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 multi-site 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
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-2G-330T, IL-6 C-174G, and IFN-γ AUTR5644T SNPs in lcSSc susceptibility and IL-1R (IL-1 receptor) Cpit1970T, 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 1.
2.2. Role of 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 is 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.

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

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

1: 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.

2.3. 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 STAT4+/+ mice. In contrast, STAT4−/−/ TSK+ mouse model did not significantly ameliorate the fibrotic phenotype (44). Regulation of T cell activation and toler-
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 mouse model (46).

2.4. Experimental interventions in scleroderma animal models
Interventions that ameliorate fibrogenesis and affect IL-6 are summarised in Table III.

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

<table>
<thead>
<tr>
<th>Animal models</th>
<th>Interventions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19 KO C57BL/6 mice (37)</td>
<td>BLM SC injection</td>
<td>↓ dermal thickness, ↓ lung fibrosis</td>
</tr>
<tr>
<td>CD19 KO C57BL/6 mice-derived B cells (37)</td>
<td>ECM breakdown product stimulation via TLR-4</td>
<td>↓ mRNA &amp; protein levels of IL-4, IL-6, IL-10, IFN-γ, TGF-β, MIP-2</td>
</tr>
<tr>
<td>CD19 KO TSK+ mice (38)</td>
<td>N/A</td>
<td>↓ skin fibrosis</td>
</tr>
<tr>
<td>CD19 KO TSK+ mice-derived B cells (38)</td>
<td>N/A</td>
<td>↓ hyper-γ-globulinemia, ↓ autoantibody production, ↓ IL-6 secretion</td>
</tr>
<tr>
<td>TSK+ (39)</td>
<td>BAFF receptor antagonist</td>
<td>↓ autoantibody production</td>
</tr>
<tr>
<td>TSK+ mice-derived B cells (39)</td>
<td>BAFF stimulation</td>
<td>↑ ability to produce IL-6</td>
</tr>
</tbody>
</table>

1. 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.

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-kB) 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 enhanced 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 stimulates 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). Roscovicine- 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
(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 homodimerization 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; ScI70) antibody production by autologous peripheral blood 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 (HL-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 -α 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

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 III. Experimental interventions affecting fibrogenesis and IL-6.

<table>
<thead>
<tr>
<th>Animal models</th>
<th>Characteristics</th>
<th>Interventions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLM-induced dermal sclerosis mice and TSK+ mice (47)</td>
<td>Overt free radical production</td>
<td>IV Edaravone (a free radical scavenger)</td>
<td>↓ dermal thickness, lung fibrosis</td>
</tr>
<tr>
<td>BLM-induced dermal sclerosis mice and TSK+ mice (48)</td>
<td>N/A</td>
<td>IP Sirolimus</td>
<td>↓ skin fibrosis</td>
</tr>
<tr>
<td>BLM-induced lung fibrosis mice (50)</td>
<td>N/A</td>
<td>Col V prophylaxis</td>
<td>↓ lung inflammation</td>
</tr>
<tr>
<td>BLM-induced dermal sclerosis mice (52)</td>
<td>N/A</td>
<td>Anti-TGF-β antibody treatment</td>
<td>↓ IL-4, IL-6 levels</td>
</tr>
</tbody>
</table>

↓: 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.
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 (20). 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).

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.**

<table>
<thead>
<tr>
<th>Authors, year (ref.)</th>
<th>Interventions</th>
<th>Patients</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Targeted therapy on IL-6 or IL-6 producing B cells</strong></td>
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</tbody>
</table>
| 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 Vesmoter 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 20 SSc (16 dcSSc 4 lcSSc) Group II: Iloprost + CyA 2.5 MKD | 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 42.0±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 Mean 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/m² 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±3.8.13 (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 Rodman 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, antecediolipin, 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 protease 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-proCNP) 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), procalcitonin (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 deSSc 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) correlates 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. Rituxi-


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