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REVIEW

Biomarkers in the Pathogenesis, Diagnosis, and Treatment of Systemic Sclerosis

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Abstract: Systemic sclerosis (SSc) is a complex autoimmune disease characterized by vascular damage, vasoinstability, and decreased perfusion with ischemia, inflammation, and exuberant fibrosis of the skin and internal organs. Biomarkers are analytic indicators of the biological and disease processes within an individual that can be accurately and reproducibly measured. The field of biomarkers in SSc is complex as recent studies have implicated at least 240 pathways and dysregulated proteins in SSc pathogenesis. Anti-nuclear antibodies (ANA) are classical biomarkers with well-described clinical classifications and are present in more than 90% of SSc patients and include anti-centromere, anti-Th/To, anti-RNA polymerase III, and anti-topoisomerase I antibodies. Transforming growth factor-β (TGF-β) is central to the fibrotic process of SSc and is intimately intertwined with other biomarkers. Tyrosine kinases, interferon-1 signaling, IL-6 signaling, endogenous thrombin, peroxisome proliferator-activated receptors (PPARs), lysophosphatidic acid receptors, and amino acid metabolites are new biomarkers with the potential for developing new therapeutic agents. Other biomarkers implicated in SSc-ILD include signal transducer and activator of transcription 4 (STAT4), CD226 (DNAX accessory molecule 1), interferon regulatory factor 5 (IRF5), interleukin-1 receptor–associated kinase-1 (IRAK1), connective tissue growth factor (CTGF), pyrin domain containing 1 (NLRP1), T-cell surface glycoprotein zeta chain (CD3ζ) or CD247, the NLR family, SP-D (surfactant protein), KL-6, leucine-rich α2-glycoprotein-1 (LRG1), CCL19, genetic factors including DRB1 alleles, the interleukins (IL-1, IL-4, IL-6, IL-8, IL-10 IL-13, IL-16, IL-17, IL-18, IL-22, IL-32, and IL-35), the chemokines CCL (2,3,5,13,20,21,23), CXC (8,9,10,11,16), CX3CL1 (fractalkine), and GDF15. Adiponectin (an indicator of PPAR activation) and maresin 1 are reduced in SSc patients. A new trend has been the use of biomarker panels with combined complex multifactor analysis, machine learning, and artificial intelligence to determine disease activity and response to therapy. The present review is an update of the various biomarker molecules, pathways, and receptors involved in the pathology of SSc.

Keywords: biomarker, systemic sclerosis, scleroderma, cytokine, autoantibody

Introduction

Biomarkers are analytic indicators of the presence of a biological or disease process and/or activity of that process within an individual that can be accurately and reproducibly measured.[1–11] Thus, a biomarker could be a physical measure such as blood pressure, a cytokine such as IL-1, a cell or cellular manifestation such as a membrane receptor, a metabolite such lactate, or other reproducible indicator of a biologic or medical process. Biomarkers have become an increasingly important aspect of systemic sclerosis (SSc) research and clinical care.[12] The field of biomarkers in SSc is complex and evolving as recent studies have implicated at least 240 pathways and numerous dysregulated proteins in the pathogenesis of SSc.[5–14]

SSc is a complex autoimmune disease characterized by prominent fibrosis of the skin, macro- and micro-vascular damage, vasoinstability, and decreased peripheral blood perfusion with ischemia, inflammation, and exuberant fibrosis of internal organs including the lungs, kidneys, bowel, heart, and esophagus.[15–17] The presence of skin thickening of the fingers extending proximal to the metacarpophalangeal joint is adequate to be classified as SSc. However, if proximal skin thickening is not present, the weighted addition of the following findings: skin thickening of the fingers, finger tip lesions (ischemic ulcerations

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Table 1 Biomarkers in Systemic Sclerosis (SSc)

Clinical Biomarkers for SSc	Autoantibody Biomarkers for SSc	HLA antigens and Immunogenetic Biomarkers for SSc
• Skin thickening of the fingers • Skin thickening extending proximal to the metacarpophalangeal joint • Finger tip lesions (ischemic ulcerations or pitting scars), • Telangiectasia • Abnormal nail fold capillaries • Interstitial lung disease • Pulmonary arterial hypertension • Raynaud's phenomenon • Gastroesophageal reflux disease • Esophageal dysmotility	• Speckled pattern antinuclear antibodies • Anti-RNA polymerase III antibodies • Anti-centromere antibodies • Anti-topoisomerase antibodies • Anti-Th/To antibodies • Anti-endothelial or fibroblast antibodies • Anti-angiotensin II type I receptor antibodies • Anti-endothelin-1 type A receptor antibodies • Anti-platelet-derived growth factor receptor (anti-PDGFR) antibodies • Anti-extracellular matrix (ECM) protein antibodies • Anti-G-protein-coupled receptor antibodies • Anti-eIF2B antibodies • Anti-RuvBL1/2 complex antibodies • Anti-U3RNP antibodies • Anti-BICD2 antibodies • Anti-Ku antibodies • Anti-PM/Scl antibodies • Anti-U11/U12 RNP antibodies	**HLA-Antigens associated with SSc and/or SSc-associated antibodies** • HLA-DR1, DR2, DR3, DR5, and DR52. • HLA-DRB1*04:01,*04:05 • HLA-DQB1 02:01,*0301,*0302, *0501, *0601 **Non-HLA associated loci** • KIR locus variants • Polymorphisms of the complement C4 gene **Potential immune-virus mimic interactions** • Human herpesvirus-6 (HHV-6) • Mimiviridae • Phycodnaviridae

or pitting scars), telangiectasia, abnormal nail fold capillaries, interstitial lung disease, pulmonary arterial hypertension, Raynaud's phenomenon, and/or SSc-related autoantibodies (Table 1) may still permit the diagnosis of SSc.[18] SSc is presently formally classified into two main forms: 1) limited cutaneous SSc characterized by mostly distal skin thickening and the presence of anti-centromere antibodies (Figure 1), and 2) diffuse cutaneous SSc with widespread distal and proximal cutaneous changes usually with the presence of anti-topoisomerase antibodies, anti-RNA-III polymerase antibodies or other anti-nucleolar pattern antinuclear antibodies.[18–21] Nail fold capillaroscopy, which is now formally used to classify SSc, is a noninvasive and reliable method to detect diagnostic microvascular involvement in SSc.[17,18,22] The presence of an ANA, recurrent Raynaud's phenomenon, and nail fold capillaroscopic abnormalities predict progression to definite SSc.[21] Anti-topoisomerase I antibodies also predict the development of diffuse cutaneous involvement with SSc and digital ulcers in the first 3 years of disease, as well as severe interstitial lung disease (ILD) (Figure 2).[21,23]

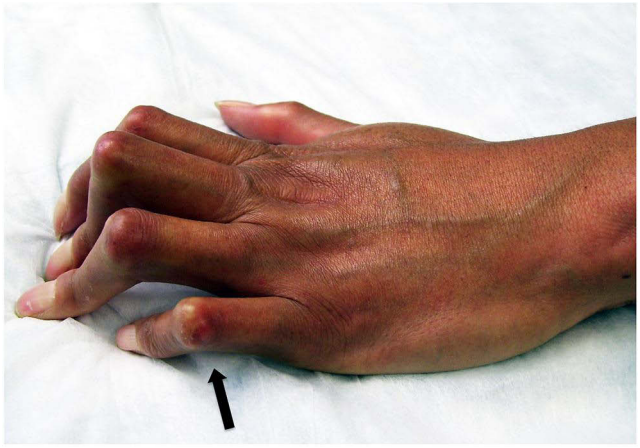

Figure 1 Sclerodactyly in SSC. The tightened skin extends beyond the metacarpophalangeal joints confirming the diagnosis of SSc. Also note contractures at the proximal interphalangeal joints and calcinosis cutis at the interphalangeal joint of the 5th digit (arrow).

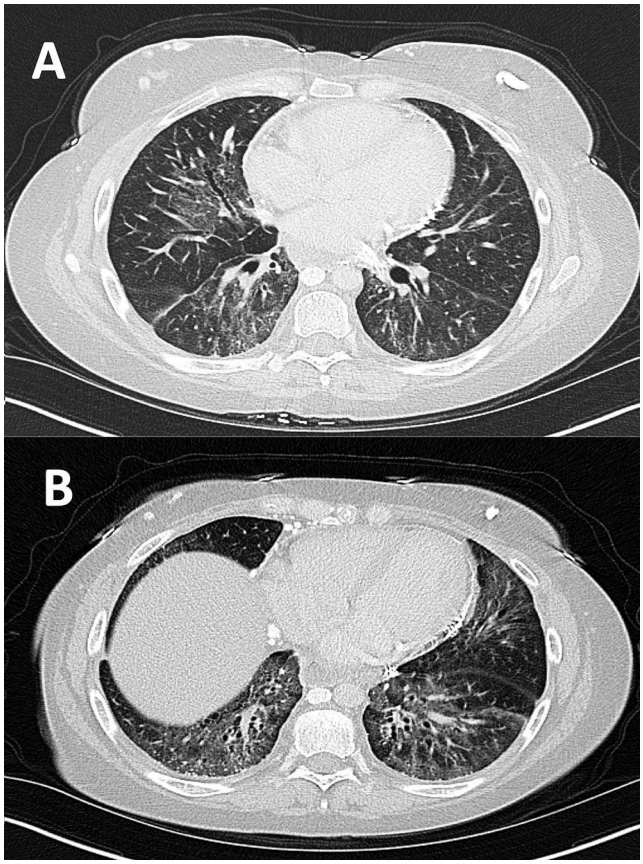

Figure 2 Computed tomographic (CT) Image of Interstitial Lung Disease (ILD) in SSc. (**A**) Interstitial lung disease (ILD) with bilateral anterior upper lobe reticular opacities and dependent ground glass opacities and bronchiectasis. Central airways are clear. (**B**) Similar scattered ground glass opacities most predominant in the lower lobes with extensive bronchiectasis and cystic changes.

Mortality in SSc is significantly increased and usually related to life-threatening manifestations of SSc including interstitial lung disease, scleroderma renal crisis, pulmonary arterial hypertension, cardiac involvement, secondary malignancy, and infections from tissue necrosis, aspiration pneumonia, and immunosuppression.[1,24]

Autoantibodies as Biomarkers in SSc

Autoantibodies are the most commonly used biomarkers in SSc and are most specifically useful for the diagnosis, classification, and prognosis of SSc (Table 1); further, biomarkers can be potential therapeutic targets.[2–4,21,25] Anti-nuclear antibodies (ANA) are present in more than 90% of SSc patients with anti-centromere, anti-Th/To, and anti-topoisomerase I antibodies considered as classical biomarkers occurring 60% of SSc and defining patients with well-described clinical classifications.[18,21] Other autoantibodies are present in the majority of patients with SSc and besides ANA include autoantibodies directed against endothelial or fibroblast antibodies, angiotensin II type 1 receptor, endothelin-1 type A receptor, platelet-derived growth factor receptor (anti-PDGFR) and extracellular matrix (ECM) proteins.[26–30] More complex autoantibody systems in SSc against G-protein-coupled receptors, growth factors and respective receptors have also been described.[4] Ten percent of SSc patients are ANA-negative, but novel antibodies including anti-elF2B, anti-RuvBL1/2 complex, anti-U11/U12 RNP, Anti-U3RNP, anti-BICD2, Anti-Ku, and Anti-PM/Scl can be seen in both ANA positive and negative patients.[7,31] The simultaneous presence of two different SSc-specific autoantibodies such as anti-centromere antibodies and anti-RNA polymerase III antibodies is so unusual that they are for practical purposes regarded as mutually exclusive, although there are always cases that may break the rule.[32] For instance, a recent study by Clark et al demonstrated that only 5% of SSc patients had ≥2 any autoantibody positivity and only 2.3% had ≥2 SSc-specific antibody positivity, with the most common combination being anti-U1RNP and anti-topoisomerase I antibodies.[33]

ANA may not be just biomarkers of disease in SSc but may have a pathogenic role through immune-complex-mediated mechanisms and molecular mimicry. ANA (particularly anti-topoisomerase-I and anti-RNA polymerase III antibodies) appear to be transported into the cell with direct interaction with intercellular components and receptors, targeting intracellular topoisomerase and RNA polymerase by the corresponding antibodies.[34,35] Anti-RNA polymerase III is a biomarker predicting rapid skin thickness progression, gastric antral vascular ectasia, SSc-associated cancers, scleroderma renal crisis, and possibly autoimmune syndromes associated with silicone breast implants.[21,36] Further supporting this concept of pathogenic autoantibodies in SSc is the response of certain SSc patients to select anti-B cell therapies and the role of activated B cells in the successful allogeneic bone marrow transplant to treat SSc.[37,38]

Immunogenetic and HLA Antigens as Biomarkers in SSc

SSc has been reported previously to be weakly associated with a restricted number of class II antigens in the major histocompatibility complex (MHC), especially HLA-DR1, DR2, DR3, DR5, and DR52 (Table 1).[39] However, these HLA-DR specificities that are weakly associated with SSc are in linkage disequilibrium with HLA-DQ alleles that are strongly associated with the specific autoantibodies peculiar to SSc. Thus, anticentromere antibodies occur most frequently in the presence of HLA-DQB1*0501 (DQ5), DQB1*0301 (DQ7) and similar DQB1 alleles characterized by a tyrosine or glycine residue in the outermost domain, specifically in position 26. Anti-topoisomerase I antibodies have been associated with the presence of HLA-DQB1*0301 (DQ7), DQB1*0302 (DQ8), DQB1*0601 and other DQB1 alleles evincing in position 30 a tyrosine residue. Anti-RNA polymerase III antibodies have been associated with HLA- DQB1*02:01, DRB1*04:05, DRB4*01, and DQB1*04:01.[40,41] Recently, Class 1 HLA antigens have also been associated with SSc, in particular, the haplotype HLA-B*44:03-HLA-C*16:01 that interacts with the KIR locus suggesting that genetic modulation of lymphocyte activation also contributes to SSc onset.[42,43] Specific KIR2 phenotypes appear to also promote human herpesvirus-6 (HHV-6) infection and reactivation and HHV-6 reactivation has been associated with fibrosis and the development and severity of SSc.[44] Similarly, the immunodominant peptides of topoisomerase 1, fibrillarin, and centromere protein A are homologous to viral protein sequences from the Mimiviridae and Phycodnaviridae families, suggesting a virus-immune receptor interaction that may trigger specific autoantibody production and the subsequent development of SSc.[45]

Similarly, higher copy number polymorphisms of the complement C4 gene appear to provide less risk to developing SSc, and lower copy numbers of C4A and C4B provide augmented risk of developing SSc, with the serum levels of C4 protein paralleling the gene copy numbers and decreased or increased risk for developing SSc.[46] Thus, C4 genetics are another immunogenetic factor that independently decreases and increases the risk of SSc along with amino acid variants of HLA-DRB1 and HLA-DQB1.

Other Biomarkers in SSc

Since the immune system and healing mechanisms of the human body are generally activated in SSc, multiple molecules are increased or decreased in SSc (Table 2) depending on the presence, activity, and therapy of the disease.[1–10] Toll-like receptors (TLRs) recognize pathogens and internal activation signals resulting in activation of multiple pathways that finally result in inflammation and alternations in innate immunity that occur with SSc.[47] Internal activation signals include damage-associated molecular patterns (DAMPs) that are intracellular molecules released under significant tissue injury or cellular stress and bind as endogenous ligands on TLRs.[48] DAMPs-TLR interaction on fibroblasts directly activates these collagen-producing cells to generate large amounts of collagen contributing to ECM expansion typical of SSc and the complications of SSc.[47] Further, ligand activation of TLR on dendritic cells results in increased production of Th17-related cytokines including IL-1β, IL-17F, IL-21 IL-22, and IL-33 resulting in aberrant T cell polarization and profibrotic inflammation.[49]

Transforming growth factor-b (TGF-β) is central to the process of fibrosis as well as dysregulation of the immune system toward inflammation.[50–52] Injured or stressed cells produce TGF-β that recruits and stimulates macrophages that secrete more TGF-β that then upregulates genes responsible for ECM production and progressive fibrosis.[53,54] Serum cytokeratin 17 (CK17), marginal zone B1 protein (MZB1) and leucine-rich α2-glycoprotein-1 (LRG1) are potential biomarkers for SSc, with CK17 negatively associated with SSc disease severity, with higher CK17 values being protective.[10] Endostatin has been associated with vascular manifestations in SSc and is specifically elevated in progressive SSc and has been considered as a marker of SSc severity and potentially as a therapeutic target.[55] Periostin is secreted by fibroblasts and epithelial cells and is

Table 2 Non-Antibody Protein Biomarkers in SSc

BioMarker	In SSc	SSc Severity/Activity Association	Therapeutic Target
• Toll-like receptors (TLRs)	Elevated activation	Positive	Potential
• Damage-associated molecular patterns (DAMPs)	Elevated	Positive	Potential
• Transforming growth factor-b (TGF-β)	Elevated	Positive, ILD	Potential
• Cytokeratin 17 (CK17)	Elevated	Negative, Less active disease	Potential
• Marginal zone B1 protein (MZB1)	Elevated	Positive, ILD	Potential
• Leucine-rich α2-glycoprotein-1 (LRG1)	Elevated	Positive, ILD, Extensive skin fibrosis	Potential
• Endostatin	Elevated	Positive, Vascular manifestations	Potential
• Basic Fibroblast growth factor (FGF)	Reduced	Negative	Potential
• Platelet-activating factor acetylhydrolase-β subunit (PAF-AHβ)	Reduced	Negative	Potential
• Periostin	Elevated	Positive, severity, skin fibrosis, cardiac fibrosis	Potential
• CC chemokine 2 (CCL2 or MCP-1)	Elevated	Positive, severity, skin fibrosis	Potential
• MicroRNA miR-138	Reduced	Negative, severity, skin fibrosis	Potential
• MicroRNA miR-27a	Reduced	Negative	Potential
• Serum soluble suppression of tumorigenicity 2 (ST2) receptor	Elevated	Positive, hand and articular involvement	Potential
• Angiopoietin-1 (Ang-1)	Reduced	Negative, Vascular manifestations	Potential
• Angiopoietin-2 (Ang-2)	Elevated	Positive, Vascular manifestations	Potential
• Protein sialic acid–binding Ig-like lectin 1 (SIGLEC-1)	Elevated	Positive	Potential
• Type 1 Interferons	Elevated	Positive, ILD, Musculoskeletal	Yes
• Interleukin 6 (IL-6)	Elevated	Positive, ILD	Yes
• Receptor-activated tyrosine kinases	Elevated	Positive, ILD	Yes
• Janus kinases (JAK)	Elevated	Positive, ILD, skin	Yes
• Signal transducer and activator of transcription (STAT) proteins	Elevated	Positive	Potential
• Intercellular adhesion molecule 1 (ICAM-1),	Elevated	Positive, Vascular manifestations, skin	Potential
• Vascular cell adhesion molecule 1 (VCAM-1)	Elevated	Positive, Vascular manifestations, skin	Potential
• Tissue thrombin	Elevated	Positive, Vascular manifestations, skin	Yes
• Tissue inhibitor of matrix metalloproteinase 4 (TIMP-4).	Elevated	Positive, Vascular manifestations	Potential
• Peroxisome proliferator-activated receptors (PPAR- γ)	Reduced	Negative, Vascular manifestations	Potential
• Adiponectin	Reduced	Negative, ILD, Extensive skin fibrosis	Potential
• Lysophosphatidic acid (LPA) and LPA receptors	Elevated	Positive	Yes

(*Continued*)

Table 2 (Continued).

BioMarker	In SSc	SSc Severity/Activity Association	Therapeutic Target
• Tissue inhibitors of matrix metalloproteinases (TIMP-4).	Elevated	Positive	Potential
• Semaphorins (Sema3A-F)	Elevated	Positive, Vascular manifestations	Potential
• Interleukins (1, 4, 10 13, 16, 17B, 17E, 17F, 18, 21.22, 32, 35)	Elevated	Positive	Potential
• Chemokines CCL (2,3,5,13,20,21,23)	Elevated	Positive	Potential
• Chemokines CXC (8,9,10,11,16)	Elevated	Positive	Potential
• Chemokine CX3CL1 (fractalkine)	Elevated	Positive	Potential
• Growth differentiation factor-15 (GDF-15)	Elevated	Positive	Potential
• Maresin 1	Reduced	Negative, Digital ulcers	Potential
• Galectin 3	Reduced	Negative, Extensive skin fibrosis, diffuse cutaneous SSc	Potential
• Soluble thrombomodulin (sTM)	Elevated	Positive, Specifically pulmonary arterial hypertension	Potential
• Cluster of differentiation 163 (CD163)	Elevated	Positive, Pulmonary arterial hypertension, Digital ulcers	Potential
• Salusin-alpha	Elevated	Positive, Associated with therapy	Potential
• Nuclear receptor subfamily 4A	Reduced	Negative	Potential
• Amino acid, lipid, and tricarboxylic acid metabolites	Variable	Variable	Potential
• Fecal calprotectin	Elevated	Positive, Gastrointestinal disease	Yes

Abbreviation: ILD, Interstitial lung disease.

associated with cell adhesion, fibrosis, angiogenesis, survival, and matrix remodeling.[56,57] Circulating levels of periostin are elevated in SSc and are associated with disease duration, skin fibrosis, and cardiomyopathy.[58] CC chemokine 2 (CCL2) has been implicated in the development of fibrosis in SSc.[59]

MicroRNAs (miRNAs) are short nucleotide sequences involved in cellular regulation. The microRNAs miR-138 and miR-27a suppress major pathways involved in epithelial to mesenchymal cell transition and subsequent fibrosis, and the relative expression of miR-138 and miR-27a are significantly lower in patients with SSc compared to controls, while only miR-138 is further depressed in diffuse cutaneous SSc, thus potentially both could be used as a diagnostic biomarker with miR-138 specifically to predict severity.[60–62] The suppression of tumorigenicity 2 (ST2) receptor binds IL-33 and the serum soluble ST2 (sST2) suppresses IL-33 signaling.[63] Elevated serum levels of sST2 serum levels are associated with higher articular disease activity and greater hand dysfunction in SSc, indicating that sST2 might be a biomarker to predict SSc articular involvement.[64]

Angiopoietins (Ang-1 and Ang-2) interact with the specific tyrosine kinase receptor Tie2 to modulate endothelial cell activation, vascular modeling, and angiogenesis.[65] Ang-1 is decreased and Ang-2 increased in SSc patients relative to controls, and this imbalance may contribute to both vascular ablation and the formation of abnormal new blood vessels.

An increased activation and expression of type 1 interferons are typical in SSc and appear similar to interferon abnormalities observed in systemic lupus erythematosus and may similarly provide a potential therapeutic target.[66–68] Increased IFN-I is associated with anti-U1-RNP antibodies, anti-topoisomerase antibodies, and more aggressive lung, skin, and skeletal muscle involvement.69.70 Dendritic cells in the tissues of patients with SSc are typically activated and

produce IFN-α and CXCL4 with the unexpected presence of TLR8, an RNA-sensing TLR.[50,69] CXCL4 modulates IFN-I responses by metabolic reprogramming of dendritic cells and an increased fibrotic phenotype.[70] Similarly, an increased expression of IFN-I–associated genetic loci correlates with a higher ILD progression rate.[54,68] The IFN-regulated protein sialic acid–binding Ig-like lectin 1 (SIGLEC-1) is upregulated in SSc compared to controls but does not associate with specific complications.[71]

Drugs that interfere with INF-1 signaling and thus the downstream activation of tyrosine kinases, inflammation, and fibrotic processes have considerable potential for the therapy of SSc.[72] Anifrolumab is a monoclonal antibody directed against IFN-I receptor subunit 1 and has been shown to be effective in systemic lupus erythematosus.[68] In early clinical trials, anifrolumab has also been shown to clinically decrease fibrosis in SSc and is associated with upregulation of type III collagen degradation markers and downregulation of T cell–associated proteins both suggesting a reduced fibrotic state.[73]

Tyrosine kinases that are activated downstream by INF-1 signaling include receptor-activated tyrosine kinases (for example, receptor kinases activated by growth factors - platelet-derived growth factor, fibroblast growth factor, or vascular endothelial growth factor) and nonreceptor tyrosine kinases (c-Abl, Src, Janus, and STATs).[74] Receptor-activated tyrosine kinases and their ligands are implicated in the migration, proliferation, ECM secretion, differentiation, and contraction of fibroblast precursors, tissue fibroblasts and myofibroblasts in SSc, and thus are intimately involved in the fibrotic process.[75–77] Nintedanib, an inhibitor of receptor activated tyrosine kinases, has been shown to be effective in reducing pulmonary fibrosis in SSc and down-regulating fibrotic processes, confirming the role of receptor activated tyrosine kinases in the pathologic processes of SSc.[74–77]

Similarly, downstream from INF-1 signaling in SSc is increased levels of IL-6 in SSc that ultimately activate nonreceptor tyrosine kinases especially Janus kinases (JAKs) and signal transducer and activator of transcription (STAT) proteins resulting in fibroblast differentiation, proliferation, and ECM and collagen production, providing promising therapeutic targets.[51,78,79] In line with these observations, tocilizumab, an IL-6 receptor blocker shown to be effective in ILD of SSc, reduces biomarkers of inflammation, ECM turnover, and macrophage activation, including collagen degradation and formation neoepitopes.[80,81] Further, there is early evidence that the JAK inhibitors tofacitinib and baricitinib reduce fibrosis in SSc in both lungs and skin, consistent with the known role of JAK/STAT activation in fibrosis in SSc.[82–86]

SSc manifests extensive endothelial injury, upregulation of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin, a von Willebrand factor (vWF), tissue factor (TF), and tissue thrombin resulting in local hypercoagulability.[85,86] Enhanced expression of thrombin stimulates endothelial cells and fibroblasts, resulting in enhanced collagen production, reduced matrix metalloproteinases, and expansion of the ECM contributing to the fibrosis of SSc. SSc is characterized by increased endogenous thrombin potential, thrombin expression, and endothelial damage biomarkers as well as lower thrombomodulin, fibrinolysis, and platelet levels associated with vasculopathy and digital ulcers.[84,85] These findings suggest that thrombin inhibitors might be useful for SSc, and early human studies using the thrombin inhibitor dabigatran preliminarily demonstrated reasonable safety and reduced skin thickness, suggesting that thrombin inhibitors are a viable therapeutic possibility.[86]

Peroxisome proliferator-activated receptors (PPARs) are ligand-responsive transcription factors of the nuclear hormone receptor family.[87] PPAR- γ is in particular an anti-inflammatory receptor that down-regulates activation of macrophages, lymphocytes, and dendritic cells. PPAR-γ reduces fibrogenesis by interfering with the TGFβ pathway by inhibiting the TGFβ driven differentiation of fibroblasts into active myofibroblasts, thus limiting collagen and ECM formation and decreasing tissue contraction.[88,89] Thus, impaired PPAR-γ expression or function may contribute to the excessive fibroblast activation and fibrosis of SSc. Expression of PPAR-γ is markedly diminished in skin tissues, lung biopsies, and fibroblasts from patients with SSc, and PPAR-γ appears to be downregulated by excessive expression of TGF-ß.[90] Adiponectin, a marker for PPAR-γ activation is also decreased in SSc, and these reduced levels are associated with the degree of skin fibrosis, suggesting that both adiponectin and membrane PPAR-γ are potential biomarkers for SSc.[91] However, circulating PPAR-γ is increased in SSc, suggesting a ligand scavenger effect that prevents membrane PPAR-γ activation thus promoting fibrogenesis.[92] PPAR-γ agonists reduce lung fibrosis and skin thickness in animal models of SSc, suggesting that the PPAR-γ pathway and PPAR-γ agonists are important potential therapeutic approaches.[93]

Lysophosphatidic acid (LPA) is a lipid mediator that is generated by the enzyme lysophospholipase (autoxain) wherever there is cell injury or inflammation, and LPA binds to G protein-coupled LPA receptors ($LPAR_{1–6}$).[94]

$LPAR_1$ after activation mediates the physiological effects of LPA including migration, survival, and infiltration of macrophages, activation of inflammasomes, release of proinflammatory mediators, including IL-1 and IL-18, promoting differentiation of mesenchymal cell to myofibroblasts, and collagen excretion.[52] Circulating LPA is increased in SSc patients compared to controls and thus is a natural biomarker and target for treatment of SSc.[94,95] Treatment of SSc patients with an $LPAR_1$ inhibitor resulted in decreases in skin scores and a reduction in LPA-related activation genes, indicating a potentially useful intervention, although more clinical trials are required.[96]

SSc is generally characterized by the abnormal accumulation of ECM.[1,97] ECM catabolism is regulated by matrix metalloproteinases (MMP-1 to MMP-28) whose activity is in turn inhibited by tissue inhibitors of MMPs (TIMP-1 to TIMP-4).[98,99] In this line, the inhibitor, TIMP-4, is increased in SSc patients with respect to healthy subjects.[100] Similarly, semaphorins (Sema3A-F) have anti-angiogenic effects and are increased in SSc patients.[101,102]

The cytokines, interleukins and chemokines are often elevated in SSc. IL-1, IL-4, IL-6, IL-10 IL-13, IL-16, IL-18, IL-22, IL-32, and IL-35 have all been reported to be elevated in SSc.[103–105] IL-17A is not specifically increased, while IL-17B, IL-17E, and IL-17F are higher SSc.[106] The chemokines CCL2,3,5,13,20,21,23, CXC8,9,10,11,16, CX3CL1 (fractalkine), and growth differentiation factor-15 (GDF-15) are all increased in SSc.[11,104,107–111] Adiponectin and maresin 1 are reduced, while galectin 3 is higher in SSc patients.[112–114] Thrombomodulin (TM) and cluster of differentiation 163 (CD163) are elevated in SSc.[115,116] Salusin-alpha, a regulator of secretion of pro-inflammatory cytokines and vascular smooth muscle proliferation, is increased in SSc.[117] The nuclear receptor subfamily 4A (including NR4A1, NR4A2, and NR4A3) are important down regulators of inflammation and fibrosis in SSc, and enhancing the expression of these receptors may be an approach to treating SSc.[118]

Recently, metabolic profiling has also be applied to SSc, demonstrating alterations in homocysteine, proline, alpha-N-phenylacetyl-L-glutamine, glutamine, asymmetric dimethylarginine, citrulline and ornithine, kynurenine, tryptophan, acylcarnitines associated from long-chain fatty acids, and tricarboxylic acids such as citrate and succinate with differences between the different SSc subtypes.[119]

In the following sections the role of biomarkers relevant to specific organ system disease in SSc will be discussed.

Interstitial Lung Disease (ILD) in SSc (SSc-ILD)

SSc is frequently complicated with interstitial lung disease (ILD) as a major contributing cause of both mortality and disability.[15,16] Approximately 65–85% of patients with SSc develop ILD (SSc-ILD) of variable severity and 25–30% develop aggressive disease that is associated with the significant mortality of 40% over a 10-year period.[1,19,120,121] Lung involvement results in an estimated 35% of all SSc-related deaths.[122] Risk factors of SSc-ILD include male sex, diffuse cutaneous SSc, African American heredity, and the presence of anti–Scl-70 (anti-topoisomerase I) antibodies.[123,124]

Lung endothelial damage is central to the pathogenesis of ILD. The typical histologic pattern in SSc-ILD is nonspecific interstitial pneumonia unlike idiopathic pulmonary fibrosis that is usually a usual interstitial pneumonia pattern.[1] SSc-ILD is characterized by inflammation early in the disease, extensive endothelial dysfunction, and increased deposition of ECM, especially collagen produced by activated myofibroblasts in resident tissues.[1,12,20] The increased ECM increases the stiffness of lung tissues resulting in restrictive lung disease with reduction of lung compliance, decreased lung volumes and decreased diffusion capacity resulting in impaired exercise tolerance, dyspnea, fatigue, hypoxia, increased pulmonary artery pressures, work disability, and reduced life expectancy. The pathological process is believed to be initiated by repetitive epithelial and endothelial cell injury with activation of the immune system, recruitment of fibroblasts, and phenotypic transformation of the fibroblast to a myofibroblast that then secretes excessive ECM resulting in fibrosis.[125] The initial endothelial and epithelial injuries are likely to be autoimmune and inflammatory in nature but could be induced also by pathogens and environmental factors.[126] Apoptosis occurs in certain epithelial cells denuding the alveoli, and simultaneously other epithelial cell transition into myofibroblasts with reduced apoptosis, loss of polarity, increased migration, and increased production of ECM, including collagen.[53] The reduced apoptosis of myofibroblasts may cause abnormal persistence of these active cells, contributing the progressive fibrosis.[53]

SSc-ILD is detected after diagnosis by high-resolution CT (HR-CT) and progression by both HR-CT and pulmonary function tests (PFT) (Figure 2).[127–129] Quantitative CT has also been used to detect early SSc-ILD and differentiate it from interstitial pneumonia and to more precisely follow the progression of SSc-ILD.[130] Recently, intercostal ultrasound

has also been used to screen for SSc-ILD to detect characteristic B-lines and subpleural disease.[131] Significant or progressive SSc-ILD usually prompts therapy. The PFT-based OMERACT (outcome measures in rheumatic diseases) detects progression of SSc-ILD defined as ≥10% decline in forced vital capacity (FVC) or ≥5% to <10% decline in FVC with ≥15% relative decline in DLCO.[129,132] It should be realized, however, that with effective therapy for SSc-ILD, the lung fibrosis is stabilized or the rate of decline of lung function is reduced, rather than complete restoration of lung function.[20] Thus, the emphasis for earlier diagnosis and prompt therapy of SSc-ILD before significant irreversible lung damage has accumulated.

The general treatment guidelines for SSc-ILD have recently been reviewed and revised.[133–136] Drugs used to treat SSc-ILD include non-specific immunosuppressives (cyclophosphamide, mycophenolate), specific immunosuppressive drugs including anti-IL-6 agents (tocilizumab), anti-B-cell drugs (rituximab), and antifibrotic agents (nintedanib - a tyrosine kinase inhibitor).[20,137,138] The non-specific immunosuppressives, mycophenolate, an inhibitor of the synthesis of guanosine nucleotides, and cyclophosphamide, an alkylating agent, decrease proliferation of fibroblast, T-helper cells, and B-cells, and thus, have significant anti-fibrotic effects.[139,140] Indeed, mycophenolate is currently considered the standard and basic underlying therapy for SSc-ILD.[20] Tocilizumab is increasingly used for SSc-ILD. Tocilizumab inhibition of the IL-6 receptor decreases myofibroblast activation and reduces M2 macrophage polarization, both important to the antifibrotic effects of tocilizumab.[141,142] B cell depletion suppresses pro-fibrotic macrophage differentiation and thus inhibits fibrosis, providing an additional rationale for unapproved but often used anti-B cell agents such as rituximab in SSC-ILD.[143] The anti-fibrotic drug nintedanib, a tyrosine kinase inhibitor, inhibits the receptors of PDGF, FGF, and vascular endothelial growth factor (VEGF), reducing fibrosis.[75] However, the anti-fibrotic pirfenidone appears to have less beneficial effects in SSc and is presently not approved for treatment of this entity.[144] JAK inhibitors have been used and seem to be effective for refractory cases of SSc-ILD, but remain off-label.[51] For rapidly progressive disease non-responsive to these agents or for very early SSc-ILD, an alternative is autologous haematopoietic stem cell transplant (AHSCT) and for end-stage lung disease lung transplantation.[14,145,146]

SSc-ILD has been especially associated with anti-topoisomerase I antibody (anti-Scl-70 antibody), anti-U11/U12 antibodies, nucleolar pattern antinuclear antibodies (including anti-RNA-polymerase III, anti-NOR-90, anti-PM/Scl-75, anti-U3-RNP /Fibrillarin anti- antibodies) (Table 3).[1,147,148] Anti-PM/Scl defines SSc patients with high frequency of ILD, calcinosis, dermatomyositis skin changes, and severe myositis.[21] Further autoantibodies against anti-phosphatidylinositol-5-phosphate 4-kinase type 2 beta (PIP4K2B) and AKT serine/threonine kinase 3 (AKT3) have been tied to increased lung fibrosis in SSc.[149]

Table 3 Biomarkers in SSc-Interstitial Lung Disease (SSc-ILD)

BioMarker	SSc-ILD	SSc-ILD Severity/ Activity Association	Therapeutic Target
• Nucleolar pattern antinuclear antibodies	Elevated	Positive	Yes
• Anti-topoisomerase-I antibodies	Elevated	Positive	Yes
• Anti-RNA-polymerase III antibodies	Elevated	Positive	Yes
• Anti-anti-NOR-90 antibodies	Elevated	Positive	Yes
• Anti-anti-Th/To antibodies	Elevated	Positive	Yes
• Anti-U3-RNP/Fibrillarin antibodies	Elevated	Positive	Yes
• Anti-PM/Scl-100 antibodies	Elevated	Positive	Yes
• Anti-U11/U12 antibodies	Elevated	Positive	Yes
• Anti-phosphatidylinositol-5-phosphate 4-kinase type 2 beta (PIP4K2B)- antibodies	Elevated	Positive	Yes
• Anti-AKT serine/threonine kinase 3 (AKT3) antibodies	Elevated	Positive	Yes

(*Continued*)

Table 3 (Continued).

BioMarker	SSc-ILD	SSc-ILD Severity/ Activity Association	Therapeutic Target
• Transforming growth factor-b (TGF-β)	Elevated	Positive	Potential
• Marginal zone B1 protein (MZB1)	Elevated	Positive	Potential
• Leucine-rich α2-glycoprotein-1 (LRG1)	Elevated	Positive	Potential
• DNAX accessory molecule 1 (CD226)	Elevated	Positive	Potential
• Interleukin 6 (IL-6)	Elevated	Positive	Yes
• Janus kinases (JAK)	Elevated	Positive	Yes
• Signal transducer and activator of transcription 4 (STAT4) proteins	Elevated	Negative	Potential
• Interferon regulatory factor 5 (IRF5)	Elevated	Positive	Potential
• Interleukin-1 receptor–associated kinase-1 (IRAK1)	Elevated	Positive	Potential
• Connective tissue growth factor (CTGF)	Elevated	Positive	Potential
• Pyrin domain containing 1 (NLRP1,3)	Elevated	Positive	Potential
• T-cell surface glycoprotein zeta chain (CD3ζ or CD247)	Elevated	Positive	Potential
• Krebs von den Lungen-6 (KL-6)	Elevated	Positive	Potential
• Interleukin-8 (IL-8)	Elevated	Positive	Potential
• Leucine-rich alpha-2 glycoprotein 1 (LRG1)	Elevated	Positive	Potential
• Chemokine [C-X-C motif] ligand 3 (CXCL3)	Elevated	Positive	Potential
• Chemokine [C-X-C motif] ligand 4 (CXCL4)	Elevated	Positive	Potential
• Chemokine [C-C motif] ligand 2 (CCL 2)	Elevated	Positive	Potential
• Chemokine [C-C motif] ligand 18 (CCL18)	Elevated	Positive	Potential
• Chemokine [C-C motif] ligand 19 (CCL18)	Elevated	Positive	Potential
• Matrix metalloproteinase-7 (MMP7)	Elevated	Positive	Potential
• Matrix metalloproteinase-12 (MMP12)	Elevated	Positive	Potential
• C-reactive protein	Elevated	Positive	Potential
• Chitinase-3–like protein 1(YKL-40)	Elevated	Positive	Potential
• Sirtuins (SIRT1 and SIRT3)	Elevated	Positive	Potential
• Surfactant protein D (SP-D)	Elevated	Positive	Potential
• Cancer antigen 15–3 (Ca15-3)	Elevated	Positive	Potential
• Intercellular adhesion molecule 1 (ICAM-1)	Elevated	Positive	Potential
• Cold-inducible RNA-binding protein (CIRP)	Elevated	Positive	Potential
• Adiponectin	Reduced	Negative	Potential
• HLA-DRB1 alleles	Present	Positive	Uncertain

Transforming growth factor-b (TGF-β) is produced by stressed or injured lung cells that stimulate and recruit tissue macrophages that amplify TGF-β production that then up upregulates genes responsible for ECM production and progressive fibrosis.[53,54,113,150] Other biomarkers typically implicated in SSc-ILD include STAT4, CD226 (DNAX accessory molecule 1), interferon regulatory factor 5 (IRF5), interleukin-1 receptor–associated kinase-1 (IRAK1), connective tissue growth factor (CTGF), pyrin domain containing 1 (NLRP1), T-cell surface glycoprotein zeta chain (CD3ζ) or CD247, the NLR family, SP-D (surfactant protein), KL-6 (Krebs von den Lungen-6), IL-8, LRG1, and CCL19, as well as genetic factors including DRB1 alleles.[1,7,55,127,151–156] The biomarkers most associated with active lung disease and progression specifically in SSc-ILD are KL-6, SP-D (surfactant protein), C-reactive protein, and CCL19 although other non-specific biomarkers can also be elevated.[1,152] Th2-lymphocytes produce IL-13 and IL-4 that stimulate fibroblasts and activate the pro-fibrotic M2 macrophages that induce TGF-β, platelet-derived growth factor (PDGF), and fibroblast growth factors (FGF) inducing myofibroblast activation.[104] The biomarker chemokines including CCL18, CX3CL1 and CXCL4 with and without RNA complexes have recently been associated with SSc-ILD.[59,108,152,155,157]

IL-6 is important in the progression of SSc-ILD and is secreted by myofibroblasts, M1 macrophages, and B-cells.[15,143] IL-6 enhances the expression of IL-4 and IL-13-receptors increasing polarization of M2 macrophage and enhancing fibrosis.[158,159] This central role is further supported by tocilizumab inhibition of the IL-6 receptor decreasing myofibroblast activation and reduced M2 macrophage polarization, resulting in a portion of the demonstrated antifibrotic effects of tocilizumab in the setting of SSc-ILD.[141] B-cell activation is also common in SSc, and B cells increase a number of angiogenic factors.[160] B cell depletion suppresses pro-fibrotic macrophage differentiation and thus inhibits fibrosis, providing rationale for anti-B cell agents, such as rituximab in SSC-ILD.[143]

Of the above biomarkers, autoantibodies and C-reactive protein are the only biomarkers typically used in contemporary routine clinical practice. In that line, if anti-topoisomerase-I antibodies are present and the anticentromere antibodies are not present, there is an increased incidence of progressive SSc ILD.[1,124,161] However, various other biomarkers are being explored for clinical use including KL-6, CCL18 (chemokine [C-C motif] ligand 18), MMP7 (matrix metalloproteinase-7), MMP12 (matrix metalloproteinase-12), IL-6, CXCL4 (chemokine [C-X-C motif] ligand 4), CXCL3 (chemokine [C-X-C motif] ligand 4), and chitinase-3–like protein 1(YKL-40) as recently reviewed.[1,132] MMP-12 are raised in SSc-ILD compared with SSc without ILD and correlated with the degree of pulmonary fibrosis.[162] Sirtuins are NAD-dependent protein deacetylases that regular angiogenesis; SIRT1 and SIRT3 correlate with the degree of lung fibrosis in SSC.[162] The chemokine CCL2 is increased in SSc and predicts long-term progression of SSc-ILD.[163] The cold-inducible RNA-binding protein (CIRP) was also associated with SSc-ILD and may be a marker of disease activity and response to therapy.[164] Jee et al have recently described a composite biomarker index consisting of SP-D, Ca15-3 and ICAM-1 that identifies SSc-ILD.[165] The use of biomarker panels, composite biomarker measures, machine learning, and artificial intelligence is a growing trend in the field.[165–167]

In conclusion, multiple arms of the immune system are activated in SSc-ILD, providing many candidate biomarkers and potential therapeutic targets with the trend being the use of biomarker panels with combined complex multifactor analysis, machine learning, and artificial intelligence to determine disease activity and response to therapy.

Vascular Injury in SSc Including Pulmonary Arterial Hypertension (PAH)

The most obvious clinical manifestation of vascular disease in SSc is Raynaud's phenomenon (RP) and digital ischemia.[168,] RP is followed by telangiectases, ischemic digital ulcers, pitting scars, periungual microvascular abnormalities, pulmonary arterial hypertension (PAH) (Figure 3), and cardiac disease affecting function and exercise tolerance.[15,169–171] All are considered outcomes of vascular injury in SSc as recently reviewed by Pattanaik et al.[172]

PAH occurs in 7% to 19% of SSc patients depending on the population and duration of the disease.[19] Risk factors for PAH include severe Raynaud's phenomenon, severe digital ischemia, cutaneous telangiectasia, chronic disease, late onset of disease, older age, postmenopausal status, reduced diffusion capacity (DLCO < 50%), DLCO/alveolar volume less than 70%, forced vital capacity/DLCO less than 1.6 and an elevation in right ventricular systolic pressure greater than 2 mmHg/year.[173] Screening should include specific autoantibodies (anti-topoisomerase I (SCL-70), anti-centromere and anti-RNA polymerase III and antiphospholipid antibodies), pulmonary function tests, echocardiography, serum N-terminal pro-brain natriuretic peptide (NT-proBNP), nail fold capillaroscopy, and initial high-resolution CT to exclude

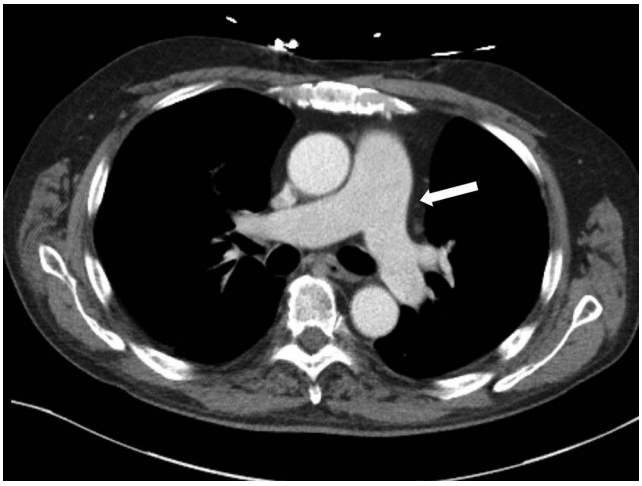

Figure 3 Computed tomographic (CT) Image of the Mediastinum. CT angiographic image of thorax in a SSc patient demonstrating a markedly dilated pulmonary artery (arrow) without thrombus consistent with pulmonary artery hypertension (PAH).

ILD, and if there is a question of PAH, a right heart catheterization to determine PA pressure.[174–178] Treatment of PAH in SSc has recently reviewed with guidelines and includes a stepwise approach using single agents or combined therapy with phosphodiesterase type 5 (PDE-5) inhibitors (including sildenafil and tadalafil), soluble guanylate cyclase (sGC) stimulators (including riociguat), endothelin receptor antagonists (including bosentan, ambrisentan, and macitentan), prostacyclin analogs (epoprostenol, treprostinil and iloprost), or selective prostacyclin IP receptor agonists (selexipag) supported by anticoagulants, diuretics, digoxin, and calcium channel blockers where appropriate.[133,174,178,179]

Raynaud's phenomenon (RP) can occur without a systemic disease (primary RP), but RP is an almost universal SSc-associated phenomenon with a characteristic 3-phase color change associated with symptoms of pain, burning, ischemia, and in some cases, ischemic ulcers and/or necrosis of the digits.[163,180,181] Bernero et al have demonstrated that a large proportion of initially primary RP progress to secondary RP – that is they eventually develop a definite autoimmune disease such as SSc or other autoimmune disease.[181] The pathophysiology of RP is similar to the other mechanisms of vascular injury in SSc discussed in detail in this section. New methods to quantify RP beyond nailfold capillaroscopy include laser Doppler imaging, laser speckle contrast analysis, thermal imaging, and color and spectral Doppler imaging.[182,183] Pharmacologic therapy for RD has recently been reviewed and is generally initiated first with calcium channel blockers, and if ineffective followed by PDE-5 inhibitors or fluoxetine with endothelin-1 receptor blockers and intravenous prostanoids reserved for the most resistant cases.[133–136,182,184]

Vascular injury and subsequent fibrosis may occur from the activation and apoptosis of endothelial cells, specific autoantibodies, infectious agents, endogenous hypercoagulability, reactive oxygen species, as well as other causes providing many potential biomarkers (Table 4).[84,185] Once activated endothelial cells secrete endothelin-1 (ET-1), von Willebrand factor (vWF), nitric oxide, and endothelial nitric oxide synthase, resulting in instability of the vascular tone with less vasodilation and more vasoconstriction causing tissue ischemia and hypoxia.[186–188] Endothelin-1 stimulates fibroblasts to convert to activated myofibroblasts with increased ECM secretion, intimal hyperplasia, luminal narrowing, reduced capillary blood flow vessel obliteration, and ischemia.[189] Local secretion of von Willebrand factor causes platelet aggregation, hypercoagulability, and fibrin deposition leading to terminal vascular damage.[186–188] Myofibroblasts are also created by the endothelial-to-mesenchymal transition.[128] Activated endothelium also expresses increased adhesion molecules and specific chemokines, recruiting immune cells and perivascular infiltrates leading to further inflammation and fibrosis.[186–190] Notably, angiogenesis is also reduced due to imbalanced cytokines including endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-9, endoglin, ET-1) and angiostatic (pentraxin 3 (PTX3), MMP-12, endostatin, angiostatin, semaphorin3E (Sema3E), and Slit2) factors and the dysfunction and impaired recruitment of endothelial progenitor cells (EPCs).[169,191–193]

Table 4 Biomarkers in SSc-Pulmonary Arterial Hypertension (PAH)

BioMarker	SSc-PAH	SSc-PAH Severity/Activity Association	Improves with Therapy
• N-terminal pro-brain natriuretic peptide (NT-proBNP),	Elevated	Positive	Yes
• L-selectin (CD62L)	Elevated	Positive	Yes
• P-selectin	Elevated	Positive	Yes
• Intercellular adhesion molecule 1 (ICAM-1)	Elevated	Positive	Yes
• Vascular cell adhesion molecules (VCAMs)	Elevated	Positive	Yes
• Platelet and endothelial Adhesion Molecule-1 (PECAM-1)	Elevated	Positive	Yes
• Vascular endothelial growth factor (VEGF-A, -B, -C, and –D)	Elevated	Positive	Yes
• Tissue thrombin	Elevated	Positive	Yes
• Transforming growth factor-b (TGF-β)	Elevated	Positive	Yes
• Endothelin-1 (ET-1)	Elevated	Positive	Yes
• Endostatin	Elevated	Positive	Yes
• Angiostatin	Elevated	Positive	Uncertain
• Matrix metalloproteinase-9 (MMP-9)	Decreased	Negative	Yes
• Matrix metalloproteinase-12 (MMP-12)	Elevated	Positive	Uncertain
• Tissue Inhibitor of matrix metalloprotease-4 (TIMP-4)	Elevated	Positive	Uncertain
• Neuropilins (NRP1-2)	Elevated	Positive	Uncertain
• Slit glycoproteins (Slit1-3)	Elevated	Positive	Uncertain
• IL-18 binding protein levels	Elevated	Positive	Uncertain
• Interleukin-32 (IL-32_)	Elevated	Positive	Uncertain
• Macrophage migration inhibitory factor (MIF)	Elevated	Positive	Uncertain
• Chemokine [C-X-C motif] ligand 15 (CXCL15)	Elevated	Positive	Uncertain
• Chemokine [C-X-C motif] ligand 16 (CXCL16)	Elevated	Positive	Uncertain
• Chemokine [C-C motif] ligand 20 (CCL 20)	Elevated	Positive	Uncertain
• Chemokine [C-C motif] ligand 21 (CCL21)	Elevated	Positive	Uncertain
• Chemokine [C-C motif] ligand 23 (CCL23)	Elevated	Positive	Uncertain
• Growth differentiation factor 14 (GDF14)	Elevated	Positive	Uncertain
• Resistin	Elevated	Positive	Uncertain
• Adipsin	Elevated	Positive	Uncertain
• Interferon-gamma	Elevated	Positive	Uncertain
• Interferon type 1	Elevated	Positive	Uncertain
• Thrombomodulin (TM)	Elevated	Positive	Yes

Enhanced endogenous thrombin generation potential and higher thrombin peak are present in SSc, accompanied by increased inflammatory markers, increased factor VIII activity, blood eosinophilia, thrombocytopenia, reduced VCAM-1, and lower thrombomodulin, indicating an important role for the thrombin system in vascular injury of SSc.[84] Dabigatran, a direct thrombin inhibitor, is well tolerated in SSc patients with ILD and appears to show some improvements, but long-term clinical trials still need to be performed.[86]

Peroxisome proliferator-activated receptor-gamma (PPAR) is an important regulator of fibroblast growth, ECM formation, and connective tissue remodeling.[87–92] PPAR interferes with the TGFβ pathway by inhibiting the ability of TGFβ to induce fibroblasts from differentiating into myofibroblasts and inhibiting collagen production via the transcriptional coactivator system.[89] PPAP activity and expression of adiponectin, a sensitive and specific index of PPAR activity, are both reduced in SSc and associated with more progressive skin fibrosis.[91] Circulating levels of PPAR are increased in SSc, particularly in diffuse cutaneous SSc with increased skin fibrosis, suggesting a defect in PPAR expression in SSc that interferes with activation of membrane bound PPAR enhancing fibrosis.[92] PPAR agonists are effective in reducing fibrosis in animal models of SSc; thus, activation of PPAR and levels of adiponectin have potential as biomarkers for fibrotic activity in SSc.[93,194]

Selectins are molecules that permit cell trafficking and cell homing. L-selectin (CD62L) is expressed on leukocytes, P-selectin is expressed on platelets, and E-selectin is present on endothelial cells and megakaryocytes, but there have been inconsistent results regarding selectins in SSc[104] immunoglobulin-like cell adhesion molecules including intercellular adhesion molecules (ICAMs), vascular cell adhesion molecules (VCAMs), and junctional adhesion molecules (JAMs). Circulating levels of ICAM-1 and VCAM-1 are higher in SSc patients with digital ulcers than without but may not be predictive of the occurrence of new DUs in SSc.[195] Similarly, VCAM-1 can be elevated in SSc both with and without PAH.[190] JAMs are adhesion molecules on endothelial cells, fibroblasts, epithelial cells, and blood cells, and circulating levels have been associated with microvascular disease and digital ulcers.[102,190] Soluble VCAM-1, ICAM-1, and P-selectin are elevated in SSc-PAH compared to controls and with effective therapy with bosentan return to normal values.[196]

The vascular endothelial growth factor (VEGF) family includes the VEGF-A, -B, -C, and –D, and placental growth factor and are elevated in both the blood and skin in SSc.[8,66,166,195,197,] VEGF levels also correlate with PAH but are lower in patients with digital ulcers.[129,198,199] TGF-β remains important to all the manifestations of SSc including PAH.[200] Endoglin (CD105) is an accessory receptor for TGF-β and higher circulating endoglin correlated with digital ulcers, suggesting that this was a biomarker for vascular injury in SSc.[201]

Endothelin-1 (ET-1) is secreted by endothelial cells and activated smooth muscle cells, fibroblasts, epithelial cells, and inflammatory cells.[202] Increased ET-1 is present in SSc-PAH when compared to SSc patients without PAH and healthy controls.[203] Treatment with bosentan decreased ET-1 in SSc patients with PAH to levels present in SSc without PAH, indicating that this biomarker could detect the severity of vascular injury and the response to bosentan therapy.[203]

Pentraxin 3 is a receptor produced by activated endothelial cells, macrophages, smooth muscle cells, dendritic cells, and fibroblasts.[204] However, levels of pentraxin 3 have only variable associations with vasculopathy in SSc[204] Endostatin, is an angiostatic peptide that blocks VEGF activity and has been associated with PAH, scleroderma renal crisis, and cardiac involvement[11] Angiostatin antagonizes a number of growth factors, including VEGF and is elevated in patients with more advance vascular disease in SSc[11,205] Matrix metalloproteinases break down ECM and levels of MMP-9 are decreased in SSc-PAH and upregulated with bosentan therapy, while MMP-12 was increased patients with digital ulcers and nailfold capillary abnormalities.[206,207]

SSc-PAH is associated with elevated tissue inhibitors of metalloproteinases including TIMP-4 levels, indicating a cardiopulmonary vasculature-specific role of TIMP-4 activation in SSc.[100] Neuropilins (NRP1-2) are non-tyrosine kinase glycoprotein receptors expressed on endothelial cells and are potential biomarkers predicting PAH, nailfold capillary abnormalities, and digital ulcers.[102,166] The slit glycoproteins (Slit1-3) are implicated in angiogenesis and are increased in SSc and in patients with microvascular disease.[208] Sirtuins (SIRT1-7) NAD-dependent protein deacetylases that regulate angiogenesis; SIRT1 and SIRT3 are decreased in SSc and microvascular disease, and SIRT3 specifically is related to the presence of digital ulcers.[162] Carcinoembryonic antigen-related cell adhesion molecule (CEACAM)-positive monocytes are associated with inflammation and ILD in SSc patients.[209]

Circulating IL-18 binding protein levels are higher SSc positively correlated with PAH.[103] Similarly, IL-33 and soluble suppression of tumorigenicity 2 (ST2) are increased in SSc especially with digital ulcers and PAH.[210] IL-32 and macrophage migration inhibitory factor (MIF) are elevated in SSc patients with PAH.[211] Chemokines CCL20, CCL21, and CCL23 are also elevated in SSc-PAH.[107–109] Elevated levels of CXCL4 and reduced levels of CXCL5 have recently also been associated with SSc-digital ulcers.[212,213] Similarly, the chemokines CXCL16 and GDF15 are elevated in SSc-PAH.[110,197] CX3CL1 (fractalkine) is elevated in SSc with digital ulcers.[111] Resistin is increased in SSc with digital ulcers and in SSc-PAH.[214] Galectin 3 was also found to be higher in SSc patients with digital ulcers.[68] Adipsin, visfatin, interferon-gamma, and type 1 interferons are elevated in SSc-PAH.[66,215–217] Aptamer proteomics of serum exosomes define patterns that could distinguish primary Raynaud's disease from early SSc and with RNA networks are potential biomarkers for vascular disease in SSc.[180,218,219]

Thrombomodulin (TM), CD163, and NT-proBNP are elevated in SSc-PAH.[115,116,220] Elevated levels of maresin 1 are associated with the development of digital ulcers in SSc.[50] Elevated asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide (NO) inhibitor that affects endothelial function and is elevated in microvascular disease in SSc.[221,222] Hypochromic erythrocytes have been closely associated with the prognosis of SSC-PAH.[223] Hemoglobin and ferritin are significantly lower in patients with pulmonary hypertension (PH) in SSc compared to those with pulmonary hypertension, while uric acid and NT-proBNP are significantly higher.[224] Circulating CCL21 in SSc is a biomarker associated with PAH and the development of PAH.[108]

Many molecules have been associated with the vascular complications of SSc, thus there are many potential biomarkers for PAH and vascular disease in SSc.

The Skin in SSc

Skin thickening is universal in SSc and generally necessary for a definite diagnosis with certain exceptions[18] There are many candidate biomarkers for skin disease in SSc (Table 5). Generally, anti-topoisomerase-I and anti-RNA polymerase III antibodies are associated with more extensive and severe skin involvement, while anti-centromere antibodies are associated with limited skin involvement.[1,2,4,225] Further, autoantibodies against PIP4K2B and AKT3 have been tied to more extensive skin fibrosis in SSc.[149] In skin biopsies, TGF-β1, TGF-βR1, and TGF-βR2 expression levels are higher in SSc patients than controls.[226] IL-6 and JAK associated pathways are also implicated in the skin thickening both directly

Table 5 Biomarkers in SSc Skin Disease

BioMarker	SSc Skin Disease	SSc-skin Severity Association	Therapeutic Target
• Nucleolar pattern antinuclear antibodies	Elevated	Positive	Yes
• Anti-topoisomerase-I antibodies	Elevated	Positive	Yes
• Anti-RNA-polymerase III antibodies	Elevated	Positive	Yes
• Anti-centromere antibodies	Elevated	Negative	Yes
• Anti-phosphatidylinositol-5-phosphate 4-kinase type 2 beta (PIP4K2B)- antibodies	Elevated	Positive	Yes
• Anti-AKT serine/threonine kinase 3 (AKT3) antibodies	Elevated	Positive	Yes
• Transforming growth factor-β (TGF-β1, βR1, βR2)	Elevated	Positive	Potential
• TGF-β- associated biomarkers (OSMR, SERPINE1, CTGF)	Elevated	Positive	Potential
• Marginal zone B1 protein (MZB1)	Elevated	Positive	Potential
• Sirtuins (SIRT1 and SIRT3)	Elevated	Positive	Potential
• Macrophage-associated biomarkers (CD14, IL13RA1)	Elevated	Positive	Potential

(*Continued*)

Table 5 (Continued).

BioMarker	SSc Skin Disease	SSc-skin Severity Association	Therapeutic Target
• Periostin	Elevated	Positive	Potential
• Leucine-rich α2-glycoprotein-1 (LRG1)	Elevated	Positive	Potential
• Interleukin 6 (IL-6)	Elevated	Positive	Yes
• Janus kinases (JAK)	Elevated	Positive	Yes
• Periostin	Elevated	Positive	Potential
• CC chemokine 2 (CCL2 or MCP-1)	Elevated	Positive	Potential
• Galectin 3	Reduced	Negative	Potential
• Fibrillar collagen molecule COL4A1	Elevated	Positive	Potential
• Matricellular protein COMP	Elevated	Positive	Potential
• Sirtuins (SIRT1 and SIRT3)	Elevated	Positive	Potential
• Adiponectin	Reduced	Negative	Potential

and by response to specific inhibitors that block these pathways.[227,228] Skin gene-expression of macrophage-associated biomarkers (CD14, IL13RA1) and TGF-β- associated biomarkers (OSMR SERPINE1, CTGF) are associated with progression of skin disease in SSc.[229] Marginal zone B1 protein (MZB1) appears to be a good biomarker for skin fibrosis.[10] Circulating levels of periostin are elevated in SSc with extensive skin fibrosis.[58] Sirtuins are NAD-dependent protein deacetylases that regular angiogenesis; SIRT1 and SIRT3 correlated with the degree of skin fibrosis in SSC.[162] Adiponectin is reduced in SSc involved skin.[114] Recently, the fibrillar collagen molecule COL4A1, the matricellular protein COMP, the gene coding for spondin SPON1, TNC, and another ECM protein were upregulated in SSc skin and completely distinguished SSc from normal skin.[230]

Skin thickness in SSc often improves spontaneously over time, confounding many interventional trials as recently reported with belimumab and nintedanib.[231–233] However, contemporary treatment guidelines note that mycophenolate, cyclophosphamide, and methotrexate have been demonstrated to improve the modified Rodnan skin score and reduce skin thickness in SSc.[133,178,179,234,235] Methotrexate can be problematic in SSc since it can occasionally cause lung inflammation that can be confused with SSc-ILD.[236] Recently, the JAK inhibitor tofacitinib, although not approved for SSc, was shown to be more effective than methotrexate in decreasing the modified Rodnan skin score, skin thickness by ultrasound, and musculoskeletal symptoms and reduced interferon regulated biomarker genes in SSc.[142,237] Ziritaxestat is a small-molecule selective autotaxin inhibitor and reduced the modified Rodnan skin score in SSc and thus is a promising new agent currently under clinical trials.[238] There is some evidence that rituximab and tocilizumab also improve skin scores in SSc.[81,227,228,239,240]

Skin ulceration in SSc is classified as calcinosis-related, traumatic, or ischemic.[241] Treatment of skin ulceration in SSc includes avoiding vasoconstrictors (eg, caffeine, amphetamines, cocaine, nicotine), cold temperature, and trauma, the cessation of tobacco products, and appropriate wound care. Ulcerations from calcinosis cutis in SSc develop from hydroxyapatite crystal deposition within the subcutaneous tissues and may be treated with surgical excision or debridement, topical antibiotics, low-dose tetracyclines (minocycline or doxycycline) or topical or intravenous sodium thiosulfate.[242,243] Vasodilators to treat and prevent skin ulceration are similar as used for Raynaud's phenomenon and include calcium channel blockers (especially amlodipine and nifedipine), topical nitroglycerin ointment, and phosphodiesterase inhibitors (cilostazol, sildenafil).[244,245]

The Gastrointestinal System in SSc

The gastrointestinal system involvement in SSc is profound and includes bowel and esophageal dysmotility and fibrosis, bowel ischemia, primary sclerosing cholangitis, primary biliary cirrhosis, bacterial overgrowth, increased bowel malignancies, and bowel inflammation amongst other complications.[246] Outcomes are usually anatomically specific; however, a gastrointestinal patient reported outcome, the Scleroderma Clinical Trial Consortium GIT 2.0 has been used recently.[247] Treatment of gastrointestinal complications is typically focused on individual problems of gastroesophageal reflux (proton pump inhibitors, H2-blockers, sucralfate), stricture (dilation), dysmotility and bacterial overgrowth (erythromycin, azithromycin, metoclopramide, domperidone, cisapride).[248] In the line of biomarkers, anti-U11/12 antibodies have been associated with severe gastrointestinal dysmotility.[31] Further, there are elevated fecal levels of the inflammatory biomarker calprotectin in SSc, suggesting that fecal calprotectin could be an effective biomarker for bowel disease as it is in other inflammatory bowel diseases.[249,250]

Biomarkers of Renal Disease in SSc

Renal manifestations of SSc are dominated by scleroderma renal crisis (SRC), while true immune-mediated glomerulonephritis and interstitial nephritis that occur in SSc are usually associated with overlap disease of SSc with vasculitis and/or systemic lupus erythematosus or drug reactions rather than pure SSc.[251–253] SRC is characterized by malignant hypertension, microangiopathic hemolysis, microthrombosis, thrombocytopenia, vasospasm, and progressive renal failure that can be provoked by corticosteroids, cocaine, cyclosporine, and tacrolimus.[251,254,255] Pathologically, SRC is characterized by rather bland or subtle findings, but may demonstrate the typical "onion bulb" histopathology, hyperplasia of the juxtaglomerular apparatus, membranoproliferation, renovascular endothelial injury, intimal proliferation, thrombotic angiopathy, fibrin microthrombi, hemolysis, mesangiolysis, narrowing of renal arterioles, vasospasm, vascular occlusion, ischemia, necrosis, vascular remodeling, and eventual fibrosis with associated with hyperreninemia and accelerated hypertension.[251,256]

Diagnostic criteria for SRC are as follows: 1) systolic blood pressure (SBP) >140mm Hg, 2) diastolic blood pressure (DBP) >90 mm Hg, 3) increase in baseline SBP ≥ 30 mm Hg, 4) rise in DBP ≥20 mm Hg, 5) increase in serum creatinine by ≥50% over baseline or serum creatinine >120% of upper limit of normal for the local laboratory, 6) proteinuria ≥2+ by dipstick and confirmed by spot urine protein:creatinine ratio ≥ upper limit of normal, 7) hematuria ≥2+ on dip stick or ≥10 red blood cells/high power field, 8) platelet count <100,000/mm3, 9) hemolysis (evidenced by schistocytes, RBC fragments on peripheral blood smear and elevated reticulocyte count), and in certain studies, 10) hypertensive encephalopathy.[255,257–259] Prevention of SRC is based on avoiding the use of high-dose corticosteroids and vasoconstrictants in SSc. Therapy of SRC has recently been reviewed and is focused on the early diagnosis of SRC, prompt use of angiotensin-converting enzyme inhibitors, dialysis, plasma exchange, and other supportive measures including eculizumab or endothelin-1 blockage in completely resistant cases in anticipation that the renal function will recover.[133,251,260,261]

Risk factors for SRC (Table 6) include diffuse cutaneous involvement, rapidly progressive skin thickening, disease duration <4 years, anti-RNA polymerase III antibodies, antiphospholipid antibodies, autoantibodies to methionine sulfoxide

Table 6 Biomarkers for Predisposition to Renal Disease in SSc (RD-SSc)

Clinical Biomarkers for Predisposition to RD-SSc	Autoantibody and Immunogenetic Biomarkers for Predisposition to RD-SSc	Biomarkers for Onset of in RD-SSc
• Diffuse cutaneous involvement • Rapidly progressive skin thickening • Disease duration <4 years • Drug use: Corticosteroids, cocaine, cyclosporine, and tacrolimus	• Speckled pattern antinuclear antibodies • Anti-RNA polymerase III antibodies • Absence of anti-centromere antibodies • Anti-fibrillarin antibodies • Antiphospholipid antibodies • Autoantibodies to methionine sulfoxide reductase A • HLA-DRB types 1*0407 and 1*1304	• Hypertension • Renal insufficiency • New cardiac events (pericardial effusion, congestive heart failure, arrhythmias) • Intravascular hemolysis and anemia • Thrombocytopenia • Elevated renin • Abnormal urine

reductase A, absence of anti-centromere antibodies, new cardiac events (pericardial effusion, congestive heart failure, arrhythmias), anemia due to intravascular hemolysis, the HLA-DRB types 1*0407 and 1*1304, and prior drug use, particularly corticosteroids and vasoconstrictants.[258,259,261]

Biomarkers of SRC (Table 7) include hypertension, elevated uric acid, decreased renal function, thrombocytopenia, hemolytic anemia, and elevated serum-soluble CD147 and CD163, renin, mannose-binding lectin (MBL), endothelin-1, soluble vascular adhesion molecules, E-selectin, lipocalin-2, angiogenin, apelin, chemerin, complement components, and NT-proBNP levels.[251,254,261–264] Serum soluble CD147 (sCD147), an ECM metalloproteinase inhibitor and CD163 (sCD163), cysteine-rich scavenger receptor, have been shown to be elevated in patients with SRC.[202,264] Similarly, increased endothelin-1 levels and endothelin receptor carriage have been associated with SRC.[265–267] Similarly, soluble vascular adhesion molecules (VCAM-1) and soluble E-selectin have been associated with SRC.[258,268] NT-proBNP is a useful biomarker for SRC and predicts the need for dialysis and renal outcome.[266,269] CXCL10 is an IFN-inducible chemokine and potent chemoattractant for Th1 cells and is found to be elevated in patients with SRC.[8] IL17B is increased specifically in SSc with renal abnormalities compared to those without.[106]

Anti-fibrillarin antibodies, anti-RNA polymerase III antibodies, and speckle pattern ANA have been most closely associated with the development of SRD; however, in certain populations anti-topoisomerase antibodies have also been associated with high incidences of SRC.[251,258,267,270–272] Antiphospholipid antibodies, in particular, IgG antiphospholipid antibodies, are a significant risk factor for renal disease in SSc versus antibody negative patients.[273] Autoantibodies to methionine sulfoxide reductase A, an important enzyme in the antioxidant pathways, have been associated with the development of renal and cardiac disease in SSc.[274]

Renin, although increased in SRC and central to the pathology and treatment of SRC, is not predictive of SRC, as it is often elevated in SSc patients without SRC.[251,275] Elevated serum uric acid, a purine metabolite, can be associated with

Table 7 Biomarkers for Active Renal Disease and SRC in SSc

Clinical Biomarkers	Blood and Serum Biomarkers	Renal and Urine Biomarkers
• Extreme hypertension • Severe headache • New cardiac events (pericardial effusion, congestive heart failure, arrhythmias) • Hypertensive encephalopathy • Acute seizures	• Elevated uric acid • Elevated creatinine • Thrombocytopenia • Hemolytic anemia (schistocytes, RBC fragments, elevated reticulocytes) • Elevated serum renin • Elevated N-terminal pro-brain natriuretic peptide (NT-proBNP) • Elevated serum-soluble CD147 and CD163 • Elevated serum mannose-binding lectin (MBL) • Elevated serum endothelin-1 • Elevated serum E-selectin • Elevated serum vascular cell adhesion molecule 1 (VCAM-1) • Elevated serum lipocalin-2 • Elevated serum endostatin • Elevated serum angiogenin • Elevated serum apelin • Elevated serum chemerin • Elevated serum CXCL10 • Elevated serum C4d • Elevated IL-17B • Reduced serum C3bBbP • Reduced soluble terminal complement complex (sTCC)	• Decreased renal function (elevated creatinine, decreased glomerulofiltration rate) • Proteinuria • Hematuria • Elevated urine monocyte chemoattractant protein 1 (MCP1) • Elevated soluble vascular cell adhesion molecule 1 (VCAM-1)

inflammation, endothelial dysfunction, and renal dysfunction.[276] Gigante et al have demonstrated in SSc that uric acid is significantly associated with serum creatinine, renal artery resistivity and decreases in glomerulofiltration rate (GFR), as well as multivascular damage in SSc.[276] Similarly, disordered levels of pro-angiogenic molecules and angiogenesis inhibitors have been associated with the progression of renal microvascular damage, defective vascular repair and fibrosis in SSC. Specifically, levels of endostatin, an inhibitor of angiogenesis, have been found to be elevated in renal disease in SSc, and are associated with decreased GFR, increased renal artery resistivity, and progression of peripheral microvascular disease.[204,277] High levels of both angiogenin and lipocalin-2 are associated with decreased GFR and may be involved in pathogenesis of SRC.[265,278,279] Apelin and chemerin are adipokines that bind to receptors on endothelial cells and are elevated in SRC.[280–283] The complement system also appears to be involved in SRC, with higher levels of C4d and lower levels of C3bBbP and soluble terminal complement complex (sTCC) in SSc patients with SRC versus without SRC.[198] Mannose-binding lectin (MBL) is also involved in the complement system, and MBL levels are substantially increased in SRC compared to SSc without SRC.[263] In the urine, monocyte chemoattractant protein 1 (MCP1) and soluble adhesion molecule vascular cell adhesion molecule 1 (VCAM-1) are elevated in SSc patients with renal disease compared to those without renal disease.[284]

As summarized in Table 6 and Table 7, there are multiple biomarkers that associated with the risk for SRC in SSc or delineate the activity of SRC, and many of these biomarkers are intimately involved in both the pathogenesis and characteristics of the disorder.

Conclusion

The field of biomarkers in SSc continues to expand in scope and complexity. The sheer number of molecules, pathways, and receptors involved in the pathology of SSc reflects the many complexities and nuances of the disease. Tyrosine kinases, interferon-1 signaling, IL-6 signaling, endogenous thrombin, peroxisome proliferator-activated receptors (PPARs), lysophosphatidic acid and receptors, and amino acid metabolites have all provided new biomarkers and the potential for new therapeutic agents. Because multiple arms of the immune system and healing mechanisms are activated in SSc-ILD, and there are many candidate biomarkers and potential therapeutic targets, with the trend being the use of biomarker panels with combined complex multifactor analysis, machine learning, and artificial intelligence to determine disease activity and response to therapy. Biomarkers are likely to be of increasing importance for research as well as for the diagnosis and therapeutic approaches to SSc and associated disease manifestations.

Data Accessibility

Data can be obtained from the corresponding author on a reasonable request.

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