Introduction

Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterized by excessive collagen deposition and vascular injury in the skin and internal organs (1). The presence of specific circulating autoantibodies such as anti-topoisomerase I antibody, anticentromere antibody, and anti-RNA polymerase (RNAP) III antibody is a common identifying feature and usually precedes disease onset. Although the pathogenesis of SSc remains unclear, a variety of cells and their products are likely contributing to the development of SSc (Figure 1).

Classification of SSc is divided into two subsets: limited cutaneous systemic sclerosis (lcSSc) and diffuse cutaneous systemic sclerosis (dcSSc) (2). lcSSc is characterized by skin thickening that is restricted to areas distal to the elbows and knees. On the other hand, dcSSc involves skin thickening proximal to the elbows and the knees in addition to distal areas. On the whole, dcSSc patients have more severe internal organ involvement compared with lcSSc patients.

The representative natural course of SSc has been demonstrated by dividing these 2 subsets into early, intermediate, and late stages (3). Patients with dcSSc generally show progressive skin thickening in early stage (symptoms< 3 years). During this period, patients frequently develop joint contracture, gastrointestinal tract involvement, interstitial lung diseases (ILD), heart disease, and renal crisis. Skin thickening of dcSSc usually begin to regress at intermediate (symptoms 3-6 years) and especially at late stage (symptoms> 6 years). By contrast, skin thickening is restricted to the distal extremities for many years in lcSSc. Although the involvement of joint contracture, ILD, and renal crisis are rare, PAH or malabsorption can be detected in late stage (symptoms >10 year) but usually not in early (symptoms< 5 years) or intermediate stage (symptoms 5-10 years) of lcSSc.

ILD (also know as pulmonary fibrosis) and pulmonary arterial hypertension (PAH) are current major causes of SSc-related mortality. According to the cohort of Pittsburg university, the 10-year survival improved from 54% in the 1970s to 66% in the 1990s (4). The cause of SSc-related deaths changed from the 1970s to the 1990s. Whereas scleroderma renal crisis was significantly reduced (42% to 6%), pulmonary fibrosis was significantly increased (6% to 33%) as the cause of SSc-related death over the 30-year time period (4). Additionally, joint contracture due to extensive skin sclerosis, refractory digital ulcers, and other severe internal organ involvements result in impaired physical function.

In fact, severity of each organ involvement, natural course, therapeutic response, and prognosis are heterogeneous among SSc patients. If the extensive tissue fibrosis or severe vascular injury has once developed, it is hard to recover the disability. Therefore, early treatment should be started in patients who have high risk for disease progression. Thus, biomarkers that can predict disease severity, activity, the response to therapy, and prognosis are critical and necessary for the medical examination. Furthermore, assessing and identifying biomarkers can result in the understanding of SSc pathogenesis. Intense investigations have been performed about biomarkers of SSc (5-9). In this review, I will discuss about major candidates for biomarkers of SSc.

Serum autoantibodies

Autoantibodies are current most reliable biomarkers for diagnosis, classification, and predicting specific clinical features of SSc (8) (5). It has been well known that 80 to 90% of SSc patients have one of SSc-specific or SSc-related serum autoantibodies and rarely have more than one of those autoantibodies. Importantly, these antibodies are closely associated with clinical features of each SSc patient. Among those autoantibodies, highly-specific antibodies for SSc include anti-topoisomerase I Ab, anti-RNAP III Ab, anti-U3 RNP Ab, and

anti-Th/To Ab (Figure 2). Although anticentromere Ab is well known as SSc-specific Ab, about 30% of this Ab-positive persons do not develop SSc. Anti-U1 RNP Ab is not specific for SSc and SSc patients with this Ab often overlap partially or totally with systemic lupus erythematosus or myositis (polymyositis/dermatomyositis). Among these autoantibodies, anti-topoisomerase I Ab, anti-RNAP III Ab, and anti-U3 RNP Ab are generally detected in dcSSc patients. Anti-topoisomerase I Ab is associated with severe ILD and thereby the prognosis is generally poor. Anti-RNAP III Ab is associated with scleroderma renal crisis and is often associated with primary malignancies. Patients with anti-U3 RNP Ab can develop cardiomyopathy, myopathy, and PAH. On the other hand, anticentromere Ab and anti-Th/To Ab are usually detected in lcSSc. About 10% of anticentromere Ab-positive SSc develop PAH and anti-Th/To Ab is associated with PAH and ILD.

It is generally accepted that presence of autoantibodies precedes the development of clinical symptoms in collagen diseases including SSc. A study of 288 isolated Raynaud's phenomenon patients demonstrated that nailfold capillaroscopic findings and the presence of antinuclear antibodies were predictive factors for future development of SSc symptoms (10). Abnormal findings of nailfold capillaroscopy together with an SSc-specific autoantibodies (anticentromere, anti-Th/To, anti-topoisomerase I, and anti-RNAP III) indicate a very high probability of developing definite SSc in a prospective study of 586 patients with isolated Raynaud's phenomenon (11). In a prospective study of 266 patients with early SSc, only the presence of anti-topoisomerase I Abs was associated with differential forced vital capacity (FVC) levels, predicting the rate of decline in FVC within the first 3 years of follow-up (12). In the Australian Scleroderma Cohort Study of 451 SSc patients (69 (15.3%) patients was positive for anti-RNAP III), anti-RNAP III were independently associated with renal crisis, diffuse skin thickening, joint contractures, and malignancy diagnosed within 5 years of onset of SSc skin change (13). Thus, characterization of autoantibodies in SSc is helpful for

assessing the clinical presentation and predicting for organ involvement, complication, and prognosis.

Growth factors and cytokines

Transforming growth factor (TGF)-β

Amounts of previous findings suggest that TGF-β is the central player in the pathogenesis of SSc, via regulating tissue fibrosis, inflammation, and vascular biology (14) (15). The main producer of TGF-β is likely macrophages, but many kinds of cells including platelets, leukocytes, and fibroblasts can produce TGF-β. Integrin, plasmin, thrombin, and thrombospondin mediate latent TGF-β activation. Although the Smad pathway has crucial roles in signaling from the TGF-β receptor, non-Smad pathways are also important for TGF-β-dependent fibrogenesis (16).

The expression of TGF-β-dependent genes was overexpressed in skin lesions of SSc patients by DNA microarray analysis (17). Another DNA microarray analysis study of the skin demonstrated that the TGF-β-responsive gene signature is expressed highly in dcSSc patients but not found in patients with lcSSc, morphea, or in healthy controls (18). Investigation of affected tissue samples may be the best method to identify biomarkers, but serial biopsies are impractical. Circulating TGF-β levels are not consistent dependent on each study, this may be due to difficulties in accurate measurements of active TGF-β and its complex regulation and short half-life in biological fluids (19).

It remains unclear which isoform of TGF-β is critical for the development of SSc. A multicentre, randomized, placebo-controlled trial of recombinant Ab that neutralizing

TGF-β1 did not have significant effect for modified Rodnan total skin thickness score (MRSS) (20). However, a recent open-label trial of fresolimumab, a neutralizing antibody that targets all 3 TGF-β isoforms, markedly reduced MRSS in patients with early dcSSc (21).

Connective tissue growth factor (CTGF)

CTGF/CCN2, a cysteine-rich 40k Da multicellular growth factor, is another crucial factor for the development of SSc (22). CTGF is undetectable in normal tissue, but is expressed in fibroblasts stimulated by TGF-β and IL-4. CTGF enhances fibroblast proliferation and extracellular matrix production as a downstream mediator of TGF-β1. However, the nature of the cellular CTGF receptors and its fibrotic mechanism remain unclear. Transgenic mice overexpressing CTGF develop scleroderma-like skin fibrosis and microvascular change (23). A series of studies of mouse model suggest a two-step hypothesis for developing fibrosis in SSc: TGF-β1 induces fibrosis in the early stage and afterwards CTGF contributes maintaining the fibrotic state (24). Serum CTGF levels were significantly elevated in patients with SSc, and correlated with the extent of skin sclerosis and the severity of pulmonary fibrosis (25). N-terminal cleavage products of CTGF but not whole and C-terminal CTGF were increased in the plasma and dermal interstitial fluid of SSc patients and correlated positively with severity of skin sclerosis and negatively with disease duration (26). In a recent study, a neutralizing antibody that targets all 3 TGF-β isoforms significantly reduced CTGF gene expression in the skin of SSc patients (21).

Interleukin-6

IL-6 is a multifunctional cytokine that regulates immune responses and induces acute phase responses. Despite the critical physiological activities of IL-6, excessive production of IL-6 is pathologically involved in various immune-mediated inflammatory diseases, including rheumatoid arthritis. Additionally, IL-6 has been investigated as a potential biomarker of SSc.

Augmented IL-6 expression was observed in fibroblasts, mononuclear cells and endothelial cells of the skin in patients with early dcSSc (27). Previous studies have reported that serum IL-6 levels were significantly associated with the extent of skin thickness (27, 28). Elevated IL-6 expression in early dcSSc patients tended to associate with more severe skin involvement at 3 years and poor prognosis (27). In a recent study, a panel of 8 serum cytokines (IL-6, IL-8, IL-10, CCL2, CXCL10, CX3CL1, fibroblast growth factor 2, and vascular endothelial growth factor) were assessed by Luminex bead technology in exploratory cohorts of 74 patients with SSc and 58 patients with idiopathic pulmonary fibrosis (29). Among those cytokines, only serum IL-6 was an independent predictor of the diffusion capacity for carbon monoxide (DLco) decline in both SSc-ILD and idiopathic pulmonary fibrosis.

Chemokines

CCL2 (MCP-1)

Since chemokines can be detected easier in peripheral blood compared with cytokines or growth factors, the utility as biomarkers of SSc have been investigated in a variety of chemokines. CCL2 (monocyte chemoattractant protein-1;MCP-1) may be one of representative candidates of SSc biomarkers. CCL2 is produced by macrophages, fibroblasts, endothelial cells and other cells and predominant chemoattractant and activator of monocytes and T cells. CCL2 induces Th2 cell polarization (30) and stimulates collagen production by fibroblasts via specific receptors and endogenous upregulation of TGF-β expression. (31). Serum CCL2 levels are elevated in SSc patients and has been found to associate with the presence of ILD (32-34). Expression of CCL2 mRNA was most augmented among 4507 genes when bronchoalveolar lavage (BAL) cells from SSc inflammatory lungs were

compared with controls (35). Consistent with this, protein levels of CCL2 are also increased in BAL fluids from SSc patients with lung inflammation (35). CCL2 concentrations in BAL fluids were associated with the presence of ILD and correlated negatively with lung function parameters and positively with computed tomography scores (36). In a single center prospective study that measured CCL2, CXCL8, CXCL9, CXCL10, CXCL9 in addition to 6 cytokines (IL-2, IL-4, IL-6, IL-10, tumor necrosis factor-α, interferon (IFN)-γ), serum CCL2 levels were elevated at baseline and declined year and year, along with improvement of skin sclerosis (37). The variations of CCL2 were significantly associated with the variations of MRSS and %VC during 3 years.

CXCL4 (PF-4)

CXCL4 (platelet factor 4; PF-4) is chemotactic for neutrophils, monocytes, and fibroblasts, and may have important roles in inflammation and wound repair. Recently, a proteome-wide analysis has demonstrated that CXCL4 is the predominant protein produced by SSc-derived plasmacytoid dendritic cells (38). Plasma CXCL4 levels were remarkably elevated and highly associated with skin and lung fibrosis and PAH in patients with SSc. Additionally, their data indicated plasma CXCL4 levels are useful to predict the disease progression in SSc (38). In addition to its antiangiogenic activity, CXCL4 stimulates the expression of profibrotic cytokines including IL-4 and IL-13, and inhibits the expression of the antifibrotic IFN–γ. CXCL4 exhibited direct effects for inducing SSc phenotype both in vitro and vivo (38), suggesting its critical roles in the development of SSc.

IFN-inducible chemokine

A recent study proposed that IFN-inducible chemokine score may be a promising biomarker of SSc (39). The composite chemokine score of plasma levels of CXCL10 (IFN-γ-inducible protein 10; IP-10) and CXCL11 (IFN-inducible T cell α chemoattractant;

I-TAC) was elevated in SSc patients and showed a correlation with the IFN gene expression signature in 266 patients with SSc. This IFN-inducible chemokine score correlated with the Medsger Severity Index, particularly with the severity of lung, skin, and muscle involvement.

Adhesion molecules

Endothelial cell injury induces adhesion molecules and this may result in further endothelial damage via recruiting inflammatory cells. Several previous studies have demonstrated that SSc fibroblasts exhibit increased surface intercellular adhesion molecule (ICAM) -1 expression, suggesting an augmented potential for binding to inflammatory cells (40). Increase of circulating ICAM have been detected in patients with SSc by several studies (41-43). Circulating ICAM-1 levels were especially elevated in SSc patients with diffuse rapidly progressive disease or digital ulcers (41). Circulating ICAM-1 levels were significantly higher in dcSSc patients and were correlated with the presence of contracture of phalanges, pulmonary fibrosis, joint involvement, and increased erythrocyte sedimentation rate (43). Serum levels of ICAM-1, P-selectin, VCAM-1, and to a lesser degree, E-selectin correlated well with their in situ expression and with clinical disease activity (42). Serum levels of circulating E-selectin, ICAM-1 and VCAM-1 were elevated in patients with scleroderma renal crisis (44). Serum levels of ICAM-1, VCAM-1, and E-selectin, were initially elevated and significantly reduced after infusions of iloprost (prostacyclin analogue) in patients with SSc-related Raynaud's phenomenon (45). In a study of small population, serum levels of ICAM-1, VCAM-1, P-selectin, and platelet endothelial cell adhesion molecule -1 were elevated in SSc patients at baseline and fell to normal levels after 12 months of bosentan therapy (46).

Vascular biomarkers

Vascular injury is one of the earliest clinical features of SSc. Microangiopathy is characterized by the remarkable loss and an irregular chaotic architecture of capillaries and small vessels that lead to chronic tissue hypoxia. Despite tissue hypoxia, a sufficient compensative angiogenesis is not found in SSc. An imbalance between angiogenic and angiostatic factors might explain the pathogenetic mechanisms of SSc vasculopathy (47). Endothelial damage leads to vascular fibroproliferative lesions in multiple organs, and can result in critical organ injury such as PAH and renal crisis. Numerous molecules have been suggested as potential biomarkers for endothelial cell injury in SSc. These includes vascular endothelial growth factor (VEGF), von Willebrand factor (vWf), endothelin-1, thrombomodulin, thrombospondin, brain natriuretic peptide (BNP), N-terminal propeptide of pro BNP (NT pro-BNP), endostatin, plasminogen activator, prostacyclin, thromboxane and nitrous oxide circulating metabolites.

VEGF

To identify possible vascular biomarkers, circulating regulators of angiogenesis have been investigated in amounts of previous studies. Vascular endothelial growth factor (VEGF) in addition to platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), and placental growth factor (PlGF) were significantly elevated in patients with SSc (48). Serum levels of VEGF were significantly elevated in SSc patients and the concentrations were especially elevated in SSc patients with systemic organ involvement (49). In other studies, serum levels of VEGF were elevated and correlated with shorter disease duration (50), systolic pulmonary artery pressure (51), skin sclerosis, and the capillary density of nailfold (52). On the other hand, there is a study that did not find any association between plasma VEGF levels and disease activity (53). Thus, the associations with clinical features are different dependent on each study, although circulating VEGF levels are generally elevated in

SSc patients. It has been proposed that chronic and uncontrolled VEGF upregulation that is mediated by an orchestrated expression of cytokines is the cause of the disturbed vessel morphology in the skin of SSc (54). Furthermore, impaired VEGF receptor signaling may also be contributing to vascular disturbances in SSc (55). A recent PRISMA-driven systemic review demonstrated that lower VEGF serum levels are present in SSc patients with digital ulcers, although significantly higher serum VEGF levels are found in early SSc patients without digital ischemic manifestations (56). Therefore, VEGF may be protective against ischemic manifestations.

Von Willebrand factor

There are many studies regarding the elevation of von Willebrand factor (vWf) in peripheral blood of SSc patients and Raynaud's phenomenon patients (57-60). Whereas plasma vWf levels were significantly increased in SSc, the vWf concentration was associated with disease severity (58), early pulmonary involvement (59), and the extent of radiologically demonstrated ILD (60). In one study, serum vWf levels were able to predict the future development of elevated pulmonary arterial pressure by logistic regression models in patients with lcSSc (61). vWf cleaving protease ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) was significantly decreased in patients with SSc (62).

Angiostatic molecules

In addition to the elevations of angiogenic factors, elevated levels of circulating angiostatic molecules have been reported. These angiostatic factors include angiostatin, endoglin,

endostatin, thrombospondin, and VEGF receptor. Serum levels of endostatin were not significantly elevated but associated with the presence of giant capillaries in nailfold capillaroscopy (50). Plasma levels of endostatin was markedly elevated in patients with SSc and correlated positively with right ventricular systolic pressure (48). In multivariate analysis of a large SSc cohort, soluble endoglin levels were significantly increased in SSc patients with cutaneous ulcerations, positive for anticentromere Ab, and with abnormal diffusing capacity for carbon monoxide (DLco) divided by alveolar volume (63). Serum soluble endoglin levels were significantly elevated in patients with lcSSc compared with dcSSc and systemic lupus erythematosus patients as well as normal controls (64). In that study, patients with elevated soluble endoglin levels had telangiectasia more frequently than those with normal soluble endoglin levels. Furthermore, pulmonary artery pressure was positively correlated with soluble endoglin levels in patients with lcSSc. Endoglin gene encodes a transmembrane glycoprotein which acts as an accessory receptor for the TGF-β superfamily, and is crucial for maintaining vascular integrity. The endoglin gene mutations are responsible for one of the two types of hereditary haemorrhagic telangiectasia (Osler–Weber–Rendu syndrome), a Mendelian autosomal vascular disorder (65). Interestingly, it has been reported that the polymorphism of endoglin gene is associated with SSc-related PAH (66).

Biomarkers of interstitial lung disorders

KL-6 and SP-D

ILD has become main cause of SSc-related death in SSc. Serum biomarkers of ILD is critical for monitoring patients with SSc, since the evaluation using computed tomography (CT) scan cannot be frequently performed. Serum biomarkers of ILD has been focused on soluble proteins secreted by alveolar epithelial cells (the main targeted cells of ILD), and various inflammatory cytokines, chemokines, and other proteins. The glycoprotein Krebs von den

Lungen-6 (KL-6) and surfactant protein-D (SP-D) may be currently the most reliable serum markers for ILD. KL-6 antigen is expressed mainly by alveolar type II pneumocytes and respiratory bronchiolar epithelial cells (67), whereas SP-D is produced and secreted by alveolar type II pneumocytes and Clara cells (68). Several studies revealed that serum levels of KL-6 and SP-D are elevated in serum from patients with ILD, including SSc-related ILD (67, 69) (70). These studies suggested that serum levels of SP-D and KL-6 are serologic markers of the severity and activity of ILD in SSc (70-72). In a comparative study, SP-D was more sensitive, but less specific for ILD than KL-6 in SSc patients (72). Combined use of these two markers would be more effective for diagnosis and monitoring of ILD activity in SSc patients than single use of each marker. A cohort study of Scleroderma Lung Study Research Group demonstrated that highly sensitivity and specificity of both KL-6 and SP-D are for the determination of "alveolitis." In that study, KL-6 and SP-D were significantly correlated with maximum fibrosis scores, but not with maximum ground-glass opacities, on high-resolution CT (71). Therefore, KL-6 or SP-D does not necessarily reflect the activity of ILD in a part of patients.

CCL18

CCL18, which is also known as pulmonary and activation regulated chemokine (PARC) is constitutively expressed at high levels in the lungs and is selectively chemotactic for T cells (73). CCL18 in high concentrations directly stimulates intracellular signaling and collagen production in primary pulmonary fibroblasts (74). Serum CCL18 levels were markedly elevated in associated with the development of ILD as well as with reductions in VC and DLco, and correlated closely with the activity of ILD (75). Furthermore, CCL18 has been demonstrated as a predictive marker for the identification of patients with a higher risk of subsequent lung disease worsening in SSc (76). In an independent cohort, it has been demonstrated that serum CCL18 is a significant predictor of mortality and progression of ILD

in patients with SSc (77). Furthermore, CCL18 production by BAL cells and serum CCL18 concentrations reflected pulmonary fibrotic activity in SSc patients with ILD (78). However, a recent study reported that SP-D correlated with concomitantly obtained forced VC, while CCL18 was a predictor of short-term decline in forced VC (79). Neither SP-D nor CCL18 was a longterm predictor of forced VC course in patients with early SSc in that study.

Biomarkers of pulmonary arterial hypertension

Brain natriuretic peptide (BNP)

PAH is a life-threating organ involvement if the condition has once progressed. However, there are currently no validated biomarkers that are useful for specific diagnosis of PAH. The DLco decrease years prior to the diagnosis of PAH in patients with lcSSc (80). Serum levels of BNP and NT pro-BNP have been reported as useful biomarkers for PAH since they tend to increase in SSc patients with early PAH and correlate with estimated pulmonary arterial pressure (81, 82). BNP and NT pro-BNP are secreted by ventricular myocytes reflecting myocardial responses to stretch, hypoxia, and by certain neurohormonal stimuli. A previous study demonstrated that SSc patients with an NT pro-BNP in excess of 395 pg/mL have a very high probability of having pulmonary hypertension (sensitivity 56%, specificity 95%) and baseline and serial changes of NT pro-BNP levels estimates prognosis (83). A prospective cohort study demonstrated that a decreased the DLco/alveolar volume ratio and an increased NT pro-BNP are as predictors of PAH in SSc (84). Only plasma levels of BNP and NT pro-BNP have been included as important parameters for assessing disease severity, stability, and prognosis of PAH in the treatment guidelines of the Task Force for the Diagnosis of Treatment of PAH of the European Society of Cardiology and European Respiratory Society (85).

Endothelin-1

Endothelin-1 is a potent vasomodulator peptides produced by various cells including endothelial cells, macrophages, and fibroblasts. Endothelin-1 expression is indued by TGF-β, and its signaling via the endothelin receptor A and B on fibroblasts stimulates fibroblast migration, myofibroblast differentiation, and proliferation of smooth muscle cells. Endothelin-1 also induces vasocontriction mainly via the endothelin receptor type A. Therefore, endothelin-1 has critical roles for proliferative vasculopathy such as PAH in patients with SSc (86). In addition, endothelin-1-receptor blockers are highly effective for PAH treatment. The expression of endothelin-1 is elevated in the SSc-associated fibrotic lung tissue and was associated with the vasculature, pulmonary interstitium, and bronchial and alveolar epithelium. (87). Plasma levels of endothelin-1 has been reported as elevated in patients with SSc (88-90). There was a positive correlation between endothelin-1 levels and systolic pulmonary arterial pressure (90). Elevated plasma endothelin-1 levels were especially detected in SSc patients with advanced microangiopathy defined by capillaroscopy (91, 92). A recent prospective cohort study demonstrated that anti-endothelin 1 type A receptor autoantibodies is an independent predictor of the occurrence of new ischemic digital ulcers together with the presence at baseline of active digital ulcers or history of digital ulcers (93).

Biomarker investigation in Japanese SSc

Previously, we sought to determine if baseline serum chemokine and adhesion molecule levels could predict the progress of symptoms in Japanese early SSc patients (94, 95). Early SSc (disease duration defined by the period from the first symptom including Raynaud's phenomenon attributable to SSc to our first assessment <3 years) who had dcSSc and/or ILD were registered at nine major scleroderma centers in Japan.

In the first study, serum levels of CCL2, CCL5, CXCL8, CXCL9, and CXCL10 were significantly elevated at their first visit in 70 patients with SSc compared with healthy controls (94). In 33 patients followed for up to 4 years, the initial serum CXCL8 levels were significantly associated with the health assessment questionnaire disability index (HAQ-DI) at the fourth year (94). Therefore, serum CXCL8 level may serve as a prognostic indicator of the physical dysfunction in SSc.

In the next study, baseline concentrations of serum intercellular adhesion molecule (ICAM) -1, E-selection, and P-selectin were significantly elevated and serum L-selectin levels were significantly reduced in 92 patients with SSc compared with healthy controls (95). In 39 patients followed for up to 4 years, serum ICAM-1 concentrations at each time point were overall significantly inversely associated with the %VC of the same time and subsequent years (95) (Table 1). The initial serum ICAM-1 levels were significantly inversely associated with the %VC at the fourth year (Table). Furthermore, the initial serum P-selectin levels were significantly associated with HAQ-DI at the fourth year. In patients with SSc, ICAM-1 and P-selectin may be useful as serum prognostic indicators of respiratory dysfunction and physical disability, respectively. However, further longitudinal studies of larger populations are needed to confirm these findings.

Conclusion

Biomarkers for SSc can be divided into several groups; biomarkers to diagnose (diagnostic biomarker), to classify into disease subset (classification biomarker), to evaluate the activity (activity biomarker) or severity (severity biomarker) of specific clinical features (fibrosis, vascular injury, each organ involvement), to predict specific clinical features (predictive biomarker), and to evaluate the response to therapeutic interventions (therapeutic response

biomarker). Large, multicentre, prospective longitudinal studies of well-defined clinical cohorts must be performed to identify and validate these biomarkers in patients with SSc.

Conflict of interest: none

Table 1. Potential serum/plasma biomarkers of systemic sclerosis

Biomarker	Clinical association
CTGF (N-terminal) ↑	MRSS, ILD
IL-6 ↑	MRSS, early progressive skin sclerosis, poor prognosis, DLco decline in SSc-ILD
CCL2 ↑	ILD (lung dysfunction, CT scores), MRSS
CXCL4 ↑	MRSS, lung fibrosis, PAH, disease progression
CXCL8 ↑	Predictive for physical disability
ICAM-1 ↑	Rapidly progressive disease, digital ulcers, dcSSc, ILD, joint involvement, renal crisis, predictive for respiratory dysfunction
P-selectin ↑	Disease activity, predictive for physical disability
VEGF ↑	Systemic organ involvement, PAH, shorter disease duration, skin sclerosis, capillary density of nailfold
VEGF ↓	Digital ulcers
Von Willebrand factor ↑	Raynaud's phenomenon, disease severity, ILD, PAH
endostatin ↑	PAH
endoglin ↑	lcSSc, anticentromere Ab, cutaneous ulcer, telangiectasia, PAH.
KL-6 ↑	Severity and activity of ILD, maximum fibrosis scores on HRCT
SP-D ↑	Severity and activity of ILD, maximum fibrosis scores on HRCT
CCL18 ↑	Activity of ILD, predictive worsening of ILD and mortality
BNP/NT pro-BNP ↑	Severity, stability, and prognosis of PAH
Endothelin-1 ↑	PAH, microangiopathy defined by capillaroscopy

CTGF, connective tissue growth factor; MRSS, modified Rodnan total skin thickness score; ILD, interstitial lung disease; IL-6, Interleukin 6; DLco, diffusing capacity of carbon monoxide; CT, computed tomography; PAH, pulmonary arterial hypertension; ICAM-1; intercellular adhesion molecule 1; dcSSc, diffuse cutaneous systemic sclerosis; VEGF, vascular endothelial growth factor; lcSSc, limited cutaneous systemic sclerosis; KL-6, krebs von den Lungen-6; HRCT, high resolution CT; SP-D, surfactant protein-D.

Table 2. The associations between ICAM-1 levels and subsequent %VC in early Japanse patients with SSc

	%VC (baseline)	%VC (1 year follow-up)	%VC (2 year follow-up)	%VC (3 year follow-up)	%VC (4 year follow-up)
Log_{10}(ICAM-1 (ng/ml)) (baseline)	r=-0.41* p=0.019	r=-0.40* p=0.033	r=-0.41* p=0.036	r=-0.57** p=0.0027	r=-0.59** p=0.0009
Log_{10}(ICAM-1 (ng/ml)) (1 year follow-up)		r=-035 p=0.080	r=-0.36 p=0.079	r=-0.56** p=0.0042	r=-0.46** p=0.014
Log_{10}(ICAM-1 (ng/ml)) (2 year follow-up)			r=-0.43* p=0.028	r=-0.58** p=0.0022	r=-0.50** p=0.0074
Log_{10}(ICAM-1 (ng/ml)) (3 year follow-up)				r=-0.55** p=0.0048	r=-0.39* p=0.040
Log_{10}(ICAM-1 (ng/ml)) (4 year follow-up)					r=-0.30 p=0.12

The Pearson product-moment correlation coefficient was used to examine the relationship between two continuous variables. ICAM-1; intercellular adhesion molecule-1, VC; vital capacity, *p<0.05, **p<0.01. (Modified from Ref. 95)

Figure legends

Figure 1. A possible overall mechanism of systemic sclerosis.

A variety of products from endothelial cells, fibroblasts, and inflammatory cells are likely contributing to the development of vascular injury, tissue fibrosis, and autoantibody production. Those products are candidates for biomarkers of systemic sclerosis.

Figure 2. Autoantibodies that are specific or related to systemic sclerosis.

Anti-topoisomerase I Ab, anti-RNA polymerase (RNAP) III Ab, and anti-U3 RNP Ab are generally specific for diffuse cutaneous systemic sclerosis (dcSSc). On the other hand, anticentromere Ab and anti-Th/To Ab are usually detected in limited cutaneous systemic sclerosis (lcSSc). Anti-U1 RNP Ab can be detected in lcSSc in addition to systemic lupus erythematosus, myositis, and mixed connective tissue disease. Each antibody has significant associations with specific organ involvement. PAH, pulmonary arterial hypertension; ILD, interstitial lung disease.

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