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REVIEW ARTICLE

Novel biomarkers in systemic sclerosis

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Short title: *Novel biomarkers in systemic sclerosis*

What is not yet known on the issue addressed in the submitted manuscript

Given the limited therapeutic options and poor prognosis of many systemic sclerosis patients, a better understanding of the immune-pathophysiological profiles might aid to an adjusted therapeutic approach.

Research hypothesis

The qualitative, analytical study was performed in order to identify the possible biomarkers for diagnosis, disease progression, and complications in systemic sclerosis patients.

The novelty added by manuscript to the already published scientific literature

Recently described biomarkers for diagnosis, disease progression and complications in systemic sclerosis patients are summarized and systematized.

Abstract

Introduction. Due to the heterogeneous nature of systemic sclerosis, it is difficult to predict disease progression and complications. Despite the discovery of novel autoantibodies associated with systemic sclerosis (SSc), there is an unmet need for biomarkers for diagnosis, disease progression, and response to treatment.

Materials and methods. An analytical, qualitative study was performed with a narrative review of literature in the form of a synthesis article. Relevant primary sources published in 2020-2022 were identified and selected, using data extraction and analysis.

Results. Anti-citrullinated protein/peptide antibody could be useful in identifying patients with a more prominent joint disease. Of most interest, the anti-carbamylated protein antibodies (anti-CarP) could be a relevant biomarker related to fibrotic skin and lung disease. Positive anti-RNA (Ribonucleic acid) polymerase III antibody and antinuclear antibodies (ANA) negativity were significantly associated with GAVE (gastral antral vascular ectasia). Autoantibodies against telomeres may help identify systemic sclerosis with lung disease. Osteopontin links myeloid activation and disease progression in systemic sclerosis. CTRP (C1q tumor necrosis factor-related proteins) 9 protein levels may be biomarker of lung disease severity. CD (cluster differentiation) 21-low B cells are linked to vascular damage**.** L-tyrosine, L-tryptophan, and 1-methyl-adenosine distinguished healthy controls from SSc patients. L-leucine, L-isoleucine, xanthosine, and adenosine monophosphate differentiated between progressing and stable SSc-ILD. CECs (circulating endothelial cells) are a direct indicator of systemic vascular damage. Levels of the protein, galectin-3, are associated with heart involvement in people with systemic sclerosis. Low levels of the galectin-10 protein (Gal-10) in scleroderma patients associate with inflammation and vascular changes in the lungs, leading to pulmonary arterial hypertension (PAH). High levels of the CD146 protein may be a potential biomarker in identifying people with systemic sclerosis. Blood levels of the protein endocan increased in scleroderma patients who are at risk for pulmonary arterial hypertension. FLCs (free light chain) could be employed as useful potential biomarker of early diagnosis and to follow disease activity.

Conclusions. Novel discovered biomarkers could predict disease development, activity, and severity of diverse organ involvement, predict risk of complications of systemic sclerosis.

Keywords: systemic sclerosis, biomarkers, diagnosis, prediction of disease progression, complication.

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Introduction

Systemic sclerosis (SSc) is an autoimmune disease, which is characterized by vasculopathy, tissue fibrosis, and activation of the innate and adaptive immune system. The disease course and severity have various clinical characteristics ranging from a relatively benign condition to a rapidly progressive disease with high mortality. Clinical features of the disease are variable and consist of skin thickening, vasculopathy and internal organ involvement. Due to the heterogeneous nature of the disease, it is difficult to predict disease progression and complications. Given the limited therapeutic options and poor prognosis of many SSc patients, a better understanding of the immune-pathophysiological profiles might aid to an adjusted therapeutic approach.

Despite the discovery of novel autoantibodies associated with SSc, there is an unmet need for biomarkers for diagnosis, disease progression, and response to treatment.

The aim of the study was to investigate recently described biomarkers for diagnosis, disease progression and complications in systemic sclerosis patients.

Materials and methods

A qualitative and analytical study was performed focused on primary studies published in 2020-2022 and dedicated to the identification of biomarkers for diagnosis, disease progression, and complications in systemic sclerosis patients.

In order to realize the proposed aim, scientific publications were searched over the PubMed, NCIB, Google Search, Medscape using the key words systemic sclerosis, biomarkers, diagnosis, prediction of disease progression, complication. More than 80 reference sources have been identified and 20 were selected for analysis.

Results and discussions

Systemic sclerosis (SSc) is an autoimmune disease that is characterized by vasculopathy, tissue fibrosis, and activation of the innate and adaptive immune system. The disease course and severity have various clinical characteristics ranging from a relatively benign condition to a rapidly progressive disease with high mortality. Clinical features of the disease are variable and consist of skin thickening, vasculopathy and internal organ involvement. Due to the heterogeneous nature of the disease, it is difficult to predict disease progression and complications. Given the limited therapeutic options and poor prognosis of many SSc patients, a better understanding of the immune-pathophysiological profiles might aid to an adjusted therapeutic approach.

Despite the discovery of novel autoantibodies associated with SSc, there is an unmet need for biomarkers for diagnosis, disease progression, and response to treatment.

SSc-specific autoantibodies are currently used for diagnosis and prediction of clinical features, as other biomarkers have not yet been fully vetted. Krebs von den Lungen-6 (KL-6), surfactant protein-D (SP-D), and CCL (C Chemokine Ligand) 18 have been considered as serum biomarkers of SSc-related interstitial lung disease. Moreover, levels of circulating brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) can provide diagnostic information and indicate the severity of pulmonary arterial hypertension. Assessment of several serum/plasma cytokines, chemokines, growth factors, adhesion molecules, and other molecules may also reflect the activity or progression of fibrosis and vascular involvement in affected organs. Recently, microRNAs have also been implicated as possible circulating indicators of SSc. In table 1 are presented some biomarkers that have been shown to possess predictive potential for various manifestations of systemic sclerosis.

In this review, we focus on several new potential SSc biomarkers that have been described in 2020-2022 and discuss their clinical utility.

Similarly, to what is seen in other connective tissue diseases, most SSc patients are seropositive for antinuclear antibodies and have disease-specific autoantibodies, which can be detected prior to the development of clinical symptoms. Disease-specific autoantibody profiles support not only conclusive and phenotypic diagnoses but can also be associated with clinical manifestations and disease progression of SSc. In fact, autoantibodies such as anticentromere antibody, anti-topoisomerase I antibody, and anti-RNA polymerase III antibody are specific and included in 2013 criteria for classification.

There are also several other autoantibodies that are considered as potential predictors of course and outcomes of systemic sclerosis.

Anti-endothelial cell antibodies are found in a substantial number of SSc patients (22–86%) [2]. These antibodies have been shown to target various vascular antigens, such as ICAM-1 [3], lamin A/C, tubulinβ-chain, and vinculin [4], which are responsible for endothelial cell activation via increased oxidative stress and proinflammatory responses. Some authors found anti-endothelial cell antibodies more frequently present in SSc patients with ILD than in those without ILD [5].

Based on two large SSc French cohorts (448 SSc patients) [6] the prevalence and associated factors with the autoantibodies linked to erosive arthritis were studied. Enzyme-linked immunosorbent assay (ELISA) for IgM rheumatoid factor (RF), IgG anti-citrullinated proteins (ACPA) and IgG anticarbamylated proteins antibodies (anti-CarP) were determined. The prevalence and clinical associations of the different antibodies were investigated. RF positivity was observed in 113 patients (25%) compared to 39 (9%) for ACPA and 63 (14%) for anti-CarP antibodies. Through multivariate regression analysis, both RF and ACPA positivity resulted to be associated with RA overlap disease (OR 5.7, 95% CI 2.3–13.8 and OR 44.1, 95% CI 15.4–126.3, respectively). Additionally, ACPA was found to be significantly related to synovitis/tenosynovitis (OR 1.7, 95% CI 1.0–2.6). RF positivity was associated to a „vascular subset" (i.e., any major vascular complication) (OR 2.1, 95% CI 1.3–3.4). Moreover, anti-CarP antibodies were associated with a fibrotic subset and with digital ulcers (OR 2.0, 95% CI 1.1–3.6 and OR 1.9, 95% CI 1.1–3.4). Authors have concluded that

Table 1. *Potential Biomarkers in Systemic Sclerosis [1].*

Biomarker	Clinical association
TGF-β+	Digital ulcers, dcSSc
TGF-β-	dcSSc, mRSS (in dcSSc)
VEGF+	Systemic organ involvement, PAH, shorter disease duration, skin sclerosis, reduced capillary density of nailfold
VEGF-	Digital ulcers
CTGF+	mRSS, ILD
GDF-15+	Skin sclerosis, PAH, ILD, respiratory dysfunction (FVC, DLCO)
IL-6+	mRSS, early progressive skin sclerosis, poor prognosis, DLCO decline in SSc-ILD
BAFF+	Skin sclerosis
APRIL+	Pulmonary fibrosis
CCL2+	ILD (lung dysfunction, CT scores), mRSS
CXCL4+	mRSS, lung fibrosis, PAH, disease progression
CXCL8+	Predictive of physical dysfunction
CXCL10+	Preclinical/early SSc
CX3CL1+	dcSSc, ILD, digital ulcer
ICAM-1+	Rapidly progressive disease, digital ulcers, dcSSc, ILD, joint involvement, renal crisis, predictive of respiratory dysfunction
VCAM-1+	Systemic organ involvement, renal crisis, disease activity
E-selectin+	Systemic organ involvement, renal crisis, disease activity
P-selectin+	Disease activity, predictive of physical disability
Endostatin+	PAH
Endoglin+	lcSSc, anticentromere Ab, cutaneous ulcer, telangiectasia, PAH.
Von Willebrand factor+	Raynaud's phenomenon, disease severity, ILD, predictive of PAH
KL-6+	Severity of ILD, maximum fibrosis scores on HRCT
SP-D+	Severity of ILD, maximum fibrosis scores on HRCT
CCL18+	Activity and severity of ILD, predictive worsening of ILD and mortality
BNP/NT pro-BNP+	Severity, stability, and prognosis of PAH
Endothelin-1+	PAH, systemic organ involvement, microangiopathy defined by capillaroscopy
Type I collagen (C-terminal telopeptide)+	Skin fibrosis, mRSS, pulmonary dysfunction, CRP
Type III collagen (N-terminal peptide)+	Disease activity, mRSS, HRCT score, prognosis
MMP-7+	ILD, disease severity
MMP-9+	mRSS, dcSSc
MMP-12+	Skin sclerosis, dcSSc, ILD, nailfold bleeding, lower FVC
CRP+	Skin sclerosis, PAH, renal dysfunction, risk of progressive early ILD, worse pulmonary function
sCD163+	ILD, PAH, skin sclerosis
YKL-40+	Pulmonary involvement, higher mortality rate

Note: + – upregulated; - – downregulated; TGF-β – transforming growth factor; GDF-15 – growth differentiation factor 15; BAFF – B-cell-activating factor belonging to the tumor necrosis factor family; APRIL – a proliferation-inducing ligand; MMP – matrix metalloproteinases; BNP – brain natriuretic peptide; NT-proBNP – N-terminal-pro hormone BNP; CTGF –connective tissue growth factor; mRSS – modified Rodnan total skin thickness score; ILD – interstitial lung disease; IL-6 – interleukin 6; DLCO – diffusing capacity of carbon monoxide; CT – computed tomography; PAH – pulmonary arterial hypertension; ICAM-1 – intercellular adhesion molecule 1; dcSSc – diffuse cutaneous systemic sclerosis; VEGF – vascular endothelial growth factor; lcSSc – limited cutaneous systemic sclerosis; KL-6 – krebs von den Lungen-6; HRCT – high resolution CT; SP-D – surfactant protein-D.

ACPA could be useful in identifying patients with a more prominent joint disease and RA overlap disease. Of the most interest was found that anti-CarP antibodies could be a relevant biomarker related to fibrotic skin and lung disease.

Based on a multicenter international cohort, the clinical associations of anti-PM/Scl (polymyositis/scleroderma) antibodies in patients with SSc was evaluated, with a particular focus on unresolved issues, including scleroderma renal crisis (RC), malignancies, and functional outcome of interstitial lung disease (ILD) [7]. Using the The European Scleroderma Trials and Research group (EUSTAR) database, SSc patient were analyzed with the following outcomes: anti-PM/Scl+ without SSc-specific autoantibodies were compared with 7202 anti-PM/Scl-, and then to 155 anti-Pm/Scl+ with SSc-specific antibodies. Additional data were collected for 165 anti-PM/Scl+ SSc patients (85 from the EUSTAR registry) and compared with 257 anti-PM/Scl- SSc controls, matched for sex, cutaneous subset, disease duration and age at SSc onset.

As a result, it appears that patients with isolated anti-PM/Scl+, as compared with anti-Pm/Scl-, had higher frequency of muscle involvement, ILD, calcinosis and cutaneous signs of dermatomyositis (DM), but similar frequency of scleroderma renal crisis (SRC) and malignancies (either synchronous with SSc onset or not). The presence of muscle involvement was associated with a more severe disease phenotype. Although very frequent, ILD had a better functional outcome in cases than in controls. In patients with both anti-PM/Scl and SSc-specific antibodies, a higher frequency of typical SSc features than in those with isolated anti-PM/Scl was observed.

Authors concluded that analysis of the largest series of anti-PM/Scl+ SSc patients so far reported helps to delineate a specific clinical subset with muscle involvement, cutaneous DM, calcinosis and ILD characterized by a good functional outcome. SRC and malignancies do not seem to be part of this syndrome.

Using a cohort study of SSc patients who were seen at Stanford between 2004 and 2018 and had undergone esophagogastroduodenoscopy (EGD), the clinical features between those with and without GAVE were compared, based on a multivariable logistic regression to identify clinical correlates with GAVE [8]. A total of 225 patients with SSc who underwent EGD were included in this study and 19 (8.4%) had GAVE. Those with GAVE were more likely to have scleroderma renal crisis (SRC) (21% vs 3%; $p<0.01$), positive anti-RNA polymerase III antibody (71% vs 19%; $p<0.01$), nucleolar pattern of anti-nuclear antibody (ANA) (33% vs 11%; $p=0.04$), and negative ANA (<1:80 by immunofluorescence) (33% vs 11%; $p=0.02$). On multivariate analysis with multiple imputation, anti-RNA polymerase III positivity (OR 4.57; 95% CI (1.57 – 13.23), $p<0.01$) and ANA negativity (OR 3.75; 95% CI (1.21 – 11.62), $p=0.02$) remained significantly associated with GAVE.

In conclusion was mentioned that positive anti-RNA polymerase III antibody and ANA negativity were significantly associated with GAVE. Further studies are necessary to de-

termine whether patients with these autoantibody profiles should undergo screening endoscopies for GAVE.

Autoantibodies against proteins of the telomeres – the protective caps of chromosomes and a marker of lifespan - were found in scleroderma patients with lung disease and shorter telomeres, a new study reveals [9]. The findings suggest these autoantibodies could serve as a novel biomarker for scleroderma with lung disease. Telomeres are coverings on the tips of chromosomes that, as cells age, become shorter and work as a kind of „molecular clock".

Previous studies have reported that people with scleroderma, or systemic sclerosis (SSc), who have shorter telomeres in white blood cells (lymphocytes) are at higher risk for interstitial lung disease (ILD). ILD is an umbrella term for a group of lung disorders characterized by inflammation and scarring (fibrosis) of the lungs. It is a frequent complication of scleroderma.

Researchers at the Johns Hopkins University in Baltimore, Maryland, Cedars-Sinai Medical Center in Los Angeles, California, and the University of California in San Francisco (UCSF), tested whether scleroderma patients with shorter telomeres carry autoantibodies against the telomerase and shelterin proteins of telomeres.

Two groups of patients, one from Johns Hopkins and the other from UCSF Scleroderma Centers, were included in the study.

To test for autoantibodies against telomerase, the researchers analyzed blood samples from 200 patients of the Johns Hopkins group, and 30 healthy individuals who served as controls. The analysis revealed that six patients (3%) were positive for these autoantibodies, while all the controls were negative. In addition, seven patients had autoantibodies against telomerase or one of the six shelterin proteins. Again, no autoantibodies were found in the healthy individuals. TERF1 was the most targeted shelterin, with 22 patients (11%) testing positive for such autoantibodies. In the UCSF group, 18 of 242 patients (7.4%) also had autoantibodies against TERF1.

Combining the two groups, a total of 40 patients (9%) tested positive for anti-TERF1 antibodies, in contrast to only 1.3% in 78 controls. To assess whether anti-TERF1 autoantibodies were exclusive of scleroderma, they measured their levels in 60 patients with rheumatoid arthritis and 60 with myositis (inflammation of the muscles). The analysis showed that only one patient with rheumatoid arthritis and one with myositis (1.7% each) were positive for anti-TERF1 autoantibodies, a rate similar to that seen in healthy individuals.

To assess whether the presence of the autoantibodies was linked with shorter telomeres, the researchers then measured the telomeres' length in white blood cells from the UCSF group.

Results showed that compared to patients without autoantibodies, the telomeres were shorter than expected for their age range in significantly more patients with anti-TERF1 autoantibodies – 78% vs. 43%.

Another method for quantifying telomere length (Flow-FISH), which is more accurate and sensitive, according to the researchers, confirmed the link between anti-TERF1 autoantibodies and shorter telomeres.

Patients with anti-TERF1 autoantibodies were generally younger than those negative for these antibodies (mean age 52.6 vs. 56.4). The presence of autoantibodies was linked with a history of severe lung disease and worse lung function, as shown by the lower percent predicted diffusion capacity (DLCO), 58.0 vs. 67.9. DLCO measures how much oxygen is transferred from the lungs into the bloodstream.

Also, anti-TERF1 autoantibodies were associated with a greater risk for severe muscle disease (three times higher) and inflammatory arthritis (about two times higher).

Finally, the researchers screened 152 patients with idiopathic pulmonary fibrosis (IPF), an inflammatory lung disease also linked with shorter telomeres, for anti-TERF1 autoantibodies. They found that 11 patients were positive (7.2%), which suggests that telomere-targeting antibodies might underlie lung disease.

"We describe a novel subgroup of patients with SSc and IPF with autoantibodies targeting the telomerase/shelterin complex that in SSc is associated with short telomeres in peripheral lymphocytes and the presence of lung disease," the researchers wrote. "These autoantibodies could serve as novel biomarkers for systemic sclerosis and specifically for systemic sclerosis lung disease," they concluded.

One of the important questions around SSc is whether there exists a dependency of clinical features of systemic sclerosis (SSc) patients negative for SSc related autoantibodies (autoAbs).

In a single-center retrospective study [10] of 546 SSc patients, 4.8% were negative for ANA and 5.3% were ANA positive but negative for SSc related autoAbs. Regarding clinical features, patients negative for ANA/SSc related autoAbs (n=55) had a significantly shorter disease duration, higher proportion of the diffuse type, contracture of phalanges, diffuse pigmentation, higher modified Rodnan total skin thickness score (mRSS), and lower incidence of telangiectasia than those with ACA (n=224). On the other hand, younger disease onset, lower mRSS, and lower incidence of scleroderma renal crisis were observed in patients negative for ANA/SSc related autoAbs than in those with antiRNAP Abs (n=52). Although pitting scars were less common in patients negative for ANA/SSc related autoAbs than in those with anti-topo I Abs (n=144), their clinical features were similar. Probably, patients negative for ANA/SSc related autoAbs form a clinically distinct subset among SSc patients.

Other study have shown that osteopontin links myeloid activation and disease progression in systemic sclerosis [11]. This study highlights that osteopontin is increased in lung tissue from patients with SSc and that lung macrophages are the main source of osteopontin in SSc-associated interstitial lung disease (SSc-ILD). The authors demonstrate that serum osteopontin is increased in SSc and that higher levels of osteopontin are associated with disease progression. From a pathogenic viewpoint, this study demonstrates that osteopontin secretion by monocytes/macrophages is induced by immune complexes in an IL-6- and M-CSF-de-

pendent manner. In patients with SSc, blockade of the IL-6 receptor by tocilizumab reduces the levels of circulating osteopontin. These results suggest that macrophagic osteopontin could be a key biomarker participating in the progression of SSc ILD.

Higher-than-normal blood levels of squamous cell carcinoma antigen 1 (SCCA1), a pro-fibrotic protein, are associated with a greater risk of interstitial lung disease (ILD) among people with systemic sclerosis (SSc-ILD), a study suggests [12]. This finding points to SCCA1 blood levels as a potential biomarker for early detection of SSc-ILD, and in helping to identify scleroderma patients with a low risk of ILD. In addition, SSCA1 could be a potential therapeutic target in SSc-ILD.

ILD is a group of disorders characterized by inflammation and scarring in tissue in and around the pulmonary air sacs, hampering the lungs' ability to transfer oxygen to the bloodstream. While it is well established that SSc patients are at high risk of developing ILDs, its diagnosis remains challenging due to the potential lack of symptoms in early stages of the disease.

Increasing efforts are focused on identifying potential biomarkers of early lung involvement in SSc patients, but few have been clinically confirmed so far.SCCA1, also known as SerpinB3, is involved in inflammatory diseases and cancer. It can be detected in the blood bound to another protein called IgM (SCCA-IgM).

Higher-than-normal levels of SCCA1 are found in the lungs of people with idiopathic pulmonary fibrosis (IPF), the most common ILD, and SCCA1 levels have been associated with the extent of fibrosis and fibrotic-associated processes in IPF patients and in people with chronic liver disease. However, whether SSCA1 plays a role in lung involvement among those with SSc remains unclear.

Researchers in Italy set out to evaluate a potential link between SCCA-IgM blood levels and clinical features of SSc patients. They analyzed demographic and clinical data on 97 SSc patients (82 women and 15 men) followed at a single tertiary center. Patients had a mean age of 55.4 and had lived with the disease for a median of 12 years. Most (63.9%) were classified as having limited scleroderma, and 36.1% had diffuse scleroderma. Pulmonary involvement was measured by high resolution computed tomography (HRCT) scans and lung function tests. Heart, gastrointestinal, and skin changes were also assessed. A cut-off value for blood levels of SCCA-IgM (higher than 200 AU/ml) was determined, based on measures made using 100 healthy people.

Results showed that 41 patients (42.3%) had ILD, which was significantly linked to diffuse SSc, severe skin involvement, poorer lung function, and the presence of autoantibodies specific for scleroderma – antibodies that wrongly attack healthy cells. Median levels of SCCA-IgM were significantly higher in patients with ILD than those without (218 vs. 87.5 AU/ml).

When comparing patients under and above the cut-off value for SCCA-IgM, the team found that significantly more patients with higher SCCA-IgM levels had ILD and a lower total lung capacity – the volume of air in the lungs after a maximum inhalation. No differences in any other clinical features were found between the two groups.

Further analyses significantly associated higher SCCA-IgM blood levels with an ILD diagnosis, increasing by 10 times the risk of developing ILD.

Interestingly, patients with a more recent ILD diagnosis (less than three years ago) had significantly higher levels of SCCA-IgM than those living with the disease for a longer period. Researchers suggested this associated with the transition from an inflammatory to a fibrotic phase in the lungs of SSc patients, in which SSCA1 may have a role.

The presence of low SCCA-IgM levels in SSc patients with normal values of total lung capacity also helped to identify those with a very low risk of ILD.

„SSCA-IgM is associated with interstitial lung disease in scleroderma patients and might be used in the assessment of SSc-ILD," the researchers wrote.

High levels of a protein known as CTRP9 appear to indicate worsening lung function in patients with systemic scleroderma (SSc), according to a recent study. The discovery suggests that CTRP9 may be a useful biomarker for the loss of lung function in SSc [13].

Such biomarkers are sorely needed, researchers said, given that diseases that cause scarring of lung tissue – known as interstitial lung diseases or ILD – are linked to poor outcomes for people with SSc.

While interstitial lung disease (ILD) is the main cause of morbidity and mortality in systemic sclerosis (SSc), there is still a lack of predictive markers to assess disease progression, the findings suggesting that CTRP9 may be a potential biomarker in SSc-associated ILD.

Past research had demonstrated that fat metabolism is disrupted over the course of SSc. Moreover, the cell-signaling molecule CTRP9, made within fat cells, is associated with pulmonary complications in that disorder.

In a recent work, researchers from across the United States teamed up to investigate this relationship further, looking at how changes in CTRP9 levels correlated with changes in pulmonary function in SSc patients.

Overall, they found high levels of CTRP9 associated with declining pulmonary function. Meanwhile, low CTRP9 protein levels corresponded to a more stable disease course over time.

These results make the protein a potential disease biomarker, the team said. To arrive at this conclusion, they reviewed the records of 110 individuals included in the Northwestern Scleroderma Patient Registry and Biorepository, led by Northwestern University, in Illinois. Among the cases reviewed, 61 involved patients with limited cutaneous SSc, and 49 pertained to those with diffuse cutaneous SSc. Most patients (70) had four years' worth (48 months) of pulmonary function tests, taken at 12-month intervals. Mean disease duration was 9.7 years.

Patients with more CTRP9 in their bloodstream (over 81.1 nanograms/ml) generally had significantly worse lung function at the study's start, and at 48 months, as measured

by forced vital capacity (FVC) and DLCO – standard measures of how much air a person can exhale after a forced breath, and of predicting the lungs' capacity to transfer oxygen to blood cells.

Additionally, high CTRP9 levels were associated with greater numbers of monocytes, cells known to contribute to lung fibrosis, or scarring. Low CTRP9 was associated with a decrease in monocytes, even after accounting for disease duration and treatment status. Notably, the data showed that patients with stable disease – those whose FVC changed by less than 3% over the study period – tended to have low levels of CTRP9.

Although CTRP9 levels did not appear to predict SSc progression so much as changes in lung function, the researchers suggested that the possibility cannot yet be ruled out.

"Development and progression of ILD in SSc is often early with a steep progression early on in disease", they wrote, adding that because the patients in this study had an average disease duration of about nine years, they may have already reached the „plateau" phase of their lung disease.

Other factors, such as the study's sample size and variability regarding disease duration and treatment status cannot be excluded, the investigators added. Future studies, they mentioned, should examine CTRP9's prognostic value in detail.

The group, involving researchers from the University of California San Francisco, the Yale School of Medicine, in Connecticut, the University of Michigan, and the University of Rochester Medical Center, in New York, in addition to Northwestern, now plans to study *CTRP9* gene variants, to determine how the gene's expression, or activity, might be altered in SSc with interstitial lung disease.

Their current findings, they concluded, „support a novel role for CTRP9 as a prognostic biomarker, and potentially a therapeutic target for SSc-associated lung disease."

Levels of a subtype of immune B-cells in the blood are increased in some people with scleroderma and associated with lower respiratory function, higher blood pressure in the lungs, and more kidney damage, a study reports [14].

Both inflammation and vascular complications in systemic sclerosis patients are influenced by B-cells, a part of the body's adaptive immune system. B-cells play a critical role in autoimmune disorders by producing both antibodies that target the body's own tissues, called autoantibodies, and signaling molecules called cytokines, which promote inflammation and fibrosis, or scarring.

A recent study found that B-cells with lower levels of a cell surface protein called CD21 were more prevalent in those with scleroderma. These cells, known as CD21-low cells, also described in people with systemic lupus erythematosus and other disorders, appear unreactive (anergic) to substances that would normally induce an immune response and are found in inflamed tissues.

This prompted researchers at Sapienza University in Rome to see whether the cells could be used to predict vascular complications in scleroderma. The team analyzed 74 scleroderma patients – 40 with limited scleroderma and 34 with diffuse disease, mean age 54.5 – and 20 healthy people (controls). Overall, the percentage of B-cells with reduced levels of CD21 was higher in people with scleroderma than in the healthy controls. Yet only one third of the patients showed increased percentages of such CD21-low B cells, suggesting that these may represent a particular subtype of scleroderma cases, the researchers said. These cells were more prone to a process called apoptosis, which refers to „programmed" cell death – as opposed to death caused by injury. Apoptosis, which involves the genetically determined elimination of cells, is a factor in many neurodegenerative diseases.

Greater proportions of CD21-low cells in people with scleroderma were associated with higher systolic pulmonary arterial pressure (sPAP), lower carbon monoxide diffusing capacity (DLCO) – a measure of respiratory function – and lower levels of vascular endothelial growth factor, which is essential for the formation of blood vessels.

Those with more CD21-low cells had stiffer renal arteries, compared with the other participants. Renal arterial stiffness is predictive of kidney damage, which is known to occur in scleroderma cases. Next to the to the alteration of B cell subpopulations, the results point towards a potential role of CD21-low B cells in the pathogenesis (disease progression) of some vascular manifestations of the disease.

No difference was noted between patients who developed digital ulcers and those who did not, suggesting that CD21-low B-cells might only operate on deeper, or visceral, tissue. It can be assumed that CD21-low have a selective homing for internal organs, however additional research is required to evaluate these assumptions.

Herein, were investigated how levels of serum metabolites correlated with different stages of SSc and SSc-ILD [15]. Serum samples of patients with SSc without ILD, stable and progressive SSc-ILD as well as of healthy controls (HC) were analyzed using liquid targeted tandem mass spectrometry. The best discriminating profile consisted of 4 amino acids (AA) and 3 purine metabolites. L-tyrosine, L-tryptophan, and 1-methyl-adenosine distinguished HC from SSc patients. L-leucine, L-isoleucine, xanthosine, and adenosine monophosphate differentiated between progressing and stable SSc-ILD. In SSc-ILD, both, L-leucine and xanthosine negatively correlated with changes in FVC% predicted.

Additionally, xanthosine was negatively correlated with changes in DLCO% predicted and positively with the prognostic GAP index. Validation of L-leucine and L-isoleucine by an enzymatic assay confirmed both the sub-stratification of SSc-ILD patients and correlation with lung function and prognosis score.

Serum metabolites may have potential as biomarkers for discriminating SSc patients based on the presence and severity of ILD. Confirmation in larger cohorts will be needed to appreciate their value for routine clinical care.

Skin biopsy samples from patients with systemic sclerosis (SSc) may help to identify potential biomarkers and pathways implicated in the disease pathogenesis, according

to study results published in *Arthritis Research and Therapy* [16].

In this study, the researchers used a high-throughput mass spectrometry technique to analyze samples from affected and unaffected areas of the skin from 7 patients (5 women). Proteins were extracted from the cryopulverized samples and analyzed to reveal differentially expressed proteins, which led to the identification of 2149 proteins. The samples were compared, revealing that 169 of the proteins were significantly differentially expressed in the affected vs unaffected tissues. Further analysis performed on these proteins identified many involved pathways that were associated with SSc pathogenesis including platelet activation and extracellular matrix (ECM)-receptor interaction. After these analyses, 15 proteins were selected for validation through affected/unaffected comparison, of which 5 were confirmed to be significantly differentially expressed in SSc-affected vs -unaffected skin biopsies.

Based on the results, the researchers confirmed that ECM proteins are a key part of SSc development. Three proteins that interacted with ECM receptors were overexpressed in the comparison analysis, which were previously suspected to be part of SSc pathogenesis, could serve as potential biomarkers for the disease.

Additional studies could include data from new patients with SSc to confirm the use of these proteins as biomarkers; these molecules isolated from easily accessible tissue may also provide an easier way for clinicians to diagnose patients and prevent painful procedures.

Endothelial damage and fibro-proliferative vasculopathy of small vessels are pathological hallmarks of systemic sclerosis (SSc). The consequence is the detachment of resident elements that become circulating endothelial cells (CECs). The aim of a study dedicated to this topic was to evaluate the potential of CECs as biomarker in SSc [17].

The study enrolled 50 patients with limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) subset of SSc, who underwent clinical evaluation to establish the organ involvement. CECs were measured by flow-cytometry utilizing a polychromatic panel.

An evident difference was observed in CEC counts comparing controls to SSc patients (median 10.5 vs. 152 cells/ml, $p < 0.0001$) and for the first time, between the two subsets of disease (median lcSSc 132 vs. dcSSc 716 CEC/ml, $p < 0.0001$). A significant correlation was established between CECs and some SSc clinical parameters, such as digital ulcers, skin, and pulmonary involvement, presence of Scl-70 antibodies, nailfold videocapillaroscopy patterns and EUSTAR activity index. After 12 months, CECs correlated with clinical worsening of patients, showing that a number higher than 414 CEC/ml is a strong negative prognostic factor (RR5.70).

The results indicate that CECs are a direct indicator of systemic vascular damage. Therefore, they can be used as a reliable marker of disease severity.

Levels of the protein, galectin-3, are associated with heart involvement in people with systemic sclerosis (SSc), according to a recent pilot study [18]. The protein could be used as a biomarker to detect and address cardiac problems more quickly. Another protein, called soluble suppression of tumorigenicity-2 (sST2), was also evaluated, but did not show any relationship to heart involvement. The study results imply that galectin-3 may be a useful and simple biomarker for the screening and early identification of SSc patients with cardiac involvement, while the usage of sST2 for the same purpose was not supported by the data.

SSc is marked by too much collagen, a protein found in connective tissue that helps to repair wounds, being produced, resulting in excessive scarring, or fibrosis, of the skin and internal organs.

Cardiac fibrosis can lead to heart dysfunction and poorer outcomes, or prognosis, so it is important to identify patients at risk for heart complications.

"Cardiac involvement implies poor prognosis in SSc, thus its early, biomarker-based screening would be crucial", the team wrote.

To determine usefulness of such biomarkers, the researchers evaluated 36 women and 4 men with SSc being treated at a University of Pécs center in Hungary. The patients' median age was 57.3. Levels of the two fibrosis-related proteins were measured before an echocardiogram and lung function tests were performed. Galectin-3 promotes fibrosis and mediates collagen production, while sST2 blocks interleukin-33, a protein that protects against fibrosis.

These proteins were chosen because previous studies showed they correlated with heart involvement and could predict mortality in heart failure patients. Galectin-3 has also been proposed as a blood biomarker of scleroderma.

Results from the lung tests showed that higher galectin-3 levels correlated with worse lung function.

Specifically, people with more galectin-3 had lower measures in forced expiratory volume (how much air can be exhaled in one breath), and diffusing capacity of carbon monoxide (how much oxygen travels from the lungs to the bloodstream).

An echocardiogram revealed that galectin-3 was also associated with several indicators of heart dysfunction.

Higher galectin-3 levels correlated with both left ventricle systolic dysfunction, or the heart's inability to contract appropriately during heartbeats, and diastolic dysfunction, the heart's inability to relax after a heartbeat.

High levels of the protein were also seen with mitral valve regurgitation, which occurs when the heart's mitral valve, responsible for keeping blood flowing in the right direction, doesn't fully close, allowing blood to travel backwards. Galectin-3 levels also correlated with disease duration, results showed.

In contrast, sST2 was not correlated with any clinical, lung, or echocardiographic measurements, a contrast to previous studies in heart failure patients.

Based on the results, the team concluded that Galectin-3 may be a useful biomarker for the screening and early diagnosis of SSc patients with cardiac involvement. However, compared to earlier research, the team was unable to show

any relationship between sST2 levels and the clinical characteristics of the disease or the echocardiographic markers of the [heart] mechanics in SSc patients.

Researchers noted that the study lacked a healthy control group, meaning they couldn't define normal galectin-3 or sST2 blood levels. Another study limitation was that, while galectin-3 and sST2 indirectly suggest the presence of cardiac fibrosis, they did not prove that fibrosis was present in these patients. "Circulating biomarker levels require careful interpretation in relation to [cardiac] involvement", the researchers wrote.

High levels of a form of the CD146 protein, normally found on endothelial cells – those that line blood vessel walls – may be a potential biomarker in identifying people with systemic sclerosis (SSc), a study suggests [19].Certain forms of the protein are implicated in lung scarring (pulmonary fibrosis) and in controlling skin thickness, having potential as therapeutic targets.

CD146 is involved in the formation of new blood vessels – a process called angiogenesis – inflammation, and controlling vascular permeability, or the capacity of certain cells and molecules to cross the vessel's wall. While the two major forms of CD146 are located in the cell's membrane, the protein also can exist in a soluble form in the blood.

Recent research suggests that measuring the levels of this soluble form of CD146, known as sCD146, could serve as a biomarker of disease activity in SSc, also known as scleroderma. Moreover, data from an animal model of SSc showed that sCD146 injections reduced disease severity. However, little is known about the mechanisms regulating sCD146 formation and whether different variants of the protein exist and play a role in SSc.

To answer this, an international team led by researchers at Aix-Marseille University, in France, conducted lab (*in vitro*) tests as well experiments in animal models of SSc. The lab tests revealed that two forms of CD146 – a short and a longer form, both present at the membrane of endothelial cells – can generate a shed form of the soluble protein. Enzymes called metalloproteinases cleaved the membrane proteins. This process represented about 75% of the sCD146 production. The remaining 25% of the soluble protein was not dependent on the action of metalloproteinases. The researchers went on to identify two new forms of sCD146 – called I5-13-sCD146 and I10-sCD146 – that arose as a result of alternative RNA splicing, a process that allows a single gene to produce a number of different messenger RNA (mRNA) sequences and as a consequence, different proteins. Of note, mRNAs are the molecules that carry genetic information to the sites where proteins are produced. The levels of sCD146 variants in blood samples from 117 SSc patients were then measured and compared with those of 81 participants serving as controls. Results showed that the sCD146 form that resulted from cleavage of the membrane protein and the splice variant I5-13-sCD146 had significantly higher levels in the blood of patients with SSc. The splice variant was particularly high in people with pulmonary fibrosis when compared with patients without lung disease. In contrast, levels of I10-sCD146 were decreased in SSc samples, as were those of cleaved sCD146 in participants with pulmonary hypertension, a disease associated with high blood pressure in the blood vessels that supply the lungs. The scientists then tested the effects of the different sCD146 forms when injected under the skin (subcutaneously) in a mouse model of SSc.

Compared with control animals, sCD146 and I10-sCD146 reduced the thickness of the dermis, the thicker layer of the skin. Later experiments linked this effect with CD146's role as a potentiator of blood vessels formation. No such effects were seen with I5-13-sCD146. Instead, lab tests suggest that it plays a role in promoting fibrosis, which is in agreement with its levels being particularly high among SSc patients with pulmonary fibrosis.

Overall, these findings suggest that "variants of sCD146, and in particular the novel I5-13-sCD146 splice variant, could thus constitute novel biomarkers and/or molecular targets for the diagnosis and treatment of SSc, but also of other angiogenesis – or fibrosis-related pathologies [diseases]", the investigators concluded.

Blood levels of the protein endocan may be a potential biomarker in identifying people with scleroderma who are at risk for pulmonary arterial hypertension (PAH), a study reported [20].

Scleroderma, or systemic sclerosis (SSc), can lead to fibrosis (scarring) in multiple organs, inflammation, and damage to blood vessels. In some patients, these changes can cause a rare disorder known as PAH, in which the blood pressure in the arteries of the lungs is higher than normal.

Although many studies have investigated PAH in scleroderma patients, biomarkers helping to establish a PAH risk are lacking. Proangiogenic hematopoietic cells (PHCs) that form in the bone marrow are a type of stem cell that circulates in the bloodstream. These cells can become heart muscle cells and smooth muscle cells, as well as endothelial cells - those that line blood vessel walls and endothelial precursor cells. However, their role in scleroderma remains unclear.

Endocan, a protein expressed by endothelial cells, has been suggested as a marker of blood vessel (vascular) stress. Additionally, vitamin D and several pro-inflammatory molecules are known to modulate the interaction between bone marrow-derived cells and changes in endothelial cell function.

„Taken together, it may be conceived that PHCs and endocan could be involved in the development of PAH, but their interrelationships have not been investigated so far in SSc," the researchers wrote. A team from Italy investigated the association among PHC cells, endocan, vitamin D levels, inflammatory markers, and clinical parameters in 36 women with scleroderma and PAH. Enrolled patients had a mean age of 64.1, and a mean disease duration of 78.3 months (about six and a half years). Twelve participants had limited SSc (33.3%), while 24 (66.6%) had diffuse SSc, a more severe disease form. Also included in the study were 36 age-matched healthy women as a control group. Blood samples

were collected, and each patient underwent a complete clinical assessment, including an echocardiogram to establish cardiac health.

No differences in the numbers of progenitor cells were evident between patients and controls, but endocan levels were significantly higher in those with scleroderma, 365.6 nanograms per mL (ng/mL), relative to controls, 280.4 ng/mL.

Vitamin D levels were also higher in patients compared with controls (27.34 ng/mL vs. 22.47 ng/mL). Among the patients, 12 had vitamin D levels under 20 ng/mL, nine had levels between 20 and 30 ng/mL, and 16 had normal levels (greater than 30 ng/mL).

Pro-inflammatory markers like C-reactive protein, erythrocyte (red blood cell) sedimentation rate, and fibrinogen were also all higher in women with scleroderma than in those without this disease.

No difference was seen regarding endocan, vitamin D levels, and progenitor cell numbers between women with diffuse SSc and those with limited SSc.

In patients, results showed a significant correlation between low numbers of progenitor cells and high endocan protein levels. Elevated endocan levels also correlated with higher pulmonary arterial blood pressure (sPAP). A low progenitor cell count correlated with higher sPAP and fibrinogen, as well as low vitamin D. Higher sPAP linked with elevated fibrinogen.

A first statistical analysis evaluated each variable with progenitor cell numbers. Here, a low cell count was associated with higher endocan, fibrinogen, and sPAP. A subsequent analysis found that endocan and fibrinogen were potential predictors of progenitor cell numbers.

A second analysis also assessed parameters such as skin thickness. It ultimately showed that endocan level was the only potential predictor of elevated pulmonary arterial blood pressure.

„Our study seems to enforce the findings on the potential role of endocan as a biomarker of vascular health in SSc," the scientists wrote. „The relationships between endocan and other angiogenesis [blood vessel formation] biomarkers should be evaluated by future prospective studies in order to investigate its ability in predicting vascular involvement, including PAH."

The amount of *free light chain* (FLC) molecules in blood and urine correspond to the severity of scleroderma, adding to the evidence supporting these molecules as biomarkers for early diagnosis and disease activity.

Free light chain molecules are made during the production of antibodies and are considered to be biomarkers for B-cell activity. B-cells, a part of the immune system, tend to become self-reactive in scleroderma, or systemic sclerosis (SSc), meaning that they target the body's own tissues, causing inflammation.

Activated B-cells produce antibodies – molecules that „flag" cells and other biological molecules for the immune response – called immunoglobulins. Immunoglobulins are made of light and heavy chains (two of each). The two light chains on each immunoglobulin will both be of a certain variant, called kappa and lambda.

Free light chains are the excess light chains made by activated B-cells and released into the blood. Here, they tend to be rapidly removed from the body by the kidneys, potentially making them direct biomarkers of B-cell activity.

Despite their association with B-cells and reports that they associate with scleroderma activity and severity, as well as with scleroderma-related interstitial lung disease, few studies have evaluated their levels in this disease, and none has examined their presence in urine.

To address this knowledge gap, researchers in Italy analyzed FLC levels in the blood and urine of 72 adult SSc patients (median age, 55) and 30 healthy controls, seeking to correlate these levels with disease severity and activity [21].

People with scleroderma showed significantly higher amounts of both kappa and lambda FLCs in their blood than did the control participants, with kappa FLC levels generally higher than those of lambda.

Likewise, urine levels of kappa FLCs were significantly higher in patients than in healthy controls, but no such significant difference between the two groups was seen in lambda FLCs. Greater levels of both FLCs tended to correspond to higher measures of inflammation, as assayed by C-reactive protein levels, and a faster erythrocyte sedimentation rate – a test that measures how fast red blood cells settle at the bottom of a test tube. They also correlated with more disease activity and severity, as measured using the disease activity index (DAI) and the disease severity scale (DSS).

DAI scores rose with urinary kappa FLCs, as SSc patients with more of these had statistically higher DAI scores than those whose kappa FLC levels remained below 15.1 mg/L.

The authors suggest the usage of FLCs as a reliable and useful potential biomarker of early diagnosis and to follow disease activity, allowing for the improvement of SSC patients clinical management. Study limitations mentioned by the investigators included its relatively small sample size and the fact that enrolled patients had a long disease duration (median of 11 years).

Low levels of the *galectin-10 protein* (Gal-10) in scleroderma patients associate with inflammation and vascular changes in the lungs, leading to pulmonary arterial hypertension (PAH).

This finding, from a recent study, provides the first evidence that GAL-10 may play a role in scleroderma [21].

Galectins are a subfamily of proteins involved in regulating numerous biological processes, including inflammation and blood vessel remodeling, both of which occur in scleroderma (also known as systemic sclerosis, SSc). Four galectins – galectin-1, galectin-3, galectin-7, and galectin-9 – have been investigated in scleroderma [22, 23, 24].

Gal-10 is mainly found in eosinophils, basophils, and regulatory T-cells (Tregs) – three types of immune cells involved in balancing the inflammatory response. Tregs, for instance, regulate the workings of T-effector cells and suppress the production of inflammatory molecules. A subtype

of eosinophils helps to provide that check, limiting T-effector cells' ability to proliferate by transferring Gal-10.

Based on these and other observations, researchers with the University of Tokyo's Graduate School of Medicine explored relationships between Gal-10 levels in blood serum and scleroderma [25].

The investigators recruited 38 patients with diffuse cutaneous SSc (dcSSc), 30 with limited cutaneous SSc (lcSSc), and 20 healthy people as controls to their study. Gal-10 levels in the blood were significantly lower in all SSc patients than in controls – a median of 20.3 nanograms (ng)/mL vs. 31.2 ng/mL. The effect was greatest in those with dcSSc. These patients averaged 19.3 ng/mL of Gal-10, compared with 23.9 ng/mL among people with lcSSc.

Because of the different degrees of skin fibrosis (scarring) involved in these two SSc subtypes, this suggests that lower levels of Gal-10 might associate with more severe skin sclerosis and the development of scleroderma. The researchers identified a moderate, but significant correlation between serum Gal-10 levels and modified Rodnan total skin thickness score (mRSS) values in total SSc patients. (mRSS scores measure skin thickness in scleroderma as a way of determining disease severity).

They also found that low Gal-10 levels are significantly associated with a lower percent diffusion lung capacity for carbon monoxide, which measures how well the lungs can transfer oxygen from their air sacs to the blood, and with higher right ventricular systolic pressure (RVSP), a measure of the pressure inside the artery that supplies blood to the lungs.

These findings suggested that lower Gal-10 levels are linked to the changes in pulmonary blood vessels that can lead to PAH. Decreasing Gal-10 also corresponded to higher overall white blood cell counts – a measure of immune response – C-reactive protein (CRP) levels, and a faster erythrocyte sedimentation rate, the last two being measures of inflammation. To the researchers, this indicated that lower Gal-10 levels correspond to an impaired ability to regulate inflammation.

In support of this potential association, SSc patients with joint pain, a symptom of active inflammation, had significantly lower Gal-10 levels than those without such pain. Statistical analysis also independently linked CRP and RVS with low Gal-10, reinforcing the idea of a relationship to systemic inflammation and pulmonary vascular changes leading to PAH.

A limitation for this study is related to missing data on the sources of Gal-10, however this still allows to examine the association and role of Gal-10 and SSc. This report being the first investigating the role of Gal-10in SSc, further research can potentially explore the connection further.

Conclusions

1. Specific autoantibodies for some autoimmune diseases could be a relevant biomarker related to fibrotic skin, lung disease, joint and muscle involvement, digestive complication in the systemic sclerosis patients.
2. Appearance and severity of interstitial lung disease in the systemic sclerosis patients could be predicted using the level of CTRP9 protein, osteopontin, serum metabolites such L-tyrosine, L-tryptophan, 1-methyl-adenosine, L-leucine, L-isoleucine, xanthosine, and adenosine monophosphate, presence of antibodies against telomers.
3. Indicators of systemic vascular damage in the systemic sclerosis patients could be considered circulating endothelial cells, subset of immune B-cells, galectin-10 protein and endocan, with the latter two found to be associated with increased risk for pulmonary arterial hypertension.
4. Levels of the protein, galectin-3, are associated with heart involvement in people with systemic sclerosis.
5. Related to early diagnosis of systemic sclerosis, high levels of a form of the CD146 protein may be a potential biomarker in identifying people with systemic sclerosis and FLCs could be employed as a reliable and useful potential biomarker of early diagnosis and for following disease activity.

Declaration of conflict of interest

Nothing to declare

Authors' contribution

All authors contributed equally to the research, data analysis, and writing of the manuscript. Final manuscript was read and approved by all authors

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