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Application of Biomarkers to Clinical Trials in Systemic Sclerosis

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Abstract

Important clinical advances in the treatment of systemic sclerosis have been made, yet fibrotic disease remains largely untreatable. Optimal design of clinical trials to test new therapeutics for fibrotic disease features has suffered from dual difficulties in patient selection and patient evaluation. Patient selection for entry into trials for treatment of interstitial lung disease and/or skin fibrosis is challenged by the natural history of the disease, which stabilizes in some patients while relentlessly progressing in others, and our lack of good clinical markers to distinguish between these trajectories. Patient evaluation is made difficult, particularly in skin disease, by the inherent difficulty in quantifying the extent of disease. Biomarkers hold the potential to solve many of these problems as surrogate outcome measures and as markers for disease progression. Identified biomarkers may have the potential to graduate to surrogate outcome singly or, more likely, in combination. Predictive biomarkers are still largely unknown.

Keywords

Scleroderma; Systemic sclerosis; Surrogate outcomes; Subtypes; Biomarkers; Clinical trial; Disease status

Introduction

At a recent National Institutes of Health (NIH) grant review session, one of the other reviewers turned to me and passionately stated that the word biomarker “has no meaning,” his point being that the word has been used too broadly and is too poorly defined to have utility. However, the NIH has actually gone to considerable effort to define a biological marker (ie, biomarker) as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [1]. Although it is perhaps easier to understand, a more restricted definition proposed by Katz [2]—“a laboratory measurement that reflects the activity of a disease process”—captures only part of this NIH definition. These definitions provide an important framework for this review considering biomarkers and the application of biomarkers of several different types to systemic sclerosis (SSc) clinical trials: biomarkers of disease status, biomarkers predicting future disease, and biomarkers of disease subtype. The broader view of biomarkers includes any outcome measure that is not directly related to the patient`s clinical condition, including, for example, radiographic measures, a prominent part of the research portfolio of the Biomarker Consortium [3]. However, for the purposes of

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this review, biomarker discussion is restricted to measurements of biochemical markers in tissues and fluids from patients.

Although the discussion here focuses on the clinical utility and particularly the application of biomarkers to clinical trials, it is important to recognize the intimate relationship between biomarker development and scientific investigation into disease pathogenesis. The more closely a biomarker is associated with the presence or severity of a disease complication, the more likely it is actually involved in disease pathogenesis. Understanding this relationship between biomarker and its role, if any, in pathogenesis is also important in the validation of the biomarker as a surrogate outcome. Biomarkers that correlate highly with disease status but are not directly involved in disease pathogenesis may change with a treatment, but the change may not reflect a change in clinical disease. Thus, utilization of biomarkers in clinical trials as surrogate outcomes measures must be done thoughtfully.

Biomarkers Reflecting Disease Status, Disease Prognosis, or Disease Subtype

Biomarkers can serve several different purposes, but to date, biomarkers for SSc have been easier to develop for some purposes than for others. The first and best developed biomarkers in SSc are assessments of disease status or severity at a point in time. Such biomarkers may prove useful as surrogate outcome measures for clinical trials if they are “validated” to change with accepted clinical disease outcomes.

Several practical features are key to disease status biomarker utility and their promotion to surrogate outcome measure. Most importantly, the biomarker needs to correlate highly with some clinical measure. For markers of disease status, this typically would be the most broadly accepted clinical outcome. For skin disease, it would be the modified Rodnan skin score (MRSS), whereas for interstitial lung disease (ILD), it would be forced vital capacity (FVC). A complete understanding of the biomarker might drill down deeper, examining, for example, the relationship between radiographic evidence of fibrosis versus ground glass for a marker of ILD. However, from the standpoint of developing a surrogate outcome, the relationship to the primary accepted clinical outcome is most important. Relating a biomarker to another surrogate marker (eg, ground glass changes on a high-resolution CT of the chest, or a patient-reported outcome) will not provide the same level of confidence in the utility of the biomarker as a surrogate marker. A second important feature of a surrogate outcome for disease status is its ability to change over time and reflect change in the clinical outcome measure.

Biomarkers of disease prognosis or predictive biomarkers can be thought of simply as extensions of biomarkers of disease status. These biomarkers should correlate with the change in a primary clinical outcome measure over time rather than the point-in-time correlation of a disease status marker. These biomarkers reflect the disease trajectory, progressive or regressive, and thus might more accurately reflect disease activity. For example, a predictive biomarker would correlate with the difference in MRSS at some time in the future compared with baseline. Biomarkers predicting future disease have proven more difficult to identify; currently, we have few studies or leads into these biomarkers. However, this difficulty may lie more in practical obstacles to their discovery, requiring clinically rich longitudinal databases that permit evaluation of a biomarker tested at a point in time to predict future disease progression or regression. The natural history of SSc skin and lung disease makes such biomarkers particularly important to identify so that patients with spontaneously regressive disease are not entered into clinical trials and are not treated aggressively with disease-modifying medications.

A final group of biomarkers are designed to discover heterogeneity in patients with SSc. These studies, to date relying on microarray analyses of skin gene expression, have sought to identify disease subtypes, particularly in patients with diffuse cutaneous disease. Identifying disease heterogeneity may be a key to success in clinical trials, as similar clinical disease might be driven by different mediators and thus respond differently to medications targeting these mediators. This notion has become particularly potent as therapeutics increasingly target specific cytokines. Skin fibrosis might be driven, for example, by interleukin (IL)-13 in one patient, but by transforming growth factor (TGF)-β in another. Ideally, clinical trials in the future might include only patients showing evidence of pathway activation targeted by the trial drug.

The "closer" one can sample to the involved organ, the more likely one is to be able to anticipate finding a correlation with disease status. For example, we might anticipate that lung biopsy and bronchoalveolar fluid (BAL) are more likely to show robust biomarkers of ILD than skin or serum samples. Biomarkers for pulmonary arterial hypertension (PAH) are particularly problematic in this regard, as lung samples are not typically available. In this case, blood, as it circulates through the pulmonary system, might provide the best source for biomarkers.

Serum Biomarkers of Fibrotic Skin Disease

Several proteins known to be upregulated by TGF-β have been examined in serum/plasma. Cartilage oligomeric protein 1 (COMP) has been examined in both skin (see below) and serum (Table 1). This protein, highly regulated by TGF-β, is increased in sera from SSc patients, and the degree of elevation correlates moderately well with the MRSS (*rs*=0.57) [4]. Serum COMP levels change over time, and the change has been shown to correlate with change in the MRSS, indicating a biomarker with many of the features needed for a surrogate outcome measure. Plasma levels of osteopontin, another protein regulated by TGF-β, also correlate weakly with the MRSS, although not as strongly as COMP (*r*=0.3; *P*=0.05) [5•]. Two other proteins that are regulated by TGF-β, thrombospondin-1 (TSP-1) and matrix metalloproteinase 9 (MMP-9), are increased in SSc sera [6]. The degree that serum TSP-1 levels correlate with skin score or lung disease has not been reported, though lesional skin mRNA levels correlate well with the MRSS (r^2=0.32) [7••]. Serum MMP-9 concentrations correlate relatively well with the MRSS (*r*=0.425) [8].

Collagen levels and processing are highly upregulated by TGF-β. Carboxy terminal telopeptide of type I collagen is elevated in patients with diffuse SSc [9], and in one study, it correlated highly with the skin score (*r*=0.646) [10]. In contrast, amino-terminal procollagen I (PINP) did not correlate with the skin score in this study [10]. Amino-terminal procollagen III (PIIINP) levels are also higher in patients with diffuse disease and a predictor of poor survival [11, 12]. In the CAT-192 study, in which collagen propeptides were used as biomarkers, although both PINP and PIIINP levels were elevated in diffuse SSc patients, only PINP levels showed statistically significant changes that correlated with the MRSS (*r*=0.37) [13]. Tissue inhibitor of metalloproteinases-1 (TIMP-1) is also higher in diffuse SSc than limited SSc, though the relationship of TIMP-1 to the skin score has not been reported [14].

The relationship between inflammation and fibrosis is strongly supported by the co-upregulation of profibrotic and proinflammatory biomarkers in sera as well as skin. Chemokines and cytokines have been analyzed in SSc in several studies. Sato et al. [15] showed that interleukin (IL)-6 and IL-10 correlate highly with the MRSS in SSc (*r*=0.625 and *r*=0.663, respectively) [15]. Codullo et al. [16] showed that SSc patients express higher levels of IL-6, IL-1RA, and IL-13 CCL2, CCL3, CCL4, and CXCL8, regardless of disease

subtype, but no clear associations were seen with the MRSS or other clinical parameters. Pentraxin 3, a protein closely related to C-reactive protein, has been shown to correlate with the MRSS (r=0.34) [17].

sCD30, a member of the tumor necrosis factor (TNF) receptor family (TNFR8), is an inflammatory marker elevated in many diseases. sCD30 levels have been shown to correlate moderately with the MRSS (rs=0.53) [18]. Elevated sCD30 and IL-6, particularly in patients with diffuse SSc, were confirmed by Scala et al. [19], though correlations with MRSS were not provided in this study.

In addition to these biomarkers that might be considered parts of inflammatory responses, several markers more associated with B-cell and T-cell activation have been identified in SSc sera. In particular, B-cell–activating factor levels correlate weakly with the skin score (r=0.41) [20]. In addition, levels of a T-cell product, soluble cytotoxic T-lymphocyte antigen-4, correlate with the MRSS (r=0.37) [21].

In at least some studies, markers of vascular injury also correlate with the skin score. These are described more completely below, including von Willebrand factor (vWF) and endothelin-1 (ET-1).

The large number of serum biomarkers for skin disease in diffuse SSc patients suggests that combining measures, particularly measures that are capturing different aspects of pathogenesis, might provide a successful approach to finding a serum biomarker of skin disease robust enough for utilization as a surrogate outcome measure in clinical trials. We have successfully used such an approach to develop a four-gene biomarker of skin disease in diffuse SSc based on multiple linear regression modeling, using several different genes, each of which correlates individually with the MRSS.

mRNA Biomarkers of Skin Disease

During the past several years, our group has studied mRNA expression in skin biopsies from SSc patients. Motivated by our observation that the MRSS correlates highly with the density of myofibroblasts, a cell phenotype highly induced in fibroblasts by TGF-β, in biopsies taken from the mid-forearm [22, 23], we examined expression of genes highly upregulated by TGF-β in SSc skin. We chose to examine genes known from the literature to be highly upregulated by TGF-β in fibroblasts, including plasminogen activator protein-1 (*PAI-1*), *TSP-1*, *COMP*, IV collagen (*COL4*), and connective tissue growth factor (*CCN2*). We found remarkable differences in the degree expression of each of these genes correlated with the MRSS. Expression of some genes in lesional skin correlated highly with the MRSS: *COMP* and *TSP-1* (r^2=0.41 and 0.32, respectively), while expression of other genes correlated highly only in nonlesional skin: *PAI-1* (r^2=0.3). Unfortunately, combining these measures using multiple linear regression modeling added little strength to the correlation of *COMP* alone.

When we expanded our analyses to include interferon-regulated genes, we found that the expression of several interferon-regulated genes also correlated with the MRSS. In contrast to including multiple TGF-β–regulated genes, including both TGF-β– and IFN-regulated genes in multiple linear regression models resulted in strikingly high correlations with the MRSS. The combination of four of these genes, *COMP*, *TSP-1*, *IFI44*, and *SIG1*, gave a particularly striking correlation with the MRSS (r^2=0.89). This biomarker was shown in a small group of patients to change over time with the skin score, thus partially validating it as a candidate surrogate outcome measure.

Markers of Fibrotic Lung Disease

The biomarkers showing the most promise for SSc-associated ILD are surfactant protein-D (SP-D) and Krebs von den Lungen-6 (KL-6 [Table 2]). Several different groups have examined the correlation between these biomarkers and pulmonary function tests, with generally consistent results, showing that these measures are increased in patients with SSc-associated ILD. However, the degree of correlation with pulmonary functions has been quite variable. In one study, SP-D concentrations correlated relatively strongly and inversely with FVC (r=-0.61; P<0.0001) and diffusing capacity of the lung for carbon monoxide (DLCO) (r=-0.70; P<0.0001), with KL-6 levels correlating less strongly and inversely with FVC (r=-0.33; P<0.05) and DLCO (r=-0.34; P<0.05) [24]. A subsequent study also showed that SP-D levels correlated inversely—though less strongly than reported in the first study—with FVC (r=-0.278) [25]. Finally, a recent study of patients entered into the Scleroderma Lung Study showed a much weaker and not statistically significant correlation of SP-D and KL-6 with FVC (SP-D, r=-0.15; KL-6, r=-0.17), with a statistically significant but still weak correlation of these measures with the DLCO (SP-D, r=-0.21; KL-6, r=-0.29); this study used a nonparametric correlation (Kendall tau b correlation coefficients) [26]. The differences between these correlations may have to do with any variety of factors, but the value of a biomarker as a surrogate outcome measure with an r=-0.15 will have limited value, even if it could be combined with other measures into a composite. Thus, the value of these well-studied measures remains uncertain even now.

Several chemokines and cytokine markers also have been examined as biomarkers of ILD. Serum pulmonary and activated chemokine (PARC/CCL18) levels correlate negatively with the TLC (r=-0.66 [27]) or weakly negatively with the FVC (r=-0.244 and DLCO, r=-0.326) [28]. IL-15 levels also have been shown to correlate negatively with the FVC (r=-0.040) [29]. YKL-40, the human homolog of murine chitinase 3-like-1, is induced by and mediates downstream effects of IL-13, particularly induction of alternatively activated macrophages and tissue fibrosis [30]. YKL-40 is increased in SSc patients and particularly elevated in patients with decreased DLCO but is also higher in patients with reduced FVC [31]. YKL-40 is thus particularly interesting as a possible marker for IL-13–mediated fibrosis. As mentioned above for skin, TIMP-1 levels have been shown to correlate weakly and negatively with DLCO (r=-0.28) [14]. Also as seen for skin, serum levels of pentraxin 3 correlate negatively with FVC (r=-0.44) [17].

Several markers for oxidative stress are increased in SSc-associated lung disease. 8-isoprostane is increased in the sera of SSc-associated ILD, also correlating negatively (r=-0.42) [32]. Another biomarker of oxidative stress measured in the urine, 8-isoprostaglandin-F2a, correlates with DLCO (r=-0.044) [33]. Although individually, these several correlations would be weak as surrogate outcome measure, these biomarkers might be effectively used together to develop a multiple regression model as we describe for skin mRNA expression. The several different pathways that appear to be captured with these various biomarkers, epithelial injury (KL-6, SP-D), IL-13 (YKL-40), inflammation (pentraxin 3, IL-15), and cell migration (CCL18), might provide a strong biomarker when combined.

Bronchoalveolar Lavage Biomarkers of Interstitial Lung Disease

BAL fluid provides a source for biomarkers that is more closely in contact with lung tissues. A recent study examining a broad array of interleukins showed that IL-4, IL-8, and CCL2 levels correlated negatively with the FVC (r=-0.503, r=-0.394, and r=-0.441, respectively) [34]. These correlations are higher than seen for most serum markers of ILD; however, BAL

is difficult as a source for repeated measure and may not be required to develop a robust marker for ILD.

Thus, a great need remains for biochemical surrogate outcomes in SSc-associated lung disease, although one could argue that the FVC, the current clinical outcome, is sufficiently reproducible to drive ongoing clinical trials. A greater need for SSc-associated ILD is a biomarker that predicts future disease progression versus regression. Unfortunately, no good candidates for such a biomarker currently exist.

Markers of Pulmonary Vascular Disease in Systemic Sclerosis

Vascular disease in SSc affects multiple organs, with PAH currently the most life-threatening vascular outcome and the easiest to assess quantitatively. Unfortunately, most studies examining biomarkers for this complication have relied on echocardiographic measures of pulmonary artery systolic pressure, and this modality is known to be quite vulnerable to significant error. Despite this limitation, several biomarkers of vascular disease and PAH have been studied and look hopeful.

Kuryliszyn-Moskal et al. [35] have shown that serum levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble E-selectin, vascular endothelial growth factor (VEGF), and ET-1 are higher in SSc patients. ET-1 is elevated in SSc patients with both limited and diffuse disease, and on average higher in limited SSc patients with PAH than those without [36]. ET-1 levels also correlate weakly with the skin score (r=0.2) and weakly and negatively with the DLCO (r=-0.24) measured in both SSc PAH and ILD patients [36], although these weak correlations have not been seen in all studies [37]. In another study, ET-1 levels again correlated weakly with the PAH (rs=0.34, by echocardiography) [38].

Other markers of vascular injury have been examined in SSc patients. vWF has been shown in several studies to be elevated in SSc patients. Scheja et al. [39] showed that both vWF and its propeptide correlated with skin score (r=0.39 and 0.40, respectively), but not with pulmonary or renal function. vWF, but not its propeptide, also correlated positively with pulmonary pressure (r=0.42), though the means of measuring pulmonary arterial pressure was not provided [39]. Serum intercellular adhesion molecule 1 (ICAM-1) is similarly elevated in both limited and diffuse SSc patients. However, rapidly progressive disease, defined as less than 12 months' duration with truncal involvement, showed the highest levels, suggesting that this might be a marker for disease progression [40]. s-IL2R was also shown to be elevated in SSc in this study but was not found to be associated with particular subtype or organ involvement. In one study, sCD40L correlated with pulmonary arterial pressure as estimated by Doppler echocardiography (r=0.41) [41].

These studies are consistent with recent observations by our group evaluating peripheral blood mononuclear cell gene expression and serum biomarkers in limited SSc patients with or without PAH as assessed by right heart catheterization [42••]. Serum biomarkers were assessed using a multianalyte approach measuring 90 cytokines. Remarkably, most of the biomarkers discussed above were found to show increased expression in PAH patients: IL-6, IL-10, ICAM-1, VCAM-1, sCD40, TIMP-1, VEGF, and vWF. The combination of serum levels of these cytokines permitted the clustering software to effectively distinguish limited SSc patients with and without PAH.

Biomarkers Predicting Future Disease

Identification of biomarkers of future disease remains the next great frontier in SSc biomarker development. As mentioned above, these are key for proper clinical management of patients and also for helping select patients for drug trials. Current methods for selecting

patients destined to have progressive skin or lung disease are inadequate and require larger trials, effectively blocking efficient drug development.

Currently, we may have some hints as to what these biomarkers may look like. The study cited above found that ICAM-1 is similarly elevated in both limited and diffuse SSc patients, but that patients with “rapidly progressive disease,” defined as less than 12 months’ duration with truncal involvement, showed the highest levels [40]. Unfortunately, this vague description provides little confidence but perhaps a reasonable place to start.

Biomarkers of Disease Heterogeneity

Our current, relatively rudimentary understanding of SSc pathogenesis has been provided with a needed shot in the arm by application of gene expression technology to skin samples. The extraordinary amount of information derived from such studies in some ways overshadows the many years of previous work. This noted, this is not a panacea, and the results must be analyzed very carefully to make sense of the confusing number of alterations in gene expression. Interpretation is also fraught with potential bias, as important events may not register highly at a gene expression level, whereas relatively unimportant pathogenic events might cause relatively large changes in gene expression. Despite these limitations, gene expression analysis of SSc skin, largely led by Whitfield, has made several remarkable observations and promises to provide many more important advances, particularly as these analyses are increasingly applied to samples obtained from clinical trials.

The most important aspect of these observations as applied to clinical trials is the division of patients into subtypes of disease based on gene expression. Confirming the clinical division, patients with limited versus diffuse SSc can be easily separated based on differences in gene expression. However, remarkably, patients with diffuse disease can be further separated into several well-defined groups by consistent differences in gene expression [43]. These subgroups of diffuse SSc patients appear to have different gene signatures associated with them, suggesting that some patients, for example, have a disease process that is being driven more strongly by TGF-β [44]. As these observations are applied to clinical trials in the future, we may expect to see selection of patients prior to entering therapeutic trials based on blocking of specific pathways indicated by patterns of gene expression. Eventually we anticipate a day not so distant when a skin biopsy will dictate the proper treatment of skin disease and perhaps also inform us about proper treatment of lung disease.

Other Biomarkers: Autoantibodies and Genetic Associations

Although discovery of new diagnostic biomarkers has utility in SSc, particularly in organ involvement that is otherwise challenging to evaluate, such as PAH or gastrointestinal hypomotility, this review instead focuses on biomarkers showing value as outcome measures or for other use in clinical trials. In addition, this review does not discuss SSc-associated autoantibodies or SSc genetic associations. Even though autoantibodies in some cases show useful associations with disease complications and thus can in these cases be considered (typically weak) prognostic biomarkers, they do not provide, at least as single measures, quantitative information to make them useful for assessing the extent of end organ disease. In addition, they are essentially static measures, with no evidence that changes in autoantibody titer over time are useful as outcomes for end organ demise or recovery. Genetic associations currently described in SSc patients, as for autoantibodies, are static measures. They may be found to have predictive value for future complications, but they cannot change over time to reflect the extent of end organ disease. An important proviso of these comments is that combinations of biomarkers, including genetic and/or autoantibody status, may provide far greater utility than single measures; thus, these biomarkers might serve key roles in more complex models for assessing disease status and prognosis.

Biomarkers as Surrogate Outcome Measures: The US Food and Drug Administration and the Regulatory Environment

The key role biomarkers may play in drug development in SSc has not been fully appreciated. The US Food and Drug Administration (FDA) has provided an increasingly strong regulatory framework, empowering the use of biomarkers in clinical trials. Understanding this regulatory environment requires understanding certain terminology. Typically, the FDA approves a medication based on its meeting a primary outcome measure —"the specific key measurement(s) or observation(s) used to measure the effect of experimental variables in a study" [45]. A clinical outcome measure—defined as "a characteristic or variable that reflects how a patient feels, functions or survives"—is the most widely accepted standard for primary outcome measure. However, surrogate outcomes also have been widely used and likely will play increasingly important roles in clinical trials in the future as our biological understanding of disease pathogenesis and associated biochemical changes improves.

A surrogate end point is "a biomarker intended to substitute for a clinical end point," or stated more completely in the FDA "accelerated approval" regulations, "a surrogate end point, or 'marker,' is a laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful end point that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy" [46]. A surrogate end point is "expected to predict clinical benefit (or harm, or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic or other scientific evidence" [2]. Surrogate end points may be used to support "accelerated approval" of a drug, and in some cases, surrogate end points are acceptable for full approval, such as blood pressure and serum cholesterol—surrogate outcomes for cardiovascular disease [47].

Biomarkers of disease, including radiographic, biochemical, or other measures, become surrogate markers of disease through various validation processes. Correlation between the biomarker and a clinical outcome is a first step in validation, but further validation requires scientific insight into the relationship between the biomarker and the disease process being measured, but more importantly, the responsiveness of the biomarker to change with a therapeutic intervention that affects the clinical outcome. Unfortunately, this level of rigor has been achieved only rarely for biomarker validation, and thus, few biomarkers have been accepted as surrogate outcomes for FDA approval without supporting clinical outcomes, serum cholesterol being the best example.

Thus, a biomarker is a "candidate" surrogate measure [2]. The FDA has been particularly encouraging the utilization of biomarkers as a means for increasing the pace of drug discovery ("the use of either biomarkers or surrogate markers in early phases of drug development is, from a regulatory perspective, noncontroversial") [2]. Accelerated approval, intended for serious or life-threatening illnesses without effective available therapies, permitted drug approval for the first time based on the effect of the drug on a surrogate marker and not a clinical outcome. Although accelerated approval for drugs is predicated upon follow-on trials with clinical outcomes, approval under this regulation can be based upon "unvalidated" surrogate outcomes, indicating the value the FDA places on biomarkers as potential measures for rapid approval of drugs for untreatable serious illnesses. The life-threatening nature of SSc would appear to qualify it for accelerated drug development based on a surrogate biomarker outcome approach.

Aside from the use of surrogate outcomes in early-phase development and accelerated approval, the FDA recently formulated a draft document providing guidance for qualification of "drug development tools (DDTs)" [48•]. DDTs addressed in this document

are patient-reported outcomes and biomarkers for which the FDA provides an application process for "qualification" of biomarkers that includes interacting with investigators to define benchmarks for validating the patient-reported outcome or biomarker. Qualified DDTs could then be used in investigational new drug and new drug applications by drug developers without further Center for Drug Evaluation and Research review. Appropriately, the bar is set relatively high for qualification of biomarkers as surrogate outcome measures ("robust scientific evidence is needed to justify qualification of a biomarker for broad use as a surrogate endpoint") [48•].

Conclusions

Biomarkers hold great promise as aids to clinical trials in SSc and under the current regulatory paradigms might even drive drug development. Biomarker development for such applications requires a greater awareness of their value but also a greater awareness about the kinds of studies that are required to validate biomarker utility. These include careful comparisons to well-defined clinical outcome measures and consideration for combining measures into composites based on modeling. Many biomarkers of fibrosis and vascular injury are ready for such careful characterization and should soon provide a stronger basis for accelerating drug discovery in SSc as they are promoted to surrogate outcome measure. Biomarkers for disease trajectory are still largely unknown and urgently needed as we continue to guess who will get worse and who will stabilize or improve. Microarray gene expression biomarkers may soon provide novel methods for patient selection and analysis of efficacy in clinical trials. These biomarker approaches must be integrated into future clinical trials.

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Table 1

Biomarkers of skin disease in systemic sclerosis[a]

Biomarker group	Biomarkers of skin disease–MRSS	Correlation with MRSS, *r*
Fibrotic	COMP	0.57
	Osteopontin	0.3
	Thrombospondin-1	?
	Matrix metalloproteinase-9	0.425
	TIMP-1	?
Collagen	PICP	0.646
	PINP	?
	PIIINP	?
Immune	Interleukin-6	0.625
	Interleukin-10	0.663
	Pentraxin 3	0.34
	sCD30	0.53
	BAFF	0.41
	sCTLA-4	0.37
Vascular	von Willebrand factor	0.39
	Endothelin-1	0.2

BAFF—B-cell–activating factor; COMP—cartilage oligomeric protein 1; MRSS—modified Rodnan skin score; PICP—carboxy-terminal telopeptide of type I collagen; PINP—amino-terminal procollagen I; PIIINP—amino-terminal procollagen III; sCTLA-4—soluble cytotoxic T-lymphocyte antigen-4; TIMP-1—tissue inhibitor of metalloproteinases-1

[a]Fibrotic markers refer to proteins known to be regulated by transforming growth factor-β. Collagen markers are aminoterminal (PINP, PIIINP) and carboxy-terminal (PICP) propeptides of type I (PICP) or type III collagen (PIIINP). Immune and vascular markers are known products from leukocytes and endothelial cells, respectively

Table 2

Biomarkers of lung disease in systemic sclerosis[a]

Biomarker group	Biomarkers of lung disease–FVC	Tissue	FVC correlation, *r*[b]
Epithelial cells	Surfactant protein-D (SP-D)	Serum	-0.61, -0.278, -0.15
	Krebs von den Lungen-6 (KL-6)	Serum	-0.33, -0.17
Immune	PARC	Serum	-0.66, -0.244
	Interleukin-15	Serum	-0.40
	YKL-40	Serum	?
	TIMP-1	Serum	?
	Pentraxin 3	Serum	-0.44
Oxidative stress	8-isoprostane	Serum	-0.42
	8-isoprostaglandin-F2a	Urine	-0.44
Immune	Interleukin-4	BAL	-0.503
	Interleukin-8	BAL	-0.394
	CCL2	BAL	-0.441

BAL—bronchoalveolar lavage; FVC—forced vital capacity; TIMP-1—tissue inhibitor of etalloproteinases-1

[a]Epithelial cell markers refer to proteins known to be secreted by type II pneumocytes. Immune markers are known products from leukocytes, and oxidative stress proteins are known to be increased in cells undergoing oxidative stress

[b]Columns showing several different correlations refer to different studies described in the text