6 values were higher in patients in SA, in AD, in those receiving steroids alone, and in patients without CR or LDAS ($p < 0.05$). IFNγ was associated with anti-double stranded DNA (dsDNA) antibodies positivity and immunosuppression, whereas IL-10 values were higher in patients with CR ($p < 0.05$). The IL6-IFN product was able to predict anti-double stranded DNA (anti-dsDNA) antibodies positivity (area under the receiver operating characteristic curve [AUC-ROC] = 0.705, 95% confidence interval [CI] 0.563–0.847), SA (AUC-ROC = 0.720, 95% CI 0.542–0.899), AD (AUC-ROC = 0.701, 95% CI 0.520–0.882), steroid treatment (AUC-ROC = 0.751, 95% CI 0.622–0.879), and the absence of LDAS (AUC-ROC = 0.700, 95% CI 0.558–0.834). The IL6-IFN/IL10 ratio predicted AD (AUC-ROC = 0.742, 955 CI 0.540–0.944), steroid treatment (AUC-ROC = 0.721, 95% CI 0.572–0.870), and the absence of LDAS (AUC-ROC = 0.694, 95% CI 0.536–0.853). In conclusion, IL-6, IL-10, and IFNγ might help to assess SLE serological and clinical activity. Their combination in the IL-6-IFN product and the IL-6xIFN to IL-10 ratio results in novel tools to determine and predict SA,

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Abbreviations: AD, active disease; Anti-dsDNA, anti-double-stranded DNA antibodies; APS, antiphospholipid syndrome; AUC-ROC, area under the (receiver operating characteristic) curve; CI, confidence interval; CR, complete remission; IFNγ, interferon-gamma; IL, interleukin; LDAS, low-disease activity state; RA, rheumatoid arthritis; SA, serological activity; SLE, systemic lupus erythematosus; SLICC/ACR, Systemic Lupus International Collaborative Clinics/American College of Rheumatology; TNF, tumor necrosis factor.

AD, and LDAS. Prompt detection of SLE activity might allow a rapid intervention to avoid established or chronic damage.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
The follow-up of patients with systemic lupus erythematosus (SLE) is mainly based on clinical findings and supported by serological and biochemical parameters, including complement factors and anti-double stranded DNA (anti-dsDNA) antibodies. Besides, certain cytokines might be key in SLE pathogenesis and might therefore be a promising tool for monitoring patients with SLE.

WHAT QUESTION DID THIS STUDY ADDRESS?
The role of interleukin (IL)-6, interferon-gamma (IFNγ), IL-10, and TNF as predictors of SLE clinical and serological activity, as well as their correlation with the treatment received.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
Il-6, IL-10, and IFNγ might help to assess SLE serological and clinical activity. Their combination in the IL-6-IFN product and the IL-6xIFN to IL-10 ratio results in novel tools to determine and predict serological activity, active disease, and the low-disease activity state.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
Prompt detection of SLE activity might allow a rapid intervention to attenuate the long-term immunosuppression impact and to avoid established or chronic damage.

INTRODUCTION

Systemic lupus erythematosus (SLE) is the prototypic multisystem autoimmune disease. It's extremely complex physiopathology involves almost every component of the immune system, under the influence of certain environmental and genetic factors.[1,2] This variability, implied in diverse immunological pathways, is responsible for the clinical heterogeneity of the disease, which in turn carries a diagnostic and therapeutic challenge.[3] To date, the follow-up, monitoring, and prognosis of patients with SLE are mainly based on clinical findings and supported by serological and biochemical parameters. Among the latter, complement factors along with anti-double-stranded DNA (anti-dsDNA) antibodies, are the only markers uniformly accepted for this purpose.[4] However, several studies have assessed the role of certain cytokines, such as interleukin (IL)-6, interferon-gamma (IFNγ), IL-10, or tumor necrosis factor (TNF), among others, as predictors or markers of disease activity.[5–7] Cytokines are soluble factors that coordinate and regulate the differentiation, maturation, and activation of immune cells in different inter-related pathways, and they are also involved in direct tissue injury in SLE.[7,8] Accordingly, certain cytokines might be key in

its pathogenesis and might therefore be a promising tool for monitoring patients with SLE. Hence, a better understanding of the role and meaning of these molecules could assist clinical management of patients with SLE and help understand the complexity and heterogeneity of the disease, define clinical phenotypes, and even lead to some therapeutic advances.[9,10]

In the light of the aforementioned, the aim of this study was to explore the role of IL-6, IFNγ, IL-10, and TNF as predictors of SLE clinical and serological activity, as well as their correlation with the treatment received.

METHODS

Study population, protocol, and clinical assessment

This cross-sectional study included 77 consecutively recruited female patients who met the 2012 Systemic Lupus International Collaborative Clinics (SLICC) criteria for the classification of SLE.[11] All patients were attending a scheduled appointment in the autoimmune disease outpatient unit between 2012 and 2018. The study was approved by the Puerta de Hierro University Hospital's Research

Ethics Committee, and written informed consent was obtained from all participants.

Data collection

Patients were assessed for demographical, epidemiological and clinical data, including previous diseases, SLE disease duration and immunosuppressive therapy at the time of recruitment. SLE-related factors and parameters, as well as inflammation markers, were determined: antinuclear antibodies (immunofluorescence), anti-dsDNA antibodies (enzyme-linked immunosorbent assay [ELISA]), lupus anticoagulant (Russell viper venom; confirmatory ratio 1.12), anti-cardiolipin and anti-b2 glycoprotein 1, IgM and IgG antibodies (ELISA), complement fractions (C3 and C4), erythrocyte sedimentation rate, high-sensitive hsCRP, D-dimer, and fibrinogen levels, as well as hemogram, liver, and kidney tests. The activity of SLE was assessed by the SLE Disease Activity Index (SLEDAI-2k), whereas organ damage was evaluated by the SLICC/American College of Rheumatology (ACR).[12,13]

Study outcomes and definitions

The study's primary outcomes were serological activity (SA), active disease (AD), complete remission (CR), and the Low-disease activity state (LDAS). These variables were selected according to the goals of treatment defined by the most recent SLE European League Against Rheumatism (EULAR) guidelines.[4] Plus, to understand the role of the cytokines, anti-dsDNA antibodies and different treatment schemes that patients were receiving (steroids, steroids alone, steroids with other immunosuppressant drug, or immunosuppressant drugs without steroids) were also considered.

Anti-dsDNA antibodies were considered positive if they were above 15 U/ml. Hypocomplementemia was considered when C3 and/or C4 fractions were below the laboratory normal range (90–180 and 10–40 mg/dl, respectively). Therefore, SA was defined by both the elevation of anti-dsDNA antibodies and hypocomplementemia. AD was determined by SLEDAI-2k greater than 4. CR was defined by the absence of clinical activity (SLEDAI-2k = 0), with no use of steroids and immunosuppressive drugs, whereas LDAS was based on a SLEDAI-2k less than or equal to 4 with less than or equal to 7.5 mg of prednisone, or equivalent doses, and stable and well-tolerated immunosuppressant agents.[4] The presence of residual organ damage was considered by an SLICC/ACR greater than 1.[13] Finally, antiphospholipid syndrome and antibody

positivity were based on the classification criteria and the Standardization Committee.[14,15]

Quantification of serum cytokine levels

Serum aliquots were obtained and stored at −80°C until the measurement assays were carried out. The concentrations of serum cytokines IL-6, IFNγ, IL-10, and TNF were determined by flow cytometry using the Cytometric Bead Array method (BD Bioscience) which has a detection range of 2–5000 pg/ml. Sample processing was performed according to the manufacturer's instructions. The samples were assessed with a FACScalibur flow cytometer and FCAP Array software was used for data analysis (BD Biosciences).

Statistical analysis

In the descriptive analysis, quantitative variables were expressed as mean and SD or as median and interquartile range, as appropriate, whereas qualitative variables were expressed as frequencies and percentages. First, we evaluated the differences in cytokine values for each outcome. The Kolmogorov test was used to evaluate data distribution and as data did not follow a parametric distribution, statistical analysis was performed using Spearman rank's test to analyze correlations and Mann–Whitney U test to assess differences between groups. Levene's test was used for the homogeneity of variance test. Second, the discrimination ability for the clinical, serological, and treatment outcomes was evaluated following an approach based on the area under the curve receiver operating characteristic curve (AUC–ROC) For all analyses, significance was defined as a p value of less than 0.05. Statistical analysis was performed using SPSS software.

RESULTS

Subject characteristics

The clinical characteristics of patients included in the study, the features of the patients with SLE and medications are summarized in Table 1. The median age was 47 years old and the median disease duration was 11 years. Thirteen patients (17.1%) presented SA, based on the presence of positive anti-DNA antibodies (38.2% of all patients) and low complement levels (35.1% of all patients). Median SLEDAI-2k was 2, and 53.2% of the patients were taking steroids and 36.4% immunosuppressants, including mycophenolate (22.1%), azathioprine (7.8%), methotrexate

TABLE 1 Clinical characteristics, disease-specific features, and serum cytokines of patients with SLE

Age, years (median, IQR)	47 (36.5–57)
Disease duration, years (median, IQR)	11 (5–20)
Antiphospholipid syndrome (*N*, %)	10 (13)
Lupus anticoagulant (*N*, %)	10 (14.3)
Anti-cardiolipin antibodies (*N*, %)	16 (21.1)
Anti-b2 glycoprotein 1 antibodies (*N*, %)	13 (17.3)
SLE activity (*N*, %)	
Positive anti-dsDNA antibodies	29 (38.2)
Hypocomplementemia	27 (35.1)
Serological activity	13 (17.1)
SLEDAI-2 K (median, IQR)	2 (0–4)
Active disease	13 (17.3)
Complete remission	10 (13)
Low disease activity state	50 (64.9)
Treatment (*N*, %)	
Antimalarials	63 (81.8)
Steroids	41 (53.2)
Immunosuppressive therapy	28 (36.4)
Organ damage	
SLICC (median, IQR)	2 (0.5–3)
Residual organ damage (SLICC≥1) (*N*, %)	58 (75.3%)
Laboratory parameters (mean, SD)	
Hemoglobin (12–16 g/dl)	13.12 (1.31)
Lymphocytes (1.2–4.0 10^3/μl)	1.53 (0.70)
Neutrophils (10^3/μl)	2.99 (0.15)
Platelets (150–450,103/μl)	224.9 (63.4)
Creatinine (<1.2 mg/dl)	0.8 (0.3)
D-dimer (0.1–0.5 μg/ml)	0.69 (2.02)
Fibrinogen (150–450 mg/dl)	355 (72)
ESR (0–13 mm)	19 (18)
hsCRP (0–10 mg/dl)	2.6 (5.3)
C3 complement (90–180 mg/dl)	104 (27)
C4 complement (10–40 mg/dl)	18 (9)
Serum cytokines (mean, SD)	
IL-6 (0.002–5 ng/ml)	4.66 (4.51)
IFNγ (0.002–5 ng/ml)	2.26 (2.83)
IL-10 (0.002–5 ng/ml)	11 (49.44)
TNF (0.002–5 ng/ml)	2.69 (6.76)

Abbreviations: ANA, antinuclear antibodies; anti-dsDNA, anti-double-stranded DNA antibody; ESR, erythrocyte sedimentation rate; hsCRP, high-sensitive C-reactive protein; IFNγ, Interferon-gamma; IL, interleukin; IQR, interquartile range; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; SLICC/ACR, Systemic Lupus International Collaborative Clinics/American College of Rheumatology; TNF, tumor necrosis factor.

(6.5%), or calcineurin inhibitors (1.3%). Therefore, 23.4% were receiving steroids alone, 29.9% were receiving steroids in combination with immunosuppressants, and 13%

were receiving immunosuppressants without steroids. Accordingly, 13 patients (17.3%) presented AD, 10 (13%) presented CR, and 50 presented (64.9%) LDAS. Median SLICC was 2, and 75.3% presented any residual organ damage. Ten patients (13%) met antiphospholipid syndrome criteria.

Laboratory parameters and serum cytokines

The laboratory parameters and serum cytokines values are also shown in Table 1. The IL-6, IFNγ, IL-10, and TNF mean and SD values were 4.66 ng/ml (4.51), 2.26 ng/ml (2.83), 11 ng/ml (49.44), and 2.69 ng/ml (6.76), respectively. No correlation was found between the different serum cytokines and the laboratory parameters.

Serum cytokines, disease activity and treatment

The cytokine association with anti-dsDNA antibodies positivity, SA, AD, CR, LDAS, and SLE treatment was analyzed (Tables 2 and 3). In addition, the discrimination ability to assess the different outcomes was evaluated by an AUC-ROC analysis (Tables 4 and 5).

Interleukin-6

IL-6 values were higher in the patients with SA (7.22 vs. 4.07 ng/ml, $p = 0.035$), AD (8.15 vs. 3.95 ng/ml, $p = 0.006$), and in those who were receiving steroids alone (6.79 vs. 3.94 ng/ml, $p = 0.033$; Tables 2 and 3). Besides, IL-6 was lower in patients with CR (2.58 vs. 5.04 ng/ml, $p = 0.001$) and with LDAS (3.52 vs. 6.73 ng/ml, $p = 0.008$). The discrimination ability analysis confirmed that IL-6 predicted AD (AUC-ROC = 0.701, 95% confidence interval [CI] 0.527–0.875), steroid treatment (AUC-ROC = 0.660, 95% CI 0.520–0.800), and the absence of CR (AUC-ROC = 0.768, 95% CI 0.639–0.897) or LDAS (AUC-ROC = 0.677, 95% CI 0.532–0.822; Tables 4 and 5).

Interferon-gamma

IFNγ was significantly associated with anti-dsDNA antibodies positivity (3.52 vs. 1.47 ng/ml, $p = 0.007$), steroid treatment (3.07 vs. 1.47 ng/ml, $p = 0.031$), immunosuppressive drugs alone (3.49 vs. 1.74, $p = 0.031$), and the combination of steroids and immunosuppressants (3.81 vs. 1.75 ng/ml, $p = 0.016$; Tables 2 and 3). In

TABLE 2 Cytokine's association with serological and clinical outcomes

	Anti-dsDNA antibodies positivity			Serological activity			Active disease			Complete remission			Low-disease activity status		
	Presence	Absence	p value	Presence	Absence	p value	Presence	Absence	p value	Presence	Absence	p value	Presence	Absence	p value
IL-6 (ng/ml) (mean, SD)	5.56 (6.12)	4.12 (3.17)	0.238	**7.22 (8.17)**	**4.07 (3)**	**0.035**	**8.15 (8.68)**	**3.95 (2.71)**	**0.006**	**2.58 (0.52)**	**5.04 (4.80)**	**<0.001**	**3.52 (1.80)**	**6.73 (6.89)**	**0.045**
TNF (ng/ml) (mean, SD)	1.91 (3.19)	3.16 (8.18)	0.498	2.33 (3.89)	2.78 (7.28)	0.845	1.95 (1.60)	2.84 (7.38)	0.706	1.75 (1.88)	2.86 (7.30)	0.655	2.03 (3.16)	3.90 (10.56)	0.313
IFNγ (ng/ml) (mean, SD)	**3.52 (3.73)**	**1.47 (1.71)**	**0.007**	3.06 (2.93)	2.06 (2.93)	0.297	2.27 (1.39)	2.25 (3.06)	0.983	1.97 (2.78)	2.31 (2.86)	0.741	2.23 (3.41)	2.31 (1.21)	0.894
IL-10 (ng/ml) (mean, SD)	7.28 (11.76)	13.21 (62)	0.660	3.93 (3.04)	12.62 (54.78)	0.603	3.75 (2.85)	12.48 (54.21)	0.615	**44.76 (125.7)**	**4.92 (8.15)**	**0.025**	14.72 (61.53)	4.26 (3.30)	0.442
IL-6-IFNγ (ng/ml) (mean, SD)	**17 (20.03)**	**7.35 (12.41)**	**0.028**	**21.12 (23.49)**	**8.67 (13.34)**	**0.021**	**20.34 (24.47)**	**9.1 (13.58)**	**0.047**	5.14 (7.24)	12.18 (17.33)	**0.047**	8.22 (13.18)	16.34 (20.26)	0.073
IL-6-IFNγ/IL10 ratio (mean, SD)	3.11 (4.8)	2.57 (3.6)	0.656	**5.21 (6.41)**	**2.22 (3.19)**	**0.047**	**6.77 (7.70)**	**1.98 (2.34)**	**0.002**	1.11 (0.92)	3.07 (7.25)	0.244	**1.77 (2.33)**	**4.62 (5.75)**	**0.019**

Abbreviations: dsDNA, double-stranded-DNA; IFNγ, interferon-gamma; IL, interleukin; SD, standard deviation; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor. Statistically significant results are remarked in bold.

TABLE 3 Cytokine's association with immunosuppressant SLE treatment

	Steroid treatment			Steroid treatment alone			Immunosuppressive treatment alone			Steroid and immunosuppressive treatment		
	Presence	Absence	p value	Presence	Absence	p value	Presence	Absence	p value	Presence	Absence	p value
IL-6 (ng/ml) (mean, SD)	5.67 (5.40)	3.62 (3.11)	0.081	**6.79 (7.28)**	**3.94 (2.83)**	**0.033**	4.20 (2.25)	4.86 (5.2)	0.604	4.54 (2.15)	4.70 (5.09)	0.908
TNF (ng/ml) (mean, SD)	3.61 (9.12)	1.75 (2.57)	0.294	5.01 (12.41)	1.90 (2.99)	0.353	1.94 (3.47)	3.02 (7.79)	0.575	2.86 (7.54)	2.21 (3.75)	0.752
IFNγ (ng/ml) (mean, SD)	**3.07 (3.24)**	**1.47 (2.14)**	**0.031**	2.34 (0.91)	2.23 (3.22)	0.852	**3.49 (4.10)**	**1.74 (1.91)**	**0.031**	**3.81 (4.45)**	**1.75 (1.86)**	**0.752**
IL-10 (ng/ml) (mean, SD)	6.13 (10.27)	16.04 (70.02)	0.446	4.21 (3.40)	13.31 (57.2)	0.543	7.21 (12.98)	12.66 (58.85)	0.700	8.04 (14.11)	12.01 (56.82)	0.791
IL-6-IFNγ (ng/ml) (mean, SD)	**16.26 (19.14)**	**6.07 (11.25)**	**0.017**	16.20 (21.40)	9.40 (14.21)	0.178	14.1 (16.56)	9.79 (16.26)	0.366	16.32 (17.41)	9.36 (15.79)	0.168
IL-6-IFNγ/IL-10 ratio (mean, SD)	3.51 (4.18)	1.99 (3.90)	0.200	24.27 (5.67)	2.29 (3.35)	0.147	2.57 (2.09)	2.88 (4.74)	0.804	2.81 (2.10)	2.77 (4.63)	0.975

Abbreviations: IFNγ, interferon-gamma; IL, interleukin; SD, standard deviation; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor. Statistically significant results are remarked in bold.

TABLE 4 Cytokine's discrimination ability for serological and clinical outcomes

	Anti-dsDNA antibodies positivity			Serological activity			Active disease			Absence of complete remission			Absence of Low-disease activity status		
	AUC-ROC	95% CI	p value	AUC-ROC	95% CI	p value	AUC-ROC	95% CI	p value	AUC-ROC	95% CI	p value	AUC-ROC	95% CI	p value
IL-6 (ng/ml) (mean, SD)	0.541	0.375–0.706	0.605	0.590	0.375–0.805	0.355	**0.701**	**0.527–0.875**	**0.047**	**0.768**	**0.639–0.897**	**0.011**	**0.677**	**0.532–0.822**	**0.025**
TNF (ng/ml) (mean, SD)	0.445	0.293–0.598	0.485	0.498	0.321–0.676	0.984	0.583	0.397–0.768	0.413	0.488	9.285–0.690	0.908	0.561	0.407–0.716	0.438
IFNγ (ng/ml) (mean, SD)	**0.740**	**0.607–0.872**	**0.002**	**0.705**	**0.533–0.876**	**0.036**	0.620	0.449–0.791	0.236	0.572	0.376–0.768	0.498	**0.678**	**0.544–0.822**	**0.028**
IL-10 (ng/ml) (mean, SD)	**0.687**	**0.537–0.837**	**0.017**	0.545	0.359–0.732	0.640	0.531	0.343–0.718	0.762	**0.552**	**0.335–0.769**	0.620	0.544	0.391–0.698	0.574
IL-6-IFNγ (mean, SD)	**0.705**	**0.563–0.847**	**0.010**	**0.720**	**0.542–0.899**	**0.024**	**0.701**	**0.520–0.882**	**0.047**	0.612	0.439–0.786	0.289	**0.700**	**0.558–0.834**	**0.014**
IL-6-IFNγ/IL-10 ratio (mean, SD)	0.522	9.352–0.692	0.800	0.685	0.476–0.895	0.086	**0.742**	**0.540–0.944**	**0.032**	0.622	0.457–0.787	0.307	**0.694**	**0.536–0.853**	**0.027**

Abbreviations: dsDNA, double-stranded DNA; IFNγ, interferon-gamma; IL, interleukin; SD, standard deviation; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor. Statistically significant results are remarked in bold.

TABLE 5 Cytokine's discrimination ability for immunosuppressant SLE treatment

	Steroid treatment			Steroid treatment alone			Immunosuppressive treatment alone			Steroid and immunosuppressive treatment		
	AUC-ROC	95% CI	p value	AUC-ROC	95% CI	p value	AUC-ROC	95% CI	p value	AUC-ROC	95% CI	p value
IL-6 (ng/ml) (mean, SD)	**0.660**	**0.520–0.800**	**0.034**	0.617	0.444–0.791	0.177	0.551	0.388–0.715	0.532	0.594	0.425–0.763	0.280
TNF (ng/ml) (mean, SD)	0.549	0.402–0.697	0.514	0.614	0.443–0.783	0.192	0.419	0.260–0.578	0.327	0.452	0.279–0.624	0.577
IFNγ (ng/ml) (mean, SD)	**0.726**	**0.592–0.860**	**0.003**	0.673	0.527–0.818	0.054	0.638	0.478–0.798	0.101	0.632	0.466–0.809	0.141
IL-10 (ng/ml) (mean, SD)	0.587	0.430–0.725	0.306	0.527	0.359–0.694	0.761	0.569	0.398–0.740	0.401	0.576	0.394–0.758	0.384
IL-6-IFNγ (mean, SD)	**0.751**	**0.622–0.879**	**0.001**	0.674	0.526–0.821	0.053	0.612	0.448–0.775	0.185	0.664	0.496–0.833	0.066
IL-6-IFNγ/IL-10 ratio (mean, SD)	**0.721**	**0.572–0.870**	**0.009**	0.654	0.480–0.828	0.113	0.593	0.421–0.765	0.306	0.633	0.460–0.806	0.160

Abbreviations: anti-dsDNA, anti-double stranded DNA; AUC-ROC, area under the receiver operating characteristic curve; CI, confidence interval; IFNγ, interferon-gamma; IL, interleukin; SD, standard deviation; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor. Statistically significant results are remarked in bold.

the AUC-ROC analysis, IFNγ was able to predict anti-dsDNA antibodies positivity (AUC-ROC = 0.740, 95% CI 0.607–0.872), SA (AUC-ROC = 0.705, 95% CI 0.533–0.876), LDAS (AUC-ROC = 0.678, 95% CI 0.544–0.822), and steroid treatment (AUC-ROC 0.726, 95% CI 0.592–0.860; Tables 4 and 5).

Interleukin-10

IL-10 values were significantly higher in the patients who presented CR (44.76 vs. 4.92 ng/ml, p = 0.025; Tables 2 and 3). However, discrimination ability was significant only for anti-dsDNA antibody positivity (AUC-ROC = 0.687, 95% CI 0.537–0.837) but not for CR (AUC-ROC 0.572, 95% CI 0.376–0.768; Tables 4 and 5).

Tumor necrosis factor

No significant association nor significant discrimination ability was found among TNF levels and the disease activity outcomes, anti-dsDNA positivity, or treatment (Tables 2–5).

Cytokine ratios as markers of serological activity, active disease, and complete remission

To improve the accuracy of identifying the patients with serological or clinical activity, we calculated two compound variables: the product of IL-6 with IFNγ (IL-6-IFNγ), and the IL6-IFNγ to IL-10 ratio (IL-6-IFNγ/IL-10 ratio). First, we considered that IL-6 was related to clinical disease activity and injury, whereas IFNγ was associated with anti-dsDNA antibodies positivity and immunosuppression. Second, we also analyzed the IL-10 role because it seemed to be related to CR and anti-dsDNA antibodies positivity.

IL-6-IFNγ was higher in the patients with anti-dsDNA antibodies positivity (17 vs. 7.35, p = 0.03), SA (21.12 vs. 8.67, p = 0.021), AD (20.34 vs. 9.1, p = 0.047), and steroid treatment (16.26 vs. 6.07, p = 0.017; Tables 2 and 3). Accordingly, IL-6-IFNγ was able to predict anti-dsDNA antibodies positivity (AUC-ROC = 0.705, 95% CI 0.563–0.847), SA (AUC-ROC = 0.720, 95% CI 0.542–0.899), AD (AUC-ROC = 0.701, 95% CI 0.520–0.882), steroid treatment (AUC-ROC = 0.751, 95% CI 0.622–0.879), and the absence of LDAS (AUC-ROC = 0.700, 95% CI 0.558–0.834; Tables 4 and 5, Figure 1).
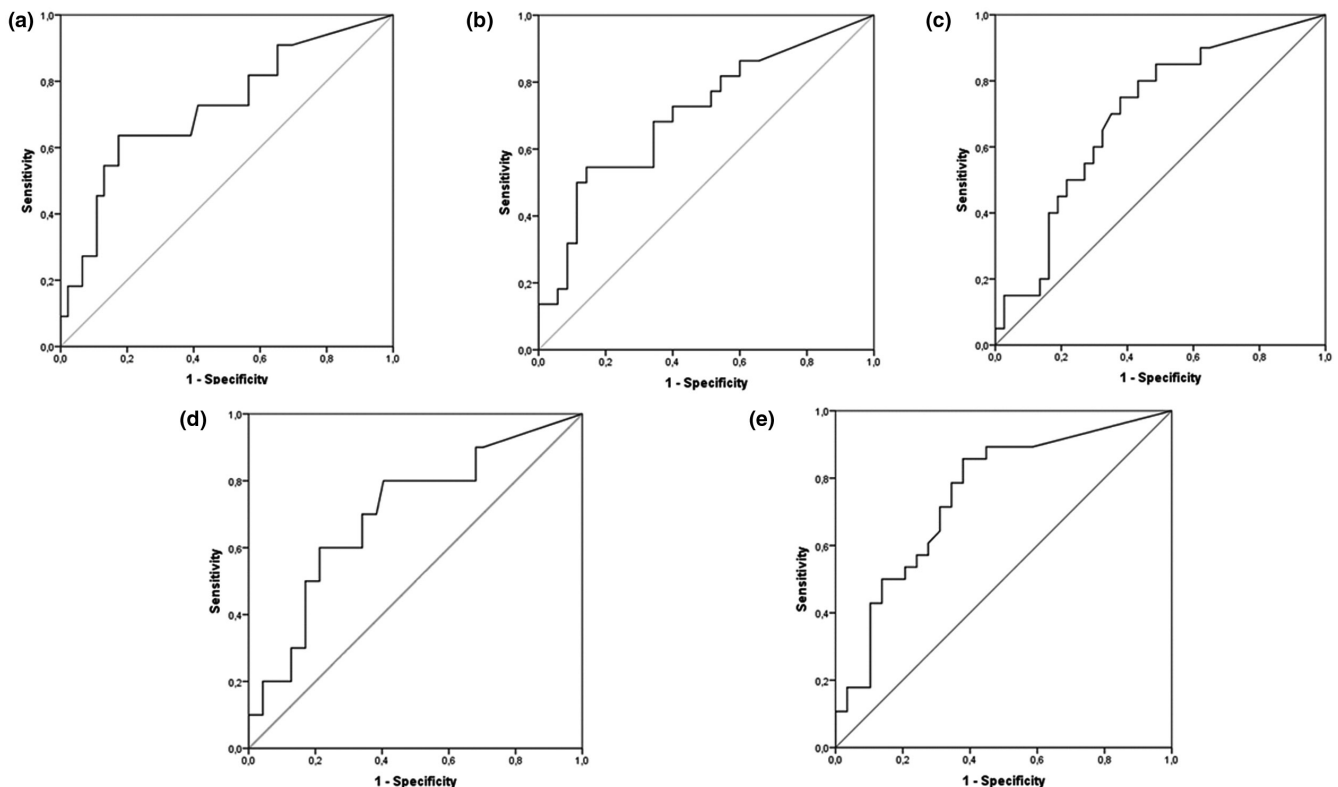
FIGURE 1 The IL-6-IFN product discrimination ability for clinical and serological outcomes. The figure represents the discrimination ability of the IL-6-IFN product for anti-double stranded DNA antibodies positivity (a), serological activity (b), active disease (c), steroid treatment (d), and the absence of the low disease activity status (e)
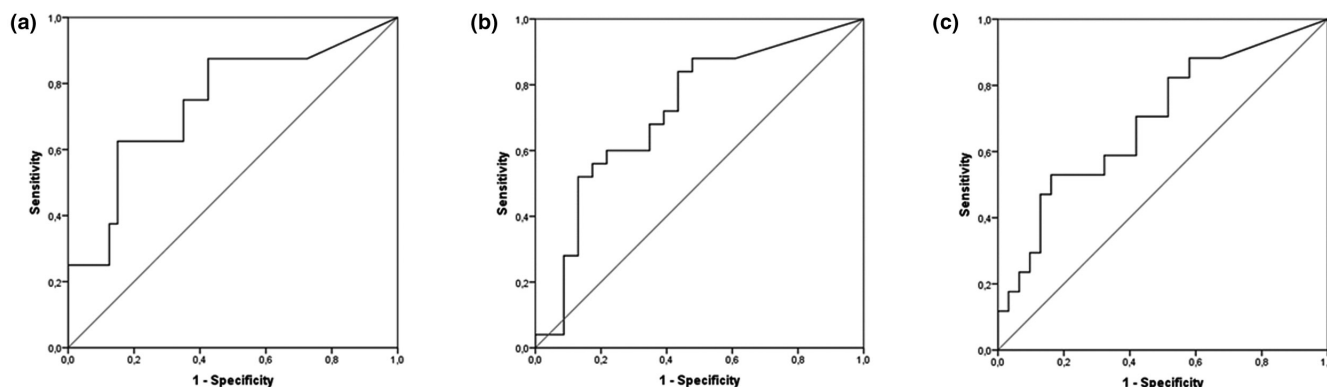
FIGURE 2 The IL-6-IFNγ/IL-10 ratio discrimination ability for clinical outcomes. The figure represents the discrimination ability of the IL-6-IFNγ/IL-10 ratio for active disease (a), steroid treatment (b), and the absence of the low disease activity status (c)

On the other hand, the IL-6-IFNγ/IL-10 ratio was associated with SA (5.21 vs. 2.22, $p = 0.047$), AD (6.77 vs. 1.98, $p = 0.002$), and with the absence of LDAS (1.77 vs. 4.62, $p = 0.019$; Tables 2 and 3). The AUC-ROC analysis for the outcomes was as follows: AD (AUC-ROC = 0.742, 95% CI 0.540–0.944), steroid treatment (AUC-ROC = 0.721, 95% CI 0.572–0.870), and the absence of LDAS (AUC-ROC = 0.694, 95% CI 0.536–0.853; Tables 4 and 5, Figure 2).

DISCUSSION

In this study, we explored the role of IL-6, IL-10, IFNγ, and TNF as markers of SLE activity and subsequent immunosuppressive treatment. Besides, we propose the IL-6-IFNγ product and the IL-6-IFNγ/IL-10 ratio as potential markers of SA, AD, and the absence of CR or the LDAS, widely applied and useful clinical criteria in patients with SLE.[4] These findings might help to understand SLE complex physiopathology, and might provide useful tools for monitoring SLE activity and prompt flares' diagnosis, before strong inflammatory activity or irreversible damage has been established.

IL-6 is a protein produced by a wide spectrum of cell types, such as macrophages, endothelium, and fibroblasts, in response to IL-1, and TNF.[8] Its pleiotropic effects include production of acute-phase reactants, neutrophil production in bone marrow, T-cell differentiation, B-cell maturation, and the synthesis and secretion of immunoglobulins.[6–8,16] Being one of the key molecules in the immune response, IL-6 has a main role in the pathology of certain autoimmune diseases, such as rheumatoid arthritis (RA).[17] In SLE, previous studies have shown that IL-6 is produced by autoreactive T-cells, promoting B-cell activation and autoantibody production.[18] Besides, IL-6 has proven to be higher in patients with active SLE, in lupus nephritis, and to be elevated in the cerebrospinal fluid of patients with neuropsychiatric involvement.[18–20] As a consequence, IL-6 is considered a key mediator in active SLE, whose values are correlated with SLEDAI and clinical activity.[6–8,21–23] In our cohort, IL-6 was related to SA, AD, LDAS, and steroid treatment, confirming its main role in SLE pathogenesis and the consequent clinical implications. In addition, the fact that there was no association with the use of Immunosuppressive treatment in our study might prove that the IL-6 synthesis sources and pathways implied are so diverse that the single action or mechanism of one drug is not enough to attenuate its production in SLE.

TNF is another strong proinflammatory cytokine synthesized by macrophages, NK and T lymphocytes, typically after bacterial infections.[5,6,8] Its effects go beyond the immune system (endothelial cell inflammation or neutrophils activation) because TNF induces a catabolic state and cell apoptosis and it is one of the main cytokines causing fever. Similar to IL-6, its role in certain autoimmune diseases' pathogenesis has been proved after the expanded use of anti-TNF agents.[24] However, evidence regarding the role of TNF in human SLE has been conflicting because some studies have shown that serum TNF levels are elevated in patients with SLE, whereas others have shown otherwise.[25–28] In our cohort, we did not find any relationship between TNF and SLE treatment, clinical or serological indices. These findings probably show that the role of this cytokine in SLE might not be as simple or direct as in other diseases, such as RA or inflammatory bowel disease. Anti-TNF agents are in fact a frequent cause of drug-induced lupus.[29,30]

On the other hand, IFNγ combines pro-inflammatory with immunoregulatory functions.[6–8] It is mostly produced by dendritic cells and stimulates the antiviral Th1 pathway, promoting B-cells differentiation, monocytes proliferation, and modulating cytokine production, or signaling responses of certain T cells. In SLE, and due to these

effects on B and T cells' function, IFNγ has been correlated with clinical and serological activity, as well as with damage scores.[5,31] Besides, its role in SLE pathogenesis is supported by the development of SLE-like syndromes after IFN use.[29,30] Probably because of this same mechanism, IFNγ might be responsible of anti-dsDNA antibodies synthesis, as seen in our population. Consequently, IFNγ values were also related to steroid and immunosuppression.

Finally, IL-10 is an immunomodulatory molecule produced by regulatory T cells and macrophages.[8,27] It is a down-regulatory cytokine that inhibits the antigen-presenting capacity of monocytes by Major Histocompatibility Complex class II, as well as the macrophage production of certain pro-inflammatory cytokines, such as IL-1, TNF, and IL-12. In addition, IL-10 might inhibit the proliferation of certain CD4+ T cells and the production of TNF. Again, the role of IL-10 in SLE pathogenesis has shown to be controversial because some reports support that this cytokine is related to AD and damage index whereas others have not.[21,27,32–34] In our study, IL-10 values were associated with CR, but, at the same time, were able to identify anti-dsDNA positivity, which could appear/seem contradictory. An interesting report by Mc Carthy et al. showed that IL-10 levels were higher in patients with SLE than in controls.[27] Besides, IL-10 was related to disease activity. However, after evaluating the proinflammatory cytokines to IL-10 ratios, they confirmed that the excess in IL-10 production may be a protective response to balance the effect of the proinflammatory cytokines. Similarly, others have proposed that this controversy, and the difficulties to concrete the role of IL-10 in SLE, might be conditioned by the different roles that IL-10 can display depending on the cell source, the Th1/Th2 imbalance, the timing of production, duration, and range levels of IL-10 expression.[32,35] In sum, our results confirm that higher IL-10 levels might be an immunomodulatory response to SLE autoimmunity, expressed in anti-dsDNA antibodies synthesis. As a consequence, IL-10 was related to CR in our cohort.

In the light of the aforementioned results and pathophysiological considerations, we evaluated two variables to assess, with higher accuracy, SLE activity, and the ensuing need for immunosuppression. First, IL-6-IFNγ was composed of IL-6, as a measure of SLE clinical activity, and IFNγ, as a synonym of autoantibody synthesis, according to our results. This compound variable was able to identify anti-dsDNA antibodies positivity, SA, AD, steroid treatment, and the absence of LDAS, robust SLE outcomes, with the following advantages. On the one hand, IL-6-IFN could predict, at the same time, outcomes that were assessed by its components individually. On the other hand, this variable presented high accuracy, even more than IL-6

or IFNγ themselves. Thus, it should be emphasized that all AUC-ROCs were above 0.700, which is the cutoff point to consider a model useful to predict an event.[36] Finally, we also considered IL-10 as an anti-inflammatory and protective cytokine in the IL-6-IFNγ/IL-10 ratio. As expected, and considering the complexity of IL-10 functions, the discrimination ability of the ratio was lower when compared to the IL-6-IFN product. However, it is noteworthy that it was the stronger predictor of AD. Altogether, whereas these findings have to be studied furtherly and in prospective cohorts, we believe that these tools might be able to identify SLE activity and flares. Besides, being in the initial stages of SLE pathogenesis, these cytokine profiles could allow an early diagnosis, before the anti-dsDNA antibodies rise, complement consumption, and, more importantly, before the establishment of the inflammatory cascade or organic damage.

Nevertheless, the limitations of this study have to be considered. In addition to being a single-center, observational, and retrospective study, with a relatively small population size; cytokines and outcomes were measured at the same time. Consequently, the real impact and effect of cytokines have not been measured before the clinical onset, clarifying its true role. Moreover, the influence of certain drugs and their mechanisms, as well as other potential confounding factors, such as age or disease duration, could not be evaluated due to the paucity of patients. For this same reason, we were not able to analyze the cytokine's profiles and significations among the different clinical manifestations, as others have performed.[18,20,23] Despite this, our study presents plausible results and, therefore, we consider that our data are generalizable and can be extrapolated to real clinical practice. Finally, we might consider that cytokine quantification is not a routine in clinical practice, being a potential pitfall for its potential use. Nevertheless, after the COVID-19 pandemic outbreak and evidence supporting tocilizumab use, serum IL-6 quantification has been widely generalized.[37,38] Therefore, and if our results are validated in other cohorts, cytokine analysis could be also extended to patients with SLE.

In conclusion, certain cytokines as Il-6, IL-10, and IFNγ might help to assess SLE serological and clinical activity. Furthermore, their combination in the IL-6-IFNγ product and in the IL-6-IFNγ/IL-10 ratio results in novel tools to determine and predict SA, AD, and the LDAS. Prompt detection of SLE activity might allow a rapid intervention in order to attenuate further immunosuppression and avoid stablished or chronic damage.

CONFLICT OF INTEREST

The authors declared that no competing interests for this work.

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