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Review

The Role of Biomarkers in the the Pathogenesis, Clinical Manifestations, and Therapeutic Outcome of Systemic Sclerosis

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Abstract: Systemic sclerosis (SSc) is a complex autoimmune disorder characterized by progressive fibrosis and obliterative vasculopathy affecting the skin and various internal organs, including the kidneys, lungs, cardiovascular system, and gastrointestinal tract. The disease manifests in two major clinical subtypes: limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc), distinguished primarily by the extent of skin involvement and the pattern of internal organ involvement. Biomarkers, serving as quantifiable indicators of biological processes in SSc, hold significant potential for refining disease classification, predicting progression, assessing therapeutic responses, and evaluating clinical outcomes. Unlike other autoimmune diseases, SSc lacks highly specific biomarkers. Given its heterogeneity and multifactorial pathogenesis, the development of a composite biomarker panel may represent the most effective approach for future diagnostic and longitudinal monitoring strategies in SSc.

Keywords: systemic sclerosis; complex pathogenesis; biomarkers; mortality

1. Introduction

Systemic sclerosis (SSc) is a rare, chronic autoimmune disease characterized by a broad spectrum of organ involvement. This inflammatory connective tissue disorder is primarily defined by vasculopathy, immune dysregulation, and progressive interstitial and perivascular fibrosis [1]. The exact etiology remains elusive, and predicting clinical outcomes or treatment responses poses a significant challenge for clinicians [2].

SSc is classified into two major subtypes: limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc), each exhibiting distinct disease trajectories [3]. The prognosis of SSc is largely determined by the presence and severity of interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH), both of which are leading contributors to disease-related mortality [4].

A comprehensive understanding of SSc pathophysiology is essential for anticipating organ manifestations, disease progression, and clinical outcomes. Vasculopathy represents an early pathogenic event, characterized by endothelial injury, dysfunction, perivascular inflammation, dysregulated apoptosis, and platelet activation and aggregation. Raynaud's phenomenon often precedes overt disease onset and may remain the predominant or sole symptom for years before cutaneous or visceral involvement emerges [5].

Although many aspects of the pathomechanism of systemic sclerosis (SSc) have been elucidated, accurately predicting disease prognosis, organ involvement, and mortality remains a significant challenge.

Biomarkers in SSc represent valuable tools for assessing disease development, clinical course, therapeutic response, and long-term outcomes. Various autoantibodies, chemokines, immunological alterations, and markers of tissue hypoxia and organ dysfunction have been previously described as potential indicators of disease progression [5].

Although more than 300 different autoantibodies have been identified across various autoimmune diseases, only a limited number of them fulfill the criteria of true disease-specific biomarkers. Table 1. These include features such as high specificity, strong association with clinical phenotype, and utility in diagnosis, prognosis, or therapeutic monitoring. For example, anti-double stranded DNA (anti-dsDNA) antibodies are well-established in systemic lupus erythematosus (SLE), serving as both diagnostic and disease activity markers. In contrast, for many other autoimmune conditions, including systemic sclerosis, the availability of such reliable, specific biomarkers remains limited. This distinction underscores the critical need to evaluate not only the presence of autoantibodies, but also their clinical relevance and biomarker validity, which forms a central focus of this review

This review aims to provide an updated overview of the most relevant biomarkers in systemic sclerosis, organized according to their functional and clinical relevance. We summarize the roles of vasculopathy-related factors, fibrosis-associated mediators, pulmonary and renal involvement markers, neurovascular guidance molecules, gastrointestinal and malignancy-associated biomarkers, and their utility in clinical practice. Understanding the contribution of these biomarkers is essential for the development of personalized medicine strategies and targeted therapeutic approaches in SSc.

Table 1. Biomarkers in the Immune System and the Pathomechanism [1–6].

Type	Molecule	Associated Clinical Manifestation/Role
Cytokines	IL-α, IL-F	Digital ulcers
Cytokines	IL-1β), IL-13	Pulmonary arterial hypertension (PAH)
Cytokines	IL-18BPA, IL-17A, IL-17B, IL-17E, IL-12	Systolic pulmonary arterial pressure (SPAP)
Cytokines	IL-4, IL-6, IL-13, IL-10, IL-17A, IL-17B, IL-17E, IL-12	Pulmonary arterial hypertension (PAH) and cardiac manifestation
Cytokines	TGF-β, CTGF, CXC, CXCL4	Skin fibrosis
Chemokines	CXCL10	Early onset of systemic sclerosis (SSc) and decreased CXCL10 → T helper 1 (TH1) → T helper 2 (TH2) shift
Chemokines	CX3CL1	Digital ulcers (DU) and pulmonary fibrosis
Chemokines	CCL2, CXCL4	Interstitial lung disease (ILD) severity

Abbreviations: IL: Interleukin; IL-α: Interleukin-alpha; IL-1β: Interleukin-1 beta; IL-4: Interleukin-4; IL-6: Interleukin-6; IL-10: Interleukin-10; IL-12: Interleukin-12; IL-13: Interleukin-13; IL-17A/B/E: Interleukin-17A/B/E; IL-18BPA: Interleukin-18 binding protein isoform A; TGF-β: Transforming Growth Factor-beta; CTGF: Connective Tissue Growth Factor; CXCL4: Chemokine (C-X-C motif) ligand 4; CXCL10: Chemokine (C-X-C motif) ligand 10; CX3CL1: Chemokine (C-X3-C motif) ligand 1; CCL2: Chemokine (C-C motif) ligand 2; DU: Digital ulcers; PAH: Pulmonary arterial hypertension; SPAP: Systolic pulmonary arterial pressure; ILD: Interstitial lung disease; SSc: Systemic sclerosis; TH1/TH2: T helper type 1/T helper type 2.

2. Vascular Pathogenesis and Vasculopathy-Related Biomarkers

Multiple immune-mediated mechanisms contribute to vascular abnormalities, which are strongly associated with microvascular damage affecting the lungs, cardiovascular system, kidneys, and gastrointestinal tract. Early detection of vasculopathy using nailfold capillaroscopy, along with the identification of aberrant vasoregulatory mediators, may facilitate risk stratification and predict disease severity in SSc [6].

SSc-specific autoantibodies not only correlate with distinct subtypes of scleroderma but also hold prognostic significance in vascular pathology. Pulmonary arterial hypertension (PAH) has been associated with anti-RNA polymerase III, anti-centromere, autoantibodies targeting the Th/To ribonucleoprotein complex (anti-Th/To), anti-U1 ribonucleoprotein (RNP), and autoantibodies directed against the U3 ribonucleoprotein complex (anti-U3 RNP)

antibodies. These autoantibodies contribute to disease pathogenesis by driving an inflammatory cascade orchestrated by chemokines, adhesion molecules, and immune cells, including T-cells and monocytes. The breakdown of self-tolerance is evidenced by the presence of autoreactive immune cells, a reduction in regulatory T-cells, and the production of autoantibodies by plasma cells [7–9].

Angiostatic and angiogenic mediators initiate inflammation and immune activation within the endothelium and perivascular tissue. Dysregulation of the Th1/Th2 axis is a key feature of SSc pathogenesis. Although autoantibody production suggests a Th2-predominant response, multiple cytokines associated with Th1 activation are also implicated. Strong evidence supports the role of interleukin-6 (IL-6) inhibition, as treatment with tocilizumab has demonstrated improvements in both cutaneous and pulmonary vasculopathy [10]. Additionally, a broad range of cell adhesion molecules, including P-selectin and E-selectin, have been described in the pathophysiology of SSc [11]. Figure 2.

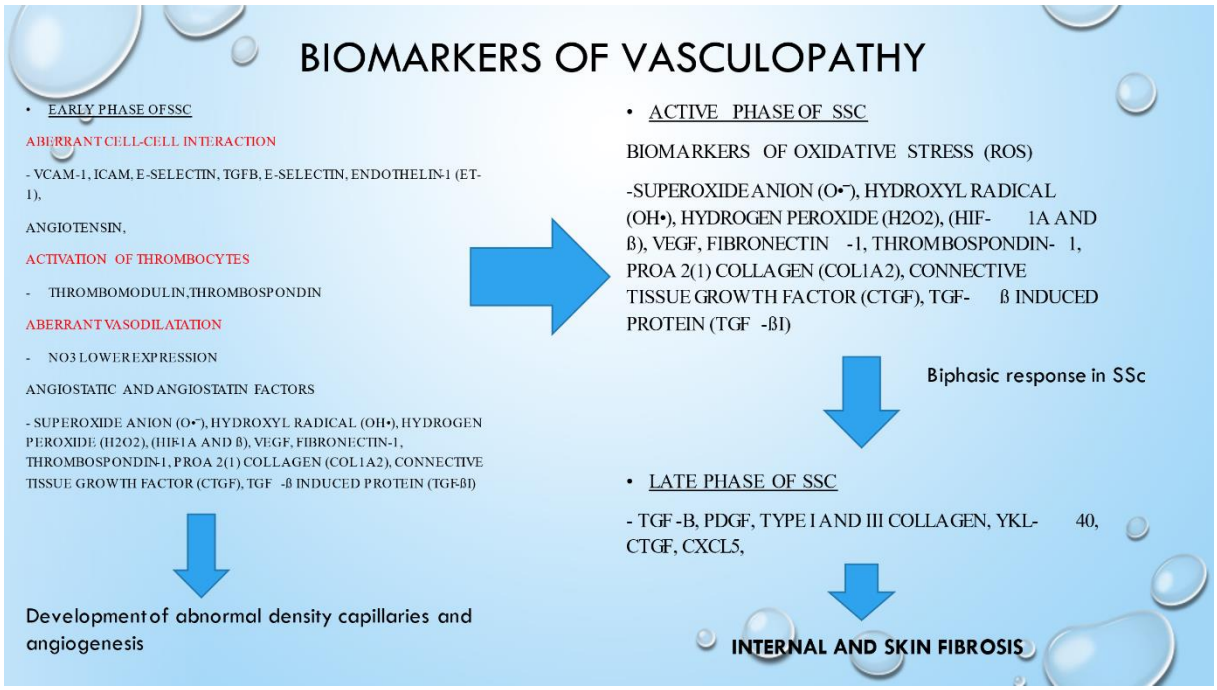

Figure 2. Biomarkers of Vasculopathy [21–37].

Abbreviations: SSc—Systemic sclerosis; VCAM-1—Vascular Cell Adhesion Molecule-1; ICAM-1—Intercellular Adhesion Molecule-1; E-selectin—Endothelial-leukocyte adhesion molecule; TGF-β—Transforming Growth Factor-beta; B-selectin—(possibly P-selectin); ET-1—Endothelin-1; NO—Nitric oxide; ROS—Reactive Oxygen Species; O_2^-—Superoxide anion; OH^-—Hydroxyl radical; H_2O_2—Hydrogen peroxide; HIF-1A—Hypoxia-Inducible Factor 1-alpha; HIF-1B—Hypoxia-Inducible Factor 1-beta; VEGF—Vascular Endothelial Growth Factor; Fibronectin-1—Extracellular matrix protein; Thrombospondin-1—Matricellular glycoprotein; TGF-βI—TGF-beta Induced protein (TGFBI, βIG-H3); COL1A2—Collagen Type I Alpha 2 chain; COL1A1—Collagen Type I Alpha 1 chain; COL3A1—Collagen Type III Alpha 1 chain; CTGF—Connective Tissue Growth Factor; PDGF—Platelet-Derived Growth Factor; CXCL5—Chemokine (C-X-C motif) ligand 5; YKL-40—Chitinase-3-like protein 1 (CHI3L1).

3. Fibrosis and Profibrotic Biomarkers in SSc

Fibrosis represents another fundamental pathological hallmark of SSc. Vascular immune dysregulation acts as a critical driver of fibrotic remodeling. Monocyte/macrophage activation, plasmacytoid dendritic cells, mast cells, neutrophils, as well as cytokines such as IL-6 and interleukin-13 (IL-13), contribute to both vascular damage and fibrosis [12,13]. On one hand, endothelial injury, intimal proliferation, hypoxia, and oxidative stress (ROS) alter the function of fibrocytes, endothelial cells, epithelial cells, pericytes, and adipocytes, ultimately inducing the transdifferentiation of fibroblasts into myofibroblasts, which secrete excessive collagen and fibrillin, leading to extracellular matrix (ECM) stiffening and tissue contraction [14]. On the other hand, fibroblast activation is driven by chronic inflammation, type I interferon (IFN-I), Th2 cytokines, M2 macrophages, Toll-like receptors, and transforming growth factor-beta (TGF-β), as well as direct stimulation by B-cells and autoantibodies [15].

Multiple signaling pathways, epigenetic modifications, and microRNAs (miRNAs) play a crucial role in the fibrotic progression of SSc. Notably, reduced expression or deletion of *Friend leukemia virus integration 1* (FLI1) has emerged as a key molecular signature in patients with SSc, highlighting its potential as a therapeutic target [13].

The deficiency of caveolin-1, a membrane-associated scaffolding protein in mesenchymal cells, plays a crucial role in upregulating vascular endothelial growth factor (VEGF) signaling and the expression of additional profibrotic mediators in SSc [14].

Hypoxia has been shown to activate profibrotic pathways; however, various intrinsic regulatory factors—such as prostanoids—possess antifibrotic properties. Notably, while certain prostanoids counteract fibrosis, prostaglandin F (PGF) appears to promote fibrotic remodeling, particularly in pulmonary tissues of SSc patients. In addition, bioactive membrane lipids such as lysophosphatidic acid (LPA) are elevated in SSc and may represent a promising therapeutic target in patients with pronounced fibrosis [15].

The pathogenesis of fibrosis in SSc is driven by a complex interplay of aberrant cell-cell interactions, cellular transitions, dysregulated differentiation, and a network of profibrotic signaling pathways. These include Transforming Growth Factor-beta (TGF-β), cellular Abelson tyrosine kinase (c-Abl), and Early Growth Response 1 (Egr-1) signaling cascades, along with excessive fibroblast activation and collagen deposition [16].

Aging is associated with intricate molecular and cellular alterations that contribute to the onset and progression of chronic diseases. In addition to environmental exposures, genetic predisposition, chronic stress, and detrimental lifestyle factors, several intrinsic aging mechanisms have been implicated in disease pathogenesis. These include chronic inflammation propagated by reactive oxygen species (ROS), oxidative stress, and increased cellular turnover and proliferation [17]. In autoimmune and rheumatic disorders, late-onset disease is often associated with a more aggressive clinical course and poorer prognosis.

Key pathogenic mechanisms linking aging to SSc include telomere shortening and dysfunction, leading to genomic instability, a feature exacerbated in SSc. The progressive decline in DNA repair mechanisms further facilitates the accumulation of somatic mutations in affected tissues [18]. Cellular senescence, characterized by an altered secretory phenotype, creates a proinflammatory microenvironment enriched in cytokines, chemokines, and proteases that drive fibrotic remodeling [19]. This senescent phenotype has been observed in endothelial cells and pulmonary smooth muscle cells, contributing to endothelial-mesenchymal transition, impaired vasodilation, and defective angiogenesis in SSc [20]. Figure 2.

4. Biomarkers of Pulmonary Involvement: ILD and PAH

The early identification of a progressive phenotype in SSc-associated interstitial lung disease (SSc-ILD) is crucial for improving patient prognosis. While high-resolution computed tomography (HRCT) remains a cornerstone of ILD assessment, its utility in detecting early-stage disease is limited [21]. In contrast, serum biomarkers offer a promising approach for diagnosing and monitoring different stages of ILD. Krebs von den Lungen-6 (KL-6), matrix metalloproteinase 7 (MMP-7), and matrix metalloproteinase 12 (MMP-12) are associated with early ILD, whereas surfactant proteins A and D (SP-A, SP-D), anti–topoisomerase I antibodies (anti-Scl-70, anti-centromere, anti-Ro52, C-reactive protein (CRP), connective tissue growth factor (CTGF), growth differentiation factor-15 (GDF-15), and C-C motif chemokine ligand 2 (CCL2) reflect disease progression. Additionally, monocyte chemoattractant protein-1 (MCP-1) has been linked to clinical disease course. Collectively, these biomarkers facilitate the early detection of ILD before the onset of progressive fibrotic lung disease [22,23].

Several biomarkers of pulmonary arterial hypertension (PAH) in SSc have been identified, primarily reflecting endothelial dysfunction, oxidative stress, cellular hypoxia, fibrosis, and microthrombosis affecting the cardiac vasculature [24,25]. Right heart catheterization (RHC) remains the gold standard for confirming PAH diagnosis [26,27]. However, non-invasive biomarkers, including chemerin, receptor for advanced glycation end products (RAGE), insulin-like growth factor-binding protein 7 (IGFBP-7), SP-D, vascular cell adhesion molecule-1 (VCAM-1), serum cardiac troponins (cTn), protein ST2, galectin-3 (GAL-3), endothelin-1 (ET-1), and N-terminal pro–B-type natriuretic peptide (NT-proBNP), have demonstrated clinical utility in assessing hemodynamic impairment [28,29].

The early detection of PAH progression is critical for improving survival outcomes. Numerous biomarkers have been proposed for evaluating PAH severity, prognosis, and mortality risk. NT-proBNP is the most widely used prognostic marker; however, it lacks sufficient sensitivity for identifying early-stage PAH (functional class I) or precapillary PAH [26–31]. Figure 3.

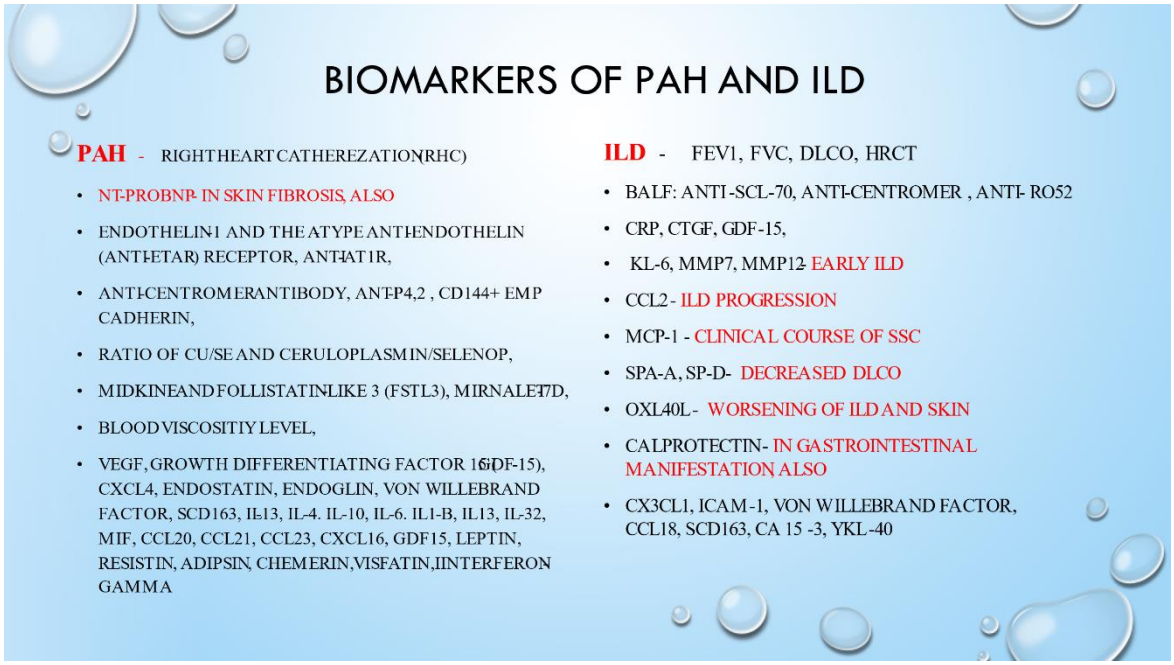

Figure 3. Biomarkers of PAH and ILD [7–17].

Abbreviations: PAH—Pulmonary Arterial Hypertension; RHC—Right Heart Catheterization; NT-proBNP—N-terminal pro b-type Natriuretic Peptide; ETAR—Endothelin Type A Receptor; AT1R—Angiotensin II Type 1 Receptor; EMP—Endothelial Microparticles; CU—Copper; SE—Selenium; SELENOP—Selenoprotein P; FSTL3—Follistatin-like 3; VEGF—Vascular Endothelial Growth Factor; GDF-15—Growth Differentiation Factor 15; CXCL4—C-X-C Motif Chemokine Ligand 4; ICAM-1—Intercellular Adhesion Molecule 1; CCL2—C-C Motif Chemokine Ligand 2; CCL18—C-C Motif Chemokine Ligand 18; CX3CL1—C-X3-C Motif Chemokine Ligand 1; SCD163—Scavenger Receptor CD163; MMP7—Matrix Metalloproteinase 7; MMP12—Matrix Metalloproteinase 12; CRP—C-Reactive Protein; CTGF—Connective Tissue Growth Factor; KL-6—Krebs von den Lungen-6; ILD—Interstitial Lung Disease; FEV1—Forced Expiratory Volume in 1 s; FVC—Forced Vital Capacity; DLCO—Diffusing Capacity of the Lung for Carbon Monoxide; HRCT—High-Resolution Computed Tomography; BALF—Bronchoalveolar Lavage Fluid; SPA-A—Surfactant Protein A; SPD—Surfactant Protein D; OX40L—OX40 Ligand; YKL-40—Chitinase-3-like protein 1 (CHI3L1); CA 15-3—Cancer Antigen 15-3.

5. Neurovascular Guidance Molecules and Microangiopathy

Neurovascular crosstalk has been implicated in dysregulated cell proliferation and aberrant cell-cell interactions, contributing to pathological processes such as tumorigenesis, metastasis, and autoimmune disorders. In systemic sclerosis (SSc), vascular dysfunction is influenced by axonal signaling and neurovascular guidance molecules (NGMs) [32]. The disruption of neuroendothelial homeostasis in SSc is evidenced by elevated serum levels of semaphorin 3E (Sema3E) and Slit2, highlighting their role in microvasculopathy [33].

Several NGMs, including ephrins, netrins, Slit glycoproteins (Slits), and semaphorins (Sema3 family, Sema3C), as well as nonribosomal peptides (NRPs) and members of the sirtuin family (SIRT1, SIRT3), have been identified as potential biomarkers, although their diagnostic accuracy varies [34]. These molecules are crucial in different stages of SSc and are associated with digital ulcer formation and microvascular stasis. Notably, lower serum levels of neuropilin-1 (NRP1) and elevated Sema3E correlate with digital ulcers, whereas higher Sema3E levels are linked to the absence of digital ulcers, and increased Slit2 concentrations have been observed in SSc [35,36].

Soluble neuropilin-1 (sNRP1) has emerged as a promising biomarker for early SSc diagnosis, as its elevated levels indicate disease onset. In contrast, in later disease stages, decreasing serum sNRP1 concentrations may serve as an indicator of microvascular disease progression [37].

6. Biomarkers of Scleroderma Renal Crisis

Scleroderma renal crisis (SRC) is a rare but potentially life-threatening complication of systemic sclerosis (SSc) [38]. Early diagnosis remains challenging due to the heterogeneous presentation of the disease. Genetic predisposition—specifically HLA-DRB11407 and HLA-DRB11304, as well as GPATCH2L and CTNND2—along with serological markers such as anti-RNA polymerase III and anti-topoisomerase I (ATA), and the presence of the diffuse cutaneous SSc (dcSSc) phenotype, have been identified as predictive factors for SRC [39].

Although there are no highly specific biomarkers for SRC, several candidates, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), complement C3b (C3b), chemerin, and E-selectin, have been implicated in its pathogenesis. Additionally, several clinical risk factors have been associated with an increased likelihood of SRC development, including anemia, pericardial effusion, congestive heart failure, rapidly progressing skin thickening, tendon friction rubs, large joint contractures, cardiomegaly, proteinuria, and the use of high-dose corticosteroids [38,40].

7. Gastrointestinal Biomarkers in SSc

Gastrointestinal (GI) involvement is a common and often debilitating feature of SSc. Autoimmune-mediated inflammation, microvascular abnormalities, and progressive fibrosis contribute to the disruption of normal gut motility [41]. Chronic gastric hypomotility and impaired intestinal peristalsis, along with prolonged bacterial colonization, can lead to persistent inflammation and disruption of the intestinal barrier's tight junctions. This dysbiosis, along with alterations in secondary metabolite production, may trigger systemic musculoskeletal inflammation through aberrant immune responses. Additionally, immunosuppressive therapies used in SSc management may themselves contribute to GI dysfunction [42,43].

Several pro-inflammatory biomarkers have been implicated in early SSc-related GI involvement, including lipopolysaccharides (LPS), fecal calprotectin, claudin-3, and interleukin-6 (IL-6) [44]. Biomarkers play a crucial role in detecting abnormal motility, intestinal barrier dysfunction, and even malignant transformation in the small intestine; however, definitive diagnosis remains challenging, often requiring invasive diagnostic procedures [42–44].

8. Malignancy Risk and Onco-Associated Biomarkers

Systemic sclerosis (SSc) is associated with an increased risk of malignancy. In addition to age and environmental exposures, several novel risk factors have been identified [45,46]. Oncogenic gene profiles, impaired transcriptional regulation, and dysregulated gene expression—often driven by histone modifications—are implicated in carcinogenesis. Moreover, epigenetic alterations, including aberrant Deoxyribonucleic Acid (DNA) methylation, histone modifications, and dysregulated microRNA expression, contribute to both the pathogenesis of SSc and its association with malignancy. Telomere shortening and chromosomal instability further exacerbate this risk [45–49].

Specific signaling pathways have been implicated in the link between SSc and malignancy. The phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) and Wingless-related integration site/beta-cateni n (Wnt/β-catenin) pathways have been shown to drive the expression of connective tissue growth factor (CTGF), a key mediator of fibrosis in both the skin and lungs [50,51]. Chronic inflammation, aberrant immune cell differentiation, and immune dysfunction also contribute to tumorigenesis in autoimmune diseases. Environmental exposures, such as silica, organic solvents, and industrial pollutants, have been implicated in the pathogenesis of SSc, and many of these agents are also well-established carcinogens [52,53].

Furthermore, immunosuppressive therapies used in SSc treatment, including cyclophosphamide and, to a lesser extent, mycophenolate mofetil, have been associated with an increased cancer risk. Chemotherapy, immune checkpoint inhibitor therapy, and radiation have also been linked to SSc-associated skin toxicity, extensive fibrosis, and morphea [54,55]. There is a well-documented association between SSc and paraneoplastic syndromes, particularly in patients with anti-RNA polymerase III (Anti-POLR3) and anti–nucleolar organizing region 90 kDa antibodies (anti-NOR90) autoantibodies, as well as elevated levels of 2-hydroxyglutarate (2-HG) and α-ketoglutaric acid (α-KG), which play key roles in oncogenesis [56,57].

Lung cancer incidence in systemic sclerosis (SSc) is increased, particularly in male patients, those with a longer duration of autoimmunity, and individuals diagnosed at a younger age. Chronic inflammation, the presence of pulmonary fibrosis, and anti-Scl70 autoantibodies are key predisposing factors for bronchogenic carcinoma in SSc [58]. In addition to these risk factors, paraneoplastic biomarkers are essential for the early detection of lung cancer. However, the identification of bronchial carcinoma in its early stages remains challenging, as conventional imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT) scans have limited sensitivity in this context [59].

Beyond lung cancer, SSc is associated with an elevated risk of breast cancer, esophageal carcinoma, and, notably, hematological malignancies [60,61]. Figure 4.

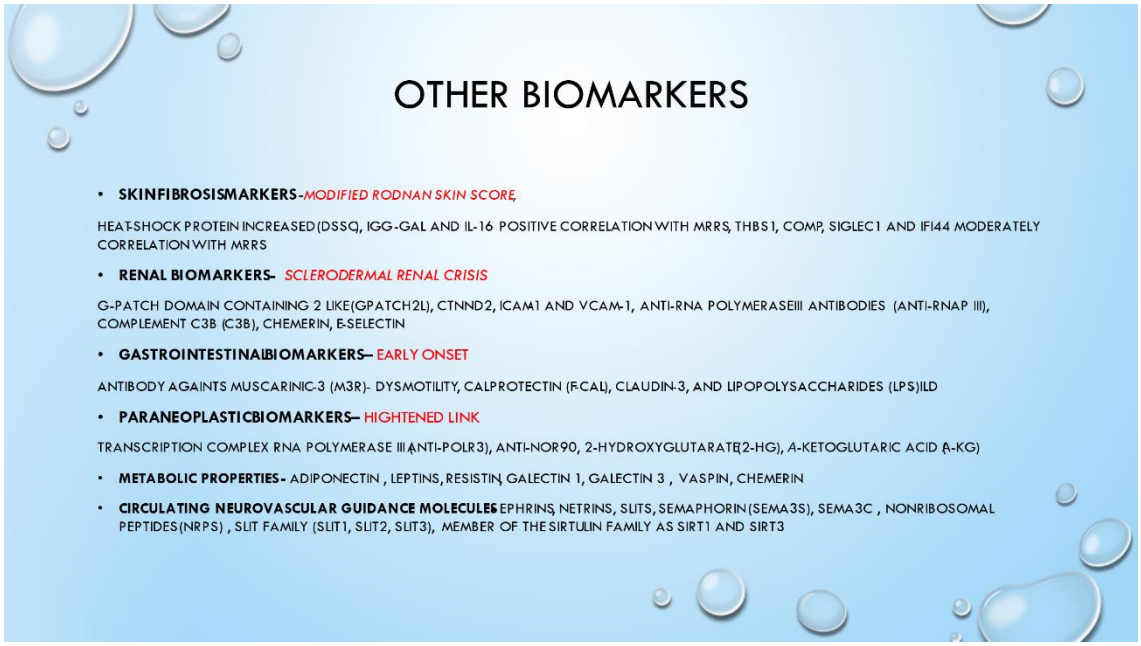

Figure 4. Other Biomarkers [38–61].

Abbreviations: MRSS—Modified Rodnan Skin Score; DSSC—Heat-shock Protein Increased in Systemic Sclerosis; IgG-GAL—Immunoglobulin G—Galectin; IL-16—Interleukin-16; THBS1—Thrombospondin-1; COMP—Cartilage Oligomeric Matrix Protein; SIGLEC1—Sialic Acid-Binding Ig-Like Lectin 1; IFI44—Interferon-Induced Protein 44; GPATCH2L—G-Patch Domain Containing 2 Like; CTNND2—Catenin Delta-2; ICAM-1—Intercellular Adhesion Molecule 1; VCAM-1—Vascular Cell Adhesion Molecule 1; RNAP III—RNA Polymerase III; C3B—Complement Component 3b; M3R—Muscarinic Acetylcholine Receptor M3; F-CAL—Fecal Calprotectin; LPS—Lipopolysaccharides; POLR3—Polymerase (RNA) III; NOR90—Nucleolar Organizer Region 90; 2-HG—2-Hydroxyglutarate; A-KG—Alpha-Ketoglutaric Acid; NRP1—Neuropilin-1; SLIT1, SLIT2, SLIT3—Slit Family Proteins 1, 2, 3; SIRT1—Sirtuin 1; SIRT3—Sirtuin 3; SEMA3A, SEMA3C—Semaphorin 3A, 3C; SEMA3S—Semaphorin 3S.

9. Conclusions and Future Direction

Systemic sclerosis is a multifaceted autoimmune disease characterized by complex pathogenesis, heterogeneous clinical manifestations, progressive vasculopathy, and extensive fibrosis. Unlike other autoimmune disorders, SSc lacks specific, universally accepted biomarkers. Given the complexity of the disease, a comprehensive biomarker panel is crucial for improving early diagnosis, prognostication, and therapeutic strategies in SSc. This review has highlighted key categories of biomarkers in SSc, including those related to vasculopathy, fibrosis, pulmonary and renal complications, neurovascular signaling, gastrointestinal involvement, and malignancy risk. While some biomarkers such as autoantibodies and natriuretic peptides are already integrated into clinical practice, many others remain in the investigational phase. Although numerous biomarkers have been identified and are being investigated in clinical research, only a limited number are currently accepted and used in routine practice for the diagnosis of systemic sclerosis. Antinuclear antibodies (ANA), anti-Scl-70, and anticentromere antibodies are helpful in the diagnostic process, while KL-6 in interstitial lung disease (ILD) and NT-proBNP in pulmonary arterial hypertension (PAH) are among the few biomarkers that have gained widespread clinical use. The future of biomarker development in SSc lies in multi-omics approaches, high-throughput technologies, and large-scale longitudinal studies that validate their utility across diverse patient populations. Integrating molecular biomarkers with imaging, clinical phenotypes, and digital health tools may enable personalized and precision medicine approaches. Ultimately, a better understanding of SSc-specific biomarkers will not only facilitate earlier diagnosis and improved risk assessment but also support the development of targeted therapies tailored to individual disease pathways. Collaboration between translational researchers and clinicians will be essential to transform these advances into tangible benefits for patients with systemic sclerosis.

Take-Home Messages:

(1) Systemic sclerosis (SSc) is a progressive autoimmune disease associated with significant morbidity and mortality.

(2) Identifying the most relevant biomarkers remains a major challenge due to disease heterogeneity.

(3) Biomarker specificity and sensitivity vary, influencing their clinical utility in diagnosis and prognosis.

(4) Breakthrough treatment options are still lacking, and current therapeutic strategies remain largely symptomatic.

(5) A comprehensive biomarker panel is essential for improving early diagnosis, risk stratification, and personalized management of SSc.

Author Contributions

Each author have made substantial contributions to the conception or design of the work; and has approved the submitted version (and version substantially edited by journal staff that involves the author's contribution to the study); and agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.

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Informed Consent Statement

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Data Availability Statement

No new data were generated or analyzed in this study. All data discussed are from previously published sources, which are cited in the references.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Denton, C.P.; Kanna, D. Systemic sclerosis. *Lancet* **2017**, *390*, 1685–1699. https://doi.org/10.1016/S0140-6736(17)30933-9.
2. Ingegnoli, F.; Ughi, N.; Mihai, C. Update on the epidemiology, risk factors, and disease outcomes of systemic sclerosis. *Best Pract. Res. Clin. Rheumatol.* **2018**, *32*, 223–240. https://doi.org/10.1016/j.berh.2018.08.005.
3. van den Hoogen, F.; Khanna, D.; Fransen, J.; et al. Classification Criteria for Systemic Sclerosis: An ACR-EULAR Collaborative Initiative. *Arthritis Rheum.* **2013**, *65*, 2737–2747. https://doi.org/10.1002/art.38098.
4. Guillén-Del-Castillo, A.; Meseguer, M.L.; Fonollosa-Pla, V.; et al. Impact of interstitial lung disease on the survival of systemic sclerosis with pulmonary arterial hypertension. *Sci. Rep.* **2022**, *12*, 5289. https://doi.org/10.1038/s41598-022-09353-z.
5. Ko, J.; Noviani, M.; Chellamuthu, V.R.; et al. The Pathogenesis of Systemic Sclerosis: The Origin of Fibrosis and Interlink with Vasculopathy and Autoimmunity. *Int. J. Mol. Sci*. **2023**, *19*, 14287.https://doi.org/10.3390/ijms241814287.
6. Raschi, E.; Privitera, D.; Bodio, C.; et al. Scleroderma-Specific Auto-antibodies Embedded in Immune Complexes Mediate Endothelial Damage: An Early Event in the Pathogenesis of Systemic Sclerosis. *Arthritis Res. Ther.* **2020**, *22*, 265. https://doi.org/10.1186/s13075-020-02360.

7. Jiang, Y.; Turk, M.A.; Pope, J.E. Factors Associated with Pulmonary Arterial Hypertension (PAH) in Systemic Sclerosis (SSc). *Autoimmun. Rev.* **2020**, *19*, 102602. https://doi.org/10.1016/j.autrev.2020.102602.
8. Young, C.; Lau, A.W.Y.; Burnett, D.L. B Cells in the Balance: Offsetting Self-Reactivity Avoidance with Protection against Foreign. *Front. Immunol.* **2022**, *13*, 951385.https://doi.org/10.3389/fimmu.2022.951385.
9. Rosa, I.; Romano, E.; Fioretto, B.S.; et al. Autoantibodies as putative biomarkers and triggers of cell dysfunctions in systemic sclerosis. *Curr. Opin. Rheumatol.* **2025**, *37*, 51–63. https://doi.org/10.1097/BOR.0000000000001035.
10. Al Dulaijan, B.; Huang, S.; Lin, C.J.F.; et al. Impact of Scleroderma-Associated Autoantibodies on Clinical Outcome Assessments: Post Hoc Analysis from a Randomised, Double-blind, Placebo-controlled, Phase 3 Trial of Tocilizumab in Scleroderma. *ACR Open Rheumatol.* **2025**, *7*, 11782. https://doi.org/10.1002/acr2.11782.
11. Bazsó, A.; Szodoray, P.; Shoenfeld, Y.; et al. Biomarkers reflecting the pathogenesis, clinical manifestations, and guide therapeutic approach in systemic sclerosis: A narrative review. *Clin. Rheumatol*. **2024**, *43*, 3055–3072. https://doi.org/10.1007/s10067-024-07123-y.
12. Brown, M.; O'Reilly, S. The Immunopathogenesis of Fibrosis in Systemic Sclerosis. *Clin. Exp. Immunol.* **2019**, *195*, 310–321.https://doi.org/10.1111/cei.13238.
13. Al-Adwi, Y.; Westra, J.; van Goor, H.; et al. Macrophages as Determinants and Regulators of Fibrosis in Systemic Sclerosis. *Rheumatology* **2023**, *62*, 535–545.https://doi.org/10.1093/rheumatology/keac410.
14. Kulshrestha, R.; Singh, H.; Pandey, A.; et al. Caveolin-1 as a critical component in the pathogenesis of lung fibrosis of different etiology: Evidences and mechanisms. *Exp. Mol. Pathol.* **2019**, *111*, 104315. https://doi.org/10.1016/j.yexmp.2019.104315.
15. Pattanaik, D.; Brown, M.; Postlethwaite, B.C.; et al. Pathogenesis of Systemic Sclerosis. *Front. Immunol.* **2015**, *6*, 272. https://doi.org/10.3389/fimmu.2015.00272.
16. Lafyatis, R. Transforming Growth Factor β—At the Centre of Systemic Sclerosis. *Nat. Rev. Rheumatol.* **2014**, *10*, 706–719. https://doi.org/10.3389/fimmu.2015.00272.
17. Yang, M.M.; Boin, F.; Wolters, P.J. Molecular underpinnings of aging contributing to systemic sclerosis pathogenesis. *Curr. Opin. Rheumatol*. **2025**, *1*, 86–92. https://doi.org/10.1097/BOR.0000000000001061.
18. Blackburn, E.H.; Epel, E.S.; Lin, J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. *Science* **2015**, *350*, 1193–1198. https://doi.org/10.1126/science.aab3389.
19. Miller, K.N.; Victorelli, S.G.; Salmonowicz, H.; et al. Cytoplasmic DNA: Sources, sensing, and role in aging and disease. *Cell* **2021**, *184*, 5506–5526. https://doi.org/10.1016/j.cell.2021.09.034.
20. Di Micco, R.; Krizhanowsky, V.; Baker, D.; et al. Cellular senescence in aging: From mechanisms to therapeutic oppurtunitites. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 75–95. https://doi.org/10.1038/s41580-020-00314-w.
21. Ledda, R.E.; Campochiaro, C. High resolution computed tomography in systemic sclerosis: From diagnosis to follow-up. *Rheumatol. Immunol. Res*. **2024**, *5*, 166–174. https://doi.org/10.2478/rir-2024-0023.
22. Di Maggio, G.; Confalonieri, P.; Salton, F.; et al. Biomarkers in systemic sclerosis. An overview. *Curr. Issues Mol. Biol*. **2023**, *45*, 7775–7802. https://doi.org/10.3390/cimb45100490.
23. Hoffmann-Vold, A.-M.; Allanore, Y.; Alves, M.; et al. Progressive interstitial lung disease in patients with systemic sclerosis-associated interstitial lung disease in the EUSTAR database. *Ann. Rheum. Dis.* **2021**, *80*, 219–227. https://doi.org/10.1136/annrheumdis-2020-217455.
24. Perelas, A.; Silver, R.M.; Arrossi, A.V.; et al. Systemic sclerosis-associated interstitial lung disease. *Lancet Respir. Med.* **2020**, *8*, 304–320. https://doi.org/10.1016/S2213-2600(19)30480-1.
25. Volkmann, E.R.; Wilhalme, H.; Assassi, S.; et al. Combining Clinical and Biological Data to Predict. Progressive Pulmonary Fibrosis in Patients with Systemic Sclerosis Despite Immunomodulatory Therapy. *ACR Open Rheumatol.* **2023**, *online ahead of print*. https://doi.org/10.1002/acr2.11598.
26. Bruni, C.; De Luca, G.; Lazzaroni, M.-G.; et al. Screening for pulmonary arterial hypertension in systemic sclerosis: A systematic literature review. *Eur. J. Intern. Med.* **2020**, *78*, 17–25. https://doi.org/10.1016/j.ejim.2020.05.042.

27. Yaqub, A.; Chung, L. Epidemiology and risk factors for pulmonary hypertension in systemic sclerosis. *Curr. Rheumatol. Rep.* **2013**, *15*, 302. https://doi.org/10.1007/s11926-012-0302-2.
28. Sanges, S.; Rice, L.; Tu, L.; et al. Biomarkers of haemodynamic severity of systemic sclerosis-associated pulmonary arterial hypertension by serum proteome analysis. *Ann. Rheum. Dis.* **2023**, *82*, 365–373. https://doi.org/10.1136/ard-2022-223237.
29. Kiely, D.G.; Lawrie, A.; Humbert, M. Screening strategies for pulmonary arterial hypertension. *Eur. Heart. J. Suppl.* **2019**, *21*, K9–K20. https://doi.org/10.1093/eurheartj/suz204.
30. Hojda, S.E.; Chis, I.C.; Clichici, S. Biomarkers in Pulmonary Arterial Hypertension. *Diagnostics* **2022**, *12*, 3033. https://doi.org/10.3390/diagnostics12123033.
31. Vasile, M.; Avouac, J.; Sciarra, I.; et al. From VEDOSS to established systemic sclerosis diagnosis according to ACR/EULAR 2013 classification criteria: A French-Italian capillaroscopic survey. *Clin. Exp. Rheumatol.* **2018**, *113*, 82–87.
32. Romano, E.; Rosa, I.; Fioretto, B.S.; et al. Circulating neurovascular guidance molecules and their relationship with peripherial microvacular impairment in sytsemic sclerosis. *Life* **2022**, *12*, 1056. https://doi.org/10.3390/life12071056.
33. Adams, R.H.; Eichmann, A. Axon guidance molecules in vascular patterning. *Cold Spring Harb. Perspect. Biol.* **2010**, *2*, a001875. https://doi.org/10.1101/cshperspect.a001875.
34. Romano, E.; Rosa, I.; Fioretto, B.S.; et al. A new avenue in the pathogenesis of systemic sclerosis: The molecular interface between the endothelial and the nervous systems. *Clin. Exp. Rheumatol.* **2019**, *37* (Suppl. 1), 133–140.
35. Chora, I.; Romano, E.; Manetti, M.; et al. Evidence for a derangement of the microvascular system in patients with a very early diagnosis of systemic sclerosis. *J. Rheumatol.* **2017**, *44*, 1190–1197. https://doi.org/10.3899/jrheum.160791.
36. Mazzotta, C.; Romano, E.; Bruni, C.; et al. Plexin-D1/Semaphorin 3E pathway may contribute to dysregulation of vascular tone control and defective angiogenesis in systemic sclerosis. *Arthritis Res. Ther.* **2015**, *17*, 221. https://doi.org/10.1186/s13075-015-0749-4.
37. Romano, E.; Chora, I.; Manetti, M.; et al. Decreased expression of neuropilin-1 as a novel key factor contributing to peripheral microvasculopathy and defective angiogenesis in systemic sclerosis. *Ann. Rheum. Dis.* **2016**, *75*, 1541–1549. https://doi.org/10.1136/annrheumdis-2015-207483.
38. Cole, A.; Ong, V.H.; Denton, C.P. Renal Disease and Systemic Sclerosis: An Update on Scleroderma Renal Crisis. *Clin. Rev. Allergy Immunol.* **2022**, *64*, 378–391. https://doi.org/10.1007/s12016-022-08945-x.
39. Shah, R.C.; Morrisroe, K.; Stevens, W.; et al. Scleroder, a renal crisis, an increasingly rare but persistently challenging condition: A retrospective cohort study. *Rheumatol. Adv. Pract.* **2024**, *8*, rkae131. https://doi.org/10.1093/rap/rkae131.
40. Trang, G.; Steele, R.; Baron, M.; et al. Coricosteroids and the risk of scleroderma renal crisis: A systematic review. *Rheumatol. Int.* **2012**, *32*, 645–653. https://doi.org/10.1007/s00296-010-1697-6.
41. Nassar, M.; Ghernautan, V.; Nso, N.; et al. Gastrointestinal involvement in systemic sclerosis: An updated review. *Medicine* **2022**, *101*, e31780. https://doi.org/10.1097/MD.0000000000031780.
42. Bandini, G.; Alunno, A.; Ruaro, B.; et al. Significant gastrointestinal unmet needs in patients with systemic sclerosis: Insights from a large international patient survey. *Rheumatology* **2024**, 63, e92–e93. https://doi.org/10.1093/rheumatology/kead486.
43. Hu, S.; Zuo, X.; Li, Y. Coexistence of systemic sclerosis and ankylosing spondylitis: A case report and literature review. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* **2018**, *43*, 1263–1265. https://doi.org/10.11817/j.issn.1672-7347.2018.11.016.
44. Hamberg, V.; Wallman, J.K.; Mogard, E.; et al. Elevated fecal levels of the inflammatory biomarker calprotectin in early systemic sclerosis. *Rheumatol. Int.* **2023**, *5*, 961–967. https://doi.org/10.1007/s00296-022-05264-4.
45. Maria, A.T.J.; Partouche, L.; Goulabchand, R.; et al. Intriguing relationships between cancer and systemic sclerosis: Role of the immune system and other contributiors. *Front. Immunol.* **2019**, *9*, 3112. https://doi.org/10.3389/fimmu.2018.03112.
46. Lepri, G.; Catalano, M.; Bellando-Randone, S.; et al. Systemic Sclerosis Association with Malignancy. *Clin. Rev. Allergy Immunol.* **2022**, *63*, 398–416. https://doi.org/10.1007/s12016-022-08930-4.

47. Dolcino, M.; Pelosi, A.; Fiore, P.F.; et al. Gene Profiling in Patients with Systemic Sclerosis Reveals the Presence of Oncogenic Gene Signatures. *Front. Immunol*. **2018**, 9, 449. https://doi.org/10.3389/fimmu.2018.00449.
48. Artlett, C.M.; Black, C.M.; Briggs, D.C.; et al. Telomer reduction in scleroderma patients: A possible cause for chromosomal instability. *Br. J. Rheumatol*. **1996**, *35*, 732–737. https://doi.org/10.1007/s00296-007-0472-9.
49. Ciechomska, M.; van Laar, J.M.; O'Reilly, S. Emerging role of epigenetics in systemic sclerosis pathogenesis. *Genes. Immun.* **2014**, *15*, 433–439. https://doi.org/10.1038/gene.2014.44.
50. Peng, Y.; Wang, Y.; Zhou, C.; et al. PI3K/Akt/mTOR Pathway and Its Role in Cancer Therapeutics: Are We Making Headway? *Front. Oncol*. **2022**, 12, 819128. https://doi.org/10.3389/fonc.2022.819128.
51. Galluzzi, L.; Spranger, S.; Fuchs, E.; et al. WNT signaling in cancer immunsurveillance. *Trends Cell Biol.* **2018**, *29*, 4–65. https://doi.org/10.1016/j.tcb.2018.08.005.
52. Marie, I.; Gehanno, J.F. Enviromental risk factors of systemic sclerosis. *Semin. Immunpathol.* **2015**, *37*, 463–473. https://doi.org/10.1007/s00281-015-0507-3.
53. Tashkin, D.P.; Roth, M.D.; Clements, P.J.; et al. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): A randomised controlled, double-blind, parallel group trial. *Lancet Respir. Med*. **2016**, *4*, 708–719. https://doi.org/10.1016/S2213-2600(16)30152-7.
54. Park, B.; Vemulapalli, R.C.; Gupta, A.; et al. Docetaxel-induced systemic sclerosis with internal organ involvement masquerading as congestive heart failure. *Case Rep. Immunol.* **2017**, *2017*, 4249157. https://doi.org/10.1155/2017/4249157.
55. Peng, H.; Wu, X.; Wen, Y.; et al. Association between systemic sclerosis and risk of lung cancer: Results from a pool of cohort studies and Mendelian randomization analysis. *Autoimmun. Rev*. **2020**, *19*, 102633. https://doi.org/10.1016/j.autrev.2020.102633.
56. Duffau, P.; Dimicoli, S.; Gensous, N.; et al. Anti-NOR90 antibody associated with paraneoplastic systemic sclerosis. *Clin. Exp. Rheumatol.* **2022**, *40*, 2002–2003. https://doi.org/10.55563/clinexprheumatol/r6fqel.
57. Didier, K.; Sobanski, V.; Robbins, A.; et al. Impact of autoantibody status on stratifying the risk of organ involvement and mortality in SSc: Experience from a multicentre French cohort of 1605 French patients. *RMD Open* **2024**, *10*, e004580. https://doi.org/10.1136/rmdopen-2024-004580.
58. Hochhegger, B.; Marchiori, E.; Sedlaczek, O.; et al. MRI in lung cancer: A pictorial essay. *Br. J. Radiol.* **2011**, *84*, 661–668. https://doi.org/10.1259/bjr/24661484.
59. Derk, C.T. Association of breast cancer development in patients with systemic sclerosis: An explorating study. *Clin. Rheumatol.* **2004**, *26*, 1615–1619. https://doi.org/10.1007/s10067-007-0546-9.
60. Anilkumar, M.; Alkhayyat, M.; Grewal, U.S.; et al. Higher risk of neoplastic progression of Barrett's esophagus in patients with systemic sclerosis. *Gastroenterol. Rep.* **2021**, *9*, 595–596. https://doi.org/10.1093/gastro/goaa096.
61. Colaci, M.; Giuggioli, D.; Vacchi, C.; et al. Haematological Malignancies in Systemic Sclerosis Patients: Case Reports and Review of the World Literature. *Case Rep. Rheumatol.* **2017**, *2017*, 6230138. https://doi.org/10.1155/2017/6230138.