REVIEW ARTICLE

Biomarkers reflecting the pathogenesis, clinical manifestations, and guide therapeutic approach in systemic sclerosis: a narrative review

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Abstract

Systemic sclerosis (SSc) is a progressive autoimmune disorder that mainly affects the skin. There are other clinical manifestations as renal, pulmonary, cardiovascular, and gastrointestinal tract involvements. Based on the skin involvement there are two subtypes of SSc, as limited cutaneous SSc (lSSc) which involves the acral part of the body and diffuse cutaneous SSc (dSSc) resulting in significant skin thickening of the body. Despite of the extensive research the pathomechanism is not fully clarified, how Ssc develops, moreover identifying biomarkers to predict the clinical outcome and prognosis still remains challenging. Circulating biomarkers can be crucial to define the diagnosis, to predict the prognosis and monitor the clinical course. However, only some patients are responsive to the therapy in SSc, and there is a need to reach the ideal therapy for any individual to prevent or slow down the progression in early stages of the disease. In this narrative review, our purpose was to summarize the potential biomarkers in Ssc, describe their role in the diagnosis, pathomechanism, clinical course, organ manifestations, as well as the response to the therapy. Biomarkers assessment aids in the evaluation of disease progression, and disease outcome.

Keywords Biomarkers · Organ manifestations · Prognosis · Systemic sclerosis

Introduction

Systemic sclerosis (Ssc) is a multiorgan autoimmune disease with cutaneous and organ manifestations. Ssc is characterized by vascular abnormalities, humoral and cellular immune disturbances, and extensive skin and organ fibrotic processes. Despite of the expanding knowledge of the defective immune mechanism in the background, to predict the clinical course and outcome of the treatment of Ssc faces difficulties. The dysregulated immune system could facilitate the development of organ manifestations through impaired vascularity and lead to chronic inflammation and irreversible fibrosis. Predictive molecular and cellular tools—as biomarkers—are needed to measure, investigate, and evaluate the development of the disease, pathologic pathways, and pharmacologic responses to the treatment. Therefore, biomarkers as "indicators" have a very strong predictive, diagnostic, and prognostic role. There are several concepts to classify the biomarkers in the systemic diseases. Reviews classify the biomarkers based on their molecular functions, while others focus on their role in the pathological processes.

Our aim was to analyze the variability of the biomarkers in Ssc, identify their entity, and facilitate to understand their state, role, and importance in the diagnostics and disease outcome evaluation. Based on the diversity of the biomarkers, it is not easy to establish a strong and clear biomarker hyrarchy in the diagnosis, follow-up, or outcome of the disease for clinicians.

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We aimed to establish a hierarchy of the biomarkers in Ssc based on their function in the (1) immune system, (2) pathogenesis, and (3) clinical course, organ manifestation, and disease activity.

The pathomechanism and progressivity of systemic sclerosis

Several environmental and genetic interactions predispose to the appearance of the disease. Besides the cellular and humoral immune abnormalities, inflammatory cytokines, the distortion in the balance of the growth factors and the autoantibodies result to the fibroproliferative vasculopathy and finally, the cutaneous and visceral fibrosis [1]. However, there is a complex cascade which results in vascular injury and fibrotic process in Ssc, three pathways caused by immunological alteration and phenotypical manifestations, such as (1) vascular abnormalities, (2) autoimmune/immune attacks, and (3) fibrosis.

The importance of the genetic and epigenetic background is continuously increasing. The HLA genes in the SSc pathomechanism are proved to have the strongest association with the antibodies and predisposing factors [2]. There are some differences between the African-American (HLA-DRB1*08.04, HLA-DRB1*11.02) and European-American (DPB1*13.01, HLA-DRB1*07.01) cohorts. While the HLA-DRB1*08.04, HLA-DRB1*11.02 alleles are associated with the development of SSc, the HLA-DRB1*11.02 alleles are related to the anti-fibrillarin antibody onset. In the European-American cohort, the DPB1*13.01, HLA-DRB1*07.01 refers to anti-topoisomerase-1 (ATA) and anti-centromere (ACA) antibodies [3]. In the Ssc myofibroblast and non-myofiboblasts, the neuroblastoma breakpoint family (NBPF) genes are highly expressed. The mutations of potassium channel genes—KCNK5, ABCC—are related to the PAH in Ssc [4]. The pathogenic association in Ssc could be grouped in three major pathways, such as genes, associated with vascularization (eNOS, ACA, ET-1, ETR-A/B), immune or inflammatory genes (STAT4, IRF5, CD247), and genes associated with fibrotic processes (MIF, CTGF, Fibrillin-1, SPARC) [5].

Moreover, there are many other tissue-specific transcription factors (ELF1, MGA) are overexpressed while KLF4 and ID4 are downregulated in Ssc blood cells [6]. Zou et al. have studied chromosome regions of SSc, and their findings proved the number genetic loci were associated with high prevalence of Choctaw Indians [7].

The molecular mimicri hypothesis is also supported as homologous sequences of the autoantibodies of SSc and the viral proteins (Mimiviridae and Pycodnaviridae families) [3].

The other important molecular pathway is the epigenetic modifications, which lead to the pathognomic molecular alteration in the fibroblasts and drive the activation of profibrotic factors (HOTAIR/EZH2/NOTCH) by mi-RNA-34a. The abnormality of the chromatin tools of dendritic cells has a prominent and accountable role in the epigenetic process in Ssc patients [8].

Both the innate and adaptive immune system have a significant impact on the pathogenesis of Ssc. Among others, type 1 interferon (T1 IFN), fibroblast growth factors (FGFs), and its receptors (FGFRs) contribute to the profibrotic process by the FGF9/FGFR3 abnormality [9].

Interaction of the genetic predisposition and environmental stimuli (viruses, organic solvents, oxidative stress, autoantibodies) triggers the immune cells' activation, phenotypical alteration of vascular cells, and fibroblasts.

Distinct, crucial steps in the pathomechanism are hallmarks of the progressivity of Ssc. In the local microvascular functional dysregulation, the microvascular damage can be persistent. Various autoimmune processes, fibrotic mechanisms, and related tissue hypoxia lead to systemic fibrosis [10].

Endothelial cells are activated and undergo structural changes. Behind the vascular damage, angiogenesis is also a crucial step in the vasculopathy in SSc, and vasculogenesis is a defective alteration driven by pro-angiogenetic factors and lack of anti-angiogenic factors. These vascular structural abnormalities are catalyzed by adhesion molecules and associated with tissue hypoxia. Also, the imbalance between vasoconstriction and vasodilatation is due to vascular damage and hypercoagulation by enhanced expression of specific molecules (endothelin) and suppressed amount and function of prostacyclin and nitric oxides, among others [11].

Altogether, there are six morphological features of the microvascular patterns, driven by tissue-specific molecules and autoantibodies. In the very early pattern, only some microvascular alteration can be detected. Later, in the attraction of fibrotic elements and transmigration of inflammatory cells, growth factors lead to the increased microvascular damage and tissue fibrosis (early and early-active phase). In the remarkable active phase, a complex fibroproliferative and occlusive interaction of inflammatory and autoimmune elements is identified. In the late phase, driven by tissue hypoxia and microvascular damage, the extensive fibrosis is the prominent feature of SSc [10, 12].

In the early phase of Ssc, cell adhesion is often stimulated by activated progenitor cells and increased expression of adhesion molecules. The increased release of growth factors results in cell migrations and platelet aggregations which are related to the structural changes of the vascularity and results in increased permeability and giant capillarity with hemorrhages and edema [13].

In the immune or active phase, more extensive activation of the innate and adaptive immune system, pro-inflammatory cytokines, increased cell death, adhesion molecules, and damage-associated molecular patterns altogether lead to the vascular damage.

The activation of endothelial cells through the endothelin-1 and chemokines stimulates the inflammatory cells, the inflammation cascade. The impaired balance of Th17/Treg cells and Th2 cell dominancy triggers a chain of inflammatory sequelae and the overproduction of inflammatory cytokines (IL-8, IL-4, IL-13, CCL2, MMP-1) [14].

On the contrary, the anti-inflammatory responses are mainly reduced in Ssc. Lower percentages of regulatory T cells, regulatory B cells, natural killer cells (NK-cells), and reduced interleukin-10 (IL-10) secretion are observed in Ssc [1, 10–15].

The molecular and cellular dysregulation leads to endothelial cell activation, vascular occlusion, vasculogenesis, and tissue hypoxia by fibroblasts, T and B cells, and endothelial cell activity. IL-4 and IL-13 induce B cell proliferation leading to the production of immunglobulins, adhesion molecules, and inflammatory cytokines [16].

Besides T cell abnormalities, B cells also contribute to the progression of Ssc. B cells secrete IL-6, which became one of the most relevant therapeutic targets. The presence of specific autoantibodies—which can be present in most of the SSc patients—is also a strong evidence that B cells play an important pathogenic role. The dysregulation and abnormal function of B cells also represent in the clinical manifestation. The activation and antibody production of B cells promote further cytokine and macrophage activation and correlate the disease progression and contribute in both the vascular and fibrotic phase of the disease [15, 16].

Dendritic cells (DCs) also have a critical pathognomonic role in the Ssc pathophysiology. DCs contribute to antigen presentation and activate naïve T cells. Interferon-α (IFN-α), chemokine ligand 4 (CXCL4) secretion is stimulated by toll-like receptor-8 (TLR8). TLR8 is expressed by plasmacytoid DCs (pDCs) and enhances the profibrotic processes in the skin. IFN-α is promoted by pDCs and correlates the development and progression of Ssc [17].

The overstimulation of monocytes, M2 macrophages, mast cells, and therefore the excessive TGFß, IL-4, IL-6, IL-13, platelet-derived growth factor (PDGF), TNF-α production stimulates directly other profibrotic factors and chemoattractive and intercellular adhesion molecules. However, a wide spectrum of pro-inflammatory cells can be detected in these inflammatory processes. DCs, monocytes, M2 macrophages, mast cells, and type 2 helper cells (Th2) contribute mostly in the early phase of the inflammation. IL-4 and IL-13 produced by Th2 cells activate macrophages and fibroblasts to produce TGFß, as well [18, 19].

The obliterative vasculopathy and the fibroblast activation are connected strongly by the immune cells and cytokines mentioned above.

In the late phase of Ssc, the fibrotic processes, increased TGFß production results in collagen synthesis and fibroblast proliferation. Activation of the circulating fibrocytes could migrate from the bloodstream and accumulate in the surrounding tissue. On the other side, the inflammatory cascade directly inhibits the anti-inflammatory factors, as the synthesis of metalloproteinase 1 and 3 (MMP1, MMP3) [1, 18, 20]. Despite of the prominent role in late phase, the TGFß could be elevated in the early, active phase of the disease, especially in the skin, as well. TGFß activates the proinflammatory cytokines and regulates adhesion molecules, however; in the late phase, it activates or dysregulates the normal fibroblasts. In fibroblast activation, resident fibroblasts, pre-adipocytes, endothelial cells, mesenchymal stem cells, and fibrocytes trans-differentiate by activation through TGFß. As a result of the transactivation, myofibroblasts are activated, and further pro-inflammatory cytokines are secreted rapidly and continuously [21]. Myofibroblasts are the source of the main extracellular matrix elements such as elastin, collagens, fibronectin, and proteoglycans. The presence of myofibroblasts is not specific but prognostic for connective tissue diseases, especially for SSc. The loss of normal apoptosis of the immune cells is also a key process in the development of SSc. Therefore, the abnormally activated myofibroblasts survive which results in prolonged fibrosis and increased rigidity of the tissues [22].

Taking together, from the tissue injury and vasculopathy to the fibrosis, the inflammation and autoimmune processes could not be easily distinctive as fibroblasts, and the immune cells maintain the immune response and fibrosis, also. The loss of balance of the vasoconstriction and vasodilatation and the loss of molecular control of angiogenic and angiostatic factors determine the clinical feature and prognosis of Ssc.

Clinical manifestations and screening tools

The skin involvement is still the hallmark of SSc. The cutaneous involvement defines two forms, such as limited or diffuse cutaneous scleroderma which can be associated with different extent of body rigidity. As the modified Rodnan skin scores (mRSS) gives highly variable results by the clinicians, the high-frequency ultrasound seems to be a more specific and useful tool to detect skin alterations [23, 24].

Musculoskeletal manifestations are strongly connected with the skin involvement. The progression of the disease is associated with the hand, foot, and further the elbow deformity, and one of the most progressive symptoms, acrosclerosis. SSc and rheumatoid arthritis can overlap in 25%

of patients, based on two French studies, and the authors confirmed that the presence and co-existence of rheumatoid factor (RF), anti-citrullinated proteins (ACPA), and anti-carbamylated protein (anti-CarP) antibodies predict a worse prognosis manifested in vascular progression, synovitis, tenosynovitis, digital ulcers (DU), and interstitial lung diseases (ILD) [25, 26].

The neurological manifestations are not rare in this disease. As a result of the derailed immune mechanisms, fibrosis can spread, and both sensory and motor polyneuropathies are observed. Polyneuropathy, trigeminus neuralgia, and mononeuritis multiplex were also reported in a wide range of SSc patients [27].

Vascular abnormalities are very significant symptoms in SSc from the early phase of the disease. These abnormalities are very specific, as well. Raynaud's phenomenon could be the leading symptom in the early onset and during the progression of the disease too [28]. The worsening of the vasculopathy could manifest in digital ulcers, internal organ involvements as PAH, or malabsorption. Calcinosis is also a specific clinical sign in SSc which is usually reported on the extensor part of the extremities [29]. While the anti-PM/Scl70 antibodies overlap refers a good prognosis, male sex, lower diffusing capacity of lung for carbon monoxide (DLCO < 70%), cardiovascular manifestation, and elevated C-reactive protein (CRP level) (> 5 mg/l) are all reported as indicators for worse outcome [30, 31].

PAH and ILD are still the two main causes of the death in SSc. Regarding vascular abnormalities, mostly arterial stiffness results in hemodynamical changes in the main arterial brunches. Otherwise, pulmonary arterial hypertension (PAH) and inflammatory lung disease (ILD) are characterized by both micro-and macrovascular abnormalities. The DETECT algorithm, echocardiography, and cardiac magnetic resonance imaging (MRI) are potential essential detecting tools in SSc to characterize the stage and phenotype of the cardio-pulmonary manifestation, such as arrythmias, non-ischaemic cardiomyopathy, increased diastolic dysfunction, and myocarditis [32].

SSc-ILD shows different manifestations. Chest x-ray, as well as lung ultrasound, lung density detected by high-resolution computer tomography (HRCT) scan, bronchoalveolar lavage (BAL) can follow disease progression. In BAL fluid (BALF)—which is not routinely performed in Ssc—various biomarkers could be identified. Worsening of pulmonary fibrosis, bronchiectasis, decreased lung diffusing capacity, and the presence of neutrophils in the BAL are also negative prognostic factors [33].

Concerning gastrointestinal (GI) manifestations, esophageal reflux disease, dilatation, and dysmotility have a prominent impact in the prognosis. Transabdominal esophageal ultrasound or manometry usually shows a slower peristalsis or esophageal dilatation [34]. The role of altered gut microbiome has a deep impact in the developing of Ssc and other immune-mediated disorders such as psoriatic arthritis, inflammatory bowel disease (IBD)-related spondyloarthritis, and coeliakia [35]. The dysregulation of the balance of the gut microbiome, such as increased number of Fusobacterium, Ruminococcus, Lactobacillus, and reduced Faecalbacterium can result in the damage of the gut permeability. Moreover, the changes of the gut permeability initiate further immune-mediated or autoimmune responses in the joints and skin, as well. Behind the histopathological assessment by intestinal biopsy which is often complicated to apply, biomarkers could be potent tools to guide us even in the early phase of the disease [36, 37].

Search strategy

There are several studies which highlighted the importance of different biomarkers in the last decades. However, to evaluate the hierarchy of the biomarkers in SSc is still very challenging both for researchers and physicians. Our concept was to represent and specify the candidate markers of SSc (1) in the immune system, (2) in the disease pathways, and (3) in the organ manifestations or disease activity (Table 1).

Our search strategies were designed to identify the best available systematic reviews and relevant literature. However, we have constructed aim, as focusing primarily on the literature in the theme of SSc by pilot key word as "biomarkers" in the last 10 years. Although, after initial scoping, searches carried out the results, and more keywords and synonyms have gathered our development of search strategy. Although, later we restricted some terms to title only, i.e., the "biomarkers in systemic sclerosis" search term and its synonyms. We have selected, almost 30 international publication—peer reviewed original articles and reviews written in English. Searches were applied between February 2019 and January 2024. We have selected the most relevant publications and systematic literature reviews in the aforementioned time-range. This review search strategy was carried out from Google, Google Scholar, and PubMed. By using this itemized strategy, we have found the major appropriate papers and scientific results for this review on the biomarkers in SSc [38].

The diversity of biomarkers

The diversity of biomarkers in systemic sclerosis is a continuously expanding field to monitor the pathomechanism, clinical course, and therapeutic approaches. The biomarkers, as non-invasive and sensitive indicators reflect the physiological and pathological processes, disease prognosis, and the response to therapy. In detail, specific biomarkers are needed for classification, early diagnosis, distinguishing between the subtypes of the disease (lSSc

Table 1 Classification of biomarkers in systemic sclerosis

Classification	Biomarkers
I. The role of biomarkers in the diagnosis system of SSc	
I/1. Autoantibodies (diagnostic)	Anti-Scl-70, anti-CENP-A, anti-Pm-Scl, antifibrillarin, anti-Th/To, anti-RNA polymerase I and III
	RNPC3, RuvBL1 and RuvsBL2 (RuvBL1/2), eukaryotic initiation factor 2B (eIF2B), bicaudal D homolog 2 (BICD2)
II. Biomarkers in immune system and the pathomechanism	
II/1 Cytokines	Interleukin-α (IL-α), IL-β and IL-13, IL-18-binding protein isoform (IL18BPa), IL-33, IL-13, IL-4, IL-6, IL-10, IL-1, IL-17A, IL-17B, IL-17E, IL-12, IL-F, transforming growth factor-β (TGFβ), connective tissue growth factor (CTGF)
II/2 Chemokines	Chemokine-ligand 4 (CXCL4), CXCL10, CX3CL1, CCL2
II/3. Vasculopathy	
II/3.1. Early phase of SSc	IL-6, IL-4, IL-13, TGF-β, monocytes, macrophages, CXCL4, platelet-derived growth factor (PDGF), fibronectin, Serpine1, intercellular adhesion molecule 1 (ICAM-1). B-cell activating factor (BAFF), interferon-γ (IFN-γ), CXCL10, CXCL8, angiopoietin 1 and 2 (Ang-1 and 2), angiostatin, resistin, visfatin, C–C motif chemokine ligand 21(CCL21), CXCL11, Semaphorin-3E (Seam3E), IL-35
II/3.2. Active phase of Ssc	TGF-β, VEGF, endoglin, endothelin-1, IL1-α, IL-6, soluble oncostatin M receptor (sOSMR), IL-17F, IL-17E, CXCL5, CX3CL1, resistin, galectin 1, galectin 3, vaspin, chemerin, IL-33, stimulating growth factor (ST2), CXCL4
	Superoxide anion (O●−), hydroxyl radical (OH●), Hydrogen peroxide (H2O2), (HIF-1α and ß), VEGF, fibronectin-1, thrombospondin-1, Proα 2(1) collagen (COL1A2), connective tissue growth factor (CTGF), TGF-ß induced protein (TGF-ßi)
II/3.3 Late phase or fibrotic biomarkers	TGF-β, PDGF, type I and III collagen, YKL-40, CTGF, CXCL5
II/4. Metabolic properties	Adiponectin, leptins, resistin, galectin 1, galectin 3, vaspin, chemerin
II/5. Circulating neurovascular guidance molecules	Ephrins, netrins, slits, semaphorin (Sema3s), Sema3C, nonribosomal peptides (NRPs), slit family (Slit1, Slit2, Slit3), member of the sirtuin family as SIRT1 and SIRT3
III. Biomarkers in the organ manifestation or disease activity	C-reactive protein (CRP), KL-6, vascular cell adhesion molecule (VCAM-1), E-selectin, P-selectin, type III collagen
III/1. ILD or lung	CRP, CTGF, GDF-15, Il-6, CX3CL1, ICAM-1, Von Willebrand factor, Kl-6, surfactant protein (SP-D), CCL18, matrix metalloproteinas 7 (MMP-7), sCD163, CA 15–3, pulmonary surfactant A and D, YKL-40
III/2. PAH and cardiovascular system	NT-proBNP, endothelin-1 and the A-type anti-endothelin (anti-ETaR) receptor, anti-AT1R, anti-centromer antibody, anti-p4,2, CD144+EMP cadherin, ratio of Cu/Se and ceruloplasmin/SELENOP, midkine and follistatin-like 3 (FSTL3), miRNAlet-7d, blood viscosity level, VEGF, growth differentiating factor 15 (GDF-15), CXCL4, endostatin, endoglin, Von Willebrand factor, sCD163, IL-13, IL-4. IL-10, IL-6. IL1-β, IL13, IL-32, MIF, CCL20, CCL21, CCL23, CXCL16, GDF15, leptin, resistin, adipsin, chemerin, visfatin, interferon- γ (IFN-γ)
III/3. Skin fibrotic markers	Modified Rodnan skin score, thrombospondin 1 (THBS1), cartilage oligomeric matrix protein (COMP), sialic acid binding Ig like lectin 1 (SIGLEC1), interferon induced protein 44 (IFI44), HOXA distal transcript antisense RNA (HOTTIP), SPRY4-IT1, heat-shock-protein (Hsp27), agalactosyl IgG (IgG-Gal), IL-16, adiponectin, terminal differentiation-induced non-coding RNA (TINCR), membrane spanning 4-domains A4A (MS4A4A), GDF-15, BAFF
III/4. Renal involvement	G-patch domain containing 2 like (GPATCH2L), CTNND2, ICAM-1 and VCAM-1, Anti-RNA polymerase III antibodies (anti-RNAP III), complement C3b (C3b), chemerin, E-selectin
III/5. Gastrointestinal involvement	Antibody agaaints muscarinic-3 (M3R), calprotectin (F-cal), claudin-3, and lipopolysaccharides (LPS)
III/6. Biomarkers of paraneoplastic SSc	Transcription complex RNA polymerase III (Anti-POLR3), anti-NOR90, 2-hydroxyglutarate (2-HG), α-ketoglutaric acid (α-KG)

and dSSc), the co-existence of the organ manifestations with the subtypes, clinical course, and the prognosis, as well as for evaluating the therapeutic response [39]. In systemic sclerosis, sensitive and specific, validated biomarkers are not confirmed yet, despite of the overwhelmed and extensive research, except for the NT-pro-brain natriuretic peptide (NT-proBNP) in pulmonary arterial hypertension, the anti-topoisomerase (anti-Scl70) in dSSc, and the anti-centromere antibody in lSSc. However, ANA positivity is one of the criteria in the early onset systemic sclerosis [40]. The modified Rodnan skin score (mRSS) is a functional biomarker and gold standard to measure the disease extension and activity and, however, has numerous difficulties to precisely assess skin involvement. To differentiate the fibrotic skin from the borderline changes or the edema in the early phase is problematic by this assessment [23]. The initial and most critical process in SSc pathogenesis is the vascular dysfunction which leads to the development of PAH and renal crisis. The endothelial cell abnormality is demonstrated by elevated von Willebrand factor levels. The presence of adhesion molecules contributes to the development of early fibrosis and correlates with organ manifestations. VEGF is an important molecule for the assessment of disease progression, and its level is

significantly high in early SSc, as well as in cases with worsening of the vital capacity (Fig. 1) [41]. Endothelin-1 (ET-1) as a potential vasoconstrictor, stimulates the smooth muscle cells and has also an important role in obliterative vasculopathy and in Raynaud's phenomenon. ET-1 correlates strongly with levels of von Willebrand factor and adhesion molecules. The elevated plasma levels of endostatin show positive correlation with the presence of mega-capillaries, digital ulcers, and PAH [42–44].

Biomarkers can be sensitive indicators of the development, the state or progression of SSc, as well they can be used to monitor therapeutic efficacy. In summary, the aforementioned biomarkers are affordable and convenient tools in the clinical practice and aid research as well (Tables 1 and 2).

Biomarkers in the diagnosis

Generally, in the broad spectrum of biomarkers, the disease-specific autoantibodies have an important role in setting the right diagnosis and also are associated with the clinical manifestations and outcome of the disease [10, 12].
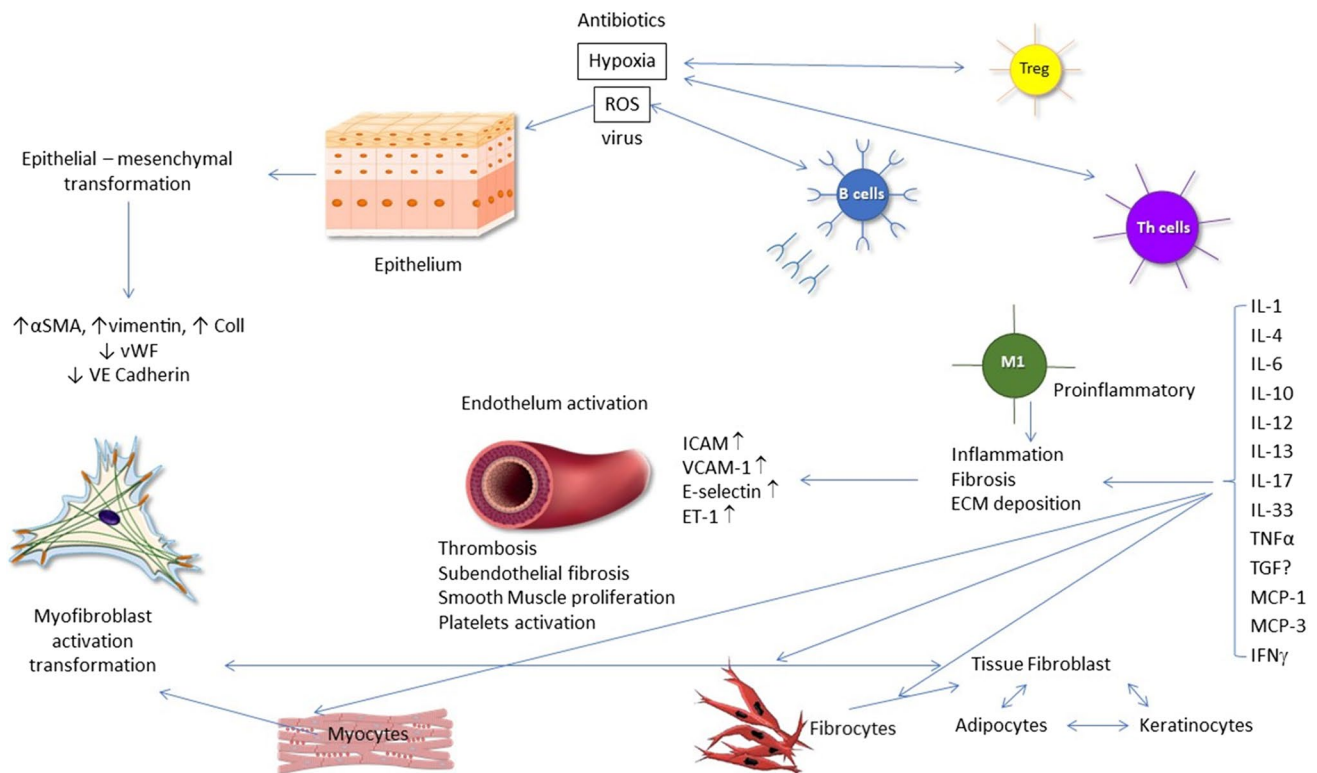

Fig. 1 A brief overview of the pathomechanism of systemic sclerosis. (αSMA, alpha-smooth muscle actin; Col1, collagen type I; ET-1, endothelin-1; ECM, extracellular matrix; ICAM, intercellular adhesion molecules; IL, interleukin; ROS, reactive oxygen species; TNFα, tumor-necrosis alpha; TGFß, transforming growth factor beta; MCP, monocyte chemoattractant protein-1; IFN, interferon; Treg, regulatory T cells; VCAM, vascular cell adhesion molecule-1; VE, vascular endothelial; vWF, von Willebrand factor)

Table 2 Treatment options in systemic sclerosis based on Campochiaro C, Allanore Y. An update on targeted therapies in systemic sclerosis based on a systematic review from the last 3 years. [156])

Treatment options

I. Vascular therapy
  I/1. Vasodilatators
    Calcium-channel blockers (CCBs)
    Beta-blockers
    Silendafil
  I/2. Prostacyclin analogues
    Iloprost (synthetic analogue of prostacyclin PGI2)
    Flolan
    Beraprost (active prostacyclin analogue)
  I/3. Vascular remodelling
    Bosenthan (anti-endothelin-R)
    Selective serotonin reuptake antagonists
    ACEI
    ARBs
  I/4. Antioxidants
    Brobucol
    Vitamin supplements
    Selenium, copper, cobalt
II. Immunomodulatory therapy
  II/1. Conventional immunomodulatory therapy
    Methotrexate
    Cyclophosphamide
    Mycophenolate mofetil
    Azathioprine
    Low-dose corticosteroid
  II/2 Biological disease-modifying antirheumatic drug (bDMARD)
    Rituximab (anti CD-20)
    Tocilizumab (anti-IL-6)
    Belimumab (anti-BAFF)
    Inebilizumab (anti-CD19)
    Romilkimab (IL-4/IL-13)
    Abatacept (CTLA4-Ig)
    Rilonacept (anti-IL-1R)
  II/3 Targeted synthetic disease-modifying antirheumatic drug (tDMARD)
    Tocaficitinib (JAK/STAT)
  II/4 Stem cell transplantation
III. Antifibrotic therapy
  Nintedanib (tyrosine kinase inhibitor)
  Imatatinib (protein-kinase inhibitor)
  Riocguat (stimulator of soluble guanylate cyclase)
  Pirfenidone (transforming growth factor beta-stimulated collagen production, unknown mechanism)
  Lenabasum (cannabinoid receptor type 2 agonist)
  Lanifibranor (peroxisome proliferator-activated receptor agonist)

Autoantibodies

To classify the biomarkers for the diagnostic and clinical categorization, for the assessment of endothelial dysfunction, fibrosis, immunological alterations, and organ manifestations are grouped as follows: autoantibodies, growth factors, cytokines, chemokines, and adhesive molecules.

The early diagnosis and identification of systemic sclerosis subtypes provide better outcome in this progressive disease. Anti-nuclear antibody (ANA) positivity, besides the presence of puffy fingers and Raynaud-phenomenon, is highly representative in the early onset systemic sclerosis. Most of the time, in the early phase of the disease, the phenotype of the two subtypes is common [45].

ANA patterns

ANA patterns (centromere, nucleolar, RNA polymerase III, Scl-70, U3-RNP, Eukaryotic initiation factor 2B (eIF2B), RuvBL1, and RuvsBL2) reflect the development of subtypes and clinical manifestation of Ssc [46–51]. The anti-Scl-70, anti-U3RNP, anti-Th/To, Bicaudal D homolog 2 (BCID2), Th/To (Rpp25/Rpp38), Ro52, eIF2B, anti-U11/U12 autoantibodies, as well as anti-Pm/Scl highly refer to interstitial pulmonary disease (ILD); however, pulmonary arterial hypertension (PAH) appears often in the presence of anti-centromere, anti-U3RNP, anti-Th/To positivity [51–54]. Ssc-myositis overlap syndromes are associated with anti-Ku, anti-RNP and anti-PM/Scl, and RuvBL1 and RuvsBL2

(RuvBL1/2) antibodies [48, 55, 56]. Correlation has been shown between malignancy and RNA-binding region containing three (RNPC3) or RNA polymerase III (RNA pol III) [57, 58] (Table 3).

Biomarkers in the immune system and pathomechanism

The complex and heterogenous pathogenesis of Ssc is characterized by vasculopathy, immune cell, and molecular mediator activation, as well as the accumulation and deposition of fibroblasts. In the genetic predisposition along with exogenous stimuli, the activation of the innate/adaptive immune system regulates the endothelial and fibroblast homeostasis, leading to the sequel of pathogenic processes [10].

Cytokines

Systemic sclerosis and its manifestations are mostly characterized by fibrosis during the disease duration. The IL1-like cytokines, as IL1α and β, were detected in SSc patients compared to healthy controls, and elevated ILα levels were observed in patients with DU; higher concentrations of ILβ and IL-13 were described in PAH. IL-18-binding protein isoform (IL18BPa) was associated with the pulmonary arterial wedge pressure (sPAP) [59]. IL-33 is correlated with the sPAP, DU, and diastolic dysfunction, as well. Remarkable elevated levels of IL-13, IL-4, IL-6, and IL-10 were detected in patients with PAH and cardiac manifestations [61, 62]. Overall, significant differences of IL-17 have not been

observed in SSc patients versus controls; however, IL-17A, IL-17B, IL-17E, and IL-17F were significantly elevated in SSc patients, and IL-17E and IL-F have been associated with DU [60–62].

There is a wide spectrum of biomarkers reflecting fibrotic processes and can aid with the therapeutic approach. TGFβ stimulates the synthesis of extracellular molecules directly and decreases the matrix metalloproteinases. At the same time, TGFβ changes the phenotype of tissue fibroblasts and initiates transformation into myofibroblast. CTGF is also a significant factor for fibrosis; however, it is not clear if TGFβ or CTGF was the better biomarker for fibrosis processes.

The PDGFα and β are also very informative and therapy-sensitive indicators and hinder the efficacy of nintedanib therapy [63–65].

Mononuclear cell infiltration is significant both in the internal organs and skin. The infiltrating phenotypically altered T cell populations release cytokines and growth factors, which usually leads to the development of extensive collagen mass. In systemic sclerosis, the pathogenic role makes IL-6 an excellent target cytokine, as tocilizumab has been proven in ILD, PAH. and musculoskeletal involvements [66]. Otherwise, lower IL-6 levels have been detected in patients with DU [67].

The other prominent pro-inflammatory cytokine, the macrophage migration inhibitory factor (MIF), has been associated with PAH [68]. IL-2 receptor has been shown to be a relevant biomarker of the disease progress and skin severity. TNF-alpha is unquestionably one of the key markers in the pathophysiology of SSc and could reflect the progression of pulmonary disease. However, it has not been clarified

Table 3 ILD-associated biomarkers

Biomarker	Function	Clinical association	Response to the therapy
KL-6 (Krebs von den Lungen-6)	Type II pneumocyte mucinosous glycoprotein	Most informative biomarkers for ILD	Yes [115]
SP-A and SP-D (surfactant protein-A and D)	Produced by type II pneumocyte	Capillary and alveolar barrier distortions	Not known [120, 121]
CCL2, CCL18 (pulmonary and activation-regulated chemokine)	T cell chemotaxis, migration	ILD progression and mortality	Not known [59, 60]
YKL-40 (chitinase-3-like protein 1)	Tissue activator	Worse ILD prognosis and mortality	Not known [118]
Calprotectin	60% soluble protein (neutrophil granulocyte, monocyte, macrophage, epithelial cells)	Gastrointestinal symptoms, ILD, more severe SSc form	Yes [123]
CXCL3 and CXCL4	Cell migration, inflammation	ILD, kidney	Not known [117]
Anti-Ro 52/TRIM21 (tripartite motif-21)	Mononuclear cells ubiquitin ligase	ILD, worse prognosis	Not known [114]
OX40L	Direct effect on MMPs expression, fibrosis	dSSc-ILD, worse prognosis	Not known [62]
MCP-1 (monocyte chemoattractant protein-1)	T cell and monocyte migration, cell adhesion	ILD progression	Not known [119]
Anti-Scl70	Anti-DNA topoisomerase antibody	ILD, FVC worsening	Not known [35, 36]

whether TNF alpha or its receptor was the more informative biomarker in this disease [69, 70]. Taken together, cytokines are excellent biomarkers reflecting vascular abnormalities and PAH in SSc.

Chemokines

Chemokines (CXCL4, CXCL10, CX3CL1) have also a significant impact in the progression of SSc. CXCL4 is a prohibitor of IFN-γ and could enhance the skin fibrosis. CXCL10 is predictive in the early onset SSc. Digital ulceration and pulmonary fibrosis are reported to be associated with CX3CL1 through its role in migration and adhesion [71]. Interstitial pulmonary disease and pulmonary arterial hypertension are highly responsible for the mortality and morbidity in SSc. Several molecules are confirmed to reflect ILD severity, and most of them are expected to be potentially useful biomarkers. The endothelial microparticles, e.g., CD144 + plays an important role in cell–cell interactions and signaling. The serum concentration of these molecules is significantly elevated in PAH. The lung-epithelial surfactant proteins are relevant diagnostic markers in ILD. KL-6 shows the fibrosis severity in ILD. ILD severity is associated with CCL-2, CXC4, and PF-4 that are produced by immune cells. CCL-18 has a pivotal role in the collagen synthesis and is a strong prognostic factor in ILD severity [72]. YKL-40 (chitinase-3-like protein 1)—as a tissue activator—is also a very important biomarker of ILD prognosis. The fecal and serum calprotectin—however is not a chemokine—is also a good biomarker both for the gastrointestinal manifestation and ILD. Furthermore, calprotectin is therapy sensitive; therefore, it could be validated for monitoring the symptoms in SSc in the future [72–74].

Chemokine alterations can reflect the pathological pathways, e.g., stable serum CCL-2 level and decreased CXCL-10 level refer to the Th1 shift to Th2 pathway. Anti-Ro52 antibodies are biomarkers of infective pulmonary diseases and predictive for worse outcome in ILD. The OX40-OX40L axis correlates with the extension of fibrosis in the lung and skin, as well [71, 75] (Table 2).

Circulating neurovascular guidance molecules

Several neural molecules have been shown to regulate vascular remodelling, as ephrins, netrins, slits, and semaphorins. The balance of neurovascular communication is essential in the neurovascular stability. In SSc, the role of secreted class III semaphorin (Sema3s) is related to angiogenesis. Sema3C has both pro- and anti-angiogenic factor functions; Sema3E has been associated with early vascular abnormalities [76].

Increased level of NRPs has been described in Ssc patients with PAH. Regarding the Slit family (Slit1, Slit2, Slit3), a Slit2-SSc association has been depicted in the early

onset as a peripheral vascular biomarker. Among the sirtuins (SIRTs), SIRT1 and SIRT3 are decreased in SSc and being related to DU [77, 78].

Metabolic properties

Adiponectin is a bifunctional hormone as having pro- and anti-inflammatory roles in different diseases. In SSc, decreased adiponectin concentrations have been found significantly increasing concentration levels which have been shown after prostaglandin analogue treatment [79]. Leptins activate the pro-inflammatory cytokines and enhance angiogenesis. However, some studies have not reported significant differences in serum leptin levels between SSc patients and controls, while others have shown increased level of leptin in SSc patients with PAH [80]. Similarly, resistin levels did not differ between the two groups; however, increased level of resistin was detected in patients with DU and PAH. Galectin 1 is associated with telangiectasias; galectin 3 refers to the development of DU [80, 81]. On the contrary, the level of vaspin was decreased in SSc patients with DU [82]. Chemerin has pro- and anti-inflammatory effects, depending on the circulating immune cells and micro-environmental background. Chemerin was significantly increased in Ssc-PAH, as well [83, 84].

Biomarkers in the early phase of Ssc

In the early phase of Ssc, the vascular dysfunction is presented by the aberrant cell–cell interaction by increased expression of adhesion molecules, such as VCAM-1, ICAM, E-selectin, and the growth factors as TGFβ, endothelin-1 (ET-1), and PDGF [85]. The permanent vasoconstriction is strongly triggered by the ET-1, angiotensin, and activation of leucocytes. The activation of thrombocytes also contributes to the vasoconstrictions and vWFAg; thrombospondin and thrombomodulin are also possible biomarkers in the early phase of Ssc [86]. On the contrary, the aberrant vasodilatation can be detected by lower concentrations of NO as well as the lower expression of NO3 gene [87]. Furthermore, the imbalance of angiostatic factors, such as angiostatin, endostatin, chemokin ligand 4 (CXCL4), thrombospondin, (IL-4) and the angiogenic factors, VEGF, PDGF, TGF-β1, PGF-2, PIGF, ET-1, MCP-1, TNF-α, IL-8, E-selectin, P-selectin, and urokinase plasminogen activator receptors reflect the development of abnormal density capillaries and angiogenesis [87, 88].

The biomarkers in the active and late phases of SSc

In the active and late phases, a broad spectrum of biomarkers can reflect various complications of the disease and predict the progression of the tissue injury.

Biomarkers of oxidative stress

The reactive oxidative species (ROS)

Reactive oxidative species (ROS) may have a great impact in the pathogenesis of Ssc, including their effect on endothelial dysfunction, fibrosis development, the innate and adaptive immune system, and the development of the autoantibodies [89]. Vasculopathy, the hallmark feature of Ssc, is signified by the perivascular mononuclear infiltration, endothelial injuries, and vascular and extracellular matrix remodelling—alteration of the vessels and capillary structure and functions. The extensive flow of reactive oxygen species, such as superoxide anion ($O\bullet^-$), hydroxyl radical ($OH\bullet$), and hydrogen peroxide (H_2O_2) produced by endothelial cells, smooth muscle cells, and fibroblasts, is responsible for the vascular abnormalities [90, 91]. The Ssc-specific vascular manifestations—the inverse reaction of capillaries—are detectable in the early phase of Ssc, and later ischaemic ulceration may be resulted by the dysregulated ROS milieu. The contractile and relaxation function of the vascular smooth muscle cells is also affected by both the ROS and the increased expression of ROS-induced contractile proteins. Besides the vasoconstriction effects, the elements of the ROS, e.g., overproduction of superoxide and H_2O_2 may drive vasodilatation resulting in the biphasic response in Ssc [92].

The previous factors and other reactive signaling molecules as NO$\bullet$ and hydrogen sulfide (H_2S) altogether interact and cause vascular dysregulation [93, 94].

The molecular response biomarkers of hypoxia

Severe hypoxia is the most potent pathogenic risk for the vascular abnormalities. There are several molecular responses to hypoxia including the expression of hypoxia-inducible factor-1 (HIF-1α and ß) and dysregulated cytokines exposure. Hypoxia leads to reduced capillar density, impaired vascular permeability, and diffusion. The upregulation of the extracellular matrix proteins and altered function of vessels are catalyzed by hypoxia [86, 95]. In Ssc, decreased HIF-1α protein levels have been measured despite the severe hypoxia [96]. This paradox mechanism could be defined by HIFα-independent pathways. The VEGF-dependent angiogenesis induced by HIF-1 could be also a possible key point in the hypoxia-induced angiogenesis and vasculopathy. VEGF could be a prominent biomarker in the chronic vascular process of Ssc as both the VEGF levels and the VEGF receptor 1 and 2 are overexpressed as well cause tissue damage. VEGF could also induce hypoxia and malnutrition, and hypoxia could maintain the upregulation VEGF, vice versa [97, 98]. In the chronic, fibrotic lesions, there are several other factors have been described, such as fibronectin-1, thrombospondin-1, proα 2(1) collagen (COL1A2), connective tissue growth factor (CTGF), and TGF-ß induced protein (TGF-ßi) [99, 100].

The anti-oxidative enzymes and its cascade

The normal differentiation and activation of B and T cells are catalyzed by anti-oxidative enzymes, such as Gpx1 and catalase. Oxidative stress can lead to the increased inflammatory cascade and IL-4, IL-13 production. Similarly, to other autoimmune disorders, Th-17 levels are also increased, and the production of the Treg cells are decreased in Ssc. These observations underline the positive effects of the anti-oxidant or anti-stress therapy in the inflammatory and autoimmune process in Ssc [101, 102]. The activity of NLRP3 is reduced by H_2O_2 scavenger catalase and could contribute to the fibrosis development. Moreover, in endothelial cells, NLRP3 activation is triggered by oxidative stress [103, 104]. The "M2-type" macrophages—as subtype of the macrophage/monocyte—polarization are strongly affected by oxidative stress through signal transducer and activator of transcription 6 (STAT6) induction [105]. The pro-fibrotic cytokines, such as TGF-ß and IL-1ß, are highly important in the fibrotic processes and are able to stimulate the elements of ROS, while increased expression of ROS triggers the fibroblast activation to express these cytokines, as well [106]. Members of the metallo-proteinases (MMPs) are also strongly linked to fibrotic processes, the pulmonary arterial hypertension, and the skin and pulmonary fibrosis. MMP-9, MMP-12, and MMP13 levels can be potential biomarkers to monitor the activity of the ROS [107–109].

Development of autoantibodies and the activation of ROS can also be associated. The H_2O_2-induced protein oxidation can lead to changes of the epitopes and trigger autoantibody production. On the other hand, the antioxidant system or enzymes are targeted by the autoantibodes, as anti-peroxiredoxin and anti-methonine sulfoxide reductase (MSRA) maintain the oxidative stress in Ssc [110–112].

Finally, oxidative stress contributes to tissue damage and the internal and skin fibrosis by increased amino acid and protein hydroperoxide (HP) levels in Ssc. Elevated eosinophilia has been shown in the skin ulcers, elevated CRP levels, cellular fibronectin, and mild anemia along with HP. Therefore, fibronectin, eosinophil cell counts, and hemoglobin levels also could be potent biomarkers for disease activity [113].

Other vascular biomarkers

The vascular biomarkers are presented in very early Ssc, as microangiopathy can appear rapidly. The small vessel damage and chronic hypoxia could be intensified by angiogenic and fibroproliferative factors, also. Antibodies against

interferon-inducible protein 16 refer to digital ischemia [114]. Endostatin is associated with giant capillarity abnormalities and clearly appears at the onset of right ventricular systolic pressure [115]. Endoglin has a remarkable role in angiogenesis, and its level is significantly elevated in patients with DU, associated with anti-centromer antibodies, ILD, and PAH. The endoglin correlates positively with telangiactasia especially hereditary hemorrhagic telangiactasia. Von Willebrand factor (vWF) and ADAMTS-13 are also a positive biomarkers for disease activity and severity in ILD and PAH [116, 117].

Markers of pulmonary hypertension (PAH) and ILD

A subset of Ssc patients with pulmonary artery hypertension and pulmonary fibrosis, reflecting interstitial lung disease, have the worst clinical outcome. These two progressive phenotypes of the disease represent the leading morbidity and mortality in Ssc.

The diagnostic biomarkers of PAH

Right heart catheterization (RHC) is essential for the diagnosis of pulmonary hypertension (PAH) in SSc, also. Although, RHC is an invasive method, it is suggested to use this procedure in cases of high-risk patients [118]. Validated non-invasive and sensitive biomarkers are essential for detecting PAH. The NT-proBNP is a sensitive but not specific marker for PAH in SSc as elevated NT-proBNP level is also associated with left ventricle dysfunction and renal insufficiency. NT-proBNP is correlated with the skin fibrosis, and its level is higher in dSSc [119, 120]. Two important biomarkers, as endothelin-1 and the A-type anti-endothelin (anti-ETaR) receptor antibody are representative for PAH, ILD, and DU. Both markers reflect sensitively for bosentan. The anti-receptor antibody (anti-AT1R) is elevated in decreased DLCO and PAH. The anti-centromer antibody, anti-p4,2, and CD144 + EMP cadherin have a strong correlation with the DLCO < 70 and PAH [121, 122]. FSTL3 expression is stimulated by heart failure and contributes to the activation of fibroblasts leading to increased cells adhesion and collagen synthesis [123]. The human lethal-7 (let-7-d) is another promising biomarker in PAH [124]. Selenium has a potential role in the oxidative stress therefore the elevated Cu/Se rate is important in patients with PAH and fibrosis, also [125].

The diagnostic process of ILD

ILD and PAH, as cardiopulmonary manifestations of SSc, are the two major causes of morbidity and mortality in SSc [126]. The mortality in patients with PAH and/or ILD is significantly higher with these comorbidities. Scleroderma renal crisis—as characterized by hypertension and renal failure—is a life-threating condition; however, its prevalence declined after the preferable introduction of angiotensin convertase inhibitor (ACE) [127]. The progressive phenotype of ILD could be identified and followed by forced expiratory volume (FEV1), forced vital capacity (FVC), and DLCO. High-resolution computed tomography (HRCT) is frequently used to clarify and detect the patterns of the pulmonary involvement [128]; however, we must take into consideration the frequented radiation exposure of the HRCT. Recently, the importance and role of the biomarkers in ILD/PAH is more emphasized in the clinical practice, as well [129].

The chemokines and other biomarkers of ILD

In BALF, behind the autoantibodies (anti-Scl-70, anti-centromer antibodies, anti-Ro52), CCL18, macrophage 2-derived protein, has been also described to be sensitive for monitoring the progression of SSc-ILD. KL-6 (Krebs von den Lungen-6), MMP7, and MMP12 are good prognostic factors in the early lung involvement or Ssc-ILD, overall [130–132]. CCL2 is related to ILD progression and poor prognosis. Some proteome-wide studies have shown that CXCL3 and CXCL4 levels were significantly higher in SSc-ILD patients, otherwise did not correlate with the severity of the disease [133]. Dichev et al. described the regulation of serum 40-kDa heparin-and-chitin binding glycoprotein (YKL-40) and plasma miR-214 levels and found that both biomarkers could distinguish between patients with SSc, dcSSc, and lcSSc [134]. The serum monocyte chemoattractant protein-1 (MCP-1) levels in the BAL are known to be a good marker to be correlated with the clinical course of ILD patients and could predict the clinical course of ILD [135]. SP-A and SP-D are elevated in patients with Ssc and correlate with decreased DLCO. SP-D was detected as could show the state of pulmonary fibrosis but did not follow the progression of the pulmonary fibrosis progression [136, 137]. Soluble OX40L also correlates with the worsening of lung and skin fibrosis. OX40L has a profibtotic effect and triggers the influx of the inflammatory cells into tissues leading to fibrosis [138]. Beyond, the proven role of calprotectin in Ssc patient with GE manifestation, calprotectin is also a promising marker in BALF connected with inflammatory pulmonary fibrosis [74] [Table 4].

Skin fibrosis markers

Besides the modified Rodnan skin score, further non-invasive but more objective biomarkers are needed to evaluate the skin involvement in SSc. The heat-shock protein, as a pro-inflammatory molecule, is increased in dSSc than in lSSc or healthy individuals [139]. IgG-Gal and IL-16

Table 4 Systemic sclerosis-specific antibodies

Biomarker	Classification	Clinical association
Anti-Scl-70	Anti-DNA topoisomerase antibody	Diffuse cutan SSc, pulmonary fibrosis [35, 36]
anti-CENP-A (anti-centromere Ab (ACA))	Anti-kinetochore protein antibody	Limited cutan SSc, arterial pulmonary hypertension (10–20%) [35, 36]
Anti-Pm-Scl	110–120 kDA nuclear and nucleolar protein antibody	PM/SSc overlap [45]
Antifibrillarin	Az U3-RNP 34 kDa nuclear protein component antibody	Diffuse cutan Ssc [35]
Anti-Th/To	RNAase P ribonucleoprotein antibody	Limited cutan SSc, pericarditis, ILD [35]
Anti-RNA polymerase I and III RNPC3	RNA polymerase antibody RNA binding region containing 3 antibodies	Diffuse cutan SSc, renal involvement, malignancy [46, 47] Malignancy, ILD, GI dysmotility, myopathy [48]
RuvBL1 and RuvsBL2 (RuvBL1/2)	ATP-binding protein belonging to the AAA + (ATPase associated with diverse cellular activities) superfamily of ATPases	Diffuse cutaneous disease, inflammatory myositis overlap [40]
Eukaryotic initiation factor 2B (eIF2B)	Cytoplasmic multimeric protein consisting of 5 subunits	Diffuse cutaneous disease, ILD [39]
Bicaudal D homolog 2 (BICD2)	94 kDa protein and one of two human homologs of Drosophila bicaudal-D	Inflammatory myositis, ILD [45]

cytokine show a positive correlation with mRSS and skin severity, and subtypes of SSc can be assessed by this molecule [140]. Inverse correlation has been established between the adiponectin and skin fibrosis or mRSS [141]. The genetic analysis of the scleroderma skin has a promising candidate biomarker pattern. The THBS1, COMP, SIGLEC1, and IFI44 are correlated moderately with the mRSS, and further analyses have confirmed that HOTTIP and SPRY4-IT1 show positive correlation with mRSS; otherwise, ANCR and SPRY4-IT1 are significant biomarkers for PAH [142].

Potential renal biomarkers

The renal manifestation is commonly appearing in SSc patients. The scleroderma renal crisis (RSC) could be a life-threatening episode in SSc. The exact role of anti-RNS polymerase III antibody is unknown. The pathogenic role of GPATCH2L, CTNND2, ICAM-1, and VCAM-1 is confirmed [143]. Additionally, there are some other molecules, such as C3b deposits and chemerin are depicted to be relevant biomarkers in several autoimmune disorders and in SSc, as well [144].

Gastrointestinal biomarkers

Calprotectin levels are highly sensitive but not specific biomarker of GI manifestation [145]. The antibody agaults Muscarinic-3 (M3R) receptor and RNA binding region containing 3 has been detected in Ssc with GI dysmotility [146, 147]. GI manifestations could be the early onset in Ssc, and the calprotectin (F-cal) is described to be presented in the early phase of the disease as well. However, F-cal has not

shown associations with the esophageal radiological alterations. Testing of the calprotectin at the time of the diagnosis or suspicion of Ssc onset can be useful [148]. In another cross-sectional study, Stec et al. have found that among the serum intestinal permeability markers as intestinal fatty acid binding protein, claudin-3 and lipopolysaccharides (LPS) were markedly different and elevated in Ssc patients with GI abnormalities. Higher levels of LPS and claudin-3 were associated with a shorter duration of the disease. Moreover, in this group, the LPS concentrations were related to ILD. Concomitant esophageal dysmotility was associated with a decrease in LPS in patients with SSc. Both calprotectin and LPS are established as early biomarkers in gastrointestinal malformations [37].

Biomarkers of paraneoplastic SSc

Individuals with systemic sclerosis have a significantly higher risk for developing cancer. Although, the development of cancers in SSc are associated with the presence of autoantibodies and several provoking and genetic factors [149]. Chronic inflammation, tissue damage, and immune-suppressive agents heightened the link between cancer development and Ssc [150]. On the other hand, SSc could appear as a paraneoplastic syndrome, as cancer-induced autoimmunity [151]. Onishy et al. have found an increased tendency of hematological, lung, liver, and bladder cancer in females and non-melanomatous cancer in males. Anti-POLR3 positive patients with diffuse scleroderma have a higher risk for breast, prostate, and tongue cancer [152]. Paraneoplastic syndrome manifestations and SSc development can happen simultaneously. The anti-NOR90 antibody is reported in lSSc

and in myelodysplastic syndromes. In anti-NOR90-positive patients, IDH1 mutation causing elevated 2-hydroxyglutarate (2-HG) levels and concomitant α-Ketoglutarate octyl ester (α-KG), dimethyl- α-KG inhibition, and elevated TGFβ levels and myofibroblast migration [153].

Recommendation for clinicians

Beyond the availability of on the numerous biomarkers we have summarized in details, there is a critical step to further evaluate their clinical implementations. Although the clinical utility of all biomarkers has been assessed in the last decades, it still remains difficult to rank the clinical usefulness of these molecules [153]. The predictive values of each biomarker could be significant; therefore, we strongly believe that several biomarkers should be used simultaneously to predict, monitor, or guide the treatment of SSc.

Although there is a great variety of biomarkers in the SSc pathogenesis, clinical course prognosis, and response to therapy, however, only some essential biomarkers are available in the clinical practice as prognostic tools for clinicians to focus on the early onset of SSc through the disease duration, which indicate the most appropriate treatment or failure the therapy [154].

Firstly, the presence of autoantibodies predicts and confirms the onset of the disease along with the clinical symptoms; therefore, the ANA patterns assist to evaluate the subtypes and the main clinical manifestations of Ssc. Moreover, there are some autoantibodies which are important to be highlighted in overlap syndromes (e.g., anti Pm/Scl 70).

Secondly, the follow-up and management of Ssc are required by multidiscipline approach. For cardiologist and pulmonologists, the vascular biomarkers are useful to predict the severity and onset of the PAH and ILD. Otherwise, the right heart catheterization (RHC) with the NT-proBNP is essential routine diagnostic procedure for the diagnosis of pulmonary hypertension (PAH), ILD, and PU. KL-6 and pulmonary surfactants A and D (PS-A, -D) are also key proteins and have a positive correlation with the pulmonary fibrosis.

The pulmonary status should be followed by forced expiratory volume (FEV1), forced vital capacity (FVC), and DLCO. High-resolution computed tomography (HRCT) is one of the most frequently useful tools to detect the patterns of the pulmonary involvement [112]. In BALF, certain autoantibodies and molecules have been also described to be sensitive for monitoring the progression of SSc-ILD.

Calprotectin levels are highly sensitive but not specific biomarker of GI manifestation and idiopathic pulmonary fibrosis.

Selenium, as a trace element nutrient and antioxidant enzyme, the Cu/Se rate is a practicable factor to predict and follow PAH and fibrosis, as well. Sclerodermal renal crisis and the high risk for cancer are associated with the presence of autoantibodies and several provoking and genetic factors; although the exact predictive biomarkers are not avaiable in the clinical routine, therefore, the regular follow-up of blood pressure, renal function, is essential. Also, the rapid progression, the late onset of the disease, can indicate parenoplastic syndrome.

There is controversial evidence of biomarkers in the current clinical practice; therefore, it is pivotal that research should be conducted to continuously evaluate "biomarker patterns" and to aid clinicians to use them in the daily clinical care [153, 154].

Conclusions

In systemic sclerosis, the importance of biomarkers is pivotal in the differential diagnosis, for classification to subgroups, to decipher manifestations, to assess disease activity, to monitor prognosis, response to therapy, and to establish personalized therapy, as well. Despite of the general scientific knowledge of the pathomechanism, breakthrough treatment options are still lacking. Only in pulmonary arterial hypertension where the molecular pathomechanism is better known, targeted therapy has been shown to slow down disease progression. However, well-defined or "clear" biomarkers to predict the prognosis have not been validated, yet. Strong biomarkers are needed to distinct the early and late phases of Ssc, as well as the vascular and fibrotic processes [155]. Unfortunately, the specificity and sensitivity of current biomarkers are variable. Finally, validated, "cost–benefit" biomarkers as well as a set of biomarkers, and biomarker-patterns to monitor response to the therapy are essential. As of today, individually tailored biomarkers are not available, as their sensitivity and specificity can differ from patient-to-patient. Several potential biomarkers for the prognosis, vascular injuries, fibroproliferative processes, and organ damages are under evaluation [156–158].

Further efforts for the evaluation of biomarker patterns are pivotal from basic research and clinical science centers in order to optimize patient follow-up and clinical care.

Compliance with ethical standards

Disclosures None.

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