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The Final Thesis

Lung Involvement in Systemic Sclerosis, Literature Review

Christopher Tobias Kappes, VI year, 4th group

Clinic of Rheumatology, Orthopaedics, Traumatology and Reconstructive Surgery

Institute of Clinical Medicine

Supervisor Assist. dr. Inesa Arštikytė

The Head of Clinic Prof. dr. Irena Butrimienė

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Email of the student

christopher.kappes@mf.stud.vu.lt

1. Summary:

Systemic sclerosis, a complex autoimmune disorder affecting multiple organ systems, progresses irreversibly if left untreated. Early treatment initiation is crucial to control systemic sclerosis, particularly for pulmonary involvement which carries the highest mortality rate. The most important factor for survival is time of treatment initiation thus late detection and the subsequent late treatment initiation worsens the prognosis. Symptoms in early stages are often non-specific and can occur without radiological evidence, whereas other patients are asymptomatic despite physical findings, necessitating diagnosis through a combination of symptoms, physical findings, pulmonary functional test and high-resolution computer tomography.

To increase diagnostic accuracy investigations have been conducted exploring the additional use of imaging and laboratory biomarkers in combination with the aforementioned methods. Sonography stands out as a promising imaging modality due to its easy accessibility and affordability. In combination with the already established diagnosis methods, sonography shows a possibility of being added as fourth method. Amongst the most promising laboratory biomarkers, the inflammatory marker axis consisting of interleukin-1ß, interleukin 17 and interleukin 23 might be a possible addition to the already established diagnostic methods due to their correlation. Krebs von den Lungen-6 in combination with cytokeratin 19 fragments conveys information about lung function status and fibrosis extent. Matrix metalloproteinase 7 is an alternative to cytokeratin 19 and just like carbohydrate antigen 15.3 is more readily available as most hospitals have established protocols for tumor marker testing. Pneumoprotein surfactant protein D can be used in monitoring disease progression and to confirm treatment efficacy.

The use of biomarkers could revolutionize the approach to treatment initiation, as currently guidelines dictate treatment initiation only upon meeting specific criteria. Immunosuppressive therapy stands as the cornerstone for treating interstitial lung disease, while the optimal treatment strategy for pulmonary artery hypertension remains a topic of debate.

Keywords: Systemic sclerosis, Pulmonary involvement, Interstitial lung disease, Pulmonary arterial hypertension, Pathology, Biomarkers, Therapy, Krebs von den Lungen-6

2. Introduction:

Systemic sclerosis (SSc) is a complex autoimmune disorder characterized by a progressive multisystem pathology, involving vasculopathy, immune dysregulation and fibrosis. In recent years pulmonary involvement has grown in significance, given the success in treating other organ manifestations, resulting in an increased mortality rate due to a delay in diagnosis and late therapy initiation (1; 2). Therapeutic interventions lack the ability to reverse fibrosis and only impede disease progression. Therefore, the identification of additional diagnostic tests and potential biomarkers for early recognition and treatment initiation has become of great importance (3).

My literature review aims to address the current understanding of pulmonary involvement in systemic sclerosis, with a focus on recent advancements in early diagnostic methods including functional, imaging and laboratory biomarkers. This emphasis was selected due to the significance of initiating treatment early and the challenges in the diagnosis of pulmonary involvement at a potentially reversible stage. The challenge of diagnosis of pulmonary involvement in SSc is the result of variability in symptom manifestation, the detection of biomarkers and their availability. Pulmonary involvement has become of greater importance over the last 25 years, as mortality from renal crisis has declined with improved treatment strategies, while interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH) have exhibited a significant increase in mortality (4).

Early diagnosis of pulmonary disease and therapy initiation, ideally at a reversible stage, has emerged as the most important prognostic predictor, as time of treatment initiation has shown to predict substantial improvements in survivability, regardless of the specific medication used (5; 6). While sensitive functional and imaging methods, for example, pulmonary function tests (PFT) and high-resolution computed tomography (HRCT), are already used in diagnosis, the exploration of additional imaging and laboratory aims to increase diagnostic accuracy (7).

3. Literature selection strategy:

The research literature selection strategy utilized a manual approach using keywords such as “systemic sclerosis”, “pulmonary involvement”, “interstitial lung disease”, “pulmonary arterial hypertension”, “biomarkers” and “therapy”. To ensure the review reflects the most recent

knowledge, specific filters were retrospectively applied, including “literature not older than 10 years”, “freely available articles/articles accessible via VU VPN on PubMed database” and “languages: English”. Although articles older than 10 years were included if they contained valuable insight to enrich understanding or support specific points. Each article underwent screening to assess its relevance to the research questions of identifying suitable biomarkers for the early detection of pulmonary involvement.

4. Clinical description:

SSc is a rare, acquired disease with an estimated prevalence ranging from 3-24 per 100.000 individuals and exhibits a global occurrence with a substantial variation in geographic distribution, supporting the potential influence of environmental factors in its pathogenesis (7). Its prevalence varies from 30 to 70 cases per million in Europe and Japan to 240 cases per million in the United States. Notably, over 50% of SSc patients in North America present with SSc pulmonary involvement with variation in severity and progression, underscoring the heterogeneous nature of the disease (2; 3).

There are two primary subtypes of SSc: limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc). These subtypes are classified based on the extent of skin involvement. Patients diagnosed with lcSSc typically exhibit sclerotic changes primarily localized to the fingers, dorsum of the hands and forearms. In contrast, patients with dcSSc present with sclerotic changes affecting not only peripheral regions but also extending to the trunk and the face. Furthermore, dcSSc is associated with a higher risk and more severe involvement of internal organs, such as the lungs, heart, gastrointestinal tract and kidneys which can result in a more aggressive disease course (8).

SSc can affect various organs and primarily affects the connective tissue, particularly the vascular and integumentary system, first. For many patients, Raynaud’s phenomenon, a vascular condition characterized by episodic color changes in the fingers or toes due to vasospasm of small blood vessels, is the earliest sign of SSc preceding the characteristic changes it is characterized by, namely thickened skin due to fibrosis. Raynaud’s phenomenon is of importance as it reflects the underlying vascular pathology characteristic of the disease. The vascular dysfunction associated with Raynaud’s phenomenon leads to endothelial injury, which is recognized as the initial trigger for the pathological process of SSc and often precedes

skin changes. As SSc advances, skin fibrosis leads to hardening and tightening of the skin, as well as joint stiffness and pain due to contractures (7).

While the pulmonary system is the most commonly affected extracutaneous organ system, depending on organ system involvement, symptoms can vary amongst SSc patients related to the specific function of the affected organ. In the early stages of pulmonary involvement, patients may be asymptomatic or present with non-specific symptoms, such as dyspnea, hypoxemia and non-productive cough (9). Among the most prominent pulmonary manifestations, ILD and PAH, there is no significant difference in early-stage symptoms to draw a satisfactory conclusion on disease manifestation. In advanced stages, differentiating between pulmonary manifestations of SSc remains challenging. Symptoms of ILD are often insidious and gradually progress with dyspnoea related to restrictive lung pattern presenting during rest or mild exertion, while in PAH dyspnea is more pronounced during physical activity and related to increased pressure in pulmonary arteries. Signs of right ventricular overload, most prominent distended jugular veins, may be seen in later stages. Both diseases eventually lead to right ventricular (RV) failure if not properly treated which can be further aggravated by cardiac involvement. In case of RV failure, prognosis indicated an estimated survival duration of 2 to 3 years (3).

5. Disease mechanism and pathology:

The pathophysiological process of SSc is characterized by vascular remodeling, fibroblast activation leading to excessive collagen production and immune system abnormalities (3). While the exact mechanism leading to SSc remains not fully understood, current research suggests that SSc may represent the phenotypic expression of different etiopathological processes, as no clear aetiological factor can be identified in the majority of cases. Environmental factors including silica and solvents have been assumed to contribute to SSc development. Genetic factors, including gene variants associated with innate immunity, as well as epigenetic factors such as histone post-translational modifications, DNA hypomethylation and dysregulation of microRNAs, have been documented to contribute to immune activation and fibrotic processes. One exception is the Erasmus syndrome, reported in 1% of SSc patients, which is defined as the development of SSc after exposure to silica (10).

The onset of fibrosis in pulmonary involvement of SSc patients may be triggered by injury to the lung epithelium or endothelial vessels in general. In SSc, an altered repair process results in

excessive production of signaling peptides, including transforming growth factor-ß (TGF-ß), endothelin-1 and platelet-derived growth factor (PDGF). These cytokines are implicated in pro-inflammatory and pro-fibrosing pathways. These factors collectively lead to the overactivity of fibroblasts, characterized by genetic dysregulation, impaired apoptosis, excessive production of extracellular matrix components and the subsequent development of vasculopathy and fibrosis. (10; 11).

It is important to note that tumor-associated antigens (TAA) may contribute to the perpetuation of inflammation, although the exact mechanism or their involvement with SSc is not yet fully understood. This association between SSc and cancer development appears to be particularly prevalent in patients with ILD, as TAAs are rarely increased in SSc patients without ILD (12; 13).

The link between TAAs and SSc is intriguing, especially considering that some observational studies have reported a higher incidence of cancer in SSc patients compared to the general population. Despite negative initial screening for neoplasia, some ILD patients developed cancer during follow-up. Notably, the types of tumors that developed varied, with cases including lung adenocarcinoma, neuroendocrine tumors, breast neoplasia or undifferentiated adenocarcinoma (14; 15).

The association between the pathology of SSc and its potential link to cancer development presents a possibility for additional incorporation of tumor biomarkers into the early diagnosis of pulmonary involvement in SSc patients.

6. Diagnostic methods:

The diagnosis of SSc is frequently suggested by a constellation of symptoms, physical findings, PFT and abnormalities observed in HRCT scans. Initial signs of SSc are closely associated with skin and blood vessel fibrosis, particularly affecting smaller blood vessels, prominently seen in the extremities. Thus, fingers are notably susceptible, often preceding SSc onset and exhibiting abnormalities such as capillary changes and digital gangrene as manifestations of vascular involvement seen in nail fold video-capillaroscopy (NVC). In the evaluation of pulmonary involvement, NVC has limited utility as it does not provide any information about status of the lungs. Specific skin changes supporting the diagnosis are sclerodactyly and flexor deformity, both of which reduce finger flexibility and function (7).

Symptoms of early pulmonary involvement in SSc are often non-specific or absent, complicating early-stage diagnosis and delaying appropriate treatment application in a potentially reversible stage. Additionally, patients can remain asymptomatic despite physical findings, the most common signs include crackles upon auscultation and interstitial thickening on chest radiography, in some cases symptoms are present without radiological evidence of pulmonary involvement. Importantly, the extent and severity of skin involvement do not necessarily correlate with pulmonary parenchymal damage. Patients with advanced skin involvement may have no pulmonary involvement while patients with few skin manifestations may have advanced pulmonary involvement (3; 16).

Due to the vast variability of symptomatic manifestations and physical findings, PFTs and HRCT are the most commonly employed methods for the diagnosis of pulmonary involvement in SSc. While PFTs may not reliably exclude early pulmonary manifestations, HRCT has become the gold standard for its sensitivity in detecting structural changes specific to SSc, such as fibrosis and ground-glass opacities (6). While alterations in PFTs often precede symptoms or radiographic changes, these changes may not accurately reflect the full extent of pulmonary involvement, as restriction in lung function can be influenced by movement restriction during inspiration caused by skin fibrosis (3). In some instances, alterations in PFTs may precede detection of abnormalities in imaging studies. However, in other cases, changes observed in imaging studies may be evident before significant alterations in lung function are detected (3; 6). Despite their utility, the variability in time of onset and symptom manifestation complicate the identification of early stages of pulmonary involvement, necessitating the exploration of additional diagnostic methods to reliably detect pulmonary manifestations in SSc patients. Nevertheless, certain specific signs or changes may raise suspicion and warrant further investigation.

PFTs can reveal early signs of lung involvement, with the reduction in diffusing capacity of the lungs for carbon monoxide (DLCO) and forced vital capacity (FVC) being notable indicators. FVC experiences the most significant decline within the first three years of SSc onset, even in asymptomatic patients. A decline of 5-10% in FVC and 10-15% in DLCO warrants further evaluation for potential lung involvement using HRCT, as both parameters correlate with the extent of lung fibrosis in an inversely proportional manner (6).

HRCT imaging is essential for reliably assessing lung involvement, as it is more sensitive than conventional chest CT scans. HRCT can identify mild or early interstitial abnormalities and detect small abnormalities indicative of early-stage involvement (6). Ground-glass

opacification of unknown etiology should prompt investigations for SSc etiology, as it often precedes lung fibrosis. Specific HRCT findings suggestive of pulmonary involvement in SSc include thickened interlobular septa, subpleural infiltrates or densities in the posterior segments of the lower lobes and interstitial reticular infiltrates (17). Particularly, changes in the posterior segments of the lower lobes are relevant, as SSc-related lung changes have a predilection for this region and thus should be investigated with special care. Traction bronchiectasis and large cystic changes indicate progressive disease and necessitate immediate treatment initiation, as pulmonary involvement has already progressed beyond a potentially reversible early stage. Subpleural honeycombing on HRCT indicates advanced disease and irreversible fibrotic changes, representing end-stage fibrosis and a severe manifestation (3; 16).

Among additional investigated imaging modalities, sonography appears promising. The number of B-lines, vertical hyperechoic reverberation artifacts, correlates linearly with fibrosis extent on HRCT. The number of B-lines detected on ultrasound has shown a significant difference between patients with and without evidence of lung involvement on HRCT, indicating potential for integration into early diagnosis of pulmonary involvement in SSc. Pleural irregularity, defined as the loss of normal hyperechoic lineal pleural contouring and associated with a general deviation from the norm rather than offering specific diagnostic insight, is another criterion under investigation, although its diagnostic specificity for pulmonary involvement in SSc remains uncertain and is currently under investigations. However, in case of detection, it may indicate a pathology that explains the deviation from the norm, warranting further follow-up (18; 19).

MRI is another imaging modality that shows promise in the early diagnosis of pulmonary involvement. It has demonstrated the ability to identify changes even before they are confirmed with HRCT. Specifically, the modalities MRI STIR and T1 signal intensity values have shown significant differences between patients with and without lung involvement, even in cases where HRCT does not indicate lung involvement. However, it is important to note that this difference could be attributed to transient accumulation of extravascular lung water in dependent areas of the lung, rather than specific SSc-related changes (20). Therefore, MRI may have a higher sensitivity but lower specificity compared to HRCT. Nevertheless, MRI may indicate the need for further diagnostic evaluation, which would have otherwise gone unnoticed in other modalities.

As a last resort, thoracoscopic lung biopsy can be considered after questionable diagnosis and exhaustion of other diagnostic procedures. However, its use is controversial due to variability

in patterns across multiple biopsies from the same patients, even among expert histopathologists. This variability further complicates the diagnostic process and often leads to delayed administration of treatment (16). Additionally, in cases of early detection of lung involvement, biopsies may not provide diagnostic values as the disease must be in an advanced stage to yield significant findings.

Due to the availability of functional biomarkers of PFTs and lung imaging biomarkers of HRCT, potential blood and bronchoalveolar lavage (BAL) biomarkers have been proposed and are currently under investigation, to increase the diagnostic accuracy of pulmonary involvement in SSc patients. This introduced a potential third diagnostic method to complete the existing two. These biomarkers must show sensitivity, either through an inverse correlation with lung function, such as a decrease in DLCO and FVC with increasing biomarkers, or a correlation with the extent of disease seen on HRCT.

BAL biomarkers provide insight into immunologic, inflammatory and infectious processes and may be important in identifying potential biomarkers of pulmonary involvement (6). However, the results are currently limited and conflicting. Some evidence suggests predictive value for clinical worsening due to the positive correlation between neutrophils and eosinophils in BAL and the extent of disease observed on HRCT. Notably, 50% of patients with normal HRCT had abnormal cellularity in BAL, suggesting BAL as a potential diagnostic method in addition to HRCT, especially if imaging biomarkers are negative (16; 21).

A potential biomarker, based on the pathologic process, is chemokine [C-X-C motif] ligand 4 (CXCL), a protein excreted by plasmacytoid dendritic cells. These cells are known for their role in immune response and regulation. Their presence in the lungs may suggest an ongoing immunologic reaction. Currently, their association with DLCO decline has been proven, although further research is warranted to elucidate their broader significance in SSc (16).

Krebs von den Lungen-6 (KL-6) is a high-molecular-weight glycoprotein of the mucin family primarily expressed on type II pneumocytes. It can be extracted from blood or BAL fluid and has been integrated into routine clinical practice in Japan following early research and validation studies, which confirmed KL-6 as a potential biomarker for pulmonary involvement in SSc patients. One of the key advantages of KL-6 is its accessibility, allowing for easy acquisition through blood or BAL samples. Its utility extends to longitudinal monitoring, offering a means to track disease progression over time (22). Furthermore, it provides valuable insight as it correlates with the degree of fibrosis and decline in lung function. Increased levels

of KL-6 also correlate with microvascular injuries which clinically manifest as the disappearance of finger pad tissue (23; 24).

Only recently, cytokeratin 19 fragment (CYFRA 21-1), expressed on type I/II pneumocytes and respiratory bronchiolar epithelial cells, has been proposed for use in combination with KL-6 to further increase diagnostic accuracy. While KL-6 demonstrates a stronger correlation with lung function decline regardless of fibrosis severity, CYFRA 21-1 correlates specifically with the extent of fibrosis. This combined approach offers a more comprehensive assessment of pulmonary involvement in SSc patients as KL-6 alone (25; 26).

In the context of potential biomarkers associated with fibrotic processes, attention has turned towards Matrix metalloproteinases (MMPs). These enzymes are responsible for the breakdown and remodeling of extracellular matrix. Elevated serum levels of MMP7 have been detected in patients with pulmonary involvement, prompting consideration for combining MMP7 measurements with KL-6 as a potential approach for identifying patients at risk of developing pulmonary manifestations in SSc (6; 16). The rationale behind this combined approach stems from the strong correlation of KL-6 with declines in lung function, regardless of fibrosis severity, whereas MMP7 provides information about fibrosis activity. The use of both KL-6 and MMP7 enables assessment of pulmonary status and function. Moreover, MMP7 may serve as a suitable replacement for CYFRA 21-1, as the latter is used as cancer biomarker and could potentially yield false positives in cancer patients (26).

Further investigation of KL-6 has suggested that similar mucins may play a role in disease pathogenesis, leading to the identification of carbohydrate antigen 15.3 (CA 15.3), a product of the same MUC1 gene encoding for KL-6, as a promising biomarker. The rationale behind proposing CA 15.3 as a biomarker lies in its inclusion in routine cancer testing panels and its strong correlation with the extent of fibrosis observed on HRCT scans, surpassing established lung parameters. Despite its promise, CA 15.3 is primarily recognized as a tumor marker associated with breast cancer and other malignancies, Therefore, hospitals and diagnostic centers regularly conduct cancer-related tests that may have established protocols for CA 15.3 testing. However, the association with oncologic and hepatic diseases limits its specificity as a biomarker for pulmonary involvement, as these disorders can influence the serum concentration of CA 15.3 (26; 27).

Other pneumoproteins, such as surfactant protein D (SP-D), a member of the lectin family, are synthesized by type II alveolar cells in lung tissue and play a crucial role in maintaining proper

pulmonary mechanical function. This specificity as a lung biomarker enables SP-D to reflect pathological processes specific to lungs better than general fibrotic and inflammatory markers. Notably, while cutaneous fibrosis tends to occur early and gradually improves, lung fibrosis progresses persistently, often until the late stage of the disease. Elevated levels of SP-D have been observed in pulmonary involvement in SSc. Consequently, SP-D can be used as a valuable tool for monitoring disease progression. correlating with Medsger score, which includes parameters such as DLCO, FVC, as well as radiological changes a. estimated pressure in pulmonary artery in echocardiography. Its detection on routine testing may prompt further investigations for pulmonary involvement in SSc (28; 29).

Other tumor-associated antigens (TAAs) CEA, CA19-9, CA15-3 and CA125 are currently being investigated for their potential use as biomarkers in SSc patients. Notably, an elevation in at least one TAA is linked to diffuse skin involvement and anti-Scl70 positivity. Additionally, SSc patients with an increased level of at least one TAA exhibit lower FVC values, alongside higher interstitial scores, compared with SSc patients without TAA elevation (30).

Furthermore, in patients with ILD, an increase in one or more TAAs corresponds to more severe lung involvement, both functionally and radiologically. This association indicates a more aggressive form of scleroderma disease, as evidenced by a direct correlation between TAA levels and HRCT scores. Conversely, there is an inverse correlation between TAA antigens and FVC values. However, it is important to note that TAA's utility in the diagnosis of pulmonary involvement in SSc patients is limited. Instead, their elevation may raise suspicion, warranting further diagnostic investigations, particularly if no malignant foci can be identified (30).

In blood serum, the currently available biomarkers utilized in clinical practice are autoantibodies, among which the most prevalent are anti-centromere antibodies, frequently detected in the sera of patients with lcSSc, and anti-topoisomerase I antibodies, commonly found in the sera of patients with dcSSc. Additionally, anti-RNA polymerase III antibodies are occasionally detected. These antibodies have been demonstrated to correlate with disease severity and activity, including pulmonary parenchymal and vascular abnormalities (16; 31).

Other potential antibodies found in the serum of SSc patients, which need further elucidation for potential diagnostic use, include antineutrophil cytoplasmic antibodies (ANCA). Positive ANCA status has been associated with a higher prevalence of ILD and features overlapping with other connective tissue diseases.

Anti-PDGFR autoantibodies play an active role in disease pathogenesis due to their stimulatory activity, leading to higher levels of type I collagen in fibroblasts. Additionally, anti-fibroblast antibodies are elevated in SSc patients and promote the production of IL-6 among other proinflammatory cytokines. Notably, anti-alpha-enolase antibodies showed an association with the prevalence of ILD (32).

The degradation of ECM is induced by MMPs. An imbalance of MMP and tissue inhibitor of metalloproteinases (TIMP) regulation could contribute to excessive ECM deposition. SSc fibroblasts exhibit a reduced MMP-1 activity compared to normal fibroblasts. Antibodies reactive to MMP-1, involved in the degradation of Type I-III collagens, and MMP-3, which degrades type V collagen, elastin and fibrillin, have been detected and correlated with the extent of fibrosis.

SSc is more frequently diagnosed in women and female sex hormones have been hypothesized to play a role in disease pathogenesis. Estrogens and 17ß-estradiol modulate immune function via estrogen receptor Era and Erß. The former were recently described in patients with systemic lupus erythematosus (SLE) and were found to be responsible for cell activation and T-lymphocyte proliferation.

Oxidative stress is known to play an important role in SSc. Increased cellular release of reactive oxygen species (ROS) has been detected in SSc monocytes, maintaining the fibrotic phenotype of SSc fibroblasts. Antibodies to methionine sulfoxide reductase A (MSRA), an antioxidant repair enzyme, have been detected in SSc patients. Serum levels of anti-MRSA antibodies correlated negatively with FVC and DLCO (32).

However, while the presence of autoantibodies found in blood serum can aid in the diagnosis of pulmonary involvement in SSc, it is important to note that their presence alone does not confirm lung manifestations. Instead, it indicated the involvement in SSc in the patient. Additional diagnostic tests, such as PFT and imaging studies, are required to confirm the presence and extent of pulmonary involvement.

An interesting association has emerged between antibodies in SSc and major histocompatibility complex (MHC) II antigens, supporting the genetic basis of pulmonary involvement in SSc patients. MHCs are a genetic region located on chromosome 6 in humans, where genes encode proteins called human leukocyte antigens (HLAs). Notably, MHC II molecules play a pivotal role in the immune system by presenting antigens to CD4+ T cells, thereby activating the

immune response. This mechanism may explain the susceptibility to autoimmune diseases observed in SSc patients in SSc.

Upon thorough analysis, only two variants have been found with statistical significance: HLA-DRB1*3 in the Han Chinese population and CTGF rs6918698 in the UK population. However, it remains uncertain at the time of writing this review whether the observed association between these genes and lung manifestations in SSc is purely coincidental or indicative of a causal correlation. Despite the multitude of reported associations, genetic biomarkers relevant to risk stratification have yet to be established.

The investigation for genetic biomarkers has expanded to include non-coding RNA molecules, namely microRNAs responsible for regulating gene expression. Notably, the downregulation of miR-181a and miR-126, along with the overexpression of miR-143 and miR-155, show promise as diagnostic tools in SSc. Patients presenting with early scleroderma pattern of microangiopathy observed on NVC demonstrated elevated serum miR-155 expression. While this finding may not immediately aid in diagnosing early pulmonary involvement, it highlights the potential for early intervention in preventing the progression of SSc before pulmonary manifestations emerge.

For patients displaying signs or symptoms of lung manifestations, miR-143 may serve as an indicator of the severity of pulmonary involvement. Notably, SSc patients with FVC values less than 70% showed significantly reduced levels of miR-143 compared to those with normal FVC values. The early decline in miR-143 levels may offer insight into risk stratification and early intervention strategies in managing SSc-related lung manifestations.

Additionally, the exclusive presence of miR-181 in the serum of patients with lcSSc suggests its potential as a biomarker for earlier and more severe pulmonary involvement in its absence, emphasizing the importance of early treatment initiation. Conversely, miR-126, an antifibrotic factor with a protective effect against lung fibrosis, was notably reduced in SSc patients, further highlighting its potential diagnostic significance (21).

Moreover, epigenetic factors may play a significant role in the pathogenesis of SSc. Four genes – F2R, FYN, PAG1 and PRKCH - which regulate methylation, have been found underexpressed in SSc patients. This underexpression results in reduced activity of Friend leukemia virus integration (Fli1) collagen transcription suppression factor. Fli1, known for its protective effect against ILD, upregulates gene expression of autoimmune regulators and CXCL13 via CpG methylation (6).

During the fibrotic process in SSc, Th1, which involves pro-inflammatory signals and cell-mediated immunity, and Th2, characterized by anti-inflammatory signals and antibody-mediated immunity, both play crucial roles. It has been observed that a mixed M1/M2 monocyte/macrophage cell population, which includes Th1/Th2-associated responses, is associated with Scl-70 antibody positivity.

Given the correlation of Scl-70 antibodies with a decline in lung function decline and the extent of fibrotic changes observed in HRCT, Th1 and Th2 responses have been investigated for their potential use as biomarkers. Flow cytometry surface marker analysis has revealed a correlation between the percentage of M1/M2 monocyte/macrophage cells and pulmonary involvement, suggesting their potential use as biomarkers.

Patients affected by SSc-related ILD and higher systolic pulmonary artery pressure were characterized by a higher percentage of circulating $CD204^+$ $CD163^+$ $CD206^+$ $TLR4^+$ $CD80^+$ $CD86^+$ and $CD14^+$ $CD206^+$ $CD163^+$ $CD204^+$ $TLR4^+$ $CD80^+$ $CD86^+$ mixed M1/M2 monocyte/ macrophage subsets. Notably, CD163 levels were significantly elevated in SSc patients compared to healthy individuals, whereas CD40+ macrophages expressed higher levels of CD206 and were associated with PAH. The association of CD163 with ILD can be attributed to its dependency on STAT3 expression.

Recently, through RNA sequencing and genotyping, a link has been identified between mixed macrophage activation signature and restricted M2 activity, leading to the downregulation of IL-6/JAK/STAT3 signaling pathway, resulting in excessive fibrosis (33).

Previous studies have highlighted increased production of TGF-ß, IL-6, and IL-1, all of which are associated with Th17 cell differentiation. Particularly, IL-6 has emerged as a predictive factor for early disease progression due to its association with collagen production, although its low specificity is attributed to elevated levels observed in diverse inflammatory conditions. Furthermore, IL-6 levels are correlated with the decline in lung function observed in PFTs and are notably elevated in the early stages of SSc (34; 35).

In exhaled breath condensate (EBC), elevated levels of IL-1ß, IL-17 and IL-23 have been recognized as potential biomarkers. Specifically, IL-17 inversely correlates with DLCO, IL-1ß is linked to radiological score of interstitial lung involvement and IL-23 demonstrates a correlation with CT scan scores of pulmonary involvement and inversely correlates with vital capacity. Collectively, these three biomarkers offer a comprehensive evaluation, completing conventional diagnostic methods such as PFT and HRCT (36).

Importantly, all aforementioned biomarkers can be obtained from EBC, a non-invasive and easily performed method for assessing lung inflammation. Moreover, the specificity of EBC is highlighted by the lack of correlation between serum and EBC levels of cytokines, indicating a distinct inflammatory reaction within each system (37).

TGF-ß has long been recognized as a key regulator of connective tissue remodeling and fibrogenesis in SSc. Another member of the TGF-ß superfamily, growth differentiation factor 15 (GDF-15), has emerged as a potential biomarker in SSc. Elevated levels of GDF-15 have been observed in all phenotypes of SSc, even preceding the occurrence of skin manifestations. Importantly, GDF-15 levels correlate with disease activity independently of skin involvement. Initially, it was thought that GDF-15 could serve as a specific biomarker for lung involvement, as serum level concentration increased with decreasing DLCO, potentially enabling detection of the disease before clinical manifestation (38; 39).

However, further investigations revealed that smoking directly affected the GDF-15 level, complicating its use as a specific biomarker for SSc. Additionally, GDF-15 is associated with the expression of proinflammatory cytokines and chemokines, such as IL-6 and CCL2, raising questions about its diagnostic value. Further studies are needed to elucidate the potential of GDF-15 as a biomarker in SSc (40).

Serum amyloid A (SAA), a 12kDa acute phase protein, has emerged as a promising biomarker in SSc, with implications for regulating the expression of TGF-ß. Elevated levels of SAA have been associated with signs and symptoms of pulmonary involvement, particularly showing correlation with measures of pulmonary function and radiologic evidence of SSc-associated ILD. Moreover, SAA has shown a significant correlation with pulmonary artery pressure, further highlighting its relevance in assessing pulmonary vascular complications (41).

One of the notable advantages of SAA is its reliability, as its level is increased regardless of gender, ethnicity, age or clinical subtype of SSc. Additionally, SAA demonstrates associations with other pro-inflammatory markers such as IL-6 and IL-8. Its release is stimulated by these markers, further emphasizing its role in the inflammatory response. Furthermore, SAA is implicated in the stimulation of MMP-1 and MMP-12 production, underscoring its involvement in tissue remodeling processes characteristic of SSc. (42).

Other potential biomarkers for diagnosing pulmonary involvement in SSc patients have been identified and are currently under investigation. The exploration of chemokines, owing to their involvement in immune system processes in SSc, has gained attention due to their integral role

in inflammation and tissue remodeling. One such chemokine, Chemokine [C-C motif] ligand 18 (CCL18), derived from macrophage 2, has chemotactic properties for various immune cells and has been identified as a potential prognostic factor (11). Notably, CCL18 exhibits selective expression in pathological tissue. However, its application in the early diagnosis of pulmonary involvement remains unclear, as its expression occurs when the disease has already manifested, and the extent to which it correlates with disease severity needs further elucidation (27; 43).

In light of this, other biomarkers such as Monocyte chemoattractant protein-1 (MCP-1 or CCL2) and macrophage inflammatory protein-1ß (MIP-1ß or CCL4) have been investigated as potential candidates for early diagnosis. These chemokines are particularly implicated in the initiation and perpetuation of fibroblast activation and ECM deposition in SSc, thus offering promise as biomarkers for identifying pulmonary involvement in its early stages. (44).

7. Treatment methods:

Treatment of pulmonary involvement in patients with SSc is not immediately initiated after diagnosis due to concerns about adverse effects of immunosuppressive therapy. Instead, treatment follows clinical guidelines and is typically initiated if patients present with pulmonary involvement exceeding 20% on HRCT along with an FVC of less than 70% on PFT. If the initial diagnosis criteria are not met but lung function has significantly decreased, defined as a significant decrease of DLCO over 15% or FVC decline exceeding 10%, treatment may be initiated on follow-up (45).

ILD, characterized primarily by fibrosis due to dysregulated immune response, prioritizes targeting inflammation in treatment. Currently, cyclophosphamide (CYC) is recommended as first-line, typically administered orally at a dose of up to 2mg/kg/day for a one-year course. However, an alternative approach suggests starting with mycophenolate mofetil (MMF) followed by CYC in combination with rituximab (RTX) as induction therapy, with MMF used as maintenance therapy due to its better safety profile. This proposal stems from observations that patients treated with CYC had a more stable disease course, with only 50% requiring further immunosuppressive therapy after treatment cessation. MMF has shown clinical stability for up to 36 months with fewer early adverse events compared to azathioprine (AZA). Hematopoietic stem cell transplantation is considered an emergency treatment option for severe diffuse SSc refractory to standard therapy (46).

To confirm PAH in SSc patients, diagnostic criteria include a mean pulmonary arterial pressure (mPAP) of 25 mmHg or greater with a pulmonary artery wedge pressure (PAWP) less than 15 mmHg on right heart catheterization. However, a specific treatment initiation point has not been established. Additional signs of right ventricular dysfunction seen on echocardiography include a tricuspid annular plane systolic excursion (TAPSE) measurement of less than 1.8 cm, a right ventricular fractional area change (RVFAC) below 35% and a systolic excursion velocity of the tricuspid valve less than 10 cm/s (46).

The target of PAH therapy is impaired endothelial function affecting vascular tone and remodeling. While most ILD patients respond to immunosuppressive therapy, other treatment options are needed for PAH. Phosphodiesterase inhibitor sildenafil and prostaglandin epoprostenol are proposed drugs for PAH treatment, with oral sildenafil as first-line therapy and epoprostenol for severe cases. Anticoagulation therapy with warfarin has been proposed for severe PAH based on evidence of pulmonary thromboembolic arterial disease, significantly prolonging survival. Tyrosine kinase inhibitors, such as imatinib and sorafenib, are currently under research for PAH treatment based on evidence of their involvement in the pathological process inhibiting vascular endothelial growth factor (VEGF) and PDGF (46; 47).

For patients with treatment-refractory lung disease, lung transplantation is considered a last resort, regardless of PAH or ILD. While SSc is not an absolute contraindication to lung transplantation, associated morbidity and organ dysfunction beyond the lung increase transplantation risks (46).

As comprehension of the underlying pathophysiology of SSc has advanced, in addition to the identification of biomarkers implicated in the disease process, there has been a growing recognition of the potential benefits of therapies targeting specific mechanisms of the disease rather than relying on general immunosuppression. These shifts factor in the use of biological agents that target specific cells or pathways involved in SSc pathogenesis.

One such target of interest is TGF-ß, a well-established key mediator of fibrogenesis. Consequently, it presents an appealing therapeutic target. However, caution is advised due to the multifunctional nature of TGF-ß, as a non-selective blockade of its signaling pathways could potentially result in severe adverse effects and even fatal outcomes.

Among the agents under investigation, fresolimumab, a human IgG4 kappa monoclonal antibody that binds and inhibits TGF-ß, has shown promise. Nonetheless, the significant

adverse effects associated with its use highlight the necessity for further research to identify and develop more appropriate biological agents for treatment (48).

Based on the biomarkers associated with pulmonary involvement in SSc patients, IL-6 has been identified as a potential therapeutic target. Tocilizumab, a monoclonal antibody targeting IL-6, has shown efficacy in slowing the progression of skin fibrosis and reducing its severity, particularly when corticosteroid therapy has been ineffective. However, its use has been linked to a higher rate of serious infection, including one death. Consequently, tocilizumab remains an option in refractory systemic sclerosis (49; 50).

8. Conclusion:

Pulmonary involvement in SSc presents a growing concern, given its substantial impact on the healthcare system and quality of life, attributed to its irreversible progression and challenges in timely diagnosis. To address these challenges, researchers have searched for additional biomarkers associated with the disease's pathological processes, aiming to add to established diagnostic methods such as PFT and HRCT.

While functional biomarkers have been exhausted with PFT, the search for additional imaging biomarkers has led to promising discoveries. Sonography emerges as a frontrunner due to its accessibility, cost-effectiveness and compatibility with established diagnostic methods. Although MRI offers heightened sensitivity, its lack of specificity may lead to increased false positives. Nonetheless, when combined with other biomarkers, MRI can prompt further investigation in cases of suspicious findings, thus contributing to early detection and disease progression monitoring.

Laboratory biomarkers, obtained primarily through BAL and blood samples, hold potential either as supplementary to existing biomarkers or as replacements, although ongoing research is still required for validation. Notably, the biomarker KL-6, utilized in clinical practice exclusively in Japan, offers insight into lung function decline, crucial in identifying pulmonary manifestations early on. When coupled with CYFRA 21-1, which provides information on fibrosis extent, a comprehensive assessment of pulmonary involvement in SSc patients is achieved.

Considering the inclusion of cancer biomarkers like CA 15.3 or the substitution of CYFRA 21-1 with MMP shows promise. The rationale behind this suggestion is grounded in the routine

cancer-related tests conducted in most hospitals, which often include established protocols for detecting tumor markers. This increases the likelihood of identifying pulmonary involvement at an early stage with the potential to detect biomarkers preceding the manifestation of signs and symptoms. However, it's important to note that further investigations are needed to exclude oncologic diseases.

The inflammatory marker axis, encompassing IL-1ß, IL-17 and IL-23 shows promise in enhancing diagnostic accuracy, potentially supplementing or even replacing traditional methods like PFT and HRCT. IL-1ß correlates with the radiological score on HRCT, IL-17 correlates with DLCO decline and IL-23 correlates with the radiological score on CT and FVC decline. This axis holds potential as an addition to PFT and HRCT, with the ability to confirm pulmonary involvement based on both radiological findings and lung function. However, further research is necessary to validate its effectiveness before considering its replacement of the established diagnostic methods.

Additional biomarkers, such as SP-D and inflammatory markers including CD206, CD163, CD14 and CD40 associated with Th1 and Th2 pathways, have been investigated for their potential relevance in SSc. Genetic biomarkers, primarily microRNAs, have also been studied, with findings indicating the downregulation of miRNA-181a and the overexpression of miR-132, miR-143, miR-145 and miR-155 in serum, suggesting their significance in the context of SSc biomarkers.

Furthermore, GDF-15 and SAA have been identified, although their potential use in the early diagnosis of pulmonary involvement in SSc is still being explored. However, it is worth noting that GDF-15 and miR-155 have shown promise in predicting disease progression, as their levels increase prior to the onset of clinical symptoms. This suggests a potential role for these biomarkers in predicting the development of pulmonary manifestation.

While autoantibodies can be tested for in SSc patients, it is important to note that their presence does not necessarily confirm pulmonary involvement. Instead, they confirm the presence and manifestation of SSc itself in patients.

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