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Biomarkers in systemic sclerosis

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Abstract

Purpose of review—To discuss recent advances in identification of biomarkers in systemic sclerosis for disease severity, prognosis, and treatment response.

Recent findings—Recent reports describe novel circulating markers of disease severity, autoantibody associations with specific manifestations including cancer, and skin gene expression-based predictors of modified Rodnan skin score progression and treatment response. Moreover, there is converging evidence that C-reactive protein and pneumoproteins such as Krebs von den Lungen-6 and chemokine ligand 18 could serve as prognostic biomarkers in systemic sclerosis-associated interstitial lung disease.

Summary—Several novel biomarkers show promise in improving the assessment of systemic sclerosis (SSc) disease severity, prognosis, and treatment response. Their potential utility in prospective selection of patients for clinical trials and in individual patient management require additional research.

Keywords

biomarkers; scleroderma; systemic sclerosis

INTRODUCTION

Heterogeneity is one of the hallmarks of systemic sclerosis (SSc, scleroderma). Reliable measures of disease activity as well as predictors of disease progression and treatment response are important for patient selection in clinical trials and to optimize individual patient outcomes. In this regard, clinical features such as diffuse vs. limited cutaneous involvement, progressive skin fibrosis, tendon friction rubs, and pulmonary function test trends are useful in estimating overall prognosis [1–5]. Specific SSc-associated autoantibodies, some of which were incorporated into the 2013 ACR/EULAR classification criteria for SSc [6], have also demonstrated prognostic value, particularly regarding organ involvement and malignancy (reviewed in [7,8]). The traditional biomarker C-reactive

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Conflicts of interest

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protein (CRP) may have a role in assessment of SSc disease activity and prediction of interstitial lung disease (ILD) progression (discussed more below), although the roles of CRP in clinical trial enrollment and patient management remain incompletely defined. Numerous other circulating factors (including proteins and microRNAs), as well as transcriptomic data from blood and skin biopsy specimens, have been characterized with reference to SSc disease manifestations, severity, prognosis, and treatment response in recent years (reviewed in [9–11]), and thus biomarker development in SSc is rapidly evolving. In this review we discuss advances in SSc biomarker identification and characterization from early-2018 to mid-2019. We focus particularly on biomarkers for monitoring disease severity (correlation with clinical evidence of fibrosis or end-organ damage), prognosis (predicting the course of a clinical manifestation over time), or response to treatment (predictive biomarkers).

CIRCULATING BIOMARKERS ASSOCIATED WITH DISEASE SEVERITY OR SPECIFIC MANIFESTATIONS

Sonic hedgehog (SHH), previously shown to have a profibrotic effect in skin [12], was measured in serum samples from 154 SSc patients (80 limited, 74 diffuse) from eight European centers and 68 matched controls, then analyzed with reference to clinical disease features [13]. SHH levels were significantly elevated in SSc patients compared with controls, and associated positively with modified Rodnan skin score (mRSS), digital ulcers, and elevated pulmonary arterial pressure (estimated by echo).

The enhanced liver fibrosis (ELF) score, consisting of three circulating markers originally validated as a biomarker for chronic liver disease, was previously shown to be elevated in a majority of SSc patients compared with healthy controls and to correlate positively with mRSS and overall disease severity and negatively with diffusion capacity of the lungs for carbon monoxide (DLCO) [14]. In a recent validation study including 247 SSc patients from six European centers, the overall ELF score again correlated positively with mRSS, disease activity and severity, and negatively with forced vital capacity (FVC) and DLCO [15]. In a multivariate analysis, increased age, increased mRSS, and decreased DLCO were independently associated with ELF score. These studies suggest a potential role of SHH and ELF measurement in monitoring skin and lung disease severity. Since these studies were cross-sectional, the predictive significance of these markers is unknown.

Antibodies against U11/U12 RNP (anti-RNPC3 antibodies) were found to be associated with an increased risk of severe gastrointestinal dysfunction (defined as requiring total parenteral nutrition) in SSc in a case–control study. This finding was tested in an independent validation cohort, in which patients with anti-RNPC3 antibodies were significantly more likely to have moderate–severe gastrointestinal dysfunction [16].

A study of cancer risk in 2383 SSc patients in comparison with a representative sample of non-SSc patients in the general US population revealed no significant increased risk of cancer in SSc overall, but an increased risk among patients with anti-RNA Pol III antibody. In addition, a decreased risk of cancer was observed in patients with limited SSc and anticentromere antibody patients [17▪]. Adding additional depth to the understanding of

specific autoantibodies and cancer risk, another recent report described the identification of antibodies against the large subunit of RNA Pol I (RPA194) and their association with decreased cancer risk [18▪]. Examining a subset of SSc patients with antibodies against RPC155, the large subunit of RNA Pol III, anti-RPA194 antibodies were found to be significantly more common in patients without cancer compared with anti-RPC155 antibody-positive, anti-RPA194 antibody-negative patients. Under-standing of these associations between specific autoantibodies and cancer risk could lead to their future use as biomarkers to inform decisions about cancer screening in SSc patients.

A study of antineutrophil cytoplasmic antibodies (ANCA's) in a large, multicenter Australian cohort showed a relatively high prevalence of ANCA positivity in SSc patients and an association with ILD and increased mortality [19]. Screening ANCA testing is routinely performed on patients in this cohort, and the investigators found that 8.9% of 1303 SSc patients tested were ANCA positive. A total of 11.2% of ANCA-positive patients were positive for anti-myeloperoxidase antibodies, 13.8% for anti-PR3 antibodies. Only three patients had evidence of ANCA-associated vasculitis during the follow-up period, but ANCA-positive patients had a higher prevalence of ILD, synovitis, pulmonary embolism, and features of overlap with other connective tissue diseases. ANCA-positive patients had significantly higher mortality than ANCA-negative patients after adjustment for age of SSc onset and sex; cause-specific mortality was not determined. Further research, including validation in other cohorts and determination of cause-specific mortality, would be needed to define a potential role of ANCA screening as a prognostic biomarker.

A summary of circulating biomarkers [excluding chemokine ligand 18 (CCL-18) and Krebs von den Lungen-6 (KL-6) which are discussed later] is shown in Table 1.

GENE EXPRESSION-BASED BIOMARKERS OF SKIN DISEASE PROGRESSION OR TREATMENT RESPONSE

The mRSS, a clinical estimate of overall skin fibrosis, is typically measured as part of the clinical assessment of SSc patients and has been a primary endpoint in clinical trials to treat diffuse cutaneous SSc (reviewed in [22]). However, prediction of mRSS progression remains quite challenging. Analyses of clinical trial results and observational cohorts have repeatedly demonstrated that a large percentage of diffuse SSc patients have stability or improvement in mRSS irrespective of targeted SSc treatment [20▪▪,23,24], highlighting a need for improved methods to predict progression. Two recent analyses of large, European multicenter observational cohorts each identified shorter disease duration and lower mRSS at baseline visit as predictors of subsequent mRSS increase [20▪▪,24]. The latter study also identified a unique mRSS progression profile associated with RNA polymerase III antibody, namely a higher and earlier peak in mRSS prior to improvement [20▪▪]. This suggests a potential prognostic role for this antibody in future mRSS-targeted clinical trials.

Skin gene expression profiling has shown potential in recent years for assessment disease activity, including changes over time (reviewed in [9,10]). In addition, associations have been observed between mRSS improvement during treatment and skin gene expression

profiles (reviewed in [10]). Here we discuss recent reports on gene expression-based predictors of disease progression and treatment response (summarized in Table 2).

Prognosis of skin disease based on skin gene expression

Analysis of skin gene expression from the placebo arm of the phase II study of Tocilizumab in diffuse SSc (faSScinate) [30] revealed multiple genes whose expression levels at baseline predicted change in mRSS at follow-up [25▪]. These observations were confirmed in a separate cohort of 20 diffuse SSc patients (some of whom were taking immunosuppressive therapy), although the correlation coefficients were generally smaller. Dividing the patients in the discovery cohort into three mRSS trajectory patterns (progressive, stable, or regressive), high expression of five genes (*CD14*, *IL13RA1*, *SERPINE1*, *OSMR*, and *CTGF*) was associated with a progressive, that is, worsening skin trajectory. The mRNA levels of these genes therefore showed potential as biomarkers to predict skin disease progression.

After applying a normalization method to reduce batch effects, differentially expressed genes in a compendium of eight previously generated, independent skin gene expression datasets (comprising 175 SSc patients and 61 healthy controls) were analyzed [26▪]. In one of these analyses, four subgroups of diffuse SSc patients were identified by nonnegative matrix factorization clustering, including a cluster enriched for inflammatory and immune cell signatures and another enriched for fibrosis signaling and a fibroblast signature. Examining skin gene expression and mRSS change from baseline to 24-week follow-up in the placebo arm of the faSScinate trial [25▪], patients in the inflammatory cluster had a significant improvement in mRSS from baseline to 24-week follow-up, suggesting that SSc skin with a prominent inflammatory gene expression profile has an improvement in skin score even without immunosuppressive treatment. Patients in the other clusters had variable mRSS progression. It should be noted that some of the patients' follow-up biopsies were in different gene expression-based clusters than their baseline biopsies, and that only four patients were analyzed for mRSS change in the inflammatory/immune cluster.

Predicting treatment responses based on skin gene expression

As a follow-up to prior work showing that high baseline inflammatory gene expression in the skin was associated with mRSS improvement during mycophenolate mofetil (MMF) treatment [31], skin gene expression profiles of a cohort of patients taking MMF were analyzed with reference to mRSS progression over time [27]. Most patients whose mRSS improved over 12 months had inflammatory or mixed inflammatory/fibroproliferative gene expression profiles in baseline skin biopsies. The inflammatory gene expression signature was reduced in follow-up biopsies after 24 months of MMF therapy. Inflammatory gene expression rebounded in three patients who discontinued MMF treatment, but remained low in three patients who remained on MMF treatment. These results suggest that patients with increased inflammatory gene expression profiles in the skin are more likely to respond favorably to MMF, although the small sample sizes and lack of randomized treatment assignments were limitations.

In SSc patients in a phase I trial of an anti-CD19 antibody Inebilizumab (MEDI-551), analysis of skin biopsy microarray data indicated an elevated plasma cell signature in SSc skin compared with healthy controls. This signature correlated with baseline mRSS, and high plasma cell signature at baseline associated with greater improvement in mRSS during Inebilizumab treatment [28▪].

Skin gene expression related to senescence, termed senescence-associated secretory phenotype (SASP), was analyzed in 12 patients with SSc-associated ILD from a single-arm clinical trial of dasatinib [29▪]. A SASP gene signature was significantly higher at baseline in patients whose mRSS improved during dasatinib treatment, compared with those whose mRSS did not improve. A greater decrease in SASP signature gene expression was also observed posttreatment in those with improving mRSS. These results suggest a role for baseline skin gene expression measurement in selection of patients for future trials of targeted immunosuppressive or antifibrotic therapies, although this approach would require prospective testing in larger patient samples for validation.

Building on prior work identifying distinct subsets of SSc patients based on skin gene expression, termed ‘intrinsic subsets [10]’, a recent report described a machine learning approach used to develop a classifier for these subsets that can be utilized for individual patient samples with the ultimate goal of using this method for risk stratification [32].Empiric testing is needed to determine the ability of this classifier to prospectively identify patients likely to progress and to respond to therapies.

While different reports on the predictive significance of skin gene expression profiles exist, it is important that the methods and transcript lists used for generating the predictive signatures are published in sufficient detail to allow independent validation. Moreover, development of skin gene expression-based predictors is complicated by the spontaneous improvement in skin fibrosis observed in many patients, which complicates interpretation of treatment effect. For example, there seems to be conflicting data on whether the observed associations of inflammatory gene expression signatures with mRSS improvement reflect the natural history of disease [26▪] or treatment effect [27].

PROGNOSTIC BIOMARKERS FOR INTERSTITIAL LUNG DISEASE

Lung involvement is the primary cause of disease-related death in SSc [33]. Lung tissue is not obtained during routine clinical care, and skin gene expression profiling shows only limited correlation with ILD severity [34] and is unlikely to be informative for predicting ILD course as the natural history of skin fibrosis and ILD is often divergent. Plasma/serum samples obtained during routine clinical care are therefore an attractive source of biomarker development in SSc-ILD. Herein, we review the recently published evidence for use of serum proteins as prognostic biomarkers in SSc-ILD.

Pneumoproteins

Pneumoproteins are linked to lung parenchymal injury and may be more specific markers for monitoring and predicting ILD course than general fibrotic and inflammatory markers, which can be influenced by extra-pulmonary fibrotic processes such as cutaneous fibrosis or

infections. Among pneumoproteins, two serum/plasma proteins have been shown to have prognostic significance for ILD course in several studies: KL-6 and CCL-18 (other name: pulmonary and activation-regulated cytokine [PARC]).

A previously published study in 50 Japanese untreated SSc-ILD patients indicated that high KL-6 levels were predictive of long-term development of end-stage lung disease, defined as % predicted FVC (FVC%) of less than 50%, requiring oxygen, or ILD-related death. A cutoff of 1273U/ml was proposed to define KL-6 positivity [35]. A follow-up study in a multiethnic observational cohort of 82 early SSc-ILD patients, which also included patients treated with immunosuppressive agents, confirmed the predictive significance of KL-6 [36▪]. In this study, higher KL-6 was predictive of short-term decline in FVC% (12 months) after correction for baseline disease severity. Using the previously defined cut-off value of 1273U/ml, SSc-ILD patients with positive KL-6 had on average 7% more decline in their annualized percentage change of FVC%. The predictive significance of KL-6 was independent of antitopoisomerase I positivity. In this study, CCL-18 was not predictive of change in FVC%.

In a European observational study of 234 SSc-ILD patients, predictive significance of KL-6 and CCL-18 was investigated [37▪]. This study also included patients that were treated with immunosuppressive agents, and a relatively small portion of patients (14.5%) had a change in FVC% more than 10% during the mean 3.2-year follow-up time. CCL-18 and KL-6 levels were dichotomized based on their correlation with baseline disease severity. Neither KL-6 nor CCL-18 were predictive of short-term (1 year) lung fibrosis worsening. However, CCL-18 was predictive of FVC decline more than 10% during the entire follow-up period, while positive KL-6 (cutoff value=923U/ml) did not show predictive significance.

Predictive significance of KL-6 and CCL-18 was also investigated in SSc-ILD patients (n=133) enrolled in the Scleroderma Lung Study II (SLSII) [38▪]. Contrary to aforementioned observational studies, the investigated SSc-ILD patients were off immunosuppressive agents at the baseline visit and were subsequently treated with either cyclophosphamide or mycophenolate according to standardized treatment protocols. The course of FVC% during the first year of study (3–12 months) when patients in both treatment arms were on active treatment was the primary outcome. In both treatment arms (mycophenolate and cyclophosphamide), higher levels of KL-6 and CCL-18 predicted worse ILD progression based on the lower levels of serially obtained FVC% after adjustment for baseline disease severity.

Table 3 summarizes results of previously published studies on predictive significance of KL-6 and CCL-18. Higher KL-6 levels showed predictive significance for worse ILD course in untreated patients [35], in an observational cohort with mixed treatment regimens [36▪], as well in the SLSII cohort where all patients were treated with immunosuppressive agents [38▪], indicating that this serum protein is a prognostic rather than predictive biomarker, predicting worse ILD course regardless of treatment regimen. Of note, KL-6 did not predict ILD course in the above mentioned European study [37▪] where a validated, conventional ELISA was used, while in the other three studies [35,36▪,38▪] KL-6 was measured using

latex-fixed anti-KL-6 monoclonal antibody with an automated analyzer. The differing assay accuracy might have influenced the discrepant results.

Clara cell secretory protein (CC16) is a marker of bronchial epithelial cell damage and another potential pneumoprotein biomarker in SSc-ILD. In a retrospective study of 106 SSc patients (half had ILD)[43], the predictive significance of baseline CC16 was investigated for a composite ILD event during the 4-year follow-up period. The outcome variable was defined as a 10% decrease in total lung capacity or FVC% from baseline, or death. The risk for this combined lung event was significantly higher in those patients with higher baseline CC16 levels.

C-reactive protein

Previous data from observational cohorts have indicated that higher baseline CRP levels are predictive of reduced survival [44] and faster FVC% decline in SSc [45]. High CRP levels were recently utilized as an enrichment criterion for a placebo-controlled tocilizumab clinical trial [30]. In this trial, early diffuse patients enrolled in the placebo arm had a relatively large mean FVC% decline of 6.3% at 12 months despite the fact that ILD was not an inclusion criterion and skin involvement was the primary focus. In the Australian Scleroderma Cohort [21], the longitudinal correlates of raised CRP (defined as ≥5mg/l) were investigated. Raised CRP was significantly associated with mRSS more than 20 and FVC% less than 80. Notably, a two-fold increase in CRP was associated with a 10% decrease in FVC between corresponding visits in the whole cohort and among those with ILD. In a retrospective Japanese study of 24 SSc-ILD patients, the predictive significance of CRP and KL-6 was investigated for treatment response after 6 monthly infusions of cyclophosphamide [39]. Unlike previously published studies in which FVC% was utilized for response assessment [46,47], good response in this study was defined as an increase of at least 6% in % predicted DLCO (DLCO%) while the remainder of patients were categorized as having poor response. Higher baseline CRP and KL-6 were significantly associated with poor response. These data cumulatively support the notion that CRP might serve as a prognostic biomarkers for worse ILD course. Future studies conducted in large, well phenotyped SSc-ILD clinical trials could contribute importantly to establishing CRP as a clinically useful biomarker in routine clinical care and clinical trials.

As evident by the above review, cross-comparison and validation of biomarkers in SSc-ILD is hampered by the fact that differing outcome measures and biomarker cutoff values are used. Although FVC is the primary outcome variable in SSc-ILD clinical trials [46–48], a widely accepted and validated definition of FVC% improvement or worsening is currently not available. Therefore, progress in this field can be accelerated if prognostic/predictive biomarker studies first show the predictive significance of the investigated candidate biomarker as a continuous variable for the undichotomized FVC% outcome and then consider other secondary analyses.

CONCLUSION

The results highlighted in this review suggest the possibility of more effective risk stratification and treatment selection for SSc patients based on circulating biomarkers and

skin gene expression profiles. However, additional research is needed to determine the utility of these biomarkers in prospective patient selection or stratification in clinical trials and for management of individual SSc patients. It may also prove beneficial to test multifaceted prediction models incorporating clinical data with serologic (including specific autoantibodies) and transcriptomic/proteomic biomarker data. In addition, with improving knowledge of genetic and epigenetic contributions to SSc progression, gene expression and serum protein profiles of SSc patients, clinical trials of novel therapies, and longitudinal outcomes of SSc patients, it is likely that important biomarkers remain to be discovered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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KEY POINTS

- A variety of circulating proteins, including autoantibodies, may have utility in monitoring SSc disease severity or predicting prognosis with regard to specific manifestations such as skin fibrosis, interstitial lung disease, or cancer.
- Multiple reports indicate an ability of skin gene expression profiles to predict prognosis or treatment response.
- For many potential biomarkers, additional research is needed for validation and to define the roles of each biomarker in clinical trial design and in the management of SSc patients in clinical practice.

Table 1.

Circulating biomarkers for severity monitoring, specific manifestations, or prognosis

Reference	Biomarker	Population	Type of marker	Summary of findings
Beyer *et al.* [13]	Sonic hedgehog	European, multicenter	Severity monitoring	Correlation with mRSS, digital ulcers, PAP
Abignano *et al.* [15]	ELF score	European, multicenter	Severity monitoring	Correlation with mRSS (positive), lung function (negative)
McMahan *et al.* [16]	U11/U12 RNP (RNPC3) antibody	US Cohorts (Johns Hopkins, Pittsburgh)	Organ manifestation	Association with gastrointestinal dysmotility
Igusa *et al.* [17▪]	[a]SSc-associated autoantibodies	US Cohort (Johns Hopkins)	Cancer risk	RNA Pol III antibody: increased risk centromere antibody: decreased risk
Shah *et al.* [18▪]	RPA194 antibody	US Cohort (Johns Hopkins)	Cancer risk	RPA194 antibody: lower risk of cancer amongst patients with RPC155 antibody
Moxey *et al.* [19]	ANCA	Australian, multicenter	Prognostic	ANCA positivity associated with higher prevalence of ILD and increased mortality
Herrick *et al.* [20▪▪]	RNA Pol III antibody	European, multicenter	Prognostic	Earlier and higher peak in mRSS amongst patients with diffuse SSc
Ross *et al.* [21]	CRP	Australian, multicenter	Severity monitoring, prognostic	High CRP associated with high mRSS and decreased baseline FVC, and decline in longitudinal FVC

ANCA, antineutrophil cytoplasmic antibody; CRP, C-reactive protein; ELF, enhanced liver fibrosis; FVC, forced vital capacity; ILD, interstitial lung disease; mRSS, modified Rodnan skin score; PAP, pulmonary arterial pressure; SSc, systemic sclerosis.

[a]Antitopoisomerase-I, anticentromere, and anti-RNA polymerase III.

Table 2.

Skin gene expression-based prognostic or predictive biomarkers

Reference	Biomarker	Population	Type of marker	Summary of findings
Stifano *et al.* [25▪]	Skin expression levels of CD14, IL13RA1, SERPINE1, OSMR, and CTGF	Placebo arm of clinical trial	Prognostic	High expression at baseline predicted worsening mRSS
Moon *et al.* [26▪]	'Clusters' based on skin gene expression profiles	Placebo arm of clinical trial	Prognostic	Improved mRSS in patients in the inflammatory/immune cluster
Hinchcliff *et al.* [27]	'Intrinsic subsets' of patients based on skin gene expression profiles	S. Cohort (Northwestern)	Predictive	mRSS improvement during MMF treatment more likely in inflammatory and mixed inflammatory/ fibroproliferative subsets
Higgs *et al.* [28▪]	Plasma cell gene expression signature in skin	Phase I trial of inebilizumab (anti-CD19)	Predictive	Increased plasma cell signature at baseline associated with greater mRSS improvement during anti-CD19 treatment
Martyanov *et al.* [29▪]	SASP gene expression signature in skin	SSc-ILD patients in single-arm trial of dasatinib	Predictive	High baseline SASP signature associated with greater mRSS improvement during treatment

ILD, interstitial lung disease; MMF, mycophenolate mofetil; mRSS, modified Rodnan skin score; SASP, senescence-associated secretory phenotype; SSc, systemic sclerosis.

Table 3.

Studies showing predictive significance of circulating Krebs von den Lungen-6 or chemokine ligand 18

KL-6		
Reference	**Population**	**Summary of findings**
Kuwana *et al.* [35]	Nippon University	Predictive of ESLD development
Salazar *et al.* [36▪]	GENISOS (US, Texas)	Predictive of worse FVC decline in 12 months
Sumida *et al.* [39]	Tokyo University	Predictive of lower DLCO
Volkmann *et al.* [38▪]	SLSII (US, multicenter clinical trial)	Predictive of worse FVC decline in 12 months
CCL-18 (PARC)		
Reference	**Population**	**Summary of findings**
Tiev *et al.* [40]	French cohort	Predictive of ILD event
Elhaj *et al.* [41]	GENISOS (US, Texas)	Predictive of worse FVC in 12 months
Hoffmann-Vold *et al.* [42]	Norwegian cohort	Annual decline in FVC
Elhai *et al.* [37▪]	French/Norwegian cohort	Predictive of 10% decline in FVC
Volkmann *et al.* [38▪]	SLSII (US, multicenter clinical trial)	Predictive of worse FVC decline in 12 months

CCL-18, chemokine ligand 18; DLCO, diffusion capacity of the lungs for carbon monoxide; ESLD, end-stage lung disease; FVC, forced vital capacity; ILD, interstitial lung disease; KL-6, Krebs von den Lungen-6; PARC, pulmonary and activation-regulated cytokine; SLS, scleroderma lung study.