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Association between clinical features and course of systemic sclerosis and serum interleukin-8, vascular endothelial growth factor, basic fibroblast growth factor, and interferon alpha

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Abstract

Background. Certain mediators, such as soluble growth factors and cytokines, among others, are implicated in the immunopathogenesis of systemic sclerosis (SSc).

Objectives. This study aimed to examine the association between serum levels of vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), interferon alpha (IFN-α), and basic fibroblast growth factor (bFGF) and the clinical presentation and course of SSc.

Materials and methods. This longitudinal, observational study included 43 patients with SSc and 24 healthy subjects. Serum concentrations of VEGF, IL-8, IFN-α, and bFGF were measured at baseline in patients previously treated for SSc. Medical history of patients was analyzed retrospectively at the time of cytokine measurement to infer clinical correlations, and during follow-up for a median of 5 years, assessing the incidence of death or cancer.

Results. The bFGF and IFN-α concentrations differed between SSc patients and controls ($p < 0.01$). In turn, organ involvement and SSc phenotypes did not impact studied cytokine concentrations, similar to systemic steroid and/or immunosuppressant use at enrollment. However, we have documented a positive correlation between the current oral steroid dose and serum levels of IL-8 and bFGF. Furthermore, patients with a VEGF level ≥95.7 pg/mL and IFN-α level ≥3.6 pg/mL required cyclophosphamide therapy more often, currently or in the past (approx. 3-fold and 4-fold, respectively). Substantially elevated VEGF and IFN-α concentrations at baseline were associated with higher cancer occurrence ($n = 4$) during follow-up, while elevated circulating IL-8 level was associated with an increased risk of death ($n = 9$).

Conclusions. The SSc group was characterized by higher serum concentrations of bFGF and IFN-α compared to healthy controls. Patients treated with cyclophosphamide or receiving higher systemic steroid doses, thus suffering from a more severe disease type, had increased cytokine levels. Elevated circulating IFN-α and VEGF levels might be correlated with cancer, whereas raised IL-8 levels may be associated with an increased risk of death. However, further research is needed to verify our findings.

Key words: systemic sclerosis, vascular endothelial growth factor, interleukin-8, interferon alpha, basic fibroblast growth factor

Background

Systemic sclerosis (SSc) is a chronic autoimmune process characterized by the dysfunction of the endothelium, defective neovascularization and pathologic tissue fibrosis.[1–6] Clinically, it is subdivided into 2 large subtypes.[2–4,7] The first type of the disease, namely diffuse cutaneous SSc (dcSSc), is characterized by proximal cutaneous involvement, whereas the second type, limited cutaneous SSc (lcSSc), is associated with skin hardening of the distal parts of the.[2–4,8,9] In dcSSc, the incidence of renal injury, pulmonary fibrosis and generalized skin involvement are more frequent. In contrast, pulmonary arterial hypertension of a primarily vascular origin is more typical for lcSSc.[2,3,9,10] Despite recent progress in understanding the etiology of SSc, the primary causes or molecular mechanisms underlying the disease onset, progression and outcomes remain to be fully elucidated.[1,11] Some researchers claim that the pathogenesis of SSc is a consequence of endothelial destruction with consecutive activation of immune cells and fibroblasts, causing an undue accumulation of extracellular matrix (ECM) proteins.[12] Certain mediators, including soluble growth factors and cytokines, are implicated in the immunopathogenesis of SSc.[1,12]

Basic fibroblast growth factor (bFGF) is a stimulating factor of angiogenesis.[1,13] It is expressed by many tissues and organs, and is a strong mitogen for cells of mesodermal and neuroectodermal origin, including fibroblasts and endothelial cells.[13,14] It has been suggested that bFGF might be released by exocytosis.[14] After its release, bFGF remains bound to the ECM, therefore, the intensity of its release might regulate angiogenesis and fibrosis.[13,14] Vascular endothelial growth factor (VEGF) is another mediator modulating various steps of angiogenesis, vasodilation and endothelial cell permeability. It activates the proliferation and migration of cells from the endothelium and causes perivascular ECM remodeling.[13,15–19] Vascular endothelial growth factor is excreted by many cells, including activated macrophages and keratinocytes,[13] and its biological effects are extremely dose- and time-dependent.[15,16,20]

Interferon alpha (IFN-α) is a cytokine that plays a fundamental role in inflammation, immunoregulation, recognition of tumor cells, and T-cell activation. Additionally, its high expression promotes cellular and humoral autoimmunity.[5,8,21]

Interleukin-8 (IL-8), the main neutrophil stimulator, is also suggested to take part in the pathogenesis of autoimmune disorders. It was found to promote pulmonary fibrosis, a complication of SSc.[22] Moreover, it is a tumor-promoting cytokine, thus it might have a prognostic and/or predictive role as a cancer biomarker.[23,24]

Objectives

Given their involvement in the pathogenetic processes relevant to SSc, we sought to assess serum concentrations of IL-8, VEGF, bFGF, and IFN-α in a group of SSc patients to determine whether the cytokine profile correlates with disease manifestation, clinical course, comorbidities, treatment modalities, and laboratory results.

Materials and methods

Study subjects

We conducted an observational, cross-sectional study with longitudinal follow-up and comparison to healthy cases.

The case group consisted of 43 adult patients with SSc (33 women and 10 men). The SSc patients were recruited during a 5-year period (2014–2018) at the Outpatient Clinic of the Second Department of Internal Medicine (University Hospital, Kraków, Poland). The individuals were diagnosed with SSc based on the 2013 European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) classification criteria or those proposed by LeRoy and Medsger for early SSc (EaSSc) diagnosis.[25,26] All SSc patients were clinically stable at enrollment, with no change in therapy during the last 6 months. The interstitial lung disease associated with SSc (SSc-ILD) was confirmed by high-resolution computed tomography (HRCT) of the lungs and was considered clinically significant if forced vital capacity (FVC), measured using spirometry, was lower than 70% of the predicted value. Probable pulmonary artery hypertension (PAH) was defined with high probability when systolic pressure values evaluated with the use of transthoracic echocardiography were greater than 45 mm Hg.[2] This noninvasive method has a 97% specificity as compared to right heart catheterization (100%).[27] Scleroderma renal crisis was diagnosed as a new onset of renal insufficiency, however, other causes must be excluded (e.g., multiple organ dysfunction syndrome, infections, etc.). Digital ulcers were described as lesions with concomitant loss of tissue, occurring at the distal parts of fingers and toes. Patients were excluded from the study if they had an active acute or chronic infection, including viral hepatitis, other active chronic inflammatory disease (e.g., rhinosinusitis), confirmed cancer, or if they were pregnant or in the postpartum period.

Scleroderma cases were followed up for a median of 5 years to record cancer and death incidence and their potential association with cytokines measured at baseline.

The healthy group was comprised of 24 individuals (14 men and 10 women), with a median age of 32 (22–40) years. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Bioethics Committee at the Jagiellonian University Medical College (approval No. KBET/235/B/2013; date of approval: September 26, 2013).

Laboratory analysis

Blood samples were obtained from a peripheral vein with minimal tourniquet pressure. Complete blood cell (CBC)

count, plasma fibrinogen, lipid profile, and serum levels of C-reactive protein (CRP), alanine and aspartate transaminases (ALT and AST, respectively), bilirubin, alkaline phosphatase, urea, and creatinine with estimated glomerular filtration rate (eGFR, using Modification of Diet in Renal Disease formula) were measured using routine laboratory techniques. Antinuclear antibodies (ANA) were evaluated using indirect immunofluorescence assay in Hep-2 cell lines (EUROIMMUN, Lübeck, Germany). They were positive if the titer was at least 1:160. We also utilized immunoblot techniques specific for autoantibodies against topoisomerase I, centromeres, PM-Scl, RNA polymerase III, Ku, NOR 90, PDGFR, fibrillarin, and Ro-52 (EUROIMMUN). After blood sample centrifugation in separation tubes at 2000 × g for 10 min at room temperature within 2 h of collection, cytokine levels were measured, and serum supernatant was immediately frozen and stored at a temperature of –70°C until analysis.

Serum cytokines

Serum levels of VEGF, IL-8, IFN-α, and bFGF were measured using Luminex ProcartaPlex Immunoassays (eBioscience, San Diego, USA), according to the manufacturer's protocol, as well as the MAGPIX® platform (Luminex Corp., Austin, USA) and xPonent software (Luminex Corp.). The array was chosen based on the earlier information about circulating biomarkers in SSc. Results below the detection threshold were assigned a value of the lower assay limits.

Statistical analyses

The results were analyzed using STATISTICA 13.3 software (TIBCO Software, Palo Alto, USA) and R software (R Foundation for Statistical Computing, Vienna, Austria). According to the Shapiro–Wilk test, continuous variables were all non-normally distributed. They are presented as median with interquartile range (25Q–75Q).

The variables were compared using the Mann–Whitney U test to check the statistical significance of the rank distribution. A one-way covariance analysis (ANCOVA) was performed to adjust for potential confounders, including sex, age and body mass index (BMI). Categorical variables were reported as percentages and compared using the χ^2 test. To evaluate the relationship between continuous variables, Spearman's rank correlation tests were performed. Independent determinants of serum cytokine levels were established using multiple linear regression models. The R^2 was assessed as a measure of the variance. The collinearity of predictors in multiple linear regression models was checked using Pearson linear correlation coefficient and variance inflation factor (VIF). The backward stepwise regression method was used to select predictors for the regression model. To calculate the odds ratio (OR) with a 95% confidence interval (95% CI), the cut-off points were determined based on receiver operating characteristic (ROC) curves, using the Youden method. We attempted to distinguish clusters of patients based on laboratory test results, including cytokine profiles that could indicate the existence of distinct disease phenotypes. The cluster analysis was performed using the k-means method. Statistical significance was set at a p-value <0.05.

Results

Characteristics of the SSc patients

Table 1 presents the demographic characteristics of the SSc patients. Table 2 demonstrates the clinical parameters and autoantibody profiles of the SSc group. Briefly, at enrollment, the median duration of the disease was 4 (range: 1–11) years, and more than half of the patients (n = 26, 60.5%) were diagnosed with dcSSc. The most frequent feature of the disease was Raynaud's phenomenon (n = 41, 95.3%). The SSc-ILD was confirmed in 69.8% of patients, whereas other symptoms of SSc, including digital ulcers, dysphagia and probable PAH, were rare (Table 2). At enrollment, 16 SSc patients (37.2%) were treated with steroids, and 7 individuals (16.3%) were receiving immunosuppressive drugs, such as cyclophosphamide (3 patients), methotrexate (2 patients), mycophenolate mofetil (1 patient), and azathioprine (1 patient). The median daily methylprednisolone dose was 0 (range: 0–4) mg (Table 2). Other medications used, such as antihypertensive drugs, vasodilators and statins, are listed in Table 2.

The ANA were detected in the serum of all SSc patients. The most frequent were anti-topoisomerase I antibodies (Scl-70), confirmed in 24 cases (55.8%), followed by anti-Ro52 and anti-centromeric antibodies (ACA) (25.6% and 23.3%, respectively). Other types of ANA, such as anti-RNA polymerase III, anti-NOR, anti-PM/Scl, anti-Ku, and anti-Th/To, had a much lower prevalence.

Table 1. Demographic and clinical characteristics of systemic sclerosis group

Variables	Patients (n = 43)
Age [years]	59 (44–65)
Male gender, n	10
Body mass index [kg/m^2]	23.6 (22.2–26.1)
Smoking in the past, n	15
Smoking [packs/year]	0 (0–5)

Categorical variables are presented as number (percentage), and continuous variables are presented as median and interquartile range (25Q–75Q).

SSc patients had higher serum bFGF and IFN-α levels than controls

Table 3 compares the serum levels of IL-8, VEGF, bFGF, and IFN-α between the control group and the whole SSc cohort. The bFGF and IFN-α levels were approx.

Table 2. Clinical characteristics and antinuclear antibodies in systemic sclerosis patients

Clinical characteristics	Patients (n = 43)
Duration of the disease [years]	4 (1–11)
Limited disease, n (%)	17 (39.5)
Diffuse disease, n (%)	26 (60.5)
Antinuclear antibodies, n (%)	43 (100)
Anti-Scl-70 antibodies, n (%)	24 (55.8)
Anti-PM/Scl antibodies, n (%)	7 (16.3)
Anti-centromeric antibodies, n (%)	10 (23.3)
Anti-NOR antibodies, n (%)	2 (4.7)
Anti-Ro52 antibodies, n (%)	11 (25.6)
RNA polymerase III antibodies, n (%)	1 (2.3)
Anti-Th/To antibodies, n (%)	1 (2.3)
Anti-Ku antibodies, n (%)	1 (2.3)
Organ involvement	
Digital ulcers, n (%)	14 (32.6)
Abnormal nailfold capillaries, n (%)	26 (60.5)
Telangiectasia, n (%)	12 (27.9)
Raynaud's phenomenon, n (%)	41 (95.3)
Dysphagia, n (%)	10 (23.3)
Interstitial lung disease, n (%)	30 (69.8)
Pulmonary arterial hypertension, n (%)	10 (23.3)
Characteristics of the treatment	
Current steroid therapy, n (%)	16 (37.2)
Current steroid dose, recalculated to methylprednisolone [mg/day]	0 (0–4)
Systemic steroid therapy [years]	0 (0–3)
Immunosuppressive treatment (currently or in the past)	
Azathioprine, n (%)	5 (11.6)
Cyclophosphamide, n (%)	16 (37.2)
Methotrexate, n (%)	12 (27.9)
Mycophenolate mofetil, n (%)	5 (11.6)
Comorbidities	
Hypertension, n (%)	18 (41.9)
Diabetes mellitus, n (%)	3 (7)
Hypercholesterolemia, n (%)	18 (41.9)
Medications	
Angiotensine converting enzyme inhibitors or angiotensin receptor antagonists, n (%)	21 (48.8)
Statins, n (%)	13 (30.2)
Beta-blockers, n (%)	10 (23.3)
Diuretics, n (%)	10 (23.3)
Calcium channel blockers, n (%)	26 (60.5)

Categorical variables are presented as number (percentage), and continuous variables are presented as median and interquartile range (25Q–75Q).

1.2-fold and 2.4-fold higher in SSc than in healthy controls ($p < 0.01$). We found a similar trend of increased serum VEGF levels in the SSc group, however, the difference was not statistically significant ($p = 0.0501$). Median IL-8 levels were similar in patients and controls. However, the highest levels of IL-8 and VEGF were observed in SSc patients (Fig. 1). Furthermore, there were no differences in the median levels of circulating IL-8, VEGF, bFGF, and IFN-α between diffuse and limited SSc phenotypes (Fig. 2) and regarding organ involvement, such as digital ulcers, abnormal nailfold capillaries, telangiectasia, SSc-ILC, or probable PAH. In addition, internal comorbidities such as arterial hypertension and hypercholesterolemia did not alter cytokine concentrations.

Serum cytokine levels at enrollment were associated with current steroid doses and were higher in patients treated with cyclophosphamide currently or in the past

Expectedly, circulating IFN-α correlated positively with biomarkers of inflammation, such as CRP ($r = 0.42$, $p = 0.005$) and white blood cell count ($r = 0.31$, $p = 0.005$). On the other hand, patients treated with systemic steroids (n = 16, 37.2%) or other immunosuppressants at enrollment (n = 7, 16.3%) did not differ in terms of studied cytokine levels (Table 4). However, we have documented a positive correlation between the current oral steroid dose and serum levels of IL-8 ($r = 0.46$, $p = 0.002$) and bFGF ($r = 0.34$, $p = 0.025$). Furthermore, patients with IL-8 ≥ 6.02 pg/mL were approx. 3 times more often treated with systemic steroids. Interestingly, patients with VEGF levels ≥95.7 pg/mL (n = 12) and IFN-α ≥ 3.6 pg/mL (n = 14) required cyclophosphamide therapy more often currently or in the past (approx. 3-fold and 4-fold, respectively).

Other medications used, such as antihypertensives, vasodilators and statins, did not influence studied cytokine levels (data not shown).

Higher circulating VEGF and IFN-α levels at baseline were associated with increased cancer risk, whereas higher IL-8 was documented in patients who died during follow-up

Circulating VEGF elevated by ≥232.5 pg/mL and an increase in IFN-α level by ≥3.6 pg/mL at baseline were linked with a higher risk of developing a neoplastic disease. During the follow-up for a median of 5 years, 4 SSc patients had been diagnosed with the following: lung cancer (n = 2), squamous cell carcinoma of unknown origin (n = 1), and multiple myeloma with concomitant breast cancer (n = 1).

Furthermore, those with IL-8 levels ≥15.3 pg/mL had a 4 times (95% CI: 1.7–10.3) higher risk of death. Nine SSc patients died during the follow-up period. The causes of death included lung cancer (n = 2), renal crisis (n = 1), multiple-organ failure (n = 1), and in 5 cases, the reason of death was unknown.

Table 3. Serum cytokine levels of the study subjects

Studied cytokine	Patients (n = 43)	Controls (n = 24)	Z	p-value
IL-8 [pg/mL]	6.9 (3.5–15.3)	5.2 (4.7–6.6)	0.949	0.3457
VEGF [pg/mL]	87 (55.5–162.9)	60.9 (44.6–84.9)	1.495	0.0501
bFGF [pg/mL]	2.2 (1.8–3.9)	1.8 (0.9–1.8)	2.654	0.0081*
IFN-α [pg/mL]	3.6 (2.3–3.6)	1.5 (0.7–1.5)	5.730	<0.0001*

Continuous variables are presented as median and interquartile range (25Q–75Q). The statistically significant results are marked with an asterisk (*).
The value of the z test statistic for the Mann–Whitney U test was presented. bFGF – basic fibroblast growth factor; IFN-α – interferon alpha; IL-8 – interleukin-8; VEGF – vascular endothelial growth factor.

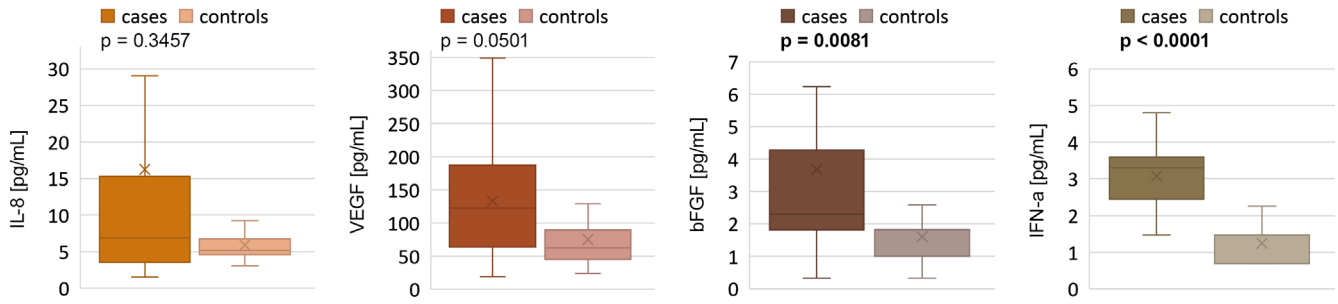

Fig. 1. Serum cytokines of the study subjects. The p-value of the Mann–Whitney U test

bFGF – basic fibroblast growth factor; IFN-α – interferon alpha; IL-8 – interleukin-8; n – number; VEGF – vascular endothelial growth factor. The chart columns represent the range (whiskers), interquartile range (box), median (line), and mean values (X mark).

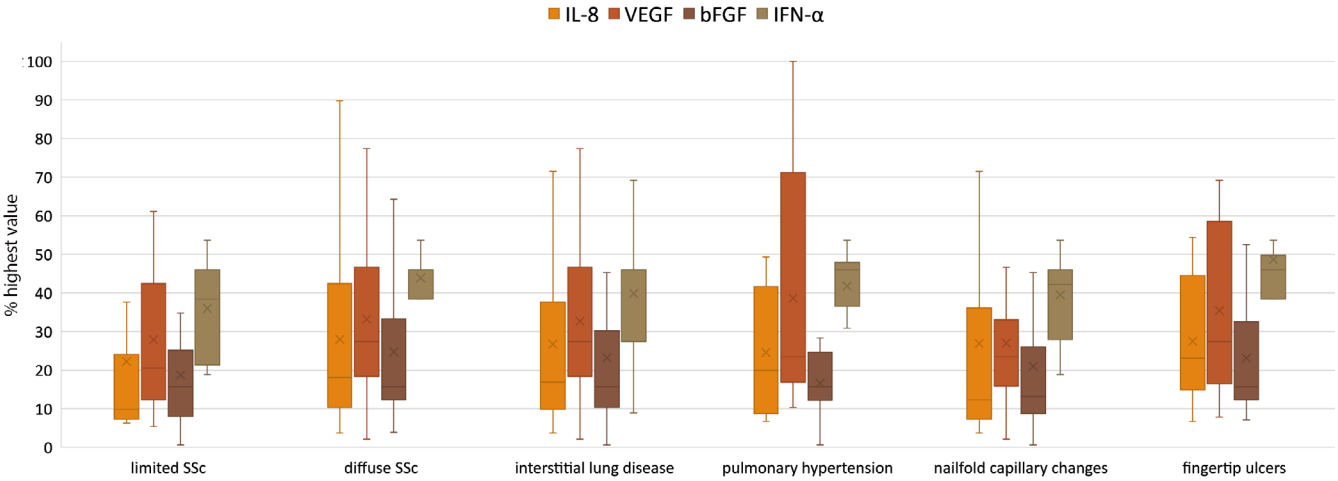

Fig. 2. Relative cytokine levels (% highest value) in different clinical manifestations of systemic sclerosis (SSc)

bFGF – basic fibroblast growth factor; IFN-α – interferon alpha; IL-8 – interleukin-8; n – number; VEGF – vascular endothelial growth factor. The chart columns represent the range (whiskers), interquartile range (box), median (line), and mean values (X mark).

Table 4. Comparison of cytokine levels between patients treated with steroids and immunosuppressants compared to those not receiving these medications at enrollment

Studied cytokine	Patients on steroids (n = 16)	Patients not receiving steroids (n = 27)	z	p-value	Patients on immunosuppressive treatment other than steroids (n = 7)	Patients not receiving immunosuppressive treatment other than steroids (n = 36)	z	p-value
IL-8 [pg/mL]	10.02 (7.09–21.03)	4.37 (2.96–9.79)	2.579	0.27	7.34 (3.54–17.85)	6.85 (3.34–14.65)	0.231	0.997
VEGF [pg/mL]	113.26 (80.11–179.08)	74.34 (51.16–144.16)	1.37	0.38	87.64 (66.62–152.39)	83.54 (55.16–162.93)	0.313	0.56
bFGF [pg/mL]	2.74 (1.81–6.35)	1.86 (1.57–3.31)	1.401	0.47	3.91 (1.81–6.18)	2.15 (1.69–3.46)	0.893	0.14
INF-α [pg/mL]	3.6 (3–3.6)	3 (1.87–3.6)	0.812	0.78	1.48 (1.48–3.6)	3.6 (3–3.6)	−1.705	0.13

Continuous variables are presented as median and interquartile range (25Q–75Q) . bFGF – basic fibroblast growth factor; IFN-α – interferon alpha; IL-8 – interleukin-8; VEGF – vascular endothelial growth factor.

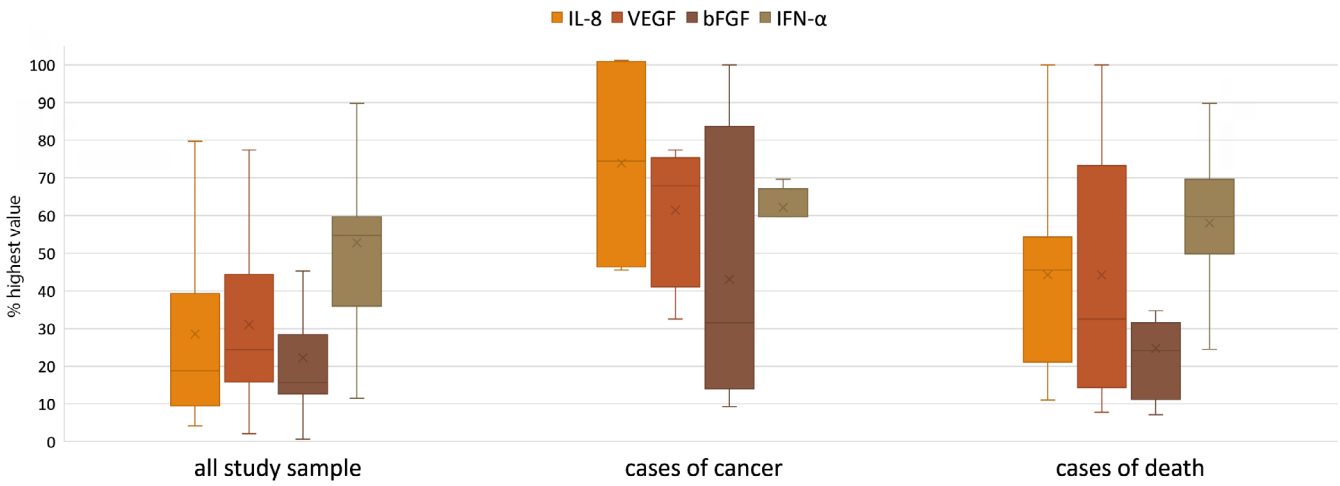

Fig. 3. Relative cytokine levels (% highest value) in different outcomes at follow-up

bFGF – basic fibroblast growth factor; IFN-α – interferon alpha; IL-8 – interleukin-8; VEGF – vascular endothelial growth factor. The chart columns represent the range (whiskers), interquartile range (box), median (line), and mean values (X mark).

The relative levels of the measured cytokines and outcomes at follow-up are presented in Fig. 3.

Independent determinants of higher serum cytokine levels in SSc patients

Multiple linear regression models demonstrated that in SSc, blood hemoglobin concentration was the most potent independent determinant of higher IL-8 levels (Table 5). On the other hand, VEGF correlated with elevated CRP, ALT, and, interestingly, higher maximal pulmonary artery pressure (mPAP) (Table 5). In addition, CRP was the most potent positive predictor of higher bFGF and IFN-α concentrations (Table 5).

Cluster analysis of SSc patients revealed 2 different phenotypes

In a cluster analysis based on laboratory test results, we have distinguished 2 clearly defined phenotypes characterized in Table 6. Both had similar clinical presentation of the disease, but cluster 2 was characterized by a higher ANA titer, increased CRP levels, and elevated levels of all the serum cytokines (Table 6). Patients in cluster 2 were also administered higher oral corticosteroid doses ($p = 0.054$). Furthermore, this cluster contained all but 1 cancer patient (Table 6).

Table 5. Multiple linear regression models showing associations between interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and interferon alpha (IFN-α), with basic laboratory variables and selected clinical features of systemic sclerosis

Studied cytokine	Predictors of cytokine concentration	β	95% CI	R^2	F	p-value
IL-8	Hb [g/dL]	0.399	(0.23–0.57)	0.20	2.89	0.045
	glucose [mmol/L]	–0.243	(–0.4––0.09)			
	lymphocytes [thous/uL]	–0.346	(–0.52––0.18)			
VEGF	CRP [mg/L]	0.228	(0.07–0.38)	0.27	2.97	0.03
	mPAP [mm Hg]	0.286	(0.13–0.44)			
	triglicerides [mmol/L]	–0.309	(–0.47––0.15)			
	ALT [U/L]	0.249	(0.09–0.41)			
bFGF	CRP [mg/L]	0.416	(0.27–0.56)	0.30	5.05	0.005
	ALT [U/L]	0.337	(0.19–0.48)			
	mPAP [mm Hg]	–0.189	(–0.33––0.05)			
IFN-α	CRP [mg/L]	0.575	(0.42–0.73)	0.36	5.01	0.006
	HDL [mmol/L]	0.260	(0.11–0.41)			
	lymphocytes [thous/uL]	0.320	(0.17–0.47)			

For all multiple regressions and all predictors, variance inflation factor (VIF) was <1.3; β – standardized regression coefficient; ALT – alanine transaminase; CI – confidence interval; CRP – C-reactive protein; Hb – hemoglobin; HDL – high density lipoprotein; mPAP – maximal pulmonary artery pressure.

Table 6. Two clusters among systemic sclerosis (SSc) patients based on the cytokine profile, clinical features and cancer frequency

Analytes and features		Cluster 1	Cluster 2	p-value
Analyte	IL-8 [pg/mL]	8.22 (4.67–11.77)	34.76 (6.76–76.29)	0.00063*
	VEGF [pg/mL]	67.58 (55.65–79.52)	209.37 (174.74–244)	<0.00001*
	bFGF [pg/mL]	2.41 (1.77–3.06)	4.84 (2.5–7.18)	0.054
	IFN-α [pg/mL]	3.02 (2.46–3.58)	3.59 (3.08–4.1)	0.033*
Feature	age [years]	54.3 (49.32–59.28)	59.31 (52.55–66.07)	0.24
	body mass index [kg/m^2]	24.67 (23.17–26.16)	24.55 (21.94–27.16)	0.93
	duration of the disease [years]	5.95 (3.35–8.55)	6.15 (2.41–9.9)	0.93
	pulmonary artery systolic pressure [mm Hg]	36.19 (33.99–38.38)	39.17 (33.66–44.68)	0.2
	daily methylprednisolone dose [mg/day]	1.47 (0.54–2.39)	4.46 (0.06–8.99)	0.054
	max. ANA [titer]	1:12,000 (1:9000–15,000)	1:18,000 (1:17,000–21,000)	0.006*
	C-reactive protein [mg/L]	7.22 (4.83–9.6)	13.73 (4.77–22.69)	0.0049*
Patients without cancer, n		28	8	0.025*
Patients with cancer, n		1	3	

Continuous variables are presented as median and interquartile range (25Q–75Q). Categorical variables are presented as numbers. The statistically significant results are marked with an asterisk (*). ANA – antinuclear antibodies; bFGF – basic fibroblast growth factor; IFN-α – interferon alpha; IL-8 – interleukin-8; VEGF – vascular endothelial growth factor.

Discussion

The present study documents that SSc patients have higher serum bFGF and IFN-α levels compared to healthy controls. Furthermore, there was an association between all studied cytokine levels and immunosuppressive treatment. Finally, elevated baseline VEGF and IFN-α levels predisposed to an increased cancer risk, whereas elevated IL-8 was associated with a higher incidence of death during follow-up for a median of 5 years. An increase in pro-angiogenic growth factors, such as bFGF and VEGF, may represent the extent of endothelial damage and an ongoing attempt at angiogenic tissue.[28] Previous studies concerning bFGF seem to be controversial. Hummers et al. and Kadono et al. noted that the levels of bFGF in SSc patients were statistically significantly higher than the values found in controls, while Distler et al. did not confirm that observation.[20,28,30] In line with our results, Hummers et al. did not find bFGF to correlate with any clinical measures, including concomitant vascular disease.[28] Other researchers have demonstrated the elevation of circulating VEGF in SSc patients, that can normalize during immunosuppressive therapy.[16,18,20,31–34] Hummers et al. indicated that higher serum levels of VEGF are more frequent in the earliest stages of the disease and in those without peripheral ulcers, therefore, VEGF plays a protective role against ischemic manifestations.[28] Choi et al. highlighted that patients with dcSSc had elevated levels of serum VEGF compared to lcSSc.[32] Compared to Hashimoto et al., they underlined that circulating VEGF levels positively correlated with the extent of skin involvement and were inversely correlated with nailfold capillary density.[32,34] However, similar to our results, no significant differences were found by Hummers et al. and Choi et al. in the levels of circulating VEGF between SSc patients with and without organ involvement.[28,32] Papaioannou et al. reported an association between serum VEGF levels and systemic pulmonary artery pressure (sPAP), suggesting a potential role of this growth factor in the pathogenesis of PAH in the course of SSc,[16] which was in line with our results. De Santis et al. confirmed an association between elevated VEGF levels and ILD.[33] Unlike previous studies, we did not find a relationship between VEGF and disease subtype or SSc duration.[16,28,29,32,34]

Also, we did not find any correlation between IFN-α, elevated in the SSc cases, and clinical manifestations of the disease or basic laboratory abnormalities. On the contrary, data presented by Kim et al. suggest that IFN-α may be higher in dcSSc as compared to lcSSc patients, and higher in cases of SSc-associated lung fibrosis, suggesting that IFN-α may play a role in tissue injury.[8] These observations are in line with results presented by Chizzolini et al.,[5,8] stating that ubiquitous antigens, including topoisomerase-1, promote the production of IFN-α, probably by interacting with Toll-like receptors.[5] Interestingly, Black et al. claimed that the treatment with IFN-α may, in fact, be deleterious in SSc.[7]

Opposite to our findings, data presented by Wu et al. show that SSc patients seem to have a higher incidence of elevated IL-8 levels compared to unaffected controls.[35] Gourh et al. stated that higher IL-8 level was associated with more severe restrictive lung disease at the baseline visit,[36] consistent with the results of Schmidt et al., who found that elevated IL-8 in bronchoalveolar lavage was correlated with SSc-ILD and worse results of lung function tests.[37] In contrast, we could not find any correlation of IL-8 with organ involvement, similar to McMahan et al., who found no significant associations between serum IL-8 levels and skin score or disease duration.[38]

In our dataset, SSc patients with elevated VEGF and IFN-α levels had a higher frequency of cancers, which was not described by other authors.[16,18,20,28,31–34] Previous investigations have confirmed that VEGF is a crucial mediator in cancer development affecting angiogenesis in patients without SSc, and is upregulated by oncogene expression, many growth factors and hypoxia.[24,39–41] In turn, IFN-α is a central immunomodulatory agent relevant in all stages of cancer development, conferring effects from an anti-tumor enhancement of the immune response in early stages to immunosuppressive function exploited through deregulated transcription of pro-tumorigenic IFN-stimulated genes, thus contributing to cancer escape in later stages.[42]

The higher incidence of death in SSc patients with elevated baseline IL-8 levels merits comment. The analogous observation was described in patients with cancers and without coexisting SSc.[23,24] Moreover, Ma et al. suggested that elevated serum IL-8 levels might help identify patients with a poor prognosis due to cancer, who may benefit from more aggressive disease management.[24] However, only 2 out of 9 fatal outcomes in our follow-up could be attributed to cancer. Further studies are needed to assess whether smoking and diabetes alter the clinical course of the disease and whether the studied cytokines may serve as biomarkers of disease progression or the need for immunosuppressive treatment as well as predictors of prognosis or risk of cancer in SSc patients.

Limitations

Our research included a limited number of patients and underpowered statistical tests used in the subgroup analyses, which may be the cause for the lack of clinical correlations that have been previously found in the datasets of other authors. Additionally, patients underwent different immunosuppressive treatments, therefore, the drugs might have impacted our results. Finally, it was challenging to provide the time of first SSc symptoms since many of them are silent or unspecific for that disease (e.g., weakness, arthralgia, dyspnea). Therefore, we could not reliably report their beginning, including the Raynaud's phenomenon.

The observational design of the study does not allow for inferring of causal relations between the studied cytokines and clinical outcomes.

Conclusions

In summary, SSc is characterized by elevated bFGF and IFN-α serum levels, regardless of organ involvement. Furthermore, elevated IFN-α and VEGF serum levels might be linked with the development of cancer, whereas elevated IL-8 levels are associated with a higher death risk during a 5-year follow-up. Further research studies are necessary to verify our findings.

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References

1. Romano E, Rosa I, Fioretto BS, Guiducci S, Manetti M, Matucci-Cerinic M. A new avenue in the pathogenesis of systemic sclerosis: The molecular interface between the endothelial and the nervous systems. *Clin Exp Rheumatol*. 2019;37 Suppl 119(4):133–140. PMID:31025932.
2. Kuszmiersz P, Pacholczak-Madej R, Siwiec A, et al. Thrombin generation potential is enhanced in systemic sclerosis: Impact of selected endothelial biomarkers. *Clin Exp Rheumatol*. 2021;39 Suppl 131(4): 13–19. doi:10.55563/clinexprheumatol/d03dnc
3. Cantero-Nieto L, Álvarez-Cienfuegos A, García-Gómez JA, Ríos-Fernández R, Robledo G, Ortego-Centeno N. Association between FGF-23 levels and risk of fracture in women with systemic sclerosis. *J Clin Densitom*. 2021;24(3):362–368. doi:10.1016/j.jocd.2020.05.010
4. Cantero-Nieto L, Alvarez-Cienfuegos A, García-Gómez JA, Martin J, González-Gay MA, Ortego-Centeno N. Role of fibroblast growth factor-23 in calcinosis in women with systemic sclerosis. *Acta Reumatol Port*. 2020;45(4):259–264. PMID:33420766.
5. Chizzolini C, Brembilla NC, Montanari E, Truchetet ME. Fibrosis and immune dysregulation in systemic sclerosis. *Autoimmun Rev*. 2011; 10(5):276–281. doi:10.1016/j.autrev.2010.09.016
6. Rentka A, Hársfalvi J, Berta A, et al. Vascular endothelial growth factor in tear samples of patients with systemic sclerosis. *Mediators Inflamm*. 2015;2015:573681. doi:10.1155/2015/573681
7. Black CM, Silman AJ, Herrick AI, et al. Interferon-alpha does not improve outcome at one year in patients with diffuse cutaneous scleroderma: Results of a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum*. 1999;42(2):299–305. doi:10.1002/1529-0131(199902)42:2<299::AID-ANR12>3.0.CO;2-R
8. Kim D, Peck A, Santer D, et al. Induction of interferon-α by scleroderma sera containing autoantibodies to topoisomerase I: Association of higher interferon-α activity with lung fibrosis. *Arthritis Rheum*. 2008;58(7):2163–2173. doi:10.1002/art.23486
9. Pacholczak-Madej R, Kuszmiersz P, Bazan-Socha S, et al. Endothelial dysfunction in patients with systemic sclerosis. *Postepy Dermatol Alergol*. 2020;37(4):495–502. doi:10.5114/ada.2019.83501
10. Ahmadi R, Hajialilo M, Ghorbanihaghjo A, et al. FGF-23, klotho and vitamin D levels in scleroderma. *Iran J Public Health*. 2017;46(4): 530–536. PMID:28540270.
11. Iannone F, Praino E, Rotondo C, et al. Body mass index and adipokines/cytokines dysregulation in systemic sclerosis. *Clin Exp Immunol*. 2021;206(2):153–160. doi:10.1111/cei.13651
12. Shirai Y, Okazaki Y, Inoue Y, et al. Elevated levels of pentraxin 3 in systemic sclerosis: Associations with vascular manifestations and defective vasculogenesis. *Arthritis Rheumatol*. 2015;67(2):498–507. doi:10.1002/art.38953
13. Kikuchi K, Hoashi T, Kanazawa S, Tamaki K. Angiogenic cytokines in serum and cutaneous lesions of patients with polyarteritis nodosa. *J Am Acad Dermatol*. 2005;53(1):57–61. doi:10.1016/j.jaad.2005.02.018
14. Lawrence A, Khanna D, Misra R, Aggarwal A. Increased expression of basic fibroblast growth factor in skin of patients with systemic sclerosis. *Dermatol Online J*. 2006;12(1):2. PMID:16638370.
15. Müller-Ladner U, Distler O, Ibba-Manneschi L, Neumann E, Gay S. Mechanisms of vascular damage in systemic sclerosis. *Autoimmunity*. 2009;42(7):587–595. doi:10.1080/08916930903002487

16. Papaioannou AI, Zakynthinos E, Kostikas K, et al. Serum VEGF levels are related to the presence of pulmonary arterial hypertension in systemic sclerosis. *BMC Pulm Med*. 2009;9:18. doi:10.1186/1471-2466-9-18
17. Ding S, Liang Y, Zhao M, et al. Decreased microRNA-142-3p/5p expression causes CD4+ T cell activation and B cell hyperstimulation in systemic lupus erythematosus. *Arthritis Rheum*. 2012;64(9):2953–2963. doi:10.1002/art.34505
18. Zhan H, Li H, Liu C, Cheng L, Yan S, Li Y. Association of circulating vascular endothelial growth factor levels with autoimmune diseases: A systematic review and meta-analysis. *Front Immunol*. 2021;12: 674343. doi:10.3389/fimmu.2021.674343
19. Allanore Y, Borderie D, Airo P, et al. Lack of association between three vascular endothelial growth factor gene polymorphisms and systemic sclerosis: Results from a multicenter EUSTAR study of European Caucasian patients. *Ann Rheum Dis*. 2007;66(2):257–259. doi:10.1136/ard.2006.054346
20. Distler O, Del Rosso A, Giacomelli R, et al. Angiogenic and angiostatic factors in systemic sclerosis: Increased levels of vascular endothelial growth factor are a feature of the earliest disease stages and are associated with the absence of fingertip ulcers. *Arthritis Res*. 2002;4(6):R11. doi:10.1186/ar596
21. De Ceuninck F, Duguet F, Aussy A, Laigle L, Moingeon P. IFN-α: A key therapeutic target for multiple autoimmune rheumatic diseases. *Drug Discov Today*. 2021;26(10):2465–2473. doi:10.1016/j.drudis.2021.06.010
22. Nielepkowicz-Goździńska A, Fendler W, Robak E, et al. Exhaled IL-8 in systemic lupus erythematosus with and without pulmonary fibrosis. *Arch Immunol Ther Exp (Warsz)*. 2014;62(3):231–238. doi:10.1007/s00005-014-0270-5
23. Schalper KA, Carleton M, Zhou M, et al. Elevated serum interleukin-8 is associated with enhanced intratumor neutrophils and reduced clinical benefit of immune-checkpoint inhibitors. *Nat Med*. 2020;26(5):688–692. doi:10.1038/s41591-020-0856-x
24. Ma Y, Ren Y, Dai ZJ, Wu CJ, Ji YH, Xu J. IL-6, IL-8 and TNF-α levels correlate with disease stage in breast cancer patients. *Adv Clin Exp Med*. 2017;26(3):421–426. doi:10.17219/acem/62120
25. 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. *Arthritis Rheum*. 2013;65(11):2737–2747. doi:10.1002/art.38098
26. LeRoy EC, Medsger TA. Criteria for the classification of early systemic sclerosis. *J Rheumatol*. 2001;28(7):1573–1576. PMID:11469464.
27. Mukerjee D, St George D, Knight C, et al. Echocardiography and pulmonary function as screening tests for pulmonary arterial hypertension in systemic sclerosis. *Rheumatology (Oxford)*. 2004;43(4):461–466. doi:10.1093/rheumatology/keh067
28. Hummers LK, Hall A, Wigley FM, Simons M. Abnormalities in the regulators of angiogenesis in patients with scleroderma. *J Rheumatol*. 2009;36(3):576–582. doi:10.3899/jrheum.080516
29. Rajkumar VS, Sundberg C, Abraham DJ, Rubin K, Black CM. Activation of microvascular pericytes in autoimmune Raynaud's phenomenon and systemic sclerosis. *Arthritis Rheum*. 1999;42(5):930–941. doi:10.1002/1529-0131(199905)42:5<930::AID-ANR11>3.0.CO;2-1
30. Kadono T, Kikuchi K, Kubo M, Fujimoto M, Tamaki K. Serum concentrations of basic fibroblast growth factor in collagen diseases. *J Am Acad Dermatol*. 1996;35(3 Pt 1):392–397. doi:10.1016/S0190-9622(96)90603-9
31. Mittag M, Beckheinrich P, Haustein UF. Systemic sclerosis-related Raynaud's phenomenon: Effects of iloprost infusion therapy on serum cytokine, growth factor and soluble adhesion molecule levels. *Acta Derm Venereol*. 2001;81(4):294–297. doi:10.1080/00015550152572976
32. Choi JJ, Min DJ, Cho ML, et al. Elevated vascular endothelial growth factor in systemic sclerosis. *J Rheumatol*. 2003;30(7):1529–1533. PMID:12858453.
33. De Santis M, Ceribelli A, Cavaciocchi F, et al. Nailfold videocapillaroscopy and serum VEGF levels in scleroderma are associated with internal organ involvement. *Auto Immun Highlights*. 2016;7(1):5. doi:10.1007/s13317-016-0077-y
34. Hashimoto N, Iwasaki T, Kitano M, Ogata A, Hamano T. Levels of vascular endothelial growth factor and hepatocyte growth factor in sera of patients with rheumatic diseases. *Mod Rheumatol*. 2003;13(2): 129–134. doi:10.3109/s10165-002-0211-8
35. Wu B, Wang W, Zhan Y, et al. CXCL13, CCL4, and sTNFR as circulating inflammatory cytokine markers in primary and SLE-related autoimmune hemolytic anemia. *J Transl Med*. 2015;13:112. doi:10.1186/s12967-015-0474-4
36. Gourh P, Arnett FC, Assassi S, et al. Plasma cytokine profiles in systemic sclerosis: Associations with autoantibody subsets and clinical manifestations. *Arthritis Res Ther*. 2009;11(5):R147. doi:10.1186/ar2821
37. Schmidt K, Martinez-Gamboa L, Meier S, et al. Bronchoalveoloar lavage fluid cytokines and chemokines as markers and predictors for the outcome of interstitial lung disease in systemic sclerosis patients. *Arthritis Res Ther*. 2009;11(4):R111. doi:10.1186/ar2766
38. McMahan Z, Schoenhoff F, Van Eyk JE, Wigley FM, Hummers LK. Biomarkers of pulmonary hypertension in patients with scleroderma: A case–control study. *Arthritis Res Ther*. 2015;17(1):201. doi:10.1186/s13075-015-0712-4
39. Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. *Oncology*. 2005;69 Suppl 3:4–10. doi:10.1159/000088478
40. Frezzetti D, Gallo M, Maiello MR, et al. VEGF as a potential target in lung cancer. *Expert Opin Ther Targets*. 2017;21(10):959–966. doi:10.1080/14728222.2017.1371137
41. Alevizakos M, Kaltsas S, Syrigos KN. The VEGF pathway in lung cancer. *Cancer Chemother Pharmacol*. 2013;72(6):1169–1181. doi:10.1007/s00280-013-2298-3
42. Vidal P. Interferon α in cancer immunoediting: From elimination to escape. *Scand J Immunol*. 2020;91(5):e12863. doi:10.1111/sji.12863