Interleukin-6 serum levels are associated with disease features and cardiovascular risk in patients with systemic sclerosis

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Abstract

Objective

Systemic sclerosis (SSc) is a chronic multisystem disease characterised by microcirculatory vascular dysfunction and progressive fibrosis of the skin and internal organs. Interleukin-6 (IL-6) is a proinflammatory cytokine that has been implicated in the pathogenesis of several autoimmune diseases and in the initiation and progression of the cardiovascular disease. In the present work we aimed to study the relationship of IL-6 with clinical manifestations and the cardiovascular risk in patients with SSc.

Methods

We carried out a cross-sectional study that included 53 individuals with SSc. A multivariate analysis was performed to study the relationship between IL-6 and disease characteristics and cardiovascular risk assessed by Systematic Coronary Risk Estimation (SCORE2) in SSc.

Results

The presence of digital ulcers, calcinosis, and anti-Scl70 antibody was associated with higher levels of IL-6. This was also the case for functional respiratory parameters where this association was found to be significant and negative after correction for covariates. In addition, the SCORE2 cardiovascular risk algorithm showed a positive and significant association with circulating IL-6.

Conclusion

IL-6 levels are associated with disease manifestations and cardiovascular risk in patients with SSc.

Key words

systemic sclerosis, scleroderma, interleukin-6, SCORE2

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Introduction

Systemic sclerosis (SSc) is a chronic multisystem disease characterised by widespread vascular dysfunction and progressive fibrosis of the skin and internal organs. It is considered a heterogeneous disease, reflected in a wide range of organic manifestations, disease progression and severity, and outcomes (1). SSc is traditionally classified based on the extent of skin involvement and the accompanying pattern of internal organ involvement, as well as the presence of overlapping features with other systemic rheumatic diseases. In this regard, the major subsets of SSc include limited cutaneous, diffuse cutaneous, SSc sine scleroderma, and SSc overlap syndrome (2). Cutaneous manifestations such as thickening and induration, and Raynaud's phenomenon, are almost universal clinical features of SSc. Other characteristics of the disease are the presence of digital ulcers and tissue loss, musculoskeletal manifestations, and gastrointestinal, pulmonary, and cardiac involvement (3, 4). Cardiovascular disease is common in SSc but often unrecognised until late in the disease. Furthermore, SSc patients are at increased risk of atherosclerosis compared with healthy subjects (5).

Interleukin-6 (IL-6) is a proinflammatory cytokine that mediates pleiotropic functions in immunologic responses during host infection, inflammatory disease, haematopoiesis, and oncogenesis. It regulates the immune response, specifically the proliferation and differentiation of T cells and the terminal differentiation of B cells (6). IL-6 also activates macrophages and osteoclasts and is considered the major inducer of most acute phase reactants (7). IL-6 has been implicated in the pathogenesis of several autoimmune diseases (8). Furthermore, IL-6 appears to have a direct causal role in the development of cardiovascular disease and is considered a future target for therapeutic interventions to prevent cardiovascular disease. This has been supported by the fact that high circulating IL-6 concentrations were associated with increased risk of coronary heart disease events in prospective observational studies (9, 10). Additionally, two large meta-analyses have confirmed the crucial role played by IL-6 in the generation of inflammation and the associated risk of cardiovascular disease (11, 12)

There has been a growing emphasis on IL-6 as a mediator in SSc due to encouraging initial clinical findings regarding the use of anti-IL-6 antibodies in diffuse SSc (13). Additionally, emerging clinical evidence suggests that elevated levels of serum IL-6 may be linked to more severe skin involvement (14) and increased progression of lung fibrosis (15). In the present work we aimed to analyse the relationship of IL-6 with SSc clinical features and cardiovascular risk. To do this end, we assessed a series of well-characterised patients with SSc.

Methods

Study participants

This was a cross-sectional study that included 53 patients with SSc. All of them were 18 years or older and met the American College of Rheumatology/European League Against Rheumatism 2013 classification criteria for systemic sclerosis (16). They had been diagnosed by rheumatologists and were periodically followed-up at rheumatology outpatient clinics of our institution. For inclusion in the present study, SSc disease duration needed to be ≥1 year. Patients who had suffered a cardiovascular event were excluded. The patients were also excluded if they were taking IL-6 inhibitors, had a history of cancer or any other chronic disease, evidence of active infection or a glomerular filtration rate <60 ml/min/1.73m^2. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and all subjects provided informed written consent (Approval code: EscleZ).

Assessments and data collection

Surveys in SSc patients and controls were performed to assess cardiovascular risk factors and medication use. Subjects completed a questionnaire and underwent a physical examination to determine anthropometric measurements and blood pressure. Medical records were reviewed to ascertain

specific diagnoses, medications, and comorbidities. Hypertension was defined as a systolic or a diastolic blood pressure higher than, respectively, 140 and 90 mmHg. Obesity, defined as a body mass index (BMI) equal to or greater than 30 kg/m2. Disease duration for SSc was defined as the time since the onset of the first SSc-related symptom other than Raynaud's phenomenon. SSc subtypes, limited and diffuse, were determined according to the distribution of skin thickness. The modified Rodnan Skin Score (mRSS) skin score was used to assess skin thickening (17). This score has been commonly used as an outcome measure in clinical trials. It rates the severity of these features from 0 (normal) to 3 (most severe) in 17 distinct areas of the body and shows an acceptable degree of intra-rater variability. Oesophageal involvement was defined as any sign of dysmotility evident on manometry. Articular involvement was determined by clinical evidence of joint swelling, deformity, contractures, and tendon friction rubs. Interstitial lung disease was defined instrumentally by forced vital capacity (FVC) ≤ 80%, forced expiratory volume in one second- FEV1/FVC ≥ 70%) and/or diffusing capacity of the lung for carbon monoxide (DLCO) < 80% and interstitial changes on chest high-resolution computed tomography. Nailfold capillaroscopy was performed as previously described (18) and scleroderma patterns were sub-graded as "early", "active" and "late" (18).

Fasting serum samples were collected and frozen at -80°C until analysis. Cholesterol, triglycerides, and HDL-cholesterol were measured using the enzymatic colorimetric assay (Roche). Lipoprotein A and lipoproteins were assessed using a quantitative immunoturbidimetric assay (Roche). Cholesterol ranged from 0.08 to 20.7 mmol/l (intra-assay coefficient of variation of 0.3%); triglycerides ranged from 4 to 1.000 mg/dl (intra-assay coefficient of variation of 1.8%); and HDL-cholesterol ranged from 3 to 120 mg/dl (intra-assay variation coefficient of 0.9%). The atherogenic index was calculated using the total cholesterol/HDL-cholesterol ratio according to the Castelli formula. LDL-cholesterol was calculated using the Friedewald formula. A standard technique was used to measure high-sensitivity C-reactive protein (CRP). The homeostatic model assessment (HOMA) method was employed to assess insulin resistance (IR), providing estimates of insulin sensitivity (%S) and β-cell function (%B) based on fasting plasma insulin, C peptide, and glucose concentrations. In this study, HOMA2, the updated computer HOMA model, was utilized (19). Human IL-6 was measured by electrochemiluminescence immunoassay method (Roche Diagnostics, Indianapolis, IN, USA). Both the intra- and inter-coefficients of variability were <10% for these assays. Cardiovascular risk score (SCORE2) was calculated according to the 2021 European Society of Cardiology guidelines on cardiovascular disease prevention in clinical practice (20). SCORE2 categorizes risk as low to moderate, high, or very high based on different age groups (<50, 50-69, and ≥70 years). The SCORE2 scoring system is designed to estimate the 10-year risk of both fatal and non-fatal cardiovascular events in individuals between the ages of 40 and 69 years. However, for healthy individuals who are 70 years or older, the SCORE2-OP (older persons) algorithm provides estimates for both 5-year and 10-year risk of fatal and non-fatal cardiovascular events.

Statistical analysis

Demographic and clinical characteristics of patients SSc were presented as mean (standard deviation) or percentages for categorical variables. For continuous variables that did not follow a normal distribution, data were reported as median and interquartile range (IQR). The association between disease-related data and IL-6 was examined using multivariable linear regression analysis, with adjustments made for confounding variables. Confounders were selected from demographics and traditional cardiovascular risk factors if their p-values were below 0.20 in the univariable analysis to IL-6. All analyses were conducted using Stata software, version 17/SE (StataCorp, College Station, TX, USA), with a two-sided significance level set at 5%. A *p*-value less than 0.05 was considered statistically significant.

Results

Demographic, laboratory, and disease-related data

A total of 53 patients with SSc (44 [83%] and 9 patients [17%] with the limited and diffuse type respectively) were included in this study. The mean age at recruitment was 60 ± 10 years. The demographic features of the patients are shown in Table I. Forty percent of them were hypertensive, 8% smoked, and 11% had a diagnosis of diabetes. Obesity was present in 32% of the patients. The median SCORE2 in the population was 3.8 (IQR2.6-6.2), and 62% were in the low or moderate cardiovascular risk category. The patients were taking statins or aspirin in about a third of the cases. Additional information on the lipid profile and insulin resistance indices is displayed in Table I.

Peripheral blood IL-6 was 2.5 (IQR 1.8-4.2) pg/ml. The disease duration was 9 (IQR 3-11) years. The median mRSS score was 4 (IQR 1-8). Both the presence of digital ulcers and calcinosis was reported in 19% of the patients. At the time the study was conducted, about one-fourth of patients (23%) were taking prednisone with a median dose of 5 (IQR 5-7.5) mg/day and 8% of the patients were taking methotrexate. Additionally, 35 (71%) patients were found to be positive for anti-centromere and 8 (16%) were positive for anti-Scl70. The remaining patients, at the time of recruitment, were negative for anticentromere or Scl-70. None of the patients were on mycophenolate mofetil of tocilizumab. Other features related to the disease are shown in Table I.

Relationship of demographics and disease related data to IL-6 serum levels

The relationship between disease characteristics and IL-6 is shown in Table II. While BMI showed an almost significant positive association with IL-6, abdominal adiposity indices, hip and circumferences abdominal pain were statistically significant and positively related to IL-6. Regarding cardiovas-

Table I. Demographics of systemic sclerosis patients.

	SSc patients (n=53)
Demographics	
Female, n (%)	49 (92)
Age, years	60 ± 10
BMI, kg/m^2	29 ± 6
Waist circumference, cm	98 ± 14
Hip circumference, cm	105 ± 11
Waist to hip ratio	0.93 ± 0.07
Cardiovascular comorbidity	
Hypertension, n (%)	21 (40)
Current smoking, n (%)	4 (8)
Diabetes, n (%)	6 (11)
BMI > 30, n (%)	17 (32)
Statins, n (%)	19 (36)
Aspirin, n (%)	17 (32)
Calcium-channel blockers, n (%)	35 (66)
SCORE 2,%	3.8 (2.6-6.2)
SCORE2 categories, n (%)	
Low to moderate	33 (62)
High	16 (30)
Very high	4 (8)
Laboratory data	
IL-6, pg/ml	2.5 (1.8-4.2)
CRP, mg/dl	2.0 (0.6-4.8)
Cholesterol, mg/dl	209 ± 33
Triglycerides, mg/dl	203 ± 90
HDL-cholesterol, mg/dl	53 ± 13
LDL-cholesterol, mg/dl	116 ± 30
LDL:HDL cholesterol ratio	2.29 ± 0.78
Non-HDL cholesterol, mg/dl	156 ± 31
Lipoprotein A, mg/dl	34 (13-85)
Apolipoprotein A1, mg/dl	163 ± 27
Apolipoprotein B, mg/dl	108 ± 25
Apo B: Apo A ratio	0.68 ± 0.21
Atherogenic index	4.2 ± 1.2
Insulin resistance indices	
Glucose, mg/dl	100 ± 22
Insulin, µU/ml	12.4 (6.1-19.3)
C-peptide, ng/ml	5.0 ± 3.2
HOMA2-IR	1.62 (0.85-2.51)
HOMA2-S%	62 (40-118)
HOMA2-B%-C-peptide	201 ± 106
Systemic sclerosis related data	
SSc type, n (%)	
Limited	44 (83)
Diffuse	9 (17)
Disease duration, years	9 (3-11)
Modified Rodnan Skin Score, units	4 (1-8)
Raynaud phenomenon, n (%)	48 (91)
Nailfold capillaroscopy pattern	
Normal	9 (19)
Early	9 (19)
Active	15 (31)
Late	2 (4)
Unclassified or not valuable	13 (27)
Digital ulcers, n (%)	10 (19)
Calcinosis, n (%)	10 (19)
Arthritis, n (%)	3 (6)
Gastric reflux, n (%)	30 (57)
Pathological oesophageal manometry, n (%)	7 (50)
Interstitial lung disease, n (%)	7 (14)
FVC, %	96 ± 16
FEV1, %	99 ± 18
DLCO, %	83 ± 16
Pulmonary hypertension, n (%)	2 (5)
Anti-centromere antibody positivity, n (%)	35 (71)
Anti-Scl70 antibody, n (%)	8 (16)
Current NSAIDs, n (%)	9 (17)
Current prednisone, n (%)	12 (23)
Prednisone, mg/day	5 (5-7.5)
Methotrexate, n (%)	4 (8)
Chloroquine, n (%)	3 (6)
Bosentan, n (%)	2 (4)
Sildenafil, n (%)	1 (2)

Data represent mean ± SD or median (IQR) when data were not normally distributed. BMI: body mass index; CRP: C reactive protein; LDL: low-density lipoprotein; IL-6: interleukin 6; HDL: high-density lipoprotein, SSc: systemic sclerosis; HOMA: homeostatic model assessment; NSAIDs: non-steroidal anti-inflammatory drugs; SCORE: Systematic Coronary Risk Assessment; FVC: forced vital capacity; FEV: forced expiratory volume; DLCO: diffusion capacity of the lung for the carbon monoxide.

cular risk factors, only current smoking revealed a significant relationship with IL-6. Although this was not the case for hypertension, diabetes or dyslipidaemia, the SCORE2 cardiovascular risk calculator showed a positive and significant association with IL-6. This relationship was observed both when the value of this calculator was analysed on a continuous or categorised basis. No associations were observed between lipid-related molecules and indices of insulin resistance and circulating IL-6 (Table II).

Diffuse SSc was associated with higher serum IL-6 levels compared to the limited type. However, when this analysis was adjusted for covariates, although significance was lost, a trend in the same direction remained. Similarly, the presence of digital ulcers and anti-Scl70 antibodies, as well as the use of methotrexate and bosentan, were significantly associated with higher levels of circulating IL-6. Interestingly, FVC and FEV1 lung function tests revealed significant and negative associations with IL-6 after multivariable adjustment (Table II).

Discussion

Serum IL-6 levels are increased in SSc patients, especially in cases of aggressive disease (21). In a recent report of 50 patients with SSc, IL-6 was augmented in the group with disease course ≥5 years, interstitial lung disease, pulmonary hypertension, and gastrointestinal involvement (22). In another study involving 68 SSc patients and 15 healthy controls, the serum IL-6 level was found to be elevated in those with diffuse cutaneous SSc, thrombocytosis, and elevated acute phase markers (23). High early IL-6 expression in diffuse SSc appeared to be associated with more severe skin involvement at 3 years and poorer long-term survival than in patients without elevated IL-6 levels (23). Our study supports the association of IL-6 with the presence of diffuse SSc, in particular with its marker, anti-Scl70. In this sense, anti-Scl70 antibodies are generally associated with diffuse cutaneous SSc and a higher risk of severe interstitial lung disease (24) or the early occurrence of digital ulcerations (25). Besides, there are conflicting data

Table II. Relationship of demographics and disease related data to IL-6 serum levels.

	IL-6 pg/ml beta coef. (95%CI), *p*			
	Univariable		Multivariable	
Demographics				
Female	-0.5 (-3-2)	0.66		
Age, years	0.02 (-0.03-0.08)	0.42		
BMI, kg/m^2	0.09 (-0.01-0.2)	0.089		
Waist circumference, cm	**0.05 (0.008-0.09)**	**0.020**		
Hip circumference, cm	**0.06 (0.003-0.1)**	**0.038**		
Waist to hip ratio	6 (-2-14)	0.16		
Cardiovascular comorbidity				
Hypertension	-0.4 (-2-0.8)	0.49		
Current smoking	**4 (2-6)**	**<0.001**		
Diabetes	-0.1 (-2-2)	0.91		
BMI > 30 kg/m^2	0.7 (-0.6-2)	0.27		
Statins	-0.7 (-2-0.6)	0.30		
Calcium-channel blockers	0.3 (-1-2)	0.68		
Aspirin	0.8 (-0.5-2)	0.25		
log SCORE 2	**0.8 (0.05-1.5)**	**0.036**		
SCORE2 categories				
Low to moderate	ref.			
High	0.6 (-0.7-2)	0.38		
Very high	**3 (0.7-5)**	**0.011**		
Laboratory data				
CRP, mg/dl	**0.2 (0.04-0.3)**	**0.015**	0.1 (-0.02-0.3)	0.089
Cholesterol, mg/dl	0.009 (-0.01-0.03)	0.35		
Triglycerides, mg/dl	0.0007 (-0.006-0.007)	0.85		
HDL-cholesterol, mg/dl	-0.2 (-0.07-0.03)	0.35		
LDL-cholesterol, mg/dl	0.01 (-0.007-0.03)	0.20		
LDL:HDL cholesterol ratio	0.7 (-0.08-1.4)	0.080	0.3 (-0.4-1)	0.37
Non-HDL cholesterol, mg/dl	0.01 (-0.006-0.03)	0.16	0.009 (-0.008-0.03)	0.29
Lipoprotein A, mg/dl	-0.0003 (-0.008-0.008)	0.95		
Apolipoprotein A1, mg/dl	-0.005 (-0.03-0.02)	0.68		
Apolipoprotein B, mg/dl	0.02 (-0.002-0.05)	0.077	0.01 (-0.006-0.04)	0.16
Apo B: Apo A ratio	2 (-0.5-5)	0.097	1 (-1-3)	0.41
Atherogenic index	0.4 (-0.2-0.9)	0.17	0.09 (-0.4-0.5)	0.68
Insulin resistance indices				
Glucose, mg/dl	0.001 (-0.03-0.03)	0.94		
Insulin, µU/ml	-0.9 (-3-1)	0.41		
C-peptide, ng/ml	-0.05 (-0.2-0.1)	0.56		
HOMA2-IR	-0.1 (-0.4-0.2)	0.42		
HOMA2-S%	0.002 (-0.006-0.01)	0.58		
HOMA2-B%-C-peptide	-0.002 (-0.008-0.003)	0.38		
Systemic sclerosis related data				
SSc type				
Limited	ref.			
Diffuse	**2 (0.3-3)**	**0.020**	1 (-0.1-3)	0.080
Disease duration, years	0.02 (-0.08-0.1)	0.73		
Modified Rodnan Skin Score, units	0.03 (-0.06-0.1)	0.55		
Raynaud phenomenon	0.9 (-1-3)	0.39		
Nailfold capillaroscopy pattern				
Early	ref.			
Active	0.4 (-2-3)	0.73		
Late	-0.9 (-5-3)	0.67		
Digital ulcers	**2 (0.06-3)**	**0.041**	**2 (0.6-3)**	**0.003**
Calcinosis	1 (-0.3-3)	0.10		
Arthritis	0.2 (-2-3)	0.88		
Gastric reflux	-0.5 (-2-0.7)	0.44		
Pathological oesophageal manometry				
Interstitial lung disease	**2 (0.3-4)**	**0.024**	1 (-0.3-3)	0.11
FVC, %	**-0.07 (-0.1-(-0.3))**	**0.001**	**-0.07 (-0.1-(-0.03))**	**0.001**
FEV1, %	**-0.06 (-0.1-(-0.03)**	**0.002**	**-0.05 (-0.08-(-0.01))**	**0.013**
DLCO, %	**-0.08 (-0.1-(-0.01))**	**0.019**	-0.03 (-0.1-0.04)	0.36
Pulmonary hypertension	0.2 (-2-3)	0.87		
Anti-centromere antibody positivity	-1 (-3-0.3)	0.11	-0.6 (-2-0.5)	0.28
Anti-Scl70 antibody	**3 (1-4)**	**<0.001**	**2 (0.2-3)**	**0.025**
Current NSAIDs	-0.5 (-2-1)	0.53		
Current prednisone	0.5 (-1-2)	0.52		
Prednisone, mg/day	-0.06 (-1-1)	0.91		
Methotrexate	**2 (0.06-4)**	**0.044**	**2 (0.05-4)**	**0.045**
Chloroquine	-0.8 (-3-2)	0.56		
Bosentan	**5 (2-8)**	**<0.001**	**5 (2-7)**	**<0.001**
Sildenafil	2 (-2-6)	0.37		

IL-6 is the dependent variable in this analysis. Oesophageal manometry assessment was available only for 14 patients. BMI: body mass index; CRP: C reactive protein; LDL: low-density lipoprotein; IL-6: interleukin 6; HDL: high-density lipoprotein, SSc: systemic sclerosis;
HOMA: homeostatic model assessment; NSAIDs: non-steroidalal anti-inflammatory drugs;
SCORE: Systematic Coronary Risk Assessment; FVC: forced vital capacity; FEV: forced expiratory volume; DLCO: diffusion capacity of the lung for the carbon monoxide.
Multivariable analysis is adjusted for smoking, BMI, and abdominal circumference.

regarding whether the presence of anti-Scl70 identifies a population of SSc patients who are more likely to have, or to develop, cancer (26). This could imply that there is a specific link between Scl-70 and serum IL-6 levels. Remarkably, we also found a relationship between IL-6 and the presence of digital ulcers. This is also in accordance with previous reports. Interestingly, sildenafil treatment has been shown to significantly reduce dermal fibroblast gene expression and cellular IL-6 release in patients with SSc (27), and tocilizumab has been reported to improve digital ulcers in small patient series (28).

In our series, high IL-6 levels were negatively associated with impaired respiratory function tests. This negative relationship is consistent with previous reports. In this sense, IL-6 was an independent predictor of decreased DLCO in a series of 74 patients with SSc. Therefore, circulating IL-6 levels have been proposed to be useful for the prediction of early disease progression in patients with mild interstitial lung disease of SSc (29). We also observed this negative association in the univariable analysis of our series. However, it was not confirmed in the multivariable analysis, probably due to the number of patients with diffuse SSc included in our study. With respect to this, two multicentre, randomised, double-blind, placebo-controlled trials demonstrated that the anti-IL-6 monoclonal antibody tocilizumab is associated with a less consistent decrease in predicted FVC in SSc patients with interstitial lung disease (30, 31).

The plasma cytokine IL-6 plays an important role in mediating inflammation and is a central stimulus for the acute-phase response. In particular, IL-6 induces the hepatic synthesis of CRP (32). For this reason, the relationship found between CPR and IL6 found in our work in SSc is expected and appears to be maintained in this disease. In addition, a small number of patients had arthritis. Thus, this relationship seems not to be mediated by the joint involvement that these patients may have.

Remarkably, in our cohort, the SCORE2 calculator was significantly and positively associated with serum

IL-6 levels. This occurred when this calculator was considered continuously or categorically. SCORE2 includes data related to age, sex, lipids, smoking, and blood pressure. This relationship between SCORE2, a predictor of cardiovascular events, and IL-6 is consistent with the previously mentioned association between this cytokine and arteriosclerotic disease (9-12).

We recognise the potential limitation of our study derived from its cross-sectional nature and the number of patients included in the evaluation. In this regard, due to the low number of patients recruited, the frequency of some organ manifestations is also small. For this reason, some associations between IL-6 and these features must be taken with caution. However, we were able to perform a multivariate analysis and adjust for covariates. This allowed us to highlight relevant associations of IL-6 with various disease manifestations and support the potential role of IL-6 as a marker of cardiovascular risk in patients with SSc. Obviously, IL-6 levels can vary over time and can be influenced by infections or other inflammatory states. For this reason, the presence of active infection and another inflammatory disease was an exclusion criterion in our work. We also acknowledge that the use of aspirin was frequent in our series. We do not have an exact explanation for this. We think this is probably because many patients had cardiovascular risk factors or because aspirin is used in the treatment of Raynaud's phenomenon. Also, SCORE2 has not been validated in patients with SS. Thus, the relationship of this tool with IL-6 could be consequence of the association of IL-6 with cardiovascular risk factors rather than a true link between the risk of suffering an event and this interleukin. Finally, although the characterisation of the patients was extensive, cardiovascular imaging techniques of the patients were not collected. For this reason, we have not been able to study the relationship of IL-6 with, for example, carotid atherosclerosis, echocardiographic parameters, etc.

In conclusion, IL-6 levels are associated with disease manifestations and cardiovascular risk in patients with SSc.

References

1. DI BATTISTA M, LEPRI G, CODULLO V *et al*.: Systemic sclerosis: one year in review 2023. *Clin Exp Rheumatol* 2023; 41: 1567-74. https://doi.org/10.55563/clinexprheumatol/ki76s5
2. SOBANSKI V, GIOVANNELLI J, ALLANORE Y *et al*.: Phenotypes determined by cluster analysis and their survival in the prospective European Scleroderma Trials and research cohort of patients with systemic sclerosis. *Arthritis Rheumatol* 2019; 71: 1553-70. https://doi.org/10.1002/ART.40906
3. ALLANORE Y, SIMMS R, DISTLER O *et al*.: Systemic sclerosis. *Nat Rev Dis Primers* 2015; 1. https://doi.org/10.1038/nrdp.2015.2
4. ARIAS-NUÑEZ MC, LLORCA J, VAZQUEZ-RODRIGUEZ TR *et al*.: Systemic sclerosis in northwestern Spain: a 19-year epidemiologic study. *Medicine* 2008; 87: 272-80. https://doi.org/10.1097/md.0b013E318189372F
5. AU K, SINGH MK, BODUKAM V *et al*.: Atherosclerosis in systemic sclerosis: A systematic review and meta-analysis. *Arthritis Rheum* 2011; 63: 2078-90. https://doi.org/10.1002/art.30380
6. CHOY EH, DE BENEDETTI F, TAKEUCHI T, HASHIZUME M, JOHN MR, KISHIMOTO T: Translating IL-6 biology into effective treatments. *Nat Rev Rheumatol* 2020; 16: 335-45. https://doi.org/10.1038/S41584-020-0419-Z
7. GAULDIE J, RICHARDS C, HARNISH D, LANSDORP P, BAUMANN H: Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci USA* 1987; 84: 7251-5. https://doi.org/10.1073/pnas.84.20.7251
8. JONES BE, MAERZ MD, BUCKNER JH: IL-6: a cytokine at the crossroads of autoimmunity. *Curr Opin Immunol* 2018; 55: 9-14. https://doi.org/10.1016/j.coi.2018.09.002
9. RIDKER PM, RIFAI N, STAMPFER MJ, HENNEKENS CH: Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 2000; 101: 1767-72. https://doi.org/10.1161/01.cir.101.15.1767
10. DANESH J, KAPTOGE S, MANN AG *et al*.: Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med* 2008; 5: 0600-10. https://doi.org/10.1371/journal.pmed.0050078
11. SWERDLOW DI, HOLMES MV, KUCHENBAECKER KB *et al*.: The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet* 2012; 379: 1214-24. https://doi.org/10.1016/S0140-6736(12)60110-X
12. SARWAR N, BUTTERWORTH AS, FREITAG DF *et al*.: Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet* 2012; 379: 1205-13. https://doi.org/10.1016/S0140-6736(11)61931-4
13. KHANNA D, DENTON CP, JAHREIS A *et al*.: Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. *Lancet* 2016; 387: 2630-40. https://doi.org/10.1016/S0140-6736(16)00232-4
14. KHAN K, XU S, NIHTYANOVA S *et al*.: Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. *Ann Rheum Dis* 2012; 71: 1235-42. https://doi.org/10.1136/annrheumdis-2011-200955
15. DE LAURETIS A, SESTINI P, PANTELIDIS P *et al*.: Serum interleukin 6 is predictive of early functional decline and mortality in interstitial lung disease associated with systemic sclerosis. *J Rheumatol* 2013; 40: 435-46. https://doi.org/10.3899/jrheum.120725
16. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *et al*.: 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis* 2013; 72: 1747-55. https://doi.org/10.1136/annrheumdis-2013-204424
17. KHANNA D, FURST DE, CLEMENTS PJ *et al*.: Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. *J Scleroderma Relat Disord* 2017; 2: 11-8. https://doi.org/10.5301/jsrd.5000231
18. SMITH V, HERRICK AL, INGEGNOLI F *et al*.: Standardisation of nailfold capillaroscopy for the assessment of patients with Raynaud's phenomenon and systemic sclerosis. *Autoimmun Rev* 2020; 19: 102458. https://doi.org/10.1016/j.autrev.2020.102458
19. WALLACE TM, LEVY JC, MATTHEWS DR: Use and abuse of HOMA modeling. *Diabetes Care* 2004; 27: 1487-95. https://doi.org/10.2337/diacare.27.6.1487
20. VISSEREN FLJ, MACH F, SMULDERS YM *et al*.: 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J* 2021; 42: 3227-337. https://doi.org/10.1093/eurheartj/ehab484
21. DUMOITIER N, CHAIGNE B, RÉGENT A *et al*.: Scleroderma peripheral B lymphocytes secrete interleukin-6 and transforming growth factor β and activate fibroblasts. *Arthritis Rheumatol* 2017; 69: 1078-89. https://doi.org/10.1002/art.40016
22. LIN X, DING M, CHEN T, MIN S, WANG D, JIANG G: Peripheral blood IL-6 levels in systemic sclerosis patients: Correlation between IL-6 levels and clinical phenotypes. *J Cosmet Dermatol* 2022; 21: 6086-91. https://doi.org/10.1111/jocd.15133
23. KHAN K, XU S, NIHTYANOVA S *et al*.: Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. *Ann Rheum Dis* 2012; 71: 1235-42. https://doi.org/10.1136/annrheumdis-2011-200955
24. HU PQ, FERTIG N, MEDSGER TA, WRIGHT TM: Correlation of serum anti-DNA topoisomerase I antibody levels with disease severity and activity in systemic sclerosis. *Arthritis Rheum* 2003; 48: 1363-73. https://doi.org/10.1002/art.10977
25. DENTON CP, KRIEG T, GUILLEVIN L *et al*.: Demographic, clinical and antibody characteristics of patients with digital ulcers in systemic sclerosis: data from the DUO Registry. *Ann Rheum Dis* 2012; 71: 718-21. https://doi.org/10.1136/annrheumdis-2011-200631
26. KANG KY, YIM HW, KIM IJ *et al*.: Incidence of cancer among patients with systemic scle-

rosis in Korea results from a single centre. *Scand J Rheumatol* 2009; 38: 299-303. https://doi.org/10.1080/03009740802642062

27. DI LUIGI L, SGRÒ P, DURANTI G *et al*.: Sildenafil reduces expression and release of IL-6 and IL-8 induced by reactive oxygen species in systemic sclerosis fibroblasts. *Int J Mol Sci* 2020; 21; 3161. https://doi.org/10.3390/ijms21093161
28. DAS NEVES MF, OLIVEIRA S, AMARAL MC, ALVES JD: Treatment of systemic sclerosis with tocilizumab. *Rheumatology* 2015; 54: 371-2. https://doi.org/10.1093/rheumatology/keu435
29. DE LAURETIS A, SESTINI P, PANTELIDIS P *et al*.: Serum interleukin 6 is predictive of early functional decline and mortality in interstitial lung disease associated with systemic sclerosis. *J Rheumatol* 2013; 40: 435-46. https://doi.org/10.3899/jrheum.120725
30. KHANNA D, LIN CJF, FURST DE *et al*.: Tocilizumab in systemic sclerosis: a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med* 2020; 8: 963-74. https://doi.org/10.1016/S2213-2600(20)30318-0
31. KHANNA D, DENTON CP, JAHREIS A *et al*.: Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. *Lancet* 2016; 387: 2630-40. https://doi.org/10.1016/S0140-6736(16)00232-4
32. PAPANICOLAOU DA, WILDER RL, MANOLAGAS SC, CHROUSOS GP: The pathophysiologic roles of interleukin-6 in human disease. *Ann Intern Med* 1998; 128: 127-37.https://doi.org/10.7326/0003-4819-128-2-199801150-00009