Curr Rheumatol Rep (2011) 13:4–12
DOI 10.1007/s11926-010-0140-z

Biomarkers in the Management of Scleroderma: An Update

Giuseppina Abignano · Maya Buch · Paul Emery · Francesco Del Galdo

Published online: 3 November 2010

Abstract Scleroderma, or systemic sclerosis, is a clinically heterogeneous disease characterized by fibroproliferative vasculopathy, tissue fibrosis affecting the skin and internal organs, and autoimmune activation. Many biomarker candidates have been identified in the past two decades; however, fully validated measures are still lacking with regard to aiding in the early diagnosis and reflecting the disease activity, severity, prognosis, and response to therapy. An ideal biomarker should be highly sensitive and specific, reflecting the current status of disease; should be related to the disease activity and/or severity in accordance with the clinical evolution; should anticipate clinical changes before they occur; and should add independent information about the risk or prognosis that is reproducible and feasible. This review focuses on the most recent and innovative approaches to identify biomarkers, such as extensive gene expression analysis and proteomics, and on markers and surrogate outcome measures closer to clinical practice, and attempts to evaluate them through the OMERACT (Outcome Measures in Rheumatology Clinical Trials) filter.

G. Abignano · M. Buch · P. Emery · F. Del Galdo (✉)
Scleroderma Research Centre, Leeds Institute of Molecular Medicine, Section of Musculoskeletal Diseases,
Chapel Allerton Hospital, University of Leeds,
Second Floor, Chapeltown Road,
Leeds LS7 4SA, UK
e-mail: f.delgaldo@leeds.ac.uk

G. Abignano
Department of Clinical and Experimental Medicine,
Rheumatology Unit, Second University of Naples,
Naples, Italy
e-mail: g.abignano@leeds.ac.uk

Keywords Biomarker · Systemic sclerosis · Scleroderma · OMERACT · Outcome measure

Introduction

Scleroderma, or systemic sclerosis (SSc), is a clinically highly heterogeneous autoimmune condition characterized by fibroproliferative vasculopathy and tissue fibrosis affecting the skin and multiple internal organs.

The extent of involvement, rate of progression, and type of organ involvement are the main determinants of morbidity and mortality [1, 2]. Thus far, these features remain not easily predictable at both the patient group level and in a given patient with regard to age at onset and clinical course.

The core clinical classification of SSc is determined by the extent of clinically involved skin fibrosis; patients are defined as having diffuse cutaneous SSc (dcSSc) or limited cutaneous SSc (lcSSc) based on skin thickening proximal (whether or not it affects the chest and abdomen) or distal to the elbows and/or knees, respectively. This classification is supported by the association with specific autoantibodies that define with specific accuracy the two types of patients; in addition, as discussed further subsequently, these autoantibodies have a meaningful clinical value correlating with mortality and risk of specific organ involvement.

Despite intense investigation and the identification of many candidate molecules in the past two decades, fully validated biomarkers that could serve as surrogate outcome measures in clinical trials or guide clinical management of the condition remain lacking.

The gold standard measure to assess skin disease activity is the modified Rodnan skin score (mRSS), currently adopted as the primary outcome measure in the vast

majority of clinical intervention studies. It measures the overall extent/amount of clinically thickened skin. Although it offers the advantage of being scored immediately with no additional cost and has been originally validated against collagen content of the skin, the method has come under increased scrutiny with regard to accuracy and especially interobserver variability [3]. It was recently suggested that proper training can realistically improve interobserver variability [4]. Nevertheless, recent studies on gene expression profiling of clinically involved versus clinically uninvolved skin have revealed that, in addition to the clinically appreciable skin thickening, a similar pathological process evidenced by a common pattern of gene expression affects clinically involved and uninvolved skin [5••]. The interpretation of this finding is somewhat challenging. It could be considered as failing to validate skin microarray in defining clinical involvement according to the gold standard (lack of criterion validity, as discussed subsequently), or it could be used to question the construct validity of the mRSS as a measure because of its inconsistency with the theoretical concept that clinically unaffected skin is healthy skin (ie, not different from normal). The consequences of this interpretation are not trivial or purely academic. In fact, most of the molecules that become targets for clinical intervention studies with a therapeutic purpose have been developed following proof-of-concept biological and molecular studies rooted in gene expression analysis, fibroblast biology, and ultimately molecular biology of the skin or other affected tissues in SSc. From this perspective, it may be paradoxical to evaluate the efficacy of an intervention assuming a measure that does not reflect these factors as the gold standard.

A comprehensive and updated list of serum biomarkers proposed in SSc was recently reviewed by Castro and Jimenez [6•]. We refer the reader to that review for a list of all the biomarkers proposed and their classification. In this review, we instead focus on the most recent and innovative approaches to identifying biomarkers, such as extensive gene expression analysis and proteomics, as well as markers and surrogate outcome measures that, with their validation, are closer to being applied in the clinical setting. Moreover, we attempt to evaluate measures and biomarkers through the filter that has been adopted internationally to evaluate the clinical meaning or applicability of a measure [7]. This filter has been developed by an informal international network originally born out of OMERACT (Outcome Measures in Rheumatology Clinical Trials), which defines a series of validity criteria that are extremely useful when attempting to identify a meaningful biomarker or outcome measure. We are strongly convinced that the unmet need of a surrogate outcome measure in SSc requires a thorough validation of any proposed biomarker, and that all the studies proposing new molecules or outcome measures in SSc should aim to consider this filter as a first step toward identifying a putative measure.

OMERACT Validity Criteria

According to the OMERACT, a measure becomes clinically applicable when it complies with the concepts of truth, discrimination, and feasibility.

Truth

The essential subject of a measure is that it measures what it is intended to. Although this may seem to be a basic attribute, a thorough evaluation of the current putative biomarkers for SSc will reveal that for many, definitive data on this essential subject are lacking. Indeed, according to the OMERACT filter, the validity criterion of truth is met when the measure is found to have face, content, construct, and criterion validity. Definitions of each of these terms are summarized in Table 1.

Discrimination

The ability of a measure to correlate with pathological status (classification/diagnosis) and disease activity (prognosis) is crucial for clinical management and for clinical intervention studies. For this purpose, an applicable measure should be reliable and sensitive in quantifying the change over the natural history of the disease and with effective treatment (Table 1).

Feasibility

Last but not least is the ease of a measure given the constraints of time, money, and interpretability. This is a crucial attribute of an applicable measure and is often decisive in determining a measure's success.

Innovative, Unbiased Approaches to Biomarker Identification

The classical approach that has been pursued for decades in studies undertaken with the aim of identifying a biological marker of disease has been "hypothesis driven." A given molecule, postulated or known to be involved in the pathogenesis of SSc, is measured in the plasma and correlated with diagnosis and clinical involvement. During the past 3 years, with the advent of powerful techniques such as genome-wide single nucleotide polymorphism (SNP) analysis, microarray analysis, and proteomics, new and unbiased (ie, not hypothesis-driven) approaches have

Table 1 OMERACT filter

Concept	Attribute(s)	Definition
Truth	Face validity	Whether or not the measure reflects what it is supposed to measure
•Is the measure truthful, and does it measure what is intended?	Content validity	Whether the measure covers the whole range of possibilities within a given disease or disease state
•Is the result unbiased and relevant?	Construct validity	Whether the measure adequately reflects the underlying construct
	Criterion validity	Whether the measure under investigation produces the same or similar results as does a gold standard
Discrimination	Reliability	Reproducibility, stability when a measure is done repeatedly
•Does the measure discriminate between situations of interest?	Sensitivity to change	Whether the measure discriminates between states at different times
Feasibility	–	Whether the measure is easy to perform, requires little time, and requires a minimal amount of equipment
•Can the measure be applied easily given constraints of time, money, and interpretability?		

OMERACT—Outcome Measures in Rheumatology Clinical Trials

revealed novel potential biomarkers and outcome measures for SSc.

Genome-Wide Association Studies for the Identification of Genetic Risk Factors in Systemic Sclerosis

During the past 15 years, many studies have pursued a candidate gene approach to identify genetic polymorphisms associated with SSc. Despite the positive results of many of these studies, this strategy has yielded a very limited and not infrequently contradictive characterization of SSc genetic risk factors [8, 9]. An important exception is the strong and confirmed association of specific HLA haplotypes with SSc, particularly *DQA1*0501* and *DQB1*1301* [10], which are shared among individuals of different racial backgrounds. Some other loci outside the HLA region have also demonstrated strong and reproducible associations with SSc susceptibility, but none of them are routinely used to predict risk of SSc in the diagnostic work-up. A completely different approach is the recent massive effort to characterize new susceptibility loci for SSc by genome-wide association studies. One such study, conducted on more than 2,000 patients and replicated in a second cohort of almost 3,000 patients, identified as the strongest susceptibility locus (besides the HLA region) an SNP in the *CD247* gene and confirmed the previously known association with *STAT4* and *IRF5* gene polymorphisms [11••]. Although they are potentially very interesting for gaining insight into the pathogenesis of SSc, genetic associations in the same loci have been found in other conditions, such as systemic lupus erythematosus (SLE) [12, 13] and rheumatoid arthritis [14]. Therefore, detailed comparative studies are needed to assess their specificity for SSc.

Nevertheless, the amount of data accrued in such a study offers an invaluable opportunity to identify haplotypes (eg, combination of SNPs) that are associated with disease subsets. A thorough, clinically oriented evaluation of the datasets undoubtedly will shed light on the permissive genetic background associated with the heterogeneity of the condition, similar to what was recently demonstrated for liver fibrosis following hepatitis C virus infection [15].

Gene Expression Profiling and Molecular Subsets in Systemic Sclerosis

Transcriptome analysis has become an extremely common and feasible research tool in the study of complex diseases. Whereas the first attempts at SSc skin microarray were aimed at identifying genes potentially involved in disease pathogenesis, the latest studies involving a greater number of patients have aimed to quantitatively and objectively capture the clinical heterogeneity in SSc by analyzing the transcriptome of the skin as well as the dermal fibroblasts, the key cellular elements of fibrosis [5••, 16, 17]. Although this approach is established and has been successfully used in cancer, it represents an extremely novel technique in SSc; furthermore, it has allowed the well-known biological heterogeneity of skin sample-derived data (classically

viewed as an impediment to consistent research) to be elegantly turned into a potential advantage.

One of the most interesting analytical approaches proposed in the study by Milano et al. [5••] is the identification of an "intrinsic signature of genes" that has a stable pattern of expression among affected or unaffected skin. As mentioned previously, the mere existence of this signature is a challenge to the face validity of mRSS as a measure of disease activity. Nevertheless, this signature enables the identification of four molecular subsets of SSc.

The diffuse proliferation group is composed solely of patients with dcSSc and is associated on average with a longer disease duration than that of the other patients analyzed in the study. The genes driving this clustering are mostly genes involved in cell cycle regulation, a new concept in SSc in itself. Of interest, immunohistochemical validation of these data showed that the cells responsible for the increased gene expression are keratinocytes from the basal layer of the epidermis, presenting a new and challenging concept in SSc pathophysiology. Indeed, the notion of epithelial cells playing a crucial role in the pathogenesis of skin fibrosis was more recently confirmed by another elegant proteomic study on SSc skin biopsies [18]. In fact, Aden et al. [18] have shown convincing data indicating that the epithelial cells in SSc are activated and express a wound healing-like phenotype.

The inflammatory group includes patients both with dcSSC and lcSSC with shorter disease duration. These patients likely reflect those clinically defined as having "early scleroderma." Interestingly, for this purpose, if validated, this set of genes could be used to better define such patients. Thus far, the definition of early SSc has been based on a given lapse of time from the onset of the first non-Raynaud's phenomenon symptom. Unfortunately, this concept is not fully agreed upon, as it is associated with several shortcomings that fail to reflect the often-subtle clinical manifestations of organ involvement in SSc. Moreover, a very interesting recent study by Valentini et al. [19••] indicated that SSc-related internal organ preclinical involvement can be detected in up to 42% of patients who present clinically with only Raynaud's phenomenon, SSc marker autoantibodies, and/or typical capillaroscopic abnormalities.

Also worth mentioning are the limited group, composed solely of lcSSc patients, and the normal-like group, in which the skin gene expression profile showed the least amount of differences with normal skin.

There is a significant unmet need with regard to biomarkers capable of capturing quantitatively the clinical heterogeneity in SSc; as such, this is potentially an extremely useful approach. Nevertheless, despite the obvious face validity of a gene signature, given the low feasibility of such testing and the good diagnostic tools already available, it is difficult to imagine such a procedure becoming part of routine clinical practice for diagnosis. On the contrary, studies on the clinical meaningfulness of the molecular subsets, as well as studies that aim to determine whether these signatures are sensitive to change over time and with therapy may validate their use in addressing clinical heterogeneity by performing molecular-guided randomization in large studies and objectively defining the early inflammatory phase, as mentioned above.

Proteomics

The use of proteomics in studying SSc is a very recent development. A few proteomic studies from the same group have focused on identifying specific molecules in the bronchoalveolar lavage of patients affected by SSc [20, 21]. Although potentially interesting, because of their poor feasibility, the predictive value of such markers should be expected to be significantly better than the current measure (ground glass opacity at high-resolution CT scan of the chest in the case of alveolitis) to justify their use in the clinical setting.

Similarly, proteomics techniques have been used to identify specific molecules in the saliva of SSc patients [22]. Two studies have used unbiased proteomic techniques, mainly with the purpose of unraveling disease mechanisms. Bogatkevich et al. [23] focused on lung fibroblasts to identify the specific profile of proteins associated with increased expression of connective tissue growth factor. Aden et al. [18] studied SSc skin biopsies to identify a specific profile of protein expression in skin affected by SSc.

Recently, a proteomic study of the secretome of explanted dermal fibroblasts that aimed to discover biomarkers of fibrogenesis used two-dimensional gel analysis followed by mass spectrometry analysis to identify a set of nine molecules that showed increased production in fibroblasts from both SSc and an unrelated (not autoimmune) fibrotic condition (nephrogenic systemic fibrosis). The approach, designed to identify common molecules linked to the profibrotic phenotype regardless of the immune activation—although it may lack sensitivity in identifying putatively important molecules in the SSc secretome—promises to be highly specific for markers of increased fibrogenesis. Because the study focused on the activity of the key cellular elements of fibrosis from the biomarker point of view, there is an obvious construct validity, with the feasibility of a serum measure unquestionable. Nevertheless, in addition to a large-scale validation of its sensitivity to identify a fibrotic condition, a full validation for discrimination

ability and sensitivity to change is needed to realize use in the clinical setting [24].

Biomarkers and Surrogate Outcome Measures with Documented Validation

Autoantibodies in Scleroderma

The presence of autoantibodies is a central defining aspect of autoimmune conditions. Connective tissue diseases (CTDs) have been classified for decades according to the presence of specific patterns of antinuclear antibodies. The autoantibodies that have been used historically as diagnostic biomarkers in SSc include anti-Scl-70 and anticentromere antibodies (ACAs). These autoantibodies are still the most useful and validated biomarkers in SSc and have been used for diagnosis and classification.

The anti-Scl-70 antibodies should be more accurately termed *antitopoisomerase I* (ATA), the 70-kDa autoantigen originally identified by Douvas et al. [25] as a major breakdown product of topoisomerase I [26]. ATA is found in about 20% of SSc patients with high disease specificity (97–100% vs healthy controls and other CTDs by immunodiffusion), in about 37% of patients with dcSSc, and in less than 10% of patients with lcSSc [27]. A recent study by Mahler et al. [28] confirmed the high specificity of ATA, especially at high titers, determining a prevalence in systemic lupus erythematosus (SLE) of less than 5%. ATA positivity also has been found to correlate with severe interstitial lung disease with musculoskeletal and cardiac involvement [29–32]. More recently, ATA titers have been found to correlate with forced vital capacity, diffusion capacity for carbon monoxide and Global Activity Score as assessed by the European Scleroderma Study Group-Activity Index (EScSG-AI) [33]. This suggests a possible use for ATA titers for prognostic purposes. From this perspective, it would be extremely useful to validate the discriminant validity of this test by measuring its sensitivity to change over time.

ACAs target centromeric proteins (CENP-A to CENP-F), of which CENP-B is reported to be the major autoantigen [34, 35]. They are seen in 20% to 30% of SSc patients [36, 37] and in up to 90% of lcSSc patients, especially those with features of CREST (calcinosis, Raynaud's phenomenon, esophageal involvement, sclerodactyly, telangiectasia) [37]. In patients with Raynaud's phenomenon, ACAs are predictive of development of lcSSc [29]. Compared with ATA, they were found to be associated with a higher risk of pulmonary hypertension rather than pulmonary fibrosis. In a recent report from the EUSTAR (European League Against Rheumatism Scleroderma Trial and Research group) database, 13% of ACA- vs 5% of ATA-positive patients had pulmonary artery hypertension (PAH) without fibrosis, 8% of ACA- versus 17.2% of ATA-positive patients had PAH with fibrosis, and 21.3% of ACA- versus 60.2% of ATA-positive patients had pulmonary fibrosis ($P<0.001$ ATA vs ACA) [32]. It also has been reported that ACA positivity correlates with a more favorable prognosis and a lower mortality rate compared with the positivity of other SSc-related autoantibodies [31]. However, ACA titers, assessed by enzyme-linked immunosorbent assay (ELISA), do not change over time and are not associated with disease activity [38]. For this reason, their use remains limited to diagnostic and classification purposes.

Although anti-RNA polymerase (ARA) II can be found in the sera of SLE, overlap syndrome, and SSc patients, ARA I to III is detected with high specificity in SSc patients (98–100%) [39, 40]. Their prevalence varies from 3.4% to 23% in different SSc cohorts [40]. In recent years, validation of the ELISA test has made the detection of ARA levels more feasible compared with immunoprecipitation, as the reported sensitivity and specificity of ELISA to identify ARA are very high (91–96% and 98–99%, respectively) [40, 41]. It has been known for years that ARA positivity correlates with diffuse cutaneous involvement with rapid skin progression and renal crisis [42] and, more recently, with rapid onset of the disease and skin thickening progression [43]. For this purpose, they are among the best predictive markers available to date for rapid skin progression. Unfortunately, no studies have assessed the discriminant validity of ARA titers over time. The only study suggesting a potential use of ARA titers to measure clinical activity was conducted by Nihtyanova et al. [44] and found significant correlation ($P=0.011$) over time between ARA titers and change in mRSS in all patients with available serial skin score (33 of 64). The same study found no correlation between change in ARA levels and change in inflammatory markers nor between absolute ARA levels (at baseline and throughout the disease course) and clinical presentation, internal organ involvement, overall severity of skin disease, immunosuppressive treatment, and mortality. Similar results on the correlation between changes in ARA levels and mRSS were previously observed in a smaller group of patients [40].

Anti-U3RNP antibodies target fibrillarin, a small protein belonging to the U3 small nucleolar ribonucleoprotein (RNP) complex, and like anti-Th/To and anti-PM-Scl antibodies, they demonstrate a nucleolar staining pattern. Originally reported in 5% to 8% of SSc patients [45], the anti-U3RNP-positive patient group had higher proportions of males (29% vs 19% in the U3RNP-negative group; $P=0.021$) and African American patients (27% vs 5% in the negative patients; $P<0.001$). Moreover, PAH was present in 31% of anti-U3RNP antibodies-positive patients and only

14% of the U3RNP-negative patients ($P<0.001$). Similarly, skeletal muscle involvement was present in 25% of anti-U3RNP antibodies-positive patients and in only 14% of the negative patients ($P=0.002$) [46]. Although statistically significant, the specificity of these markers is too low to be of benefit in a clinical setting.

Anti-Th/To antibodies recognize small RNP components of RNase P and RNase MRP, showing a nucleolar staining pattern in indirect immunofluorescence. They occur in 2% to 5% of SSc patients [29], are found in sera from patients with limited form (8.4% of lcSSc patients, 0.6% of dcSSc patients) [47] and are associated with a shorter interval between the onset of Raynaud's phenomenon and swollen hands than the ACA-positive patient group. Among lcSSc patients, they are a marker of worst survival rate. Mitri et al. [48] reported a 5- and 10-year cumulative survival rate of 61% and 49%, respectively, in this group, which is significantly worse than in ACA-positive patients (77% and 59%; $P<0.02$).

A recent single-center study confirmed the association between anti-Th/To antibodies and lcSSc but failed to confirm an association with lung disease [49]. A more extensive study would address this knowledge gap and validate their use as prognostic markers. Nevertheless, the test is limited by low feasibility, as these antibodies are usually identified by immunoprecipitation and not by ELISA.

U11/U12 RNPs are components of spliceosome found in eukaryotic cells [50]. A recent study reported a prevalence of 3.2% in two consecutive series of SSc patients, with high specificity (100%) compared with other CTDs [51]. The authors suggested the utility of anti-U11/U12 RNP antibodies as markers of severe pulmonary fibrosis, as this involvement was observed more frequently in antibody-positive than in antibody-negative SSc patients (70% vs 37%; $P<0.0001$), with a higher risk of pulmonary fibrosis-related death.

Anti-PM/Scl antibodies recognize several protein components of the human exosome, of which two proteins, PM/Scl-75 and PM/Scl-100, have been identified as the main antigens [52]. They are classified as SSc overlap-associated antibodies and are not specific for SSc. Anti-PM/Scl-positive SSc patients usually have muscle involvement and an increased risk of pulmonary fibrosis and digital ulceration, with a lower likelihood of developing PAH [53, 54]. A recent report suggests that the two types of antibodies can distinguish between the two different disease subsets. Anti-PM/Scl-75 antibodies were found in both dcSSc and overlap syndrome patients, while anti-PM/Scl-100 antibodies were found mainly in overlap syndrome patients. The profile of anti-PM/Scl-75 antibodies-positive patients usually reflects younger age, a more active disease, and joint contractures, while the anti-PM/Scl-100 antibodies-positive patients may have less frequent gastrointestinal involvement and show creatine kinase elevation [53].

Composite Measures of Disease Activity

One of the most recently proposed tools to assess the disease activity is the EScSG-AI [55–57]. The EScSG-AI is a feasible index of ten items consisting of clinical, laboratory and functional values—each with different weighting—that has been shown to have face and construct validity but has not yet been tested for sensitivity to change. A recent study of 131 consecutive SSc patients confirmed the construct validity of the EScSG-AI. The authors also developed a new 12-point activity index to better represent the interstitial and vascular lung components of the disease. Interestingly, in the same study, serum concentrations of several previously investigated biomarkers, such as vascular endothelial growth factor, B-cell-activating factor, surfactant protein D (SP-D), cross-linked collagen I carboxyterminal telopeptide, ATA I antibody titer, a proliferation-inducing ligand, soluble CD40 ligand, procollagen type I N-terminal propeptide, procollagen type III N-terminal propeptide, Krebs von den Lungen-6 antigen (KL-6), sE-selectin, P-selectin glycoprotein ligand-1 (sPSGL-1), and von Willebrand factor from plasma samples, were detected in the same cohort of 131 consecutive SSc patients, 30 healthy controls, and 51 patients with primary Raynaud's phenomenon. The study indicated that vascular endothelial growth factor, albumin, C-reactive protein and sPSGL-1 correlated with both the EScSG-AI and the new 12-point index. These complex indices may be very useful as surrogate measures that assess more than one aspect of the disease. This study is encouraging and establishes a foundation for the development of a tool that not only encompasses the varied nature of SSc disease but also allows evaluation of disease activity and change/improvement. It is clear, however, that for optimal clinical utility, any such instrument must be relatively straightforward with a minimum requirement for multiple tests.

Focused Gene Expression Studies

Another interesting, although completely different approach to constructing a composite disease activity index was recently published by Farina et al. [58]• and involves the use of skin gene expression as a surrogate outcome measure. In this study, the authors analyzed and validated the level of expression of a four-gene biomarker signature in skin biopsies against mRSS, including two transforming

growth factor-β and two interferon-inducible genes, namely cartilage oligomeric matrix protein, thrombospondin-1, interferon-inducible 44 (*IFI44*), and sialoadhesin (*Siglec-1*). The test was validated against mRSS for absolute score and for its sensitivity to detect change in mRSS over time. For this purpose, this signature remains one of the only examples of a measure with obvious face validity and a validated sensitivity to change. In this regard, if it was not for the feasibility obstacles, it could serve as a more objective and applicable surrogate outcome measure of skin disease activity in SSc.

Biomarkers and Outcome Measures of Lung Involvement

One of the biggest burdens in SSc is fibrotic involvement of the lung. To date, the only measures routinely used to quantitatively assess lung function are the diffusion capacity for carbon monoxide and forced vital capacity. Although these are good measures of overall lung function/volume, they are not specific at all for the fibrotic process, much less for active fibrogenesis. The presence of alveolitis, as evidenced by the not-fully-agreed-upon definition of ground glass on high-resolution CT scan, is the only tool available to predict progression of lung involvement.

In this regard, a very interesting study of patients involved in the Scleroderma Lung Study by Hant et al. [59] found a strong correlation between the presence of alveolitis and the concentration of SP-D and KL-6, glycoproteins expressed by type II pneumocytes (albeit in 66 SSc patients, of which only 22 were without alveolitis). Levels of SP-D and KL-6 were not only ninefold and fourfold higher in SSc patients compared with healthy controls, respectively ($P<0.0001$ for both), but their concentration was higher in patients with alveolitis (2.5-fold higher than in patients without alveolitis; $P<0.0005$ for both). Although it is unlikely that high-resolution CT scans would be replaced by these tests, a longitudinal study determining the discriminant validity and specificity of these tests may encourage their use as an outcome measure in clinical intervention trials that target progression of interstitial lung disease in SSc [59].

N-terminal-pro Brain Natriuretic Peptide as a Biomarker of Pulmonary Artery Hypertension/Vasculopathy

The assessment of vasculopathy activity is a central requirement in SSc. One of the most promising and validated biomarkers is the N-terminal-pro brain natriuretic peptide (NT-proBNP), which has been reported and validated mainly as a biomarker of pulmonary vascular involvement in SSc [60, 61]. In particular, in SSc patients, it has been found to be a marker of early detection of increased systolic pulmonary artery pressure [60], a marker of severity of PAH [62], a potential marker of response to therapy [63, 64], and even a predictor of the occurrence of PAH, together with a decreased diffusion capacity for carbon monoxide/alveolar volume ratio of less than 70% [61]. More recently, one study, conducted on 69 consecutive SSc patients, proposed NT-proBNP as a candidate marker of depressed myocardial contractility and overall cardiac involvement as assessed by pulsed tissue Doppler echocardiography [65]. Using 125 pg/mL as the cutoff concentration, the sensitivity and specificity of NT-proBNP measurements were 92% and 71%, respectively, for the detection of depressed myocardial contractility, and 94% and 78% for overall cardiac involvement in SSc patients with cardiac involvement compared with controls. Given its high feasibility, if confirmed in a larger cohort, it could be a very useful instrument to stratify SSc patients based on the risk of development of cardiac and pulmonary artery involvement. Additional potential biomarkers of PAH were recently investigated in SSc patients [66]. In this cross-sectional study, undertaken among 20 SSc patients with an echocardiographic diagnosis of secondary PAH, endothelin-1, interleukin-8, tumor necrosis factor-α, and endoglin (Eng) levels were found to be significantly elevated in SSc patients compared with healthy controls, with correlations between endothelin-1 and Eng and between interleukin-8 and Eng also reported. However, these data on the potential role as biomarkers of PAH need to be confirmed in a comparative study that includes SSc patients without PAH, as well as in a more heterogeneous cohort based on cutaneous involvement.

Conclusions

Our understanding of SSc pathophysiology has improved significantly in recent years as a complement to the established knowledge base. We are discovering an increasing list of molecules and pathways implicated in the disease process that offer the tantalizing opportunity to improve the whole-scale management of SSc, from diagnostic capability and disease and response assessment to crucially effective therapies. Insights from other diseases as well as use of more novel technologies have contributed to these advances.

Although characterized by acknowledged fundamental pathological abnormalities (vasculopathy and fibrosis with evidence of immune activation), SSc is a markedly heterogeneous disease with regard to its nature of presentation, evolution, and extent of involvement. This under-

lines the challenges faced in clinical practice and in designing effective clinical trials.

The continued development of biomarker technology and investigation will inevitably lead to real changes in the management of SSc and, therefore, patient outcomes. The future of SSc research is exciting and holds a great deal of promise.

Disclosure No potential conflicts of interest relevant to this article were reported.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Steen VD, Medsger TA: Improvement in skin thickening in systemic sclerosis associated with improved survival. Arthritis Rheum 2001, 44:2828–2835.
2. Clements PJ, Hurwitz EL, Wong WK, et al.: Skin thickness score as a predictor and correlate of outcome in systemic sclerosis: high-dose versus low-dose penicillamine trial. Arthritis Rheum 2000, 43:2445–2454.
3. Clements PJ, Lachenbruch PA, Seibold JR, et al.: Skin thickness score in systemic sclerosis: an assessment of interobserver variability in 3 independent studies. J Rheumatol 1993, 20:1892–1896.
4. Silman A, Harrison M, Brennan P: Is it possible to reduce observer variability in skin score assessment of scleroderma? The ad hoc International Group on the Assessment of Disease Outcome in Scleroderma. J Rheumatol 1995, 22:1277–1280.
5. •• Milano A, Pendergrass SA, Sargent JL, et al.: Molecular subsets in the gene expression signatures of scleroderma skin. PLoS One 2008, 3:e2696. *This remarkable study showed that according to a signature of genes that are highly similarly expressed in affected and not clinically affected skin biopsies, it is possible to define four molecular subsets of SSc.*
6. • Castro SV, Jimenez SA: Biomarkers in systemic sclerosis. Biomark Med 2010, 4:133–147. *This is one of the most recent reviews on serum biomarkers.*
7. Boers M, Brooks P, Strand CV, Tugwell P: The OMERACT filter for outcome measures in rheumatology. J Rheumatol 1998, 25:198–199.
8. Agarwal SK, Tan FK, Arnett FC: Genetics and genomic studies in scleroderma (systemic sclerosis). Rheum Dis Clin North Am 2008, 34:17–40, v.
9. Agarwal SK, Reveille JD: The genetics of scleroderma (systemic sclerosis). Curr Opin Rheumatol 2010, 22:133–138.
10. Arnett FC, Gourh P, Shete S, et al.: Major histocompatibility complex (MHC) class II alleles, haplotypes and epitopes which confer susceptibility or protection in systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. Ann Rheum Dis 2010, 69:822–827.
11. •• Radstake TR, Gorlova A, Rueda B, et al.: Genome-wide association study of systemic sclerosis identifies CD247 as a new susceptibility locus. Nat Genet 2010, 42:426–429. *This was an innovative and comprehensive study that aimed to identify genetic risk factors in SSc.*
12. Gorman CL, Russell AI, Zhang Z, et al.: Polymorphisms in the CD3Z gene influence TCRzeta expression in systemic lupus erythematosus patients and healthy controls. J Immunol 2008, 180:1060–1070.
13. Glas J, Seiderer J, Nagy M, et al.: Evidence for STAT4 as a common autoimmune gene: rs7574865 is associated with colonic Crohn's disease and early disease onset. PLoS One 2010, 5:e10373.
14. Morgan AW, Robinson JI, Conaghan PG, et al.: Evaluation of the rheumatoid arthritis susceptibility loci HLA-DRB1, PTPN22, OLIG3/TNFAIP3, STAT4 and TRAF1/C5 in an inception cohort. Arthritis Res Ther 2010, 12:R57.
15. Huang H, Shiffman ML, Friedman S, et al.: A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C. Hepatology 2007, 46:297–306.
16. Pendergrass SA, Hayes E, Farina G, et al.: Limited systemic sclerosis patients with pulmonary arterial hypertension show biomarkers of inflammation and vascular injury. PLoS One 2010, 5:e12106.
17. Sargent JL, Milano A, Connolly MK, Whitfield ML: Scleroderma gene expression and pathway signatures. Curr Rheumatol Rep 2008, 10:205–211.
18. Aden N, Shiwen X, Aden D, et al.: Proteomic analysis of scleroderma lesional skin reveals activated wound healing phenotype of epidermal cell layer. Rheumatology (Oxford) 2008, 47:1754–1760.
19. •• Valentini G, Cuomo G, Abignano G, et al.: Early systemic sclerosis: assessment of clinical and pre-clinical organ involvement in patients with different disease features. Rheumatology (Oxford) 2010 Jun 18 (Epub ahead of print). *This study revealed that preclinical SSc involvement of internal organs is present in 42% of patients who as of today's standard of care would be labeled as affected only by secondary Raynaud's phenomenon.*
20. Bargagli E, Olivieri C, Prasse A, et al.: Calgranulin B (S100A9) levels in bronchoalveolar lavage fluid of patients with interstitial lung diseases. Inflammation 2008, 31:351–354.
21. Rottoli P, Magi B, Perari MG, et al.: Cytokine profile and proteome analysis in bronchoalveolar lavage of patients with sarcoidosis, pulmonary fibrosis associated with systemic sclerosis and idiopathic pulmonary fibrosis. Proteomics 2005, 5:1423–1430.
22. Giusti L, Bazzichi L, Baldini C, et al.: Specific proteins identified in whole saliva from patients with diffuse systemic sclerosis. J Rheumatol 2007, 34:2063–2069.
23. Bogatkevich GS, Ludwicka-Bradley A, Singleton CB, et al.: Proteomic analysis of CTGF-activated lung fibroblasts: identification of IQGAP1 as a key player in lung fibroblast migration. Am J Physiol Lung Cell Mol Physiol 2008, 295:L603–L611.
24. Del Galdo F, Shaw MA, Jimenez SA: Proteomic analysis identification of a pattern of shared alterations in the secretome of dermal fibroblasts from systemic sclerosis and nephrogenic systemic fibrosis. Am J Pathol 2010, 177:1638–1646.
25. Douvas AS, Achten M, Tan EM: Identification of a nuclear protein (Scl-70) as a unique target of human antinuclear antibodies in scleroderma. J Biol Chem 1979, 254:10514–10522.
26. Shero JH, Bordwell B, Rothfield NF, Earnshaw WC: High titers of autoantibodies to topoisomerase I (Scl-70) in sera from scleroderma patients. Science 1986, 231:737–740.
27. Basu D, Reveille JD: Anti-scl-70. Autoimmunity 2005, 38:65–72.
28. Mahler M, Silverman ED, Schulte-Pelkum J, Fritzler MJ: Anti-Scl-70 (topo-I) antibodies in SLE: myth or reality? Autoimmun Rev 2010, 9:756–760.
29. Reveille JD, Solomon DH: Evidence-based guidelines for the use of immunologic tests: anticentromere, Scl-70, and nucleolar antibodies. Arthritis Rheum 2003, 49:399–412.

30. Steen VD, Powell DL, Medsger TA: Clinical correlations and prognosis based on serum autoantibodies in patients with systemic sclerosis. Arthritis Rheum 1988, 31:196–203.
31. Hesselstrand R, Scheja A, Shen GQ, et al.: The association of antinuclear antibodies with organ involvement and survival in systemic sclerosis. Rheumatology (Oxford) 2003, 42:534–540.
32. Walker UA, Tyndall A, Czirják L, et al.: Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials and Research group database. Ann Rheum Dis 2007, 66:754–763.
33. Hanke K, Dähnrich C, Brückner CS, et al.: Diagnostic value of anti-topoisomerase I antibodies in a large monocentric cohort. Arthritis Res Ther 2009, 11:R28.
34. Earnshaw W, Bordwell B, Marino C, Rothfield N: Three human chromosomal autoantigens are recognized by sera from patients with anti-centromere antibodies. J Clin Invest 1986, 77:426–430.
35. Rothfield N, Whitaker D, Bordwell B, et al.: Detection of anticentromere antibodies using cloned autoantigen CENP-B. Arthritis Rheum 1987, 30:1416–1419.
36. Cepeda EJ, Reveille JD: Autoantibodies in systemic sclerosis and fibrosing syndromes: clinical indications and relevance. Curr Opin Rheumatol 2004, 16:723–732.
37. LeRoy EC, Black C, Fleischmajer R, et al.: Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988, 15:202–205.
38. Tramposch HD, Smith CD, Senecal JL, Rothfield N: A long-term longitudinal study of anticentromere antibodies. Arthritis Rheum 1984, 27:121–124.
39. Chang M, Wang RJ, Yangco DT, et al.: Analysis of autoantibodies against RNA polymerases using immunoaffinity-purified RNA polymerase I, II, and III antigen in an enzyme-linked immunosorbent assay. Clin Immunol Immunopathol 1998, 89:71–78.
40. Kuwana M, Okano Y, Pandey JP, et al.: Enzyme-linked immunosorbent assay for detection of anti-RNA polymerase III antibody: analytical accuracy and clinical associations in systemic sclerosis. Arthritis Rheum 2005, 52:2425–2432.
41. Parker JC, Burlingame RW, Webb TT, Bunn CC: Anti-RNA polymerase III antibodies in patients with systemic sclerosis detected by indirect immunofluorescence and ELISA. Rheumatology (Oxford) 2008, 47:976–979.
42. Kuwana M, Kaburaki J, Mimori T, et al.: Autoantibody reactive with three classes of RNA polymerases in sera from patients with systemic sclerosis. J Clin Invest 1993, 91:1399–1404.
43. Cavazzana I, Angela C, Paolo A, et al.: Anti-RNA polymerase III antibodies: a marker of systemic sclerosis with rapid onset and skin thickening progression. Autoimmun Rev 2009, 8:580–584.
44. Nihtyanova SI, Parker JC, Black CM, et al.: A longitudinal study of anti-RNA polymerase III antibody levels in systemic sclerosis. Rheumatology (Oxford) 2009, 48:1218–1221.
45. Kipnis RJ, Craft J, Hardin JA: The analysis of antinuclear and antinucleolar autoantibodies of scleroderma by radioimmunoprecipitation assays. Arthritis Rheum 1990, 33:1431–1437.
46. Aggarwal R, Lucas M, Fertig N, et al.: Anti-U3 RNP autoantibodies in systemic sclerosis. Arthritis Rheum 2009, 60:1112–1118.
47. Okano Y, Medsger TA: Autoantibody to Th ribonucleoprotein (nucleolar 7-2 RNA protein particle) in patients with systemic sclerosis. Arthritis Rheum 1990, 33:1822–1828.
48. Mitri GM, Lucas M, Fertig N, et al.: A comparison between anti-Th/To- and anticentromere antibody-positive systemic sclerosis patients with limited cutaneous involvement. Arthritis Rheum 2003, 48:203–209.
49. Ceribelli A, Cavazzana I, Franceschini F, et al.: Anti-Th/To are common antinucleolar autoantibodies in Italian patients with scleroderma. J Rheumatol 2010 Aug 3 (Epub ahead of print).
50. Wassarman KM, Steitz JA: The low-abundance U11 and U12 small nuclear ribonucleoproteins (snRNPs) interact to form a two-snRNP complex. Mol Cell Biol 1992, 12:1276–1285.
51. Fertig N, Domsic RT, Rodriguez-Reyna RT, et al.: Anti-U11/U12 RNP antibodies in systemic sclerosis: a new serologic marker associated with pulmonary fibrosis. Arthritis Rheum 2009, 61:958–965.
52. Raijmakers R, Schilders G, Pruijn GJM: The exosome, a molecular machine for controlled RNA degradation in both nucleus and cytoplasm. Eur J Cell Biol 2004, 83:175–183.
53. Hanke K, Brückner CS, Dähnrich C, et al.: Antibodies against PM/Scl-75 and PM/Scl-100 are independent markers for different subsets of systemic sclerosis patients. Arthritis Res Ther 2009, 11: R22.
54. Mahler M, Raijmakers R: Novel aspects of autoantibodies to the PM/Scl complex: clinical, genetic and diagnostic insights. Autoimmun Rev 2007, 6:432–437.
55. Della Rossa A, Valentini G, Bombardieri S, et al.: European multicentre study to define disease activity criteria for systemic sclerosis. I. Clinical and epidemiological features of 290 patients from 19 centres. Ann Rheum Dis 2001, 60:585–591.
56. Valentini G, Della Rossa A, Bombardieri S, et al.: European multicentre study to define disease activity criteria for systemic sclerosis. II. Identification of disease activity variables and development of preliminary activity indexes. Ann Rheum Dis 2001, 60:592–598.
57. Valentini G, Bencivelli W, Bombardieri S, et al.: European Scleroderma Study Group to define disease activity criteria for systemic sclerosis. III. Assessment of the construct validity of the preliminary activity criteria. Ann Rheum Dis 2003, 62:901–903.
58. • Farina G, Lafyatis D, Lemaire R, Lafyatis R: A four-gene biomarker predicts skin disease in patients with diffuse cutaneous systemic sclerosis. Arthritis Rheum 2010, 62:580–588. *This is one of the few proposed composite measures shown to have a discriminant validity.*
59. Hant FN, Ludwicka-Bradley A, Wang HJ, et al.: Surfactant protein D and KL-6 as serum biomarkers of interstitial lung disease in patients with scleroderma. J Rheumatol 2009, 36:773–780.
60. Allanore Y, Ludwicka-Bradley A, Wang HJ, et al.: N-terminal pro-brain natriuretic peptide as a diagnostic marker of early pulmonary artery hypertension in patients with systemic sclerosis and effects of calcium-channel blockers. Arthritis Rheum 2003, 48:3503–3508.
61. Allanore Y, Borderie D, Avouac J, et al.: High N-terminal pro-brain natriuretic peptide levels and low diffusing capacity for carbon monoxide as independent predictors of the occurrence of precapillary pulmonary arterial hypertension in patients with systemic sclerosis. Arthritis Rheum 2008, 58:284–291.
62. Williams MH, Handler CE, Akram R, et al.: Role of N-terminal brain natriuretic peptide (N-TproBNP) in scleroderma-associated pulmonary arterial hypertension. Eur Heart J 2006, 27:1485–1494.
63. Simeoni S, Lippi G, Puccetti A, et al.: N-terminal pro-BNP in sclerodermic patients on bosentan therapy for PAH. Rheumatol Int 2008, 28:657–660.
64. Dimitroulas T, Giannakoulas G, Karvounis H, et al.: N-terminal probrain natriuretic peptide as a biochemical marker in the evaluation of bosentan treatment in systemic-sclerosis-related pulmonary arterial hypertension. Clin Rheumatol 2008, 27:655–658.
65. Allanore Y, Wahbi K, Borderie D, et al.: N-terminal pro-brain natriuretic peptide in systemic sclerosis: a new cornerstone of cardiovascular assessment? Ann Rheum Dis 2009, 68:1885–1889.
66. Coral-Alvarado P, Quintana G, Garces MF, et al.: Potential biomarkers for detecting pulmonary arterial hypertension in patients with systemic sclerosis. Rheumatol Int 2009, 29:1017–1024.