The significance of interleukin-6 and C-reactive protein in systemic sclerosis: a systematic literature review

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ABSTRACT

Objectives. *Interleukin-6 (IL-6) may play a role in the pathogenesis of SSc. C-reactive protein (CRP), an acute phase reactant induced by IL-6, may be a prognostic marker in SSc. The goal of this systematic review was to address the significance and clinical application of IL-6 and CRP in systemic sclerosis (SSc).*

Methods. *A literature search was conducted to identify English-language original articles within PubMed, Scopus, and Medline database from inception to May 30, 2013 using keywords "systemic sclerosis or scleroderma and C-reactive protein or interleukin-6".*

Results. *The search resulted in 156 relevant articles. Some single nucleotide polymorphisms and gene-gene interactions affect SSc predisposition, manifestation and expression of IL-6. Studies in animal models show IL-6 and IL-6 trans-signalling are involved in SSc disease development. Derangements of T and B cells function regulate IL-6 in SSc pathogenesis. Fibroblasts, T/B cells, monocytes, macrophages, dendritic cells and endothelial cells participate in IL-6 expression and interact with each other resulting in tissue sclerosis. Up-regulation of serum IL-6 and CRP levels are evident in SSc patients and associated with disease activity, severity, disability, worse outcome and reduced survival. Targeted IL-6 therapy in SSc has occurred in small cases series and within a multisite trial that is under way.*

Conclusions: *Studies show IL-6 and CRP are important in SSc both in pathogenesis and clinical manifestations and may be useful indicators of disease activity, severity, and poor prognosis. IL-6 could be a relevant treatment target in SSc.*

Introduction

Systemic sclerosis (SSc) or scleroderma is a systemic autoimmune rheumatic disease characterised by autoimmunity; fibrosis and dysfunction in vascular regulatory mechanisms highlighted by vasculopathy of microcirculation (1). SSc has increased extracellular matrix protein deposition due to increased fibroblast biosynthetic activity (2). SSc is rare and has a female predisposition (3, 4). It is classified into diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc) subsets according to extent of cutaneous involvement (5). Patients with dcSSc have more skin involvement and worse survival rates than lcSSc (6-9).

The pathogenesis of SSc is still obscure as there are no true animal models, but immune activation is present with complex cytokines and protein interactions. T helper 1 lymphocyte (Th1) cytokines *e.g.* interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), interleukin-1α (IL-1α), IL-2 and Th17 cytokines *e.g.* IL-17, IL-21, IL-23, IL-22 promote inflammation in SSc, while Th2 cytokines *e.g.* IL-4, IL-13, IL-6, IL-10 contribute tissue fibrosis (10). Interestingly, IL-6 has a pro-inflammatory function via Th17 differentiation in the presence of transforming growth factor-β (TGF-β)/IL-21 and inhibition of T regulatory lymphocyte (Treg) differentiation as well as fibrogenesis via stimulation of collagen production and inhibition of collagenase synthesis (10).

IL-6 also participates in the pathogenesis of a variety of chronic inflammatory disease such as rheumatoid arthritis (RA) (11) especially for systemic bone loss and structural bone damage (12). Treatment with a humanised anti-interleukin-6 receptor monoclonal antibody corrects Th17/Treg cell imbalance (13) and demonstrate efficacy and safety in

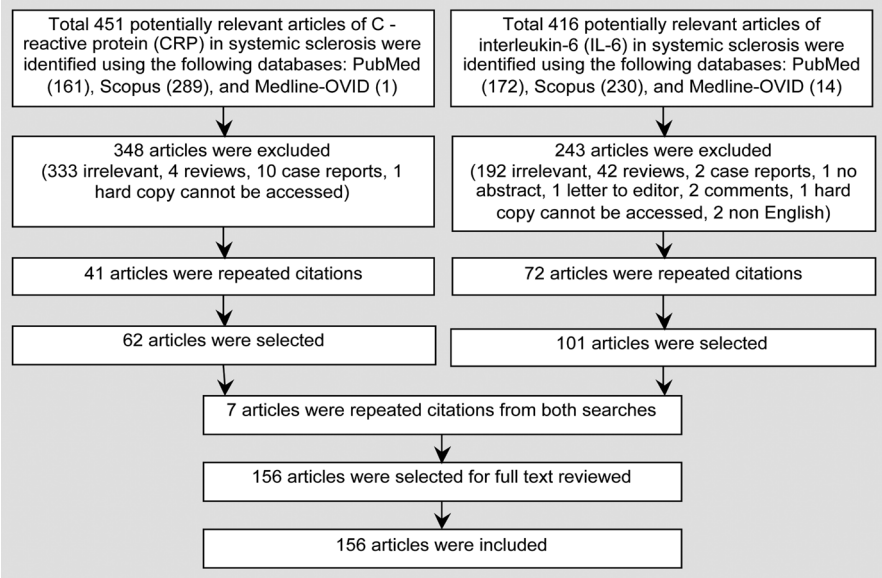

Fig. 1. Flow diagram of literature search of the systematic review of IL-6 and CRP in SSc.

RA (14). CRP, an acute phase response protein produced prominently under the transcriptional control by IL-6, serves as an assessment of disease activity in many inflammatory conditions (15). CRP is correlated with serum levels of IL-6 in RA (16) and is a surrogate marker in RA for disease activity and increased erosions (17), whereas in SLE, CRP is used more to predict active infection rather than an exacerbation of SLE (18).

We previously concisely reviewed the importance of IL-6 in SSc; constructing a diagram of signalling from various cell types in SSc (19). We have also published the importance of CRP in data from the Canadian Scleroderma Research Group (CSRG) where elevated CRP was especially prevalent in early dcSSc and was associated with SSc disease activity, severity, and poor survival (20). The purpose of this extensive systematic literature review was to address the overall and organ-specific significance of IL-6 and CRP in SSc and potential therapeutic targets that decrease IL-6 in active SSc.

Methods

We conducted a systematic review of the literature to determine the significance and clinical application of IL-6 and CRP in SSc. We searched for English-language original articles indexed in PubMed, Scopus, and Medline from the inception through May 30, 2013 using the following key words: (systemic sclerosis OR scleroderma) AND interleukin-6 (IL-6); and (systemic sclerosis OR scleroderma) AND C-reactive protein (CRP). Publications were deemed relevant to this review if they reported results from *in vitro* or *in vivo* studies, animal models, observational cohorts, clinical studies/trials, and both positive and negative studies regarding IL-6 or CRP in SSc were reviewed. Publications were excluded if they were irrelevant, review articles, letters to the editors, or did not study systemic sclerosis. Thus reports on morphea were not included.

Results

An overview of the literature search is found in Figure 1. Ultimately, 156 relevant articles were chosen for the review.

1. Genetic susceptibility to scleroderma in part of interleukin-6 and C-reactive protein

Multiple genes are involved in immune regulation affecting Th1/Th2 cytokines production and balance via regulation of T helper cell differentiation and activation. Single nucleotide polymorphisms (SNPs) variants in Signal Transducer and Activator of Transcription 4 (STAT4) and T-box expressed in T cell 21 (TBX21) that regulate Th1 cells though promotion of Th1 cytokines and suppression of Th2 cytokines are associated with SSc. The TT genotype of TBX21 rs 11650354 SNP variant has a recessive pattern for SSc susceptibility, while the A allele of STAT4 rs 11889341 has a dominant pattern. SSc patients carrying the CC genotype of TBX21 rs 11650354 have higher pro-inflammatory cytokines - interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) levels; and those with the TT genotype have elevated IL-2, IL-5, IL-4, and IL-13 levels (21). There are also significant gene-gene interactions for SNPs in cytokine genes for SSc subset susceptibility such as IL-2 G-330T, IL-6 C-174G, and IFN-γ AUTR5644T SNPs in lcSSc susceptibility and IL-1R (IL-1 receptor) Cpst1970T, IL-6 Ant565G, and IL-10 C-819T SNPs in dcSSc susceptibility (22). For IL-6, SSc patients who have homozygous GG SNPs of the -597 region of the IL-6 promoter gene (IL-6 pr) have higher disease activity and worse functional scores than the heterozygous GA patients (23). SNPs in Toll-like receptor 2 (TLR2) genes (Pro631His) are associated with anti-topoisomerase (Scl70) positivity, dcSSc subset, and the development of pulmonary artery hypertension (PAH). This variant influences TLR-2–mediated cell responses in monocyte-derived dendritic cells to produce increased levels of TNF-α and IL-6 (24). The GGC allelic combination rs2069827-rs1800795-rs2069840 of IL-6 gene has an association with overall SSc susceptibility (25) but neither selected SNPs of the IL-6 receptor (IL-6R) gene nor CRP gene showed an association with overall SSc susceptibility (26, 27).

2. Scleroderma animal models and interleukin-6

There are no exact animal models of SSc where the spectrum of autoantibodies, inflammation, fibrosis and vascular changes are fully manifest. However, SSc animal models give insights into pathogenesis of SSc and can be divided into 4 inter-related categories

2.1. Role of IL-6 trans-signalling in fibrogenesis

Mouse models studying the role of IL-6 trans-signalling in fibrogenic responses are summarised in Table I.

Table I. Role of IL-6 trans-signalling in fibrogenesis.

Animal models	Characteristics	Interventions	Results
Subcutaneous injection with BLM mouse model (28)			
WT C57BL/6 mice	↑ serum IL-6 levels ↑ expression of IL-6 mRNA in skin, cutaneous lymph nodes	Anti- mIL-6R mAb prophylaxis or treatment	↓ dermal thickness ↓ dermal numbers of myofibroblasts, and mast cells
IL-6 KO mice	↓ dermal sclerosis ↓ dermal numbers of myofibroblasts, and mast cells	N/A	N/A
N*on-treated-BLM mouse model (28)*			
WT C57BL/6 mice-derived fibroblasts	Already express α-SMA	RmIL-6 stimulation Anti-mIL-6R mAb treatment	Do not alter α-SMA expression ↑ IL-6 expression ↓ α-SMA mRNA expression
IL-6 KO mice-derived fibroblasts	↓ expressions of α-SMA	RmIL-6 stimulation	↑ α-SMA mRNA expression
IL-6 KO mice-derived fibroblasts treated with RmIL-6	↑ α-SMA mRNA expression	Anti-mouse IL-6R mAb treatment	Inhibition of α-SMA mRNA expression
Mouse model injected with rh-DNA-topo I and Freund's complete adjuvant (29)			
WT C57BL/6 mice	↑ dermal thickness ↑ lung fibrosis ↑ serum/ skin/ lung IL-4, IL-6, IL-10, IL-17, IFN-γ, TNF-α, TGF-β levels ↑ serum IgG, IgM	N/A	N/A
IL-6 KO mice	↓ dermal thickness ↓ lung fibrosis ↓ serum/skin/lung IL-17 levels, ↓ serum Ig levels ↓ BALF Th2, Th17 cells ↑ BALF Th1, Treg cells	N/A	N/A
Scl-cGVHD mouse model			
Scl-cGVHD mice (30)	Express AIF-1 ↑ induce IL-6 secretionon mononuclear cells and fibroblast chemotaxis	N/A	N/A
Scl-cGVHD mice (31)	↑ serum IL-6 levels after bone marrow transplantation	Anti-mIL-6R mAb prophylaxis	↓ Severity of Scl-cGVHD ↑ CD4+CD25+FoxP3+ Treg cells

↓: decreased; ↑: increased; BLM: bleomycin; WT: wild-type; mRNA: messenger RNA; anti mIL-6R mAb: anti-mouse-IL-6 receptor monoclonal antibody; IL-6KO: IL-6-knock-out; α-SMA: α smooth muscle actin; RmIL-6: recombinant-mouse-IL6; rh-DNA-topo I: recombinant human DNA topoisomerase I; BALF: bronchoalveolar lavage fluid; Scl-cGVHD: sclerodermatous-chronic graft-versus-host disease; AIF-1: allograft inflammatory factor-1; N/A: not available.

2.2. Role of B cell and B cell compartment in fibrogenic and inflammatory responses

B lymphocyte (B cell) is the most potent antigen-presenting cell (APC) and among several cells in immune system that secrete IL-6 (19). CD19 a cell-surface signal transduction molecule of B cell, acts as a central critical positive response regulator that lowers the B cell signalling threshold resulting in amplified signalling, clonal expansion and antibody production of B cells (32). Transgenic mice that overexpress CD19 by 20-170% lose tolerance and generate autoantibodies. SSc patients also overexpress CD19 by approximately 20%, which may contribute to their intrinsic B cell abnormalities and autoantibody production (33). B cell activating factor belonging to the tumour necrosis factor family (BAFF) is a TNF-like homeostatic cytokine that supports B cell survival and differentiation. Excessive BAFF production corrupts B-cell tolerance and leads to autoimmunity (34-36). Mouse models that examined CD19 and BAFF in fibrogenesis and inflammation were summarised in Table II.

2.3. Factors affecting T cell signalling, resulting in fibrogenesis

Cytokines-cytokine receptor interactions rely on signals through pathways such as Janus kinases (JAK; JAK1, JAK2, JAK3, and Tyk2) and STAT1-STAT-6 (40-41). Their signals yield cellular proliferation, differentiation, migration, apoptosis, and cell survival, depending on the signal, tissue, and cellular context (42). The IL-6 trans-signalling pathway is mediated by the JAK/STAT1-3 pathway (43),but the STAT4 knockout ($STAT4^{-/-}$) mice subcutaneously injected with bleomycin (BLM) showed decreased dermal sclerosis, CD4, CD8 T cells infiltration, lower levels of IFN-γ, IL-2, TNF-α, and IL-6 relative to $STAT^{+/+}$ mice. In contrast, $STAT4^{-/-}$ / TSK+/ mouse model did not significantly ameliorate the fibrotic phenotype (44).

Regulation of T cell activation and tol-

Table II. Role of CD19 and BAFF in intrinsic B cell signalling and IL-6.

Animal models	Interventions	Results
CD19 KO C57BL/6 mice (37)	BLM SC injection	↓ dermal thickness, ↓lung fibrosis ↓ numbers of mast cells, macrophages, T cells, B cells dermal infiltration ↓ serum levels & mRNA of IL-4, IL-6, IL-10, IFN-γ, TNF-α, TGF-β1, MIP-2 ↓ hyaluronan over-production, ↓ serum Ig (relatively to CD19 +/+ C57BL/6 mice)
CD19 KO C57BL/6 mice-derived B cells (37)	ECM breakdown product stimulation via TLR-4	↓ mRNA & protein levels of IL-4, IL-6, IL-10, IFN-γ, TGF-β, MIP-2 (relatively to CD19 +/+ C57BL/6 mice)
CD19 KO TSK+ mice (38)	N/A	↓ skin fibrosis
CD19 KO TSK+ mice-derived B cells (38)	N/A	↓ hyper-γ -globulinemia ↓ autoantibody production ↓ IL-6 secretion
TSK+ (39)	BAFF receptor antagonist	↓ autoantibody production ↓dermal IL-6, IL-10 mRNA expression, ↑IFN-γ expression
TSK+ mice-derived B cells (39)	BAFF stimulation	↑ ability to produce IL-6

↓: decreased; ↑: increased; CD19 KO: CD19 knock-out; MIP-2: macrophage inflammatory protein-2; Ig: immunoglobulin; ECM: extracellular matrix; TLR-4: Toll-like receptor-4; TSK+ mice: tight-skin mice; N/A: not available.

erance requires 4 signal interactions (45).

1. Antigen-Major histocompatibility complex (MHC) on antigen presenting cells (APC) interact with T cell receptor (TCR)-CD3 complexes on T cells
2. B7-1(CD80) or B7-2 (CD86) on APC interact with CD28 or cytotoxic T-lymphocyte antigen 4 (CTLA-4 or CD152) on T cells
3. Inducible T cell co-stimulator ligand (ICOSL) on APC interacts with Inducible T cell co-stimulator (ICOS) on T cells
4. Programme cell death ligand 1 & 2 (PD-L1, PD-L2) on APC interact with PD-1 on T cells

In BLM-induced dermal sclerosis and lung fibrosis mouse model, ICOS knock-out ($ICOS^{-/-}$) mice has less skin and lungs fibrosis and lower TGF-β levels; in contrast, ICOSL knock-out ($ICOSL^{-/-}$) mice had more skin and lung fibrosis and higher TGF-β levels. Double-knock out ($ICOS^{-/-}$, $ICOSL^{-/-}$) mice result the same as $ICOSL^{-/-}$ mice. So apart from the ICOS/ICOSL co-stimulatory pathway, ICOS and ICOSL might have a role in development of tissue sclerosis in this mice model (46).

2.4. Experimental interventions in scleroderma animal models

Interventions that ameliorate fibrogenesis and affect IL-6 are summarised in Table III.

3. Scleroderma fibroblasts and interleukin-6

Dermal fibroblasts from early SSc patients up-regulate IL-6 secretion several-fold more than normal fibroblasts (54-60), via the accumulation of IL-6 mRNA mediated by the constitutive binding of Nuclear Factor-Kappa B (NF-κB) to the IL-6 promoter gene (55, 59, 61-62). However, one study found SSc fibroblasts did not produce more IL-6 than controls (63). Augmentation of IL-6 mRNA and IL-6 release is modulated by TNF-α with a synergistic effect from the type II IFN -IFN-γ (54, 56, 59, 64), IL-1α (64-68), IL-1β (59, 69) and platelet derived growth factor (PDGF) (70). Type I IFN; IFN-α2, also augment TLR3 expression on SSc fibroblasts primed by TGF-β resulted in enhance TLR3-induced IL-6 production (71). SSc fibroblasts constitutively up-regulate IL-1α mRNA and express IL-1α endogenously yielding an autocrine loop with further increases in IL-6 and PDGF (65-68) which in turn stimulate SSc fibroblasts. SSc macrophages, T cells, and B cells are sources of IL-1α, IL-6, basic fibroblast growth factor (bFGF), TNF-α, and TGF-β responsible for fibroblast proliferation, PDGF secretion, and PDGF receptor (PDGFR) expression (64, 72). IL-4 and IL-13 –a Th2 cytokines promote circulating bone marrow-derived fibrocyte differentiation whereas IFN-γ, IL-12 –a Th1 cytokines inhibits fibrocyte differentiation (73). CD40 is expressed on macrophages, dendritic cells, B cells, fibroblasts, and endothelial cells. CD40L (CD154) is expressed on T cells and NK cells (74). CD40 expression from fibroblasts is higher in early SSc. Ligation of CD40 by CD40L (CD154) results in increased production of IL-6, IL-8, monocytes chemoattractant protein-1 (MCP-1) and Regulated upon Activation Normal T-cell Expressed and presumably Secreted (RANTES) (75-76). IL-6 trans-signalling of fibroblasts through the JAK2/STAT3 or extracellular signal-regulated kinase/ mitogen-activated protein kinase (ERK/ MAPK) transduction pathway is responsible for a production of procollagen type 1, α-smooth muscle actin (α-SMA) and connective tissue growth factor (CTGF) (55, 77). The expression of IL-6 in SSc fibroblasts is positively correlated with collagen production (61, 63). Roscovitine- a cyclin-dependent kinase inhibitor caused decreased IL-6 production by SSc fibroblasts below the basal levels of normal fibroblasts (78).

4. Peripheral blood mononuclear cells (PBMC), dendritic cells, T and B lymphocytes, endothelial cells and interleukin-6 in scleroderma

SSc peripheral blood mononuclear cells (PBMC) secrete more IL-6 spontaneously and when stimulated compared to controls, especially SSc PBMC from patients with early disease

Table III. Experimental interventions affecting fibrogenesis and IL-6.

Animal models	Characteristics	Interventions	Results
BLM-induced dermal sclerosis mice and TSK+ mice (47)	Overt free radical production	IV Edaravone (a free radical scavenger)	↓ dermal thickness, lung fibrosis ↓ T cells, B cells, macrophages, neutrophils dermal infiltration ↓ IL-6, TGF-β1, TNF-α, IFN-γ, anti topo-I Ab, IgG, IgM
BLM-induced dermal sclerosis mice and TSK+ mice (48)	N/A	IP Sirolimus	↓ skin fibrosis ↓ IL-4, IL-6, IL-10, IL-17, TGF-β1, IFN-γ *In human, rapamycin did not differ from treatment with methotrexate but the study was under-powered (49)
BLM-induced lung fibrosis mice (50)	N/A	Col V prophylaxis	↓ lung inflammation ↓ expression of BALF IL-6, IL-17, IL-22 *Human oral Col I trial show no skin score improvement (51)
BLM-induced dermal sclerosis mice (52)	N/A	Anti-TGF-β antibody treatment	↓ IL-4, IL-6 levels *A phase I/II trial of recombinant anti-TGF β1 antibody in early dcSSc patients was ineffective (53)

↓: decreased; BLM: bleomycin; IV: intravenous; TSK+ mice: tight-skin mice; IP: intra-peritoneal; BALF: bronchoalveolar lavage fluid; Col V: collagen type V; Col I: collagen type I.

(79-82). One study found SSc alveolar macrophages produced more IL-6 but the results were not significantly different from controls (83). IL-10 and TNF-α is increased secretion by SSc PBMC (79). TNF-α is correlated with IL-6 and IL-10 production (79). Increased levels of IL-6, soluble IL-6 receptor (sIL-6R), IL-13, macrophage inflammatory protein (MIP) -1α, and RANTES but decreased levels of IL-4, IL-10, TGF-β, and macrophage derived chemokines (MDC) have been described in SSc CD3+ T cells (79, 84). Blood SSc monocytes also spontaneously secrete IL-6 which is related to serum IL-6 levels (85). IL-6 production by stimulated NK cells are elevated in dcSSc patients (86), with increased expression of IL-6 mRNA in alveolar T cells and macrophages (87). TLR-2, 3, 4-mediated stimulation of SSc monocyte-derived dendritic cells (moDC) from early dcSSc patients result in increased IL-6, TNF-α and IL-10 production (88, 89). Membrane-bound/ and soluble IL-6 receptor (mIL-6R/ and sIL-6R) is a functional receptor of IL-6. IL-6 binds to either mIL-6R or sIL-6R causing homodimerisation of membrane-bound gp130 (mgp130) then this IL-6-IL-6R-mgp130 complex transfers IL-6 signalling. Soluble gp130 (sgp130) also binds to IL-6-IL-6R complex, therefore inhibiting binding of these complexes to mgp130, so sgp130 is an inhibitor of IL-6 signalling (43). SSc PBMC produces more IL-6 and sIL-6R but not significantly more sgp130 (80). Tran-signalling of IL-6 mediates neutrophil-dependent endothelial cells activation and apoptosis (90). Centromere protein B (CENP-B) released from apoptotic endothelial cells can bind to the surface of smooth muscle cells, and subsequently stimulate the migration of smooth muscle cells and release of IL-6 (91). Topoisomerase I (Topo 1; Scl70) antibody production by autologous peripheral blood B cells via the interactions of MHC-TCR and CD40/CD40L requires IL-2 and IL-6 for topoisomerase I Th1 and Th2 cell regulation, respectively (92). In addition, IL-6 may mediate the enhanced expression of high-affinity IL-2 receptor (HIL-2R) on SSc T cells (93). T cells can induce activation of normal fibroblasts, increasing their collagen production and the expression of several markers of fibrosis including IL-6, TGF-β, α- smooth muscle actin (α-SMA), and endothelin receptor (ET-R) by expression of allograft inflammatory factor-1 (AIF-1) (94). IL-12 -a Th1 cytokine, produced by SSc PBMC is significantly elevated in SSc patients but does not seem to correlate with Th2 cytokines *e.g.* IL-4, IL-6, IL-10 and IL-13 (95). BAFF levels were significantly elevated in both dcSSc, and lcSSc compared to controls and were higher in dcSSc than lcSSc. BAFF correlated with the skin score and ESR; where increased changes in BAFF were associated with worsening organ involvement. B cells from SSc patients produced more IL-6 and IgG when stimulated with BAFF (96).

5. Serum interleukin-6 and C-reactive protein and clinical applications in scleroderma

5.1. Serum IL-6 and CRP levels in scleroderma

Many studies found serum IL-6 (63, 79, 88, 97-107) and CRP (108-117) in SSc were significantly higher than controls. Serum IL-6 levels are 5-12 times higher than controls (97, 99). The frequency of elevated serum IL-6 and CRP levels in SSc is from 50-94% for IL-6 (118-121) and 20-70% for CRP (20, 114, 122-125). However, most patients with SSc do not have elevated CRP (20, 126). Also, some studies reported no significant differences in serum IL-6 (60, 81, 85, 127-131) and CRP between SSc patients and healthy controls (132). Few studies reported increased IL-6 levels from sources other than serum. Exhaled breath in SSc had higher IL-6, than controls. IL-6 and IL-2, -4, -10, TNF-α, and IFN-γ levels have negative correlations with diffusing capacity of the lung for carbon monoxide (DLCO) and total lung capacity (TLC) % predicted (133) A different study found no increase in IL-6 in bronchoalveolar lavage fluid (BALF) in SSc and controls (85). SSc skin biopsies have more expression of IL-6, IL-8, TNF-α, vascular cell adhesion molecule-1 (VCAM-1) and p-selectin (134).

Table IV. Correlations of serum IL-6 and CRP levels with parameters.

Serum Interleukin-6 levels		
Correlation		No correlation
Positive	Negative	
Fever (119)	FVC, DLCO %predicted (104, 159)	Clinical/laboratory findings (127)
Interstitial lung disease (79, 119)		BAL cell characteristics/ BALF
Alveolitis score (140)		IL-6 in SSc-alveolitis (85)
Arthritis/ Myositis (119)		Serum adiponectin (160)
Total body skin score (102, 129)		LAMPs (161)
Extent of skin thickening (129)		
DcSSc-mRSS, peak skin score (55)		
WBC count (157), Platelets count (55)		
ESR (104) CRP (55, 104, 124)		
Serum IgG (104, 158)		
Serum P-III-P levels (63)		
Serum IL-1RA (105)		
Serum C-reactive proteins levels		
Correlation		No correlation
Positive	Negative	
MRSS (20)	PFT parameters (20, 116)	SSc-myopathy (176)
Severity of skin, lung, joint (116)	Serum albumin (173)	Bone mineral density (177)
MRI fascial thickening, synovitis (136)	Serum vitamin D (174)	Aortic stiffness (178)
EScSG Activity Index (20, 162)	Erythrocyte deformability (175)	Arterial wall dysfunction (108)
12-point Activity Index (162)		Serum pentraxin-3 (179)
Medsger Disease Severity Index (20)		Serum amyloid P (180)
HAQ Disability Index (20)		Serum adiponectin (160)
Abnormal 6MWD (163)		Serum prolactin (181)
ESR (20, 114)		Serum YKL-40 (182)
ANA titer (114)		Serum MMP-3 (147)
Serum IL-6 (55, 104, 124)		
Serum IL-13 (164)		
Serum sCD40L (165)		
Plasma microparticles (166)		
Platelets activation markers (125)		
Serum vWf (114)		
Serum NT-proCNP (167)		
Serum COMP-C3b (168)		
Serum CTX-I (169)		
Serum TAP (170)		
Serum HSP-70 (171)		
Serum Ang-2 (172)		

dcSSc: diffuse cutaneous systemic sclerosis; mRSS: modified Rodnan skin score; WBC: white blood cells; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; IgG: immunoglobulin G; P-III-P: N-terminal procollagen-III propeptide; IL-1RA: interleukin-1 receptor antagonist; FVC: forced vital capacity; DLCO: diffusing capacity of the lung for carbon monoxide; BAL: brochoalveolar lavage; BALF: bronchoalveolar lavage fluid; LAMPs: lysosome-associated membrane proteins; MRI: magnetic resonance imaging; EScSG: European Scleroderma Study Group; HAQ: health Assessment Questionnaires; 6MWD: 6-minute walk distance; ANA: antinuclear antibody; sCD40L: soluble CD40 ligand; vWf: von Willebrand factor; NT-proCNP: amino-terminal fragment of pro C-type natriuretic peptide; COMP-C3b: complex between cartilage oligomeric protein and complement activation product C3b; CTX-1: c-terminal telopeptide of collagen type I; TAP: total anti-oxidant power; HSP-70: heat shock protein-70; Ang-2: angiopoietin-2; PFT: pulmonary function test; SSc-myopathy: scleroderma-associated myopathy; YKL-40: human cartilage glycoprotein-39; MMP-3: matrix metalloproteinase-3.

5.2. IL-6 levels in dcSSc compared to lcSSc

IL-6 levels are higher in dcSSc compared to lcSSc in some studies (55, 79, 128) and not in others (98, 105). CRP levels also varied between subsets in one but not another study (113, 114). Early dcSSc had the highest IL-6 levels (88, 104, 118) and occasionally also early lcSSc (88, 102, 118). CRP is elevated more in early dcSSc and in those with a worse prognosis (20, 110, 126, 135, 136). Both IL-6 and CRP trend to decrease overtime in SSc (20, 99). Soluble IL-6 receptor (sIL-6R) serum levels are increased in SSc patients and correlated with progression and severity of the disease (104, 137). Serum anti-IL-6 autoantibody found in SSc serum (138, 139) binds to IL-6. This IL-6 - anti-IL-6 autoantibody complex retains the original IL-6 activity and is able to bind to sIL-6R (138). The autoantibody seems to transport IL-6 in the serum (138).

5.3. Clinical applications of IL-6 and CRP in scleroderma

IL-6 levels are frequently elevated when CRP levels are in SSc (124), and also in interstitial lung disease (ILD) (102, 104, 140), pulmonary artery hypertension (PAH) (102, 141), anti-topoisomerase I positivity (102), anti-RNA polymerase III positivity (102), and CRP is elevated in those with extensive skin involvement, worse pulmonary function, higher disease ac-

Table V. Interventions base on clinical application of IL-6 and CRP in scleroderma.

Authors, year (ref.)	Interventions	Patients	Results
1. Targeted therapy on IL-6 or IL-6 producing B cells			
Shima Y. 2010 (186)	TCZ 8 mg/kg monthly x 6 months	2 dcSSc 42 yr. man with SRC 57 yr. woman with ILD	After 6 months; ≥50% ↓ Total z-score of Vesmeter hardness in both patients; ≥50% ↓ mRSS in only 1 patients
Shima Y. 2013 (187)	TCZ 8 mg/kg monthly x 16 months	1 dcSSc 59 yr. woman with SRC	After 16 months; ↓ mRSS (35→7), improved joints range of motion
Bosello S. 2010 (188)	RTX 1 g IV every 2 weeks	9 dcSSc Mean age 40.9±11 yr. Mean Dis. Dur. 4.08±6.1 yr.	After 6 months; ↓ Mean mRSS (21.1±9.0→12.0±6.1; p=0.001) No changes of FVC, DLCO %predicted; and echocardiographic parameters ↓ Mean serum IL-6 levels (3.7±5.3 pg/ml → 0.6±0.9 pg/ml; p=0.02)
2. Targeted therapy on endothelial/vascular regulatory dysfunction			
Filaci G. 1999 (100)	Group I: Iloprost Group II: Iloprost + CyA 2.5 MKD	20 SSc (16 dcSSc 4 lcSSc) Age 12 – 65 yr. Dis. Dur. <2 yr.	After 12 months; ↓ serum IL-6 levels Group I: 17.03±17.9 pg/ml → 12.3±14.9 pg/ml (p=0.3) Group II: 17.50±13.1 pg/ml → 3.8±2.5 pg/ml (p=0.007) No significant differences were observed for mean ESR & CRP
Sicinska J. 2008 (113)	PGE1 vs. placebo	50 SSc (32 dcSSc, 18 lcSSc) Mean age 49.5±12.9 yr. Mean Dis. Dur. dcSSc 10.9±7.3 yr. lcSSc 12.0±8.8 yr.	After 4 weeks; ↓ CRP levels (p=0.05) in PGE1 group
Bellisai F. 2011 (189)	Bosentan 125 mg twice daily	10 SSc with DU ± PH	↓ Serum IL-2, IL-6, IL-8, IFN-γ levels
Abou-Raya A 2007 (190)	Atorvastatin 40 mg/day vs. placebo	40 SSc Mean age 59.9±10.1 yr. Mean Dis. Dur. 9.8±5.2 yr.	After 6 months; ↓ IL-6 & CRP levels in atorvastatin group Serum IL-6: atorvastatin 26.1±11.5 pg/ml→19.4±12.1 pg/ml vs. placebo 25.5±11.8 pg/ml→25.8±11.5 pg/ml Serum CRP: atorvastatin 3.79±1.8 mg/l→3.14±1.5 mg/l vs. placebo 3.85±1.4 mg/l→3.91±1.5 mg/l
Del Papa N. 2008 (191)	Simvastatin 20 mg/day x 12 weeks	20 lcSSc Median age 59 yr. (28-65) Median Dis. Dur. 8 yr. (1-28)	After 12 weeks; ↓ Serum IL-6 levels from 42±10.3 ng/ml→25.7±7.5 ng/ml (p=0.04) After 16 weeks; Rebound serum IL-6 levels to 35±9.1 ng/ml (not different to baseline)
Alekperov R.T. 2011 (192)	Atorvastatin 10-40 mg/day vs. placebo	50 SSc	After 12 months; ↓ Serum level of CRP & IL-6 in atorvastatin group
3. Immunosuppression			
Åkesson A. 1994 (193)	CYC 2-2.5 MKD x 12 months with prednisone 30 mg/day x 10 weeks	18 SSc Median age 47 yr. (24-68) Median Dis. Dur. 2.5 yr. (0.5-17)	After 12 months; Median (range) ESR from 39 (3-113) mm/hr→17 (5-75) mm/hr (p<0.05) Median (range) CRP from 16 (12-123) mg/l→12 (12-55) mg/l (p<0.01)
De Macedo P.A. 2009 (194)	CYC 0.5-1.0 g/m^2 IV monthly x 18 months	9 dcSSc Mean age 41.7 yr. Mean Dis. Dur. 2.2 yr.	After 12 months; ↓ Mean mRSS from 37.7±4.08→29.1±8.13 (p=0.009) After 18 months; ↓ Mean CRP from 8.9 mg/dL→3.09 mg/dL (p=0.04)
4. Other modalities			
McNearney T.A. 2013 (195)	Transcutaneous electrical nerve stimulation at GI acupoints apply daily (30 min. x 3)	17 SSc (9 dcSSc) Mean age 55 ± 2.28 yr., Mean Dis. Dur. 8.8±1.1 yr.	After 2 weeks; Improved patient gastric myoelectrical activity (GMA) score Improved association between GMA and sympathovagal balance ↓ Plasma IL-6 (p<0.05)

TCZ: Tocilizumab; dcSSc: diffuse cutaneous systemic sclerosis; lcSSc: limited cutaneous systemic sclerosis; SRC: scleroderma renal crisis; ILD: interstitial lung disease; ↓: decreased; mRSS: modified Rodnan skin score; RTX: Rituximab; Dis. Dur.: Disease duration; CyA: cyclosporine A; MKD: mg/ kg/ day; PGE1: Prostaglandin E1; DU: digital ulcer; PH: pulmonary hypertension; CYC: Cyclophosphamide; GI: gastro-intestinal tract.

tivity/ severity, and poorer quality of life, PAH, RA overlap, digital ulcers, renal damage and those with primary biliary cirrhosis (PBC), calcinosis, myopathy, contractures and antibodies (topoisomerase I, RF, anticardiolipin, and anti-IL-6 IgG antibody) (20, 114, 126, 139, 141-149). The levels of IL-6 in BALF do not differ between SSc patients with and without alveolitis (150). Similarly, SSc patients with and without elevated PA pressures on echocardiography have no difference in CRP levels (151), nor did the presence vs. absence of coronary calcification (152), arthritis (153), and ILD (154, 155). One study reported lower CRP levels in SSc patients who had elevated anti-caspase-8 proteinase domain antibodies which were frequent in females and lcSSc patients (156).

Correlations of serum IL-6 and CRP levels with clinical and laboratory parameters are shown in Table IV.

IL-6 levels are usually correlated with worse SSc organ manifestations (55, 79, 102, 104, 119, 129, 140, 157-159), elevated ESR (104) and CRP (55, 104, 124) (Table IV) but there also negative publications (85, 127, 160-161). CRP is increased with disease activity (20, 162), severity (20, 116, 136), and disability (20). Elevated CRP is correlated with a lower 6-minute walk distance (6MWD) (163). CRP is also correlated with ESR (20, 114), ANA titer (114), serum IL-6 (55, 104, 124), IL-13 (164), soluble CD40L (sCD40L) and fibroblasts proliferation (165), whereas normal IL-6 is related to a lack of CD40L expression (125). In addition, CRP is correlated with plasma microparticles (166); platelets activation markers which are related to disease activity/ severity (125); serum vWf (114, 162); serum amino-terminal fragment of pro C-type natriuretic peptide (NT-proC-NP) which has vasodilatory/ anti-inflammatory activity (167); COMP-C3b – a complex between cartilage oligomeric protein and complement activation product C3b which is elevated in SSc serum (168); serum C-terminal telopeptide of collagen type I (s-CTX-I) that is associated with mRSS (169); serum total anti-oxidant power (TAP) (170); heat shock protein-70 (Hsp-70) (171) which is a biomarker for oxidative stress and tissue injury; and serum angiopoietin-2 (Ang-2) that is abnormal in SSc-ILD (172). Pulmonary function (20), albumin (173), vitamin D levels (174) and erythrocyte deformability (175) are inversely associated with CRP.

5.4. Factors not associated with CRP

Although elevated CRP levels occur in SSc-myopathy (145). An elevated CRP is not essential for the occurrence of SSc-myopathy (176). CRP is not correlated with many factors including bone mineral density (BMD) (177), aortic stiffness (178), arterial wall dysfunction (108), pentraxin 3 (PTX-3) (179), serum amyloid P (SAP) (180), prolactin (181), matrix metalloproteinase- 3 (MMP-3) (147), serum YKL-40 or human cartilage glycoprotein 39 (HC gp-39) (182) and adiponectin (160). There is only one study reporting no correlation between serum IL-6 levels and CRP (119).

5.5. IL-6 and CRP and survival

Serum IL-6 and CRP levels also have predictive values to SSc morbidity and mortality. IL-6 levels >2 pg/ml are increased in digital ulcers and avascular areas at nailfolds (183). An avascular score >1.5 on nailfold capillaroscopy (NFC) is associated with increased risk of death (184). Elevated IL-6 levels in the first year of SSc predicted worsening FVC and DLCO and death (159). IL-6 levels ≥10.1 pg/ml in early SSc predicted higher mortality in dcSSc with a 15-year survival of 30% compared with 93% in the group with low IL-6 levels (55). CRP elevation is associated with long term decline of FVC% predicted in multivariate models (116) and levels >20 mg/l aggregate with reduced survival (122). Even mild elevation of serum CRP (>8 mg/l) correlate with worse survival (20,116). One small study could not determine that an elevated CRP predicted increased mortality (185).

5.6. Recent interventions based on IL-6 and CRP in scleroderma

Tocilizumab is an anti-interleukin-6 receptor monoclonal antibody. Rituximab can also reduce IL-6 by destroying CD20 marked B cells. Both drugs have reports of softening of SSc skin but large RCTs are needed (186-188). Rituximab has a single site trial in SSc ILD (188) and there is a NIH trial of rituximab to treat PAH from SSc that is ongoing. There is a trial of tocilizumab in the treatment of early active dcSSc that is ongoing. There are other immunosuppressive and targeted therapies reported to lower IL-6 and/or CRP. Prednisone can lower both. Interventions reported changes of serum levels of IL-6 and CRP were shown in Table V.

Discussion

Not all studies are in agreement about the role of IL-6 and CRP in SSc prognosis. There needs to be both ongoing bench research combined with well conducted randomised blinded trials in order to determine if specific medications that alter IL-6 will show promise in patients with SSc.

In conclusion, we have addressed a significance of IL-6 in SSc and importance of CRP that could be used as a marker of disease activity, severity and worse outcome in SSc and treatment strategies to down regulate IL-6 will need to be proven in clinical trials.

References

1. RABQUER BJ, KOCH AE: Angiogenesis and vasculopathy in systemic sclerosis: evolving concepts. *Curr Rheumatol Rep* 2012; 14: 56-63.
2. KISSIN EY, KORN JH: Fibrosis in scleroderma. *Rheum Dis Clin N Am* 2003; 29: 351-69.
3. CHIFFLOT H, FAUTREL B, SORDET C, CHATELUS E, SIBILIA J: Incidence and Prevalence of Systemic Sclerosis: A Systematic Literature Review. *Semin Arthritis Rheum* 2008; 37: 223-35.
4. MAYES M: Scleroderma epidemiology. *Rheum Dis Clin N Am* 2003; 29: 239-54.
5. LEROY EC, BLACK C, FLEISCHMAJER R *et al*.: Scleroderma (systemic sclerosis): classification, subsets, and pathogenesis. *J Rheumatol* 1988; 15: 202-5.
6. JOVEN BE, ALMODOVAR R, CARMONA L, CARREIRA PE: Survival, causes of death, and risk factors associated with mortality in Spanish systemic sclerosis patients: results from a single university hospital. *Semin Arthritis Rheum* 2010; 39: 285-93.
7. AL-DHAHER FF, POPE JE, OUIMET JM: Determinants of morbidity and mortality of systemic sclerosis in Canada. *Semin Arthritis Rheum* 2010; 39: 269-77.
8. JACOBSEN S, HALBERG P, ULLMAN S: Mortality and causes of death of 344 Dan-

ish patients with systemic sclerosis (scleroderma). *Br J Rheumatol* 1998; 37: 750-5.
9. CZIRJÁK L, KUMÁNOVICS G, VARJÚ C *et al*.: Survival and causes of death in 366 Hungarian patients with systemic sclerosis. *Ann Rheum Dis* 2008; 67: 59-63.
10. BARAUT J, MICHEL L, VERRECCHIA F, FARGE D: Relationship between cytokine profiles and clinical outcomes in patients with systemic sclerosis. *Autoimmun Rev* 2010; 10: 65-73.
11. SMOLEN JS, ALETAHA D, REDLICH K: The pathogenesis of rheumatoid arthritis: new insights from old clinical data? *Nat Rev Rheumatol* 2012; 8: 235-43.
12. ABDEL MEGUID MH, HAMAD YH, SWILAM RS, BARAKAT MS: Relation of interleukin-6 in rheumatoid arthritis patients to systemic bone loss and structural bone damage. *Rheumatol Int* 2013; 33: 697-703.
13. SAMSON M, AUDIA S, JANIKASHVILI N *et al*.: Brief Report: Inhibition of interleukin-6 function corrects Th17/Treg cell imbalance in patients with rheumatoid arthritis. *Arthritis Rheum* 2012; 64: 2499-503.
14. NAVARRO-MILLÁN I, SINGH JA, CURTIS JR: Systematic review of tocilizumab for rheumatoid arthritis: a new biologic agent targeting theinterleukin-6 receptor. *Clin Ther* 2012; 34: 788-802.
15. PEPYS MB, HIRSCHFIELD GM: C-reactive protein: a critical update. *J Clin Invest* 2003; 111: 1805-12.
16. GABAY C, ROUX-LOMBARD P, DE MOERLOOSE P, DAYER JM, VISCHER T, GUERNE PA: Absence of correlation between interleukin 6 and C-reactive protein blood levels in systemic lupus erythematosus compared with rheumatoid arthritis. *J Rheumatol* 1993; 20: 815-21.
17. EMERY P, GABAY C, KRAAN M, GOMEZ-REINO J: Evidence-based review of biologic markers as indicators of disease progression and remission in rheumatoid arthritis. *Rheumatol Int* 2007; 27: 793-806.
18. FIROOZ N, ALBERT DA, WALLACE DJ, ISHIMORI M, BEREL D, WEISMAN MH: High-sensitivity C-reactive protein and erythrocyte sedimentation rate in systemic lupus erythematosus. *Lupus* 2011; 20: 588-97.
19. MUANGCHAN C, POPE JE: Interleukin 6 in systemic sclerosis and potential implications for targeted therapy. *J Rheumatol* 2012; 39: 1120-4.
20. MUANGCHAN C, HARDING S, KHIMDAS S, BONNER A, BARON M, POPE J; THE CANADIAN SCLERODERMA RESEARCH GROUP (CSRG): C - reactive protein (CRP) is associated with high disease activity in systemic sclerosis: results from the Canadian Scleroderma Research Group (CSRG). *Arthritis Care Res* (Hoboken) 2012; 64: 1405-14.
21. GOURH P, AGARWAL SK, DIVECHA D *et al*.: Polymorphisms in TBX21 and STAT4 increase the risk of systemic sclerosis: Evidence of possible gene-gene interaction and alterations in Th1/Th2 cytokines. *Arthritis Rheum* 2009; 60: 3794-806.
22. BERETTA L, CAPPIELLO F, MOORE JH, BARILI M, GREENE CS, SCORZA R: Ability of epistatic interactions of cytokine single-nucleotide polymorphisms to predict susceptibility to disease subsets in systemic sclerosis patients. *Arthritis Care Res* 2008; 59: 974-83.
23. SFRENT-CORNATEANU R, MIHAI C, BALAN S, IONESCU R, MOLDOVEANU E: The IL-6 promoter polymorphism is associated with disease activity and disability in systemic sclerosis. *J Cell Mol Med* 2006; 10: 955-9.
24. BROEN JCA, BOSSINI-CASTILLO L, VAN BON L *et al*.: A rare polymorphism in the gene for toll-like receptor 2 is associated with systemic sclerosis phenotype and increases the production of inflammatory mediators. *Arthritis Rheum* 2012; 64: 264-71.
25. CÉNIT MC, SIMEÓN CP, VONK MC *et al*.: Influence of the IL6 gene in susceptibility to systemic sclerosis. *J Rheumatol* 2012; 39: 2294-302.
26. CÉNIT MC, SIMEÓN CP, FONOLLOSA V *et al*.; SPANISH SCLERODERMA GROUP: No evidence of association between functional polymorphisms located within IL6R and IL6ST genes and systemic sclerosis. *Tissue Antigens* 2012; 80: 254-8.
27. WIPFF J, DIEUDÉ P, AVOUAC J *et al*.: Association study of CRP gene in systemic sclerosis in European Caucasian population. 2013 Feb. 9 [Epub ahead of print].
28. KITABA S, MUROTA H, TERAO M *et al*.: Blockade of interleukin-6 receptor alleviates disease in mouse model of scleroderma. *Am J Pathol* 2012; 180: 165-76.
29. YOSHIZAKI A, YANABA K, OGAWA A, ASANO Y, KADONO T, SATO S: Immunization with DNA topoisomerase 1 and freund's complete adjuvant induces skin and lung fibrosis and autoimmunity via interleukin-6 signaling. *Arthritis Rheum* 2011; 63: 3575-85.
30. YAMAMOTO A, ASHIHARA E, NAKAGAWA Y *et al*.: Allograft inflammatory factor-1 is over expressed and induces fibroblast chemotaxis in the skin of sclerodermatous GVHD in a murine model. *Immunol Lett* 2011; 135: 144-50.
31. LE HUU D, MATSUSHITA T, JIN G *et al*.: IL-6 blockade attenuates the development of murine sclerodermatous chronic graft-versus-host disease. *J Invest Dermatol* 2012; 132: 2752-61.
32. SATO S: CD19 is a central response regulator of B lymphocyte signaling thresholds governing autoimmunity. *J Dermatol Sci* 1999; 22: 1-10.
33. TEDDER TF, POE JC, FUJIMOTO M, HAAS KM, SATO S: The CD19-CD21 signal transduction complex of B lymphocytes regulates the balance between health and autoimmune disease: systemic sclerosis as a model system. *Curr Dir Autoimmun* 2005; 8: 55-90.
34. DAVIDSON A: Targeting BAFF in autoimmunity. *Curr Opin Immunol* 2010; 22: 732-9.
35. MOISINI I, DAVIDSON A: BAFF: a local and systemic target in autoimmune diseases. *Clin Exp Immunol* 2009; 158: 155-63.
36. MACKAY F, SILVEIRA PA, BRINK R: B cells and the BAFF/APRIL axis: fast-forward on autoimmunity and signaling. *Curr Opin Immunol* 2007; 19: 327-36.
37. YOSHIZAKI A, IWATA Y, KOMURA K *et al*.: CD19 regulates skin and lung fibrosis via Toll-like receptor signaling in a model of bleomycin-induced scleroderma. *Am J Pathol* 2008; 172: 1650-63.
38. SAITO E, FUJIMOTO M, HASEGAWA M *et al*.: CD19-dependent B lymphocyte signaling thresholds influence skin fibrosis and autoimmunity in the tight-skin mouse. *J Clin Invest* 2002; 109: 1453-62.
39. MATSUSHITA T, FUJIMOTO M, HASEGAWA M *et al*.: BAFF antagonist attenuates the development of skin fibrosis in tight-skin mice. *J Invest Dermatol* 2007; 127: 2772-80.
40. ORTMANN RA, CHENG T, VISCONTI R, FRUCHT DM, O'SHEA JJ: Janus kinases and signal transducers and activators of transcription: their roles in cytokine signaling, development and immunoregulation. *Arthritis Res* 2000; 2: 16-32.
41. O'SHEA JJ, PLENGE R: JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. *Immunity* 2012; 36: 542-50.
42. HARRISON DA: The JAK/ STAT pathway. *Cold Spring Harb Perspect Biol* 2012; 4:a011205.
43. MIHARA M, HASHIZUME M, YOSHIDA H, SUZUKI M, SHIINA M: IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clin Sci* (Lond) 2012; 122: 143-59.
44. AVOUAC J, FÜRNROHR BG, TOMCIK M *et al*.: Inactivation of the transcription factor STAT-4 prevents inflammation-driven fibrosis in animal models of systemic sclerosis. *Arthritis Rheum* 2011; 63: 800-9.
45. SHARPE AH, FREEMAN GJ: The B7-CD28 superfamily. *Nat Rev Immunol* 2002; 2: 116-26.
46. TANAKA C, FUJIMOTO M, HAMAGUCHI Y, SATO S, TAKEHARA K, HASEGAWA M: Inducible co stimulator ligand regulates bleomycin-induced lung and skin fibrosis in a mouse model independently of the inducible co stimulator/inducible co stimulator ligand pathway. *Arthritis Rheum* 2010; 62: 1723-32.
47. YOSHIZAKI A, YANABA K, OGAWA A *et al*.: The specific free radical scavenger edaravone suppresses fibrosis in the bleomycin-induced and tight skin mouse models of systemic sclerosis. *Arthritis Rheum* 2011; 63: 3086-97.
48. YOSHIZAKI A, YANABA K, YOSHIZAKI A *et al*.: Treatment with rapamycin prevents fibrosis in tight-skin and bleomycin-induced mouse models of systemic sclerosis. *Arthritis Rheum* 2010; 62: 2476-87.
49. SU TI, KHANNA D, FURST DE *et al*.: Rapamycin versus methotrexate in early diffuse systemic sclerosis: results from a randomized single-blind pilot study. *Arthritis Rheum* 2009; 60: 3821-30.
50. BRAUN RK, MARTIN A, SHAH S *et al*.: Inhibition of bleomycin-induced pulmonary fibrosis through pre-treatment with collagen type V. *J Heart Lung Transplant* 2010; 29: 873-80.
51. POSTLETHWAITE AE, WONG WK, CLEMENTS P *et al*.: A multicenter, randomized, double-blind, placebo-controlled trial of oral type I collagen treatment in patients

with diffuse cutaneous systemic sclerosis: I. oral type I collagen does not improve skin in all patients, but may improve skin in late-phase disease. *Arthritis Rheum* 2008; 58: 1810-22.
52. YAMAMOTO T, TAKAGAWA S, KATAYAMA I, NISHIOKA K: Anti-sclerotic effect of transforming growth factor-β antibody in a mouse model of bleomycin-induced scleroderma. *Clinical Immunology* 1999; 92: 6-13.
53. DENTON CP, MERKEL PA, FURST DE *et al*.: Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. *Arthritis Rheum* 2007; 56: 323-33.
54. ANTONELLI A, FALLAHI P, FERRARI SM *et al*.: Systemic sclerosis fibroblasts show specific alterations of interferon-γ and tumor necrosis factor-α-induced modulation of interleukin 6 and chemokine ligand 2. *J Rheumatol* 2012; 39: 979-85.
55. KHAN K, XU S, NIHTYANOVA S *et al*.: Clinical and pathological significance of interleukin 6 over expression in systemic sclerosis. *Ann Rheum Dis* 2012; 71: 1235-42.
56. ANTONELLI A, FERRI C, FERRARI SM *et al*.: IFN-γ and TNF-α induce a different modulation of interleukin-6 in systemic sclerosis fibroblasts compared to healthy controls. *Scand J Rheumatol* 2011; 40: 453-6.
57. DE PALMA R, D'AIUTO E, VETTORI S, CUOPPOLO P, ABBATE G, VALENTINI G: Peripheral T cells from patients with early systemic sclerosis kill autologous fibroblasts in co-culture: Is T-cell response aimed to play a protective role? *Rheumatology* 2010; 49: 1257-66.
58. GARCIA-GONZALEZ E, SELVI E, BALISTRERI E *et al*.: Cannabinoids inhibit fibrogenesis in diffuse systemic sclerosis fibroblasts. *Rheumatology* 2009; 48: 1050-6.
59. KADONO T, KIKUCHI K, IHN H, TAKEHARA K, TAMAKI K: Increased production of interleukin 6 and interleukin 8 in scleroderma fibroblasts. *J Rheumatol* 1998; 25: 296-301.
60. FEGHALI CA, BOST KL, BOULWARE DW, LEVY LS: Mechanisms of pathogenesis in scleroderma. I. overproduction of interleukin 6 by fibroblasts cultured from affected skin sites of patients with scleroderma. *J Rheumatol* 1992; 19: 1207-11.
61. ZURITA-SALINAS CS, RICHAUD-PATIN Y, KRÖTZSCH-GÓMEZ E *et al*.: Spontaneous cytokine gene expression by cultured skin fibroblasts of systemic sclerosis. correlation with collagen synthesis. *Revista de Investigacion Clinica* 1998; 50: 97-104.
62. FEGHALI CA, BOST KL, BOULWARE DW, LEVY LS: Control of IL-6 expression and response in fibroblasts from patients with systemic sclerosis. *Autoimmunity* 1994; 17: 309-18.
63. BRUNS M, HERRMANN K, HAUSTEIN U-: Immunologic parameters in systemic sclerosis. *Int J Dermatol* 1994; 33: 25-32.
64. KONDO K, OKADA T, MATSUI T *et al*.: Establishment and characterization of a human B cell line from the lung tissue of a patient with scleroderma; extraordinary high level of IL-6 secretion by stimulated fibroblasts. *Cytokine* 2001; 13: 220-6.
65. KAWAGUCHI Y, NISHIMAGI E, TOCHIMOTO A *et al*.: Intracellular IL-1α-binding proteins contribute to biological functions of endogenous IL-1α in systemic sclerosis fibroblasts. *Proc Natl Acad Sci USA* 2006; 103: 14501-6.
66. KAWAGUCHI Y, MCCARTHY SA, WATKINS SC, WRIGHT TM: Autocrine activation by interleukin 1α induces the fibrogenic phenotype of systemic sclerosis fibroblasts. *J Rheumatol* 2004; 31: 1946-54.
67. CHIZZOLINI C, RASCHI E, REZZONICO R *et al*.: Autoantibodies to fibroblasts induce a proadhesive and proinflammatory fibroblast phenotype in patients with systemic sclerosis. *Arthritis Rheum* 2002; 46: 1602-13.
68. KAWAGUCHI Y, HARA M, WRIGHT TM: Endogenous IL-1α from systemic sclerosis fibroblasts induces IL-6 and PDGF-A. *J Clin Invest* 1999; 103: 1253-60.
69. KAWAGUCHI Y, HARIGAI M, SUZUKI K *et al*.: Interleukin 1 receptor on fibroblasts from systemic sclerosis patients induces excessive functional responses to interleukin 1β. *Biochem Biophys Res Commun* 1993; 190: 154-61.
70. TAKEMURA H, SUZUKI H, FUJISAWA H *et al*.: Enhanced interleukin 6 production by cultured fibroblasts from patients with systemic sclerosis in response to platelet derived growth factor. *J Rheumatol* 1998; 25: 1534-9.
71. AGARWAL SK, WU M, LIVINGSTON CK *et al*.: Toll-like receptor 3 up regulation by type I interferon in healthy and scleroderma dermal fibroblasts. *Arthritis Res Ther* 2011; 13: R3.
72. YAMAMOTO T, KATAYAMA I, NISHIOKA K: Fibroblast proliferation by bleomycin stimulated peripheral blood mononuclear cell factors. *J Rheumatol* 1999; 26: 609-15.
73. SHAO DD, SURESH R, VAKIL V, GOMER RH, PILLING D: Pivotal advance: Th-1 cytokines inhibit, and th-2 cytokines promote fibrocyte differentiation. *J Leukoc Biol* 2008; 83: 1323-33.
74. SNANOUDJ R, DE PRÉNEUF H, CRÉPUT C *et al*.: Costimulation blockade and its possible future use in clinical transplantation. *Transpl Int* 2006; 19: 693-704.
75. KAWAI M, MASUDA A, KUWANA M: A CD40-CD154 interaction in tissue fibrosis. *Arthritis Rheum* 2008; 58: 3562-73.
76. FUKASAWA C, KAWAGUCHI Y, HARIGAI M *et al*.: Increased CD40 expression in skin fibroblasts from patients with systemic sclerosis (SSc): Role of CD40-CD154 in the phenotype of SSc fibroblasts. *Eur J Immunol* 2003; 33: 2792-800.
77. MIHARA M, MORIYA Y, OHSUGI Y: IL-6-soluble IL-6 receptor complex inhibits the proliferation of dermal fibroblasts. *Int J Immunopharmacol* 1996; 18: 89-94.
78. STEINMAN RA, ROBINSON AR, FEGHALI-BOSTWICK CA: Antifibrotic effects of roscovitine in normal and scleroderma fibroblasts. *PLoS One* 2012; 7: e48560.
79. SCALA E, PALLOTTA S, FREZZOLINI A *et al*.: Cytokine and chemokine levels in systemic sclerosis: Relationship with cutaneous and internal organ involvement. *Clin Exp Immunol* 2004; 138: 540-6.
80. HASEGAWA M, SATO S, IHN H, TAKEHARA K: Enhanced production of interleukin-6 (IL-6), oncostatin M and soluble IL-6 receptor by cultured peripheral blood mononuclear cells from patients with systemic sclerosis. *Rheumatology* 1999; 38: 612-7.
81. GIACOMELLI R, CIPRIANI P, DANESE C *et al*.: Peripheral blood mononuclear cells of patients with systemic sclerosis produce increased amounts of interleukin 6, but not transforming growth factor β1. *J Rheumatol* 1996; 23: 294-6.
82. GURRAM M, PAHWA S, FRIERI M: Augmented interleukin-6 secretion in collagen-stimulated peripheral blood mononuclear cells from patients with systemic sclerosis. *Ann Allergy* 1994; 73: 493-6.
83. CZIRJÁK L, KONCZ A, VARGA I, DÉVÉNYI K, KUMÁNOVICS G, SZÜCS G: Investigation of the alveolar macrophages and T lymphocytes in 15 patients with systemic sclerosis. *Clin Rheumatol* 1999; 18: 357-63.
84. HÜGLE T, O'REILLY S, SIMPSON R *et al*.: Tumor necrosis factor-costimulated T lymphocytes from patients with systemic sclerosis trigger collagen production in fibroblasts. *Arthritis Rheum* 2013; 65: 481-91.
85. CRESTANI B, SETA N, DE BANDT M *et al*.: Interleukin 6 secretion by monocytes and alveolar macrophages in systemic sclerosis with lung involvement. *Am J Respir Crit Care Med* 1994; 149: 1260-5.
86. HORIKAWA M, HASEGAWA M, KOMURA K *et al*.: Abnormal natural killer cell function in systemic sclerosis: Altered cytokine production and defective killing activity. *J Invest Dermatol* 2005; 125: 731-7.
87. ANDERSEN GN, NILSSON K, NAGAEVA O, RANTAPÄÄ-DAHLQVIST S, SANDSTRÖM T, MINCHEVA-NILSSON L: Cytokine mRNA profile of alveolar T lymphocytes and macrophages in patients with systemic sclerosis suggests a local Tr1 response. *Scand J Immunol* 2011; 74: 272-81.
88. VAN BON L, POPA C, HUIJBENS R *et al*.: Distinct evolution of TLR-mediated dendritic cell cytokine secretion in patients with limited and diffuse cutaneous systemic sclerosis. *Ann Rheum Dis* 2010; 69: 1539-47.
89. JIANG Y, CHEN G, ZHANG Y, LU L, LIU S, CAO X: Nerve growth factor promotes TLR4 signaling-induced maturation of human dendritic cells *in vitro* through inducible p75NTR. *Journal of Immunology* 2007; 179: 6297-304.
90. BARNES TC, SPILLER DG, ANDERSON ME, EDWARDS SW, MOOTS RJ: Endothelial activation and apoptosis mediated by neutrophil-dependent interleukin 6 trans-signaling: A novel target for systemic sclerosis? *Ann Rheum Dis* 2011; 70: 366-72.
91. ROBITAILLE G, HÉNAULT J, CHRISTIN M-, SENÉCAL J-, RAYMOND Y: The nuclear autoantigen CENP-B displays cytokine-like activities toward vascular smooth muscle cells. *Arthritis Rheum* 2007; 56: 3814-26.
92. KUWANA M, MEDSGER TA JR, WRIGHT TM: Analysis of soluble and cell surface factors regulating anti-DNA topoisomerase I autoantibody production demonstrates synergy between Th1 and Th2 autoreactive T cells. *J Immunol* 2000; 164: 6138-46.

93. KAHALEH MB, YIN T: Enhanced expression of high-affinity interleukin-2 receptors in scleroderma: Possible role for IL-6. *Clin Immunol Immunopathol* 1992; 62: 97-102.
94. DEL GALDO F, JIMÉNEZ SA: T cells expressing allograft inflammatory factor 1 display increased chemotaxis and induce a profibrotic phenotype in normal fibroblasts in vitro. *Arthritis Rheum* 2007; 56: 3478-88.
95. SATO S, HANAKAWA H, HASEGAWA M *et al*.: Levels of interleukin 12, a cytokine of type 1 helper T cells, are elevated in sera from patients with systemic sclerosis. *J Rheumatol* 2000; 27: 2838-42.
96. MATSUSHITA T, HASEGAWA M, YANABA K, KODERA M, TAKEHARA K, SATO S: Elevated serum BAFF levels in patients with systemic sclerosis: Enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum* 2006; 54: 192-201.
97. MATSUURA E, OHTA A, SUEMATSU R *et al*.: Functional disturbance of the stress-adaptation system in patients with scleroderma. *Modern Rheumatology* 2011; 21: 397-405.
98. RADSTAKE TR, VAN BON L, BROEN J *et al*.: The pronounced Th17 profile in systemic sclerosis (SSc) together with intracellular expression of TGFbeta and IFNgamma distinguishes SSc phenotypes. *PLoS One* 2009; 4: e5903.
99. MATSUSHITA T, HASEGAWA M, HAMAGUCHI Y, TAKEHARA K, SATO S: Longitudinal analysis of serum cytokine concentrations in systemic sclerosis: Association of interleukin 12 elevation with spontaneous regression of skin sclerosis. *J Rheumatol* 2006; 33: 275-84.
100. FILACI G, CUTOLO M, SCUDELETTI M *et al*.: Cyclosporin A and iloprost treatment of systemic sclerosis: Clinical results and interleukin-6 serum changes after 12 months of therapy. *Rheumatology* 1999; 38: 992-6.
101. KUCHARZ EJ, JONDERKO G, RUBISZ-BRZEZINSKA J, BRZEZINSKA-WCISŁO L: Serum interleukin-6 level in patients with systemic sclerosis: lack of correlation with aminoterminal propeptide of type III procollagen. *Clin Rheumatol* 1995; 14: 380-1.
102. 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: R147.
103. BEIRNE P, PANTELIDIS P, CHARLES P *et al*.: Multiplex immune serum biomarker profiling in sarcoidosis and systemic sclerosis. *Eur Respir J* 2009; 34: 1376-82.
104. HASEGAWA M, SATO S, FUJIMOTO M, IHN H, KIKUCHI K, TAKEHARA K: Serum levels of interleukin 6 (IL-6), oncostatin M, soluble IL-6 receptor, and soluble gp130 in patients with systemic sclerosis. *J Rheumatol* 1998; 25: 308-13.
105. CODULLO V, BALDWIN HM, SINGH MD *et al*.: An investigation of the inflammatory cytokine and chemokine network in systemic sclerosis. *Ann Rheum Dis* 2011; 70: 1115-21.
106. HARDARDÓTTIR H, VAN HELVOORT HAC, VONK MC, VAN DEN HOOGEN FHJ, DEKHUIJZEN PNR, HEIJDRA YF: Exercise in systemic sclerosis intensifies systemic inflammation and oxidative stress. *Scand J Rheumatol* 2010; 39: 63-70.
107. BEČVÁŘ R, HULEJOVÁ H, BRAUN M, ŠTORK J: Collagen degradation products and proinflammatory cytokines in systemic and localized scleroderma. *Folia Biol* 2007; 53: 66-8.
108. CYPIENE A, LAUCEVICIUS A, VENALIS A *et al*.: The impact of systemic sclerosis on arterial wall stiffness parameters and endothelial function. *Clin Rheumatol* 2008; 27: 1517-22.
109. HETTEMA ME, ZHANG D, DE LEEUW K *et al*.: Early atherosclerosis in systemic sclerosis and its relation to disease or traditional risk factors. *Arthritis Res Ther* 2008; 10:R49.
110. HETTEMA ME, BOOTSMA H, GRAAFF R, DE VRIES R, KALLENBERG CG, SMIT AJ: Skin autofluorescence, as marker of accumulation of advanced glycation end products and of cumulative metabolic stress, is not increased in patients with systemic sclerosis. *Int J Rheumatol* 2011; 2011: 417-813.
111. MONTAGNANA M, LIPPI G, VOLPE A *et al*.: Evaluation of cardiac laboratory markers in patients with systemic sclerosis. C*lin Biochem* 2006; 39: 913-7.
112. BLANN AD, ILLINGWORTH K, JAYSON MIV: Mechanisms of endothelial cell damage in systemic sclerosis and Raynaud's phenomenon. *J Rheumatol* 1993; 20: 1325-30.
113. SICINSKA J, GORSKA E, CICHA M *et al*.: Increased serum fractalkine in systematic sclerosis. down-regulation by prostaglandin E1. *Clin Exp Rheumatol* 2008; 26: 527-33.
114. ALEKPEROV RT, BARANOV AA, ABAĬTOVA NE: Clinical associations of C-reactive protein in systemic sclerosis. *Ter Arkh* 2006; 78: 30-5.
115. LIPPI G, CARAMASCHI P, MONTAGNANA M, SALVAGNO GL, VOLPE A, GUIDI G: Lipoprotein[a] and the lipid profile in patients with systemic sclerosis. *Clinica Chimica Acta* 2006; 364: 345-8.
116. LIU X, MAYES MD, PHD CP *et al*.: C-reactive protein predictsthe long-term progression of interstitial lung disease and survival in patients with early systemic sclerosis. *Arthritis Care Res* (Hoboken) February 11, 2013 [Epub ahead of print].
117. GRUSZEWSKA E, CHLU.DZINSKA A, CHROSTEK L *et al*.: Carbohydrate-deficient transferrin depends on disease activity in rheumatoid arthritis and systemic sclerosis. *Scand J Rheumatol* February 5, 2013 [Epub ahead of print].
118. LIS AD, BRZEZIŃSKA-WCISŁO LA: Interleukin-2 and interleukin-6 in serum as markers of disease progression in systemic sclerosis. *Pol Merkur Lekarski* 2001; 11: 206-9.
119. STUART RA, LITTLEWOOD AJ, MADDISON PJ, HALL ND: Elevated serum interleukin-6 levels associated with active disease in systemic connective tissue disorders. *Clin Exp Rheumatol* 1995; 13: 17-22.
120. ALECU M, GELERIU L, COMAN G, GĂLĂȚESCU L: The interleukin-1, interleukin-2, interleukin-6 and tumors necrosis factor alpha serological levels in localized and systemic sclerosis. *Rom J Intern Med* 1998; 36: 251-9.
121. SZEGEDI A, CZIRJÁK L, UNKELESS JC, BOROS P: Serum cytokine and anti-FcγR autoantibody measurements in patients with systemic sclerosis. *Acta Derm Venereol* 1996; 76: 21-3.
122. NAGY Z, CZIRJÁK L: Increased levels of amino terminal propeptide of type III procollagen are an unfavourable predictor of survival in systemic sclerosis. *Clin Exp Rheumatol* 2005; 23: 165-72.
123. HUSSEIN MR, HASSAN HI, HOFNY ERM *et al*.: Alterations of mononuclear inflammatory cells, $CD4/CD8^+$ T cells, interleukin 1β, and tumour necrosis factor α in the bronchoalveolar lavage fluid, peripheral blood, and skin of patients with systemic sclerosis. *J Clin Pathol* 2005; 58: 178-84.
124. OHTSUKA T: Serum interleukin-6 level is reflected in elevated high-sensitivity C-reactive protein level in patients with systemic sclerosis. *J Dermatol* 2010; 37: 801-6.
125. AGACHE I, RĂDOI M, DUCA L: Platelet activation in patients with systemic scleroderma – pattern and significance. *Rom J Intern Med* 2007; 45: 183-91.
126. OHTSUKA T: Relation between elevated high-sensitivity C-reactive protein and antimitochondria antibody in patients with systemic sclerosis. *J Dermatol* 2008; 35: 70-5.
127. HASEGAWA M, FUJIMOTO M, MATSUSHITA T, HAMAGUCHI Y, TAKEHARA K, SATO S: Serum chemokine and cytokine levels as indicators of disease activity in patients with systemic sclerosis. *Clin Rheumatol* 2011; 30: 231-7.
128. BEČVÁŘ R, STORK J, PESÁKOVÁ V *et al*.: Clinical correlations of potential activity markers in systemic sclerosis. *Ann N Y Acad Sci* 2005; 1051: 404-12.
129. SATO S, HASEGAWA M, TAKEHARA K: Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. *J Dermatol Sci* 2001; 27: 140-6.
130. BRUNS M, HAUSTEIN UF, HOFMANN C, HERRMANN K: Serum levels of soluble IL-2 receptor, soluble ICAM-1, TNF-alpha, interleukin-4 and interleukin-6 in scleroderma. *J Eur Acad Dermatol Venereol* 1997; 8: 222-8.
131. NEEDLEMAN BW, WIGLEY FM, STAIR RW: Interleukin-1, interleukin-2, interleukin-4, interleukin-6, tumor necrosis factor alpha, and interferon-gamma levels in sera from patients with scleroderma. *Arthritis Rheum* 1992; 35: 67-72.
132. KHURMA V, MEYER C, PARK GS *et al*.: A pilot study of subclinical coronary atherosclerosis in systemic sclerosis: Coronary artery calcification in cases and controls. *Arthritis Rheum* 2008; 59: 591-7.
133. EDMÉ JL, TELLART AS, LAUNAY D *et al*.: Cytokine concentrations in exhaled breath condensates in systemic sclerosis. *Inflammation Res* 2008; 57: 151-6.
134. KOCH AE, KRONFELD-HARRINGTON LB, SZEKANECZ Z *et al*.: In situ expression of cytokines and cellular adhesion molecules in the skin of patients with systemic sclerosis, their role in early and late disease. *Pathobiology* 1993; 61: 239-46.
135. CRUZ-DOMÍNGUEZ MP, MONTES-CORTES

DH, OLIVARES-CORICHI IM, VERA-LASTRA O, MEDINA G, JARA LJ: Oxidative stress in Mexicans with diffuse cutaneous systemic sclerosis. *Rheumatol Int* 2013 March 2 [Epub ahead of print].
136. SCHANZ S, HENES J, ULMER A *et al*.: Magnetic resonance imaging findings in patients with systemic scleroderma and musculoskeletal symptoms. *Eur Radiol* 2013; 23: 212-21.
137. LIS AD, BRZEZIŃSKA-WCISŁO LA: Soluble receptors of cytokines in sera of patients with systemic sclerosis--clinical correlation. *Wiad Lek* 2003; 56: 532-6.
138. SUZUKI H, TAKEMURA H, YOSHIZAKI K *et al*.: IL-6-anti-IL-6 autoantibody complexes with IL-6 activity in sera from some patients with systemic sclerosis. *J Immunol* 1994; 152: 935-42.
139. TAKEMURA H, SUZUKI H, YOSHIZAKI K *et al*.: Anti-interleukin-6 autoantibodies in rheumatic diseases: Increased frequency in the sera of patients with systemic sclerosis. *Arthritis Rheum* 1992; 35: 940-3.
140. DE SANTIS M, BOSELLO S, LA TORRE G *et al*.: Functional, radiological and biological markers of alveolitis and infections of the lower respiratory tract in patients with systemic sclerosis. *Respir Res* 2005; 6: 96.
141. PENDERGRASS SA, HAYES E, FARINA G *et al*.: Limited systemic sclerosis patients with pulmonary arterial hypertension show biomarkers of inflammation and vascular injury. *PLoS One* 2010; 5: e12106.
142. JINNIN M, IHN H, YAMANE K, ASANO Y, YAZAWA N, TAMAKI K: Clinical features of patients with systemic sclerosis accompanied by rheumatoid arthritis. *Clin Exp Rheumatol* 2003; 21: 91-4.
143. NISHIJIMA C, SATO S, HASEGAWA M *et al*.: Renal vascular damage in Japanese patients with systemic sclerosis. *Rheumatology* 2001; 40: 406-9.
144. HAYAKAWA I, HASEGAWA M, TAKEHARA K, SATO S: Anti-DNA topoisomerase II α autoantibodies in Japanese patients with systemic sclerosis. *Arch Dermatol Res* 2005; 297: 180-3.
145. HAYAKAWA I, HASEGAWA M, MATSUSHITA T *et al*.: Increased cutaneous T-cell-attracting chemokine levels in sera from patients with systemic sclerosis. *Rheumatology* 2005; 44: 873-8.
146. MIMURA Y, IHN H, JINNIN M *et al*.: Rheumatoid factor isotypes and anti-agalactosyl IgG antibodies in systemic sclerosis. *Br J Dermatol* 2004; 151: 803-8.
147. JINNIN M, IHN H, ASANO Y, YAMANE K, YAZAWA N, TAMAKI K: Serum matrix metalloproteinase-3 in systemic sclerosis. *Arch Dermatol Res* 2004; 296: 25-9.
148. SPERANSKIĬ AI, RIAZANTSEVA TA, GUSEVA NG, MELKUMOVA KL, IVANOVA SM: Anticardiolipin antibodies and other immunological disorders in patients with systemic scleroderma. *Revmatologiia* (Mosk) 1990; 3: 11-4.
149. MANSOUR S, BONNER A, MUANGCHAN C, HUDSON M, BARON M, POPE JE; AND THE CANADIAN SCLERODERMA RESEARCH GROUP: Low Socioeconomic Status (Measured by Education) and Outcomes in Systemic Sclerosis: Data from the Canadian Scleroderma Research Group. *J Rheumatol* 2013; 40: 447-54.
150. BOLSTER MB, LUDWICKA A, SUTHERLAND SE, STRANGE C, SILVER RM: Cytokine concentrations in bronchoalveolar lavage fluid of patients with systemic sclerosis. *Arthritis Rheum* 1997; 40: 743-51.
151. YAMANE K, IHN H, ASANO Y *et al*.: Clinical and laboratory features of scleroderma patients with pulmonary hypertension. *Rheumatology* 2000; 39: 1269-71.
152. MOK MY, LAU CS, CHIU SSH *et al*.: Systemic sclerosis is an independent risk factor for increased coronary artery calcium deposition. *Arthritis Rheum* 2011; 63: 1387-95.
153. SCHMEISER T, PONS-KÜHNEMANN J, ÖZDEN F, MÜLLER-LADNER U, DINSER R: Arthritis in patients with systemic sclerosis. *Eur J Intern Med* 2012; 23: e25-9.
154. HASEGAWA M, SATO S, YANABA K, KOMURA K, YAMAZAKI M, TAKEHARA K: Autoantibodies against phosphatidylserine-prothrombin complex in patients with systemic sclerosis. *Ann Rheum Dis* 2004; 63: 1514-7.
155. BASSYOUNI IH, AZAB NA, EL-DAKRONY EHM, FAWZI MMT, GHANOUM R, BASSYOUNI RH: Elevated serum levels of a proliferation-inducing ligand in patients with systemic sclerosis: Possible association with myositis? *Joint Bone Spine* 2011; 78: 56-61.
156. YAMAOKA T, OGAWA F, MUROI E *et al*.: Autoantibody against a protease domain of caspase-8 in patients with systemic sclerosis. *Clin Exp Rheumatol* 2008; 26: 998-1004.
157. BECVÁR R, STORK J, PESÁKOVÁ V *et al*.: Clinical correlations of potential activity markers in systemic sclerosis. *Ann N Y Acad Sci* 2005; 1051: 404-12.
158. KOMURA K, YANABA K, OGAWA F, SHIMIZU K, TAKEHARA K, SATO S: Elevation of IgG levels is a serological indicator for pulmonary fibrosis in systemic sclerosis with anti-topoisomerase I antibodies and those with anticentromere antibody. *Clin Exp Dermatol* 2008; 33: 329-32.
159. 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.
160. TOMČÍK M, ARIMA K, HULEJOVÁ H *et al*.: Adiponectin relation to skin changes and dyslipidemia in systemic sclerosis. *Cytokine* 2012; 58: 165-8.
161. HOLCOMBE RF, BAETHGE BA, STEWART RM *et al*.: Cell surface expression of lysosome-associated membrane proteins (LAMPs) in scleroderma: relationship of lamp2 to disease duration, anti-Sc170 antibodies, serum interleukin-8, and soluble interleukin-2 receptor levels. *Clin Immunol Immunopathol* 1993; 67: 31-9.
162. MINIER T, NAGY Z, BÁLINT Z *et al*.: Construct validity evaluation of the European scleroderma study group activity index, and investigation of possible new disease activity markers in systemic sclerosis. *Rheumatology* 2010; 49: 1133-45.
163. SCHOINDRE Y, MEUNE C, DINH-XUAN AT, AVOUAC J, KAHAN A, ALLANORE Y: Lack of specificity of the 6-minute walk test as an outcome measure for patients with systemic sclerosis. *J Rheumatol* 2009; 36: 1481-5.
164. HASEGAWA M, FUJIMOTO M, KIKUCHI K, TAKEHARA K: Elevated serum levels of interleukin 4 (IL-4), IL-10, and IL-13 in patients with systemic sclerosis. *J Rheumatol* 1997; 24: 328-32.
165. KOMURA K, SATO S, HASEGAWA M, FUJIMOTO M, TAKEHARA K: Elevated circulating CD40L concentrations in patients with systemic sclerosis. *J Rheumatol* 2004; 31: 514-9.
166. GUIDUCCI S, DISTLER JHW, JÜNGEL A *et al*.: The relationship between plasma microparticles and disease manifestations in patients with systemic sclerosis. *Arthritis Rheum* 2008; 58: 2845-53.
167. OLEWICZ-GAWLIK A, DANCZAK-PAZDROW-SKA A, KLAMA K *et al*.: Blood serum levels of amino-terminal pro-c-type natriuretic peptide in patients with systemic sclerosis. *Connect Tissue Res* 2010; 51: 83-7.
168. HAPPONEN KE, SAXNE T, GEBOREK P *et al*.: Serum COMP-C3b complexes in rheumatic diseases and relation to anti-TNF-α treatment. *Arthritis Res Ther* 2012; 14: R15.
169. ALLANORE Y, BORDERIE D, LEMARÉCHAL H, CHERRUAU B, EKINDJIAN OG, KAHAN A: Correlation of serum collagen I carboxy-terminal telopeptide concentrations with cutaneous and pulmonary involvement in systemic sclerosis. *J Rheumatol* 2003; 30: 68-73.
170. OGAWA F, SHIMIZU K, MUROI E, HARA T, SATO S: Increasing levels of serum antioxidant status, total antioxidant power, in systemic sclerosis. *Clin Rheumatol* 2011; 30: 921-5.
171. OGAWA F, SHIMIZU K, HARA T *et al*.: Serum levels of heat shock protein 70, a biomarker of cellular stress, are elevated in patients with systemic sclerosis: Association with fibrosis vascular damage. *Clin Exp Rheumatol* 2008; 26: 659-62.
172. TAKAHASHI T, ASANO Y, AKAMATA K *et al*.: Dynamics of serum angiopoietin-2 levels correlate with efficacy of intravenous pulse cyclophosphamide therapy for interstitial lung disease associated with systemic sclerosis. *Mod Rheumatol* 2012 Sep 13 [Epub ahead of print].
173. BARON M, HUDSON M, STEELE R *et al*.: Is serum albumin a marker of malnutrition in chronic disease? the scleroderma paradigm. *J Am Coll Nutr* 2010; 29: 144-51.
174. CARAMASCHI P, DALLA GASSA A, RUZZENENTE O *et al*.: Very low levels of vitamin D in systemic sclerosis patients. *Clin Rheumatol* 2010; 29: 1419-25.
175. VAYÁ A, TODOLÍ J, CALVO J, ROMAGNOLI M, RICART JM: Haemorheological profile in patients with systemic sclerosis. *Clin Hemorheol Microcirc* 2008; 40: 243-8.
176. TOLÉDANO C, GAIN M, KETTANEH A *et al*.: Aldolase predicts subsequent myopathy occurrence in systemic sclerosis. *Arthritis Res Ther* 2012; 14: R152.
177. FREDIANI B, BALDI F, FALSETTI P *et al*.: Clinical determinants of bone mass and

bone ultrasonometry in patients with systemic sclerosis. *Clin Exp Rheumatol* 2004; 22: 313-8.
178. MOYSSAKIS I, GIALAFOS E, VASSILIOU V *et al*.: Aortic stiffness in systemic sclerosis is increased independently of the extent of skin involvement. *Rheumatology* 2005; 44: 251-4.
179. IWATA Y, YOSHIZAKI A, OGAWA F *et al*.: Increased serum pentraxin 3 in patients with systemic sclerosis. *J Rheumatol* 2009; 36: 976-83.
180. TENNENT GA, DZIADZIO M, TRIANTAFILLIDOU E *et al*.: Normal circulating serum amyloid P component concentration in systemic sclerosis. *Arthritis Rheum* 2007; 56: 2013-7.
181. LA MONTAGNA G, MELI R, CRISCUOLO T, D'ANGELO S, VALENTINI G: Bioactivity of prolactin in systemic sclerosis. *Clin Exp Rheumatol* 2004; 22: 145-50.
182. LA MONTAGNA G, D'ANGELO S, VALENTINI G: Cross-sectional evaluation of YKL-40 serum concentrations in patients with systemic sclerosis. relationship with clinical and serological aspects of disease. *J Rheumatol* 2003; 30: 2147-51.
183. ALIVERNINI S, DE SANTIS M, TOLUSSO B *et al*.: Skin ulcers in systemic sclerosis: Determinants of presence and predictive factors of healing. *J Am Acad Dermatol* 2009; 60: 426-35.
184. KAYSER C, SEKIYAMA JY, PRÓSPERO LC, CAMARGO CZ, ANDRADE LE: Nailfold capillaroscopy abnormalities as predictors of mortality in patients with systemic sclerosis. *Clin Exp Rheumatol* 2013; 31 (Suppl. 76): S115-S117.
185. BLANN AD, SHEERAN TP, EMERY P: Von willebrand factor: Increased levels are related to poor prognosis in systemic sclerosis and not to tissue autoantibodies. *Br J Biomed Sci* 1997; 54: 5-9.
186. SHIMA Y, KUWAHARA Y, MUROTA H *et al*.: The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab. *Rheumatology* 2010; 49: 2408-12.
187. SHIMA Y, HOSEN N, HIRANO T *et al*.: Expansion of range of joint motion following treatment of systemic sclerosis with tocilizumab. *Mod Rheumatol* 2013 March 1 [Epub ahead of print].
188. BOSELLO S, DE SANTIS M, LAMA G *et al*.: B cell depletion in diffuse progressive systemic sclerosis: Safety, skin score modification and IL-6 modulation in an up to thirty-six months follow-up open-label trial. *Arthritis Res Ther* 2010; 12: R54.
189. BELLISAI F, MOROZZI G, SCACCIA F *et al*.: Evaluation of the effect of bosentan treatment on proinflammatory cytokine serum levels in patients affected by systemic sclerosis. *Int J Immunopathol Pharmacol* 2011; 24: 261-4.
190. ABOU-RAYA A, ABOU-RAYA S, HELMII M: Statins as immunomodulators in systemic sclerosis. *Ann N Y Acad Sci* 2007; 1110: 670-80.
191. DEL PAPA N, CORTIANA M, VITALI C *et al*.: Simvastatin reduces endothelial activation and damage but is partially ineffective in inducing endothelial repair in systemic sclerosis. *J Rheumatol* 2008; 35: 1323-8.
192. ALEKPEROV RT, KORZENEVA EG, ALEKSANDROVA EN, NOVIKOV AA, ANAN'EVA LP: Pleiotropic effects of statins in systemic sclerosis. *Ter Arkh* 2011; 83: 41-7.
193. ÅKESSON A, SCHEJA A, LUNDIN A, WOLLHEIM FA: Improved pulmonary function in systemic sclerosis after treatment with cyclophosphamide. *Arthritis Rheum* 1994; 37: 729-35.
194. DE MACEDO PA, BORGES CTL, DE SOUZA RBC: Cyclophosphamide: Effective in the treatment of severe cutaneous involvement in systemic sclerosis. *Revista Brasileira de Reumatologia* 2009; 49: 270-5.
195. MCNEARNEY TA, SALLAM HS, HUNNICUTT SE, DOSHI D, CHEN JD: Prolonged treatment with transcutaneous electrical nerve stimulation (TENS) modulates neuro-gastric motility and plasma levels of vasoactive intestinal peptide (VIP), motilin and interleukin-6 (IL-6) in systemic sclerosis. *Clin Exp Rheumatol* 2013; 31 (Suppl. 76): S140-S150.