It has been widely accepted that TGF-β is the central player in the pathogenic mechanism of SSc, especially in the development of tissue fibrosis [21]. The main producer of TGF-β is likely macrophages, but many kinds of cells including platelets, leukocytes, and fibroblasts can produce TGF-β. Thrombospondins, plasmin, and various cell surface integrins 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 [22].

DNA microarray analysis demonstrated that a group of TGF-β-dependent genes are overexpressed in skin lesions of SSc patients [23]. In another study, DNA microarray analysis of skin biopsies has demonstrated that the TGF-β-responsive gene signature is expressed highly in dcSSc subset but not found in patients with lcSSc, morphea, or in healthy controls [24]. Serial assessment of skin biopsy samples represents the most direct way of assessing changes in scleroderma patients at a biochemical level. Regarding the biomarkers in skin biopsy specimens, CAT-192 study reported that tissue levels of mRNA for procollagens I and III and for TGF-β1 and TGF-β2 were elevated in patients with dcSSc. Clear differences were identified between lesional and nonlesional sites as well as between SSc and healthy control samples [25].

However, circulating TGF-β levels are not consistently 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 [26].

13.3.4. Connective Tissue Growth Factor (CTGF)

CTGF/CCN2 is another critical factor for the development of tissue fibrosis [27]. CTGF is expressed in fibroblasts induced by TGF-β1 and enhances fibroblast proliferation and ECM production as a downstream mediator of TGF-β1. 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 in maintaining the fibrotic state [28]. Serum CTGF levels were significantly elevated in patients with SSc and correlated with the severity of skin sclerosis and ILD [29]. 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 the severity of skin sclerosis and negatively with disease duration [30].

13.3.5. Interleukin (IL)-6

Among various cytokines, IL-6 has been considered as a potential biomarker of disease monitoring in SSc. 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. Augmented IL-6 expression was observed in dermal fibroblasts, mononuclear cells, and endothelial cells in patients with early dcSSc [31]. Previous studies have reported that serum IL-6 levels were significantly associated with ~~skin sclerosis (~~MRSS~~)~~ [31, 32]. Elevated IL-6 expression was likely associated with poor prognosis in patients with early dcSSc [31]. In a recent study, serum levels of eight 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 [33]. In the exploratory analysis, only serum IL-6 was an independent predictor of the diffusing capacity of the lungs for carbon monoxide (DLCO) decline in both SSc-ILD and idiopathic pulmonary fibrosis.

13.3.6. Chemokine

A variety of chemokines have been reported as possible biomarkers of SSc. CCL2 (monocyte chemoattractant protein-1, MCP-1) may be one of the promising candidates. CCL2 is produced by macrophages, fibroblasts, endothelial cells, and other cells and predominant chemoattractant and activator of monocytes and T cells. Also, this chemokine induces Th2 cell polarization [34] and stimulates collagen production by fibroblasts via specific receptors and endogenous upregulation of TGF-β expression. The latter results in autocrine and/or juxtacrine stimulation of collagen gene expression [35]. Serum CCL2 levels are increased in SSc patients and have been found to correlate with the presence of ILD [36–38]. Interestingly, expression of CCL2 mRNA was most augmented among 4,507 genes when bronchoalveolar lavage (BAL) cells from SSc lung are compared with controls [39]. Consistent with this, protein levels of CCL2 are increased in BAL fluids from SSc patients with lung inflammation [39]. CCL2 concentrations in BAL fluids were associated with the presence of ILD and correlated with lung function parameters and computed tomography scores [40].

A recent proteome-wide analysis and validation has identified that CXCL4 is the predominant protein produced by plasmacytoid dendritic cells in SSc [41]. Plasma levels of CXCL4 were markedly elevated and strongly correlated with skin and lung fibrosis and pulmonary arterial hypertension in patients with SSc. In addition, plasma CXCL4 levels are likely useful to predict the disease progression in SSc [41]. In addition to its antiangiogenic activity, CXCL4 inhibits the expression of the antifibrotic cytokine interferon (IFN)-γ and upregulates profibrotic cytokines such as IL-4 and IL-13. CXCL4 exhibited direct effects for inducing SSc phenotype both in vitro and in vivo [41], suggesting its central roles in the pathogenesis of SSc.

13.4. Biomarkers of Endothelial Cell Dysfunction (Table 13.2)

Vascular involvement has been considered to be one of the earliest pathogenic features of SSc. 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 include von Willebrand factor (vWF), endothelin-1, thrombomodulin, thrombospondin, brain natriuretic peptide (BNP), N-terminal propeptide of proBNP (NT-proBNP), vascular endothelial growth factor (VEGF), endostatin, plasminogen activator, prostacyclin, thromboxane, and nitrous oxide circulating metabolites.

Table 13.2

Potential biomarkers of endothelial cell dysfunction

Biomarker	Clinical association	Sample
von Willebrand factor	Raynaud's phenomenon, disease severity, ILD	Serum/plasma
VEGF	Shorter disease duration, MRSS, capillary density of nail fold, PAH	Serum/plasma
HGF	Right ventricular systolic pressure	Serum/plasma
Endostatin	Right ventricular systolic pressure	Serum/plasma
Endoglin	lcSSc, telangiectasia, pulmonary artery pressure	Serum/plasma
ICAM-1	Rapidly progressive disease, digital ulcers, dcSSc, respiratory function	Serum/plasma

ILD interstitial lung disease, *VEGF* vascular endothelial growth factor, *MRSS* modified Rodnan total skin thickness score, *PAH* pulmonary arterial hypertension, *HGF* hepatocyte growth factor, *lcSSc* limited cutaneous systemic sclerosis, *ICAM* intercellular adhesion molecule, *dcSSc* diffuse cutaneous systemic sclerosis

13.4.1. von Willebrand Factor

In the pioneering study, von Willebrand factor (vWF) was markedly increased in the plasma of patients with SSc and patients with Raynaud's phenomenon [42]. Circulating vWF was associated with disease severity [43], pulmonary involvement [44], and the extent of radiologically demonstrated ILD [45]. However, association with clinical feature of vascular disease is not clear [46, 47]. von Willebrand factor-cleaving protease ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) was significantly decreased in patients with SSc [48].

13.4.2. Growth Factors

Multiple growth factors have been considered as regulating angiogenesis. Abnormal plasma levels of proangiostatic factors including fibroblast growth factor, placental growth factor, platelet-derived growth factor, and VEGF have been reported [49]. Levels of circulating VEGF were elevated and correlated with shorter disease duration [50], skin sclerosis, and the capillary density of nail fold [51]. On the other hand, other studies did not find any association between VEGF levels and disease activity [52]. Plasma levels of the proangiogenic and antifibrotic factor, hepatocyte growth factor, were reduced in patients with SSc, but had a remarkable positive correlation with right ventricular systolic pressure as measured by echocardiogram [49].

Higher serum concentrations of endothelin-1, E-selectin, VEGF, and vascular cell adhesion molecule (VCAM)-1 were detected in SSc patients compared with healthy controls. Elevated concentrations of endothelin-1, E-selectin, VCAM-1, and VEGF dominated in the serum of SSc patients with organ systemic involvement compared to those without systemic manifestation [53]. Serum VEGF levels were correlated with systolic pulmonary artery pressure in patients with SSc [54]. Thus, the associations with clinical features are different depending on each study, although circulating VEGF levels are generally elevated in SSc patients [55]. 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 [55]. Furthermore, impaired VEGF receptor signaling may be contributing to vascular disturbances in SSc [56].

13.4.3. Angiostatic Molecules

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 nail fold capillaroscopy [50]. Plasma levels of endostatin were markedly elevated in patients with SSc and correlated positively with right ventricular systolic pressure [49]. 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 divided by alveolar volume [56]. Serum soluble endoglin levels were significantly elevated in patients with lcSSc compared with dcSSc and systemic lupus erythematosus patients as well as normal controls [57]. 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. These findings are interesting, since 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 hemorrhagic telangiectasia (Osler–Weber–Rendu syndrome), a Mendelian autosomal vascular disorder [58], and the polymorphism is associated with SSc-related PAH [59].

13.4.4. Adhesion Molecules

SSc patients exhibit increased numbers and activation of monocytes/macrophages and T cells in the circulation and tissues [60 , 61]. Leukocyte recruitment into inflammatory sites is generally achieved using multiple cell adhesion molecules [62]. 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 T cells [63]. There are several reports that have demonstrated the increase of circulating ICAM in patients with SSc [64–66]. In one of those studies, circulating ICAM-1 levels were especially elevated in patients with diffuse rapidly progressive disease or digital ulcers [64]. Another study demonstrated that circulating ICAM-1 levels was significantly higher in dcSSc patients and was correlated with the presence of contracture of phalanges, pulmonary fibrosis, joint involvement, and increased erythrocyte sedimentation rate [66]. In one report, serum levels of ICAM-1, P-selectin, VCAM-1, and, to a lesser degree, E-selectin correlate well with their in situ expression and with clinical disease activity [65]. Japanese multicenter, prospective, observational study demonstrated that serum ICAM-1 levels were elevated and inversely associated with the current and subsequent respiratory functions in patients with early SSc [67]. In a small study, serum levels of ICAM-1, platelet endothelial cell adhesion molecule-1, P-selectin, and VCAM-1 were higher in SSc patients compared with healthy controls at baseline and fell to normal levels after 12 months of bosentan therapy [47]. In scleroderma renal crisis, mean levels of circulating E-selectin, ICAM-1, and VCAM-1 were elevated. On the other hand, serum levels of E-selectin, ICAM-1, and VCAM-1 were not increased in lcSSc patients with PAH [68]. Serum levels of E-selectin, ICAM-1, and VCAM-1 were initially elevated and significantly reduced after infusions of iloprost (prostacyclin analogue) on Raynaud's phenomenon [69].

13.5. Biomarkers of Interstitial Lung ~~Disorders~~Diseases (Table 13.3)

13.5.1. Proteins Secreted by Alveolar Epithelial Cells

Recently, ILD associated with SSc has become the main cause of SSc-related death in SSc. Serum biomarkers of ILD are critical for monitoring patients with SSc, since the evaluation using computed tomography (CT) scan cannot be frequently performed. Serum biomarkers of ILD have 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 [70], whereas SP-D is produced and secreted by alveolar type II pneumocytes and Clara cells [71]. Several studies revealed that serum levels of KL-6 and SP-D are elevated in serum from patients with ILD, including SSc-related ILD [70 , 72 , 73]. These studies suggested that serum levels of SP-D and KL-6 are serologic markers of the severity and activity of ILD in SSc [73–75]. In a comparative study, SP-D was more sensitive but less specific for ILD than KL-6 in SSc patients [75]. A cohort study of Scleroderma Lung Study Research Group demonstrated that both KL-6 and SP-D are highly sensitive and specific 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

[74]. Therefore, 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. However, some SSc patients with active ILD showed discrepancies in the serum levels of these markers. Furthermore, KL-6 or SP-D does not necessarily reflect the activity of ILD in a part of patients. KL-6 and SP-D are currently ~~only~~ utilized in clinical practice in Japan.

Table 13.3

Potential biomarkers of interstitial lung diseases

Biomarker	Clinical association	Sample
KL-6	Severity and activity of ILD, maximum fibrosis scores on HRCT	Serum/plasma
SP-D	Severity and activity of ILD, maximum fibrosis scores on HRCT	Serum/plasma
CCL18	Activity of ILD, predictive worsening of ILD	Serum/plasma

KL-6 Krebs von den Lungen-6, *ILD* interstitial lung disease, *HRCT* high-resolution computed tomography, *SP-D* surfactant protein-D

13.5.2. CCL18

Another promising biomarker of SSc-ILD is 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 [76]. CCL18 in high concentrations directly stimulates intracellular signaling and collagen production in primary pulmonary fibroblasts [77]. Serum CCL18 levels were markedly elevated in association with the development of ILD, as well as with reductions in vital capacity (VC) and diffusing capacity for carbon monoxide (DLCO), and correlated closely with the activity of ILD [78]. Furthermore, serum CCL18 levels have been demonstrated as predictive biomarker for the identification of patients with a higher risk of subsequent lung disease worsening in SSc [79]. ~~However, a~~A recent study reported that SP-D correlated with concomitantly obtained forced vital capacity, while CCL18 was a predictor of short-term decline in forced VC [80]. However, neither SP-D nor CCL18 was a long-term predictor of forced VC course in patients with early SSc in that study. Furthermore, CCL18 production by BAL cells and serum CCL18 concentrations reflected pulmonary fibrotic activity in SSc patients with ILD [81].

13.6. Biomarkers of Pulmonary Arterial Hypertension (Table 13.4)

13.6.1. Brain Natriuretic Peptide (BNP)

Pulmonary arterial hypertension has become one of the most important factors that affect morbidity and mortality. However, there are currently no validated laboratory examination or serologic markers that are useful for specific diagnosis of PAH. Therefore, biomarkers are necessary to find out early asymptomatic PAH. The DLCO decreases years prior to the diagnosis of PAH in patients with lcSSc [82]. Serum BNP and serum NT-proBNP have been reported as useful biomarkers for pulmonary hypertension since they tend to increase in SSc patients with early PAH and correlate with hemodynamic measures [83 , 84]. BNP and NT-proBNP are secreted by ventricular myocytes reflecting myocardial responses to stretch and hypoxia and by certain neurohormonal stimuli. A previous study demonstrated that SSc patients with an NT-proBNP in excess of 395 pg/mL have a very high probability of having pulmonary hypertension, and

baseline and serial changes of NT-proBNP levels are highly predictive of survival [85]. A prospective cohort study demonstrated that a decreased DLCO/alveolar volume ratio and an increased NT-proBNP are predictors of PAH in SSc [86]. Furthermore, only plasma levels of BNP and NT-proBNP 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 and Treatment of PAH of the European Society of Cardiology and European Respiratory Society [87]. However, ~~the plasma~~the serum or plasma levels of BNP ~~or~~and NT-proBNP are not specific for PAH.

Table 13.4

Potential biomarkers of pulmonary arterial hypertension

Biomarker	Clinical association or usefulness	Sample
BNP/NT-proBNP	Severity, stability, and prognosis of PAH, predictive of survival	Serum/plasma
Endothelin-1	Pulmonary arterial pressure, advanced microangiopathy defined by capillaroscopy	Serum/plasma
MRC1	lcSSc with PAH, pulmonary arterial pressure, mortality	mRNA of PBMC
IL-13	lcSSc with PAH	Serum/plasma

BNP brain natriuretic peptide, *NT* N-terminal, *PAH* pulmonary arterial hypertension

13.6.2. Endothelin-1

Endothelin-1 is a 21-amino acid polypeptide produced by various cells including endothelial cells. It is a potent vasoconstrictor and can stimulate proliferation of smooth muscle cells. It is well known that endothelin-1 has critical roles in the proliferative vasculopathy of SSc, including PAH [88]. Also, endothelin-1 receptor blockers are highly effective for the treatment of PAH. Plasma levels of endothelin-1 have been reported as elevated in patients with SSc [89 , 90]. In one study, plasma levels of endothelin-1 were elevated in SSc patients with PAH and SSc patients with anticentromere antibodies. There was a positive linear correlation between endothelin-1 levels and systolic pulmonary arterial pressure [91]. High endothelin-1 plasma levels were especially detected in SSc patients with advanced microangiopathy defined by capillaroscopy [92 , 93]. Therefore, circulating endothelin-1 levels may be a possible biomarker for evaluating SSc-related PAH (Table 13.5

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Table 13.5

Potential biomarkers of disability and disease activity

Biomarker	Clinical association	Sample
CXCL8	Subsequent HAQ-DI	Serum/plasma
P-selectin	Subsequent HAQ-DI	Serum/plasma
IFN-inducible chemokine	Medsger Severity Index (particularly with the lung, skin, muscle	Serum/plasma

score involvement)

13.6.3. Cytokines

Increased expression of nine genes (ICAM1, IFNGR1, IL1B, IL13Ra1, JAK2, AIF1, CCR1, ALAS2, TIMP2) was found in lcSSc patients with PAH by genome-wide gene expression using peripheral blood mononuclear cell (PBMC) samples [94]. Increased circulating cytokine levels of inflammatory mediators, such as TNF-α, IL-1β, ICAM-1, and IL-6, and markers of vascular injury such as VCAM-1, VEGF, and vWF were detected in lcSSc patients with PAH by multi-analyte profiling immunoassays [94].

In another study of the same group, the mRNA expression of CCR1 and JAK2 was elevated in lcSSc patients with PAH compared with controls on PBMC but mainly on $CD14^+$ cells [95]. Expression of MRC1, a marker of alternative activation of monocyte/macrophages, was also increased in lcSSc patients with PAH and correlated with pulmonary artery pressure and higher mortality. MRC1 expression was elevated in $CD14^+$ cells and was increased by IL-13 stimulation. Plasma levels of IL-13 were markedly elevated in lcSSc patients with PAH. These findings indicate that IL-13-activated monocyte/macrophages may have a critical role in the development of PAH in lcSSc, with MRC1 as an important biomarker.

13.7. Biomarkers of Disability and Disease Activity

The Disability Index of the Health Assessment Questionnaire (HAQ-DI) was originally created to examine and quantify functional capacity in patients with arthritis [96]. The HAQ-DI is determined by a self-administered questionnaire consisting of eight categories (score 0–3). Steen and Medsger have demonstrated that changes in the HAQ-DI correlate with objective physical and laboratory variations in SSc over time [97]. Other studies have also shown that high HAQ-DI is associated with increased morbidity and mortality in patients with SSc [98, 99]. The scleroderma HAQ (SHAQ) consists of the eight domains utilized in the HAQ-DI, plus the following visual analog scales: pain, patient global assessment, vascular, digital ulcers, lung involvement, and gastrointestinal involvement [97]. The Medsger Severity Index, developed through consensus methodology, defines severity in nine organ systems (the general, vascular, skin, joint/tendon, musculoskeletal, gastrointestinal, lung, heart, and kidney) [100].

In a series of Japanese multicenter prospective studies of early dcSSc, initial serum levels of CXCL8 and P-selectin were significantly associated with the HAQ-DI at the fourth year [67, 101]. Recently, IFN-inducible chemokine score has been proposed as a promising biomarker of SSc [102]. The composite chemokine score of plasma levels of IFN-γ-inducible protein 10 (IP-10/CXCL10) and IFN-inducible T cell α chemoattractant (I-TAC/CXCL11) 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 the lung, skin, and muscle involvement.

13.8. Conclusion

Clinical and serological heterogeneity of SSc and effects of medications are making identification and validation of biomarkers challenging. Large, multicenter, prospective studies of well-defined clinical cohorts must be performed to identify and validate biomarkers useful for clinical practice of SSc.

References I think that words following page number (ex. PubMed, PubMed Central PMCID:3049114) should be deleted in all references. Could you please delete them in all references?

1. Khanna D, Clements PJ, Furst DE, Chon Y, Elashoff R, Roth MD, et al. Correlation of the degree of dyspnea with health-related quality of life, functional abilities, and diffusing capacity for carbon monoxide in patients with systemic sclerosis and active alveolitis: results from the Scleroderma Lung Study. Arthritis Rheum. 2005;52(2):592–600. PubMed.

2. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89–95. PubMed.

3. Castro SV, Jimenez SA. Biomarkers in systemic sclerosis. Biomark Med. 2010;4(1):133–47. PubMed Pubmed Central PMCID: 3049114.

4. Hummers LK. The current state of biomarkers in systemic sclerosis. Curr Rheumatol Rep. 2010;12(1):34–9. PubMed Pubmed Central PMCID: 3329274.

5. Moinzadeh P, Denton CP, Abraham D, Ong V, Hunzelmann N, Eckes B, et al. Biomarkers for skin involvement and fibrotic activity in scleroderma. J Eur Acad Dermatol Venereol: JEADV. 2012;26(3):267–76. PubMed.

6. Castelino FV, Varga J. Current status of systemic sclerosis biomarkers: applications for diagnosis, management and drug development. Expert Rev Clin Immunol. 2013;9(11):1077–90. PubMed.

7. Balanescu P, Ladaru A, Balanescu E, Baicus C, Dan GA. Systemic sclerosis biomarkers discovered using mass-spectrometry-based proteomics: a systematic review. Biomarkers. 2014;19(5):345–55. PubMed.

8. Clements P, Lachenbrush P, Seibold J, White B, Weiner S, Martin R, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. J Rheumatol. 1995;22:1281–5.

9. Czirjak L, Nagy Z, Aringer M, Riemekasten G, Matucci-Cerinic M, Furst DE, et al. The EUSTAR model for teaching and implementing the modified Rodnan skin score in systemic sclerosis. Ann Rheum Dis. 2007;66(7):966–9. PubMed Pubmed Central PMCID: 1955103.

10. Hunzelmann N, Risteli J, Risteli L, Sacher C, Vancheeswaran R, Black C, et al. Circulating type I collagen degradation products: a new serum marker for clinical severity in patients with scleroderma? Br J Dermatol. 1998;139(6):1020–5. PubMed.

11. Allanore Y, Borderie D, Lemarechal H, Cherruau B, Ekindjian OG, Kahan A. Correlation of serum collagen I carboxyterminal telopeptide concentrations with cutaneous and pulmonary involvement in systemic sclerosis. J Rheumatol. 2003;30(1):68–73. PubMed.

12. Dziadzio M, Smith RE, Abraham DJ, Stratton RJ, Gabrielli A, Black CM, et al. Serological assessment of type I collagen burden in scleroderma spectrum disorders: a systematic review. Clin Exp Rheumatol. 2004;22(3):356–67. PubMed.

13. Black CM, McWhirter A, Harrison NK, Kirk JM, Laurent GJ. Serum type III procollagen peptide concentrations in systemic sclerosis and Raynaud's phenomenon: relationship to disease activity and duration. Br J Rheumatol. 1989;28(2):98–103. PubMed.

14. Diot E, Diot P, Valat C, Boissinot E, Asquier E, Lemarie E, et al. Predictive value of serum III procollagen for diagnosis of pulmonary involvement in patients with scleroderma. Eur Respir J. 1995;8(9):1559–65. PubMed.

15. Nagy Z, Czirjak L. Increased levels of amino terminal propeptide of type III procollagen are an unfavourable predictor of survival in systemic sclerosis. Clin Exp Rheumatol. 2005;23(2):165–72. PubMed.

16. Ra HJ, Parks WC. Control of matrix metalloproteinase catalytic activity. Matrix Biol: J Int Soc Matrix Biol. 2007;26(8):587–96. PubMed Pubmed Central PMCID: 2246078.

17. Kim WU, Min SY, Cho ML, Hong KH, Shin YJ, Park SH, et al. Elevated matrix metalloproteinase-9 in patients with systemic sclerosis. Arthritis Res Ther. 2005;7(1):R71–9. PubMed Pubmed Central PMCID: 1064883.

18. Pardo A, Selman M. Matrix metalloproteases in aberrant fibrotic tissue remodeling. Proc Am Thorac Soc. 2006;3(4):383–8. PubMed.

19. Serrati S, Cinelli M, Margheri F, Guiducci S, Del Rosso A, Pucci M, et al. Systemic sclerosis fibroblasts inhibit in vitro angiogenesis by MMP-12-dependent cleavage of the endothelial cell urokinase receptor. J Pathol. 2006;210(2):240–8. PubMed.

20. Manetti M, Guiducci S, Romano E, Bellando-Randone S, Conforti ML, Ibba-Manneschi L, et al. Increased serum levels and tissue expression of matrix metalloproteinase-12 in patients with systemic sclerosis: correlation with severity of skin and pulmonary fibrosis and vascular damage. Ann Rheum Dis. 2012;71(6):1064–72. PubMed.

21. Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. J Clin Invest. 2007;117(3):557–67. PubMed.

22. Moustakas A, Heldin CH. Non-Smad TGF-beta signals. J Cell Sci. 2005;118(Pt 16):3573–84. PubMed.

23. Whitfield ML, Finlay DR, Murray JI, Troyanskaya OG, Chi JT, Pergamenschikov A, et al. Systemic and cell type-specific gene expression patterns in scleroderma skin. Proc Natl Acad Sci U S A. 2003;100(21):12319–24. PubMed.

24. Sargent JL, Milano A, Bhattacharyya S, Varga J, Connolly MK, Chang HY, et al. A TGFbeta-responsive gene signature is associated with a subset of diffuse scleroderma with increased disease severity. J Invest Dermatol. 2010;130(3):694–705. PubMed Pubmed Central PMCID: 3867816.

25. Denton CP, Merkel PA, Furst DE, Khanna D, Emery P, Hsu VM, et al. Recombinant human anti-transforming growth factor beta1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. Arthritis Rheum. 2007;56(1):323–33. PubMed.

26. Dziadzio M, Smith RE, Abraham DJ, Black CM, Denton CP. Circulating levels of active transforming growth factor beta1 are reduced in diffuse cutaneous systemic sclerosis and correlate inversely with the modified Rodnan skin score. Rheumatology (Oxford). 2005;44(12):1518–24. PubMed.

27. Krieg T, Takehara K. Skin disease: a cardinal feature of systemic sclerosis. Rheumatology (Oxford). 2009;48 Suppl 3:iii14–8. PubMed.

28. Takehara K. Hypothesis: pathogenesis of systemic sclerosis. J Rheumatol. 2003;30(4):755–9. PubMed.

29. Sato S, Nagaoka T, Hasegawa M, Tamatani T, Nakanishi T, Takigawa M, et al. Serum levels of connective tissue growth factor are elevated in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. J Rheumatol. 2000;27(1):149–54. PubMed.

30. Dziadzio M, Usinger W, Leask A, Abraham D, Black CM, Denton C, et al. N-terminal connective tissue growth factor is a marker of the fibrotic phenotype in scleroderma. QJM. 2005;98(7):485–92. PubMed.

31. Khan K, Xu S, Nihtyanova S, Derrett-Smith E, Abraham D, Denton CP, et al. Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. Ann Rheum Dis. 2012;71(7):1235–42. PubMed.

32. Sato S, Hasegawa M, Takehara K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. J Dermatol Sci. 2001;27(2):140–6.

33. De Lauretis A, Sestini P, Pantelidis P, Hoyles R, Hansell DM, Goh NS, et al. Serum interleukin 6 is predictive of early functional decline and mortality in interstitial lung disease associated with systemic sclerosis. J Rheumatol. 2013;40(4):435–46. PubMed.

34. Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. Nature. 2000;404(6776):407–11. PubMed.

35. Gharaee-Kermani M, Denholm EM, Phan SH. Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. J Biol Chem. 1996;271(30):17779–84. PubMed.

36. Carulli MT, Ong VH, Ponticos M, Shiwen X, Abraham DJ, Black CM, et al. Chemokine receptor CCR2 expression by systemic sclerosis fibroblasts: evidence for autocrine regulation of myofibroblast differentiation. Arthritis Rheum. 2005;52(12):3772–82. PubMed.

37. Hasegawa M, Sato S, Takehara K. Augmented production of chemokines (MCP-1, MIP-1α, and MIP-1β) in patients with systemic sclerosis: MCP-1 and MIP-1α may be involved in the development of pulmonary fibrosis. Clin Exp Immunol. 1999;117:159–65.

38. Antonelli A, Ferri C, Fallahi P, Ferrari SM, Giuggioli D, Colaci M, et al. CXCL10 (alpha) and CCL2 (beta) chemokines in systemic sclerosis – a longitudinal study. Rheumatology (Oxford). 2008;47(1):45–9. PubMed.

39. Luzina IG, Atamas SP, Wise R, Wigley FM, Xiao HQ, White B. Gene expression in bronchoalveolar

lavage cells from scleroderma patients. Am J Respir Cell Mol Biol. 2002;26(5):549–57. PubMed.

40. Schmidt K, Martinez-Gamboa L, Meier S, Witt C, Meisel C, Hanitsch LG, et al. Bronchoalveoloar lavage fluid cytokines and chemokines as markers and predictors for the outcome of interstitial lung disease in systemic sclerosis patients. Arthritis Res Ther. 2009;11(4):R111. PubMed Pubmed Central PMCID: 2745793.

41. van Bon L, Affandi AJ, Broen J, Christmann RB, Marijnissen RJ, Stawski L, et al. Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. N Engl J Med. 2014;370(5):433–43. PubMed Pubmed Central PMCID: 4040466.

42. Kahaleh MB, Osborn I, LeRoy EC. Increased factor VIII/von Willebrand factor antigen and von Willebrand factor activity in scleroderma and in Raynaud's phenomenon. Ann Intern Med. 1981;94(4 pt 1):482–4. PubMed.

43. Herrick AL, Illingworth K, Blann A, Hay CR, Hollis S, Jayson MI. Von Willebrand factor, thrombomodulin, thromboxane, beta-thromboglobulin and markers of fibrinolysis in primary Raynaud's phenomenon and systemic sclerosis. Ann Rheum Dis. 1996;55(2):122–7. PubMed Pubmed Central PMCID: 1010106.

44. Scheja A, Akesson A, Geborek P, Wildt M, Wollheim CB, Wollheim FA, et al. Von Willebrand factor propeptide as a marker of disease activity in systemic sclerosis (scleroderma). Arthritis Res. 2001;3(3):178–82. PubMed Pubmed Central PMCID: 30710.

45. Kumanovics G, Minier T, Radics J, Palinkas L, Berki T, Czirjak L. Comprehensive investigation of novel serum markers of pulmonary fibrosis associated with systemic sclerosis and dermato/polymyositis. Clin Exp Rheumatol. 2008;26(3):414–20. PubMed.

46. Hesselstrand R, Ekman R, Eskilsson J, Isaksson A, Scheja A, Ohlin AK, et al. Screening for pulmonary hypertension in systemic sclerosis: the longitudinal development of tricuspid gradient in 227 consecutive patients, 1992–2001. Rheumatology (Oxford). 2005;44(3):366–71. PubMed.

47. Iannone F, Riccardi MT, Guiducci S, Bizzoca R, Cinelli M, Matucci-Cerinic M, et al. Bosentan regulates the expression of adhesion molecules on circulating T cells and serum soluble adhesion molecules in systemic sclerosis-associated pulmonary arterial hypertension. Ann Rheum Dis. 2008;67(8):1121–6. PubMed Pubmed Central PMCID: 2564790.

48. Mannucci PM, Vanoli M, Forza I, Canciani MT, Scorza R. Von Willebrand factor cleaving protease (ADAMTS-13) in 123 patients with connective tissue diseases (systemic lupus erythematosus and systemic sclerosis). Haematologica. 2003;88(8):914–8. PubMed.

49. Hummers LK, Hall A, Wigley FM, Simons M. Abnormalities in the regulators of angiogenesis in patients with scleroderma. J Rheumatol. 2009;36(3):576–82. PubMed Pubmed Central PMCID: 4020014.

50. Distler O, Del Rosso A, Giacomelli R, Cipriani P, Conforti ML, Guiducci S, et al. Angiogenic and angiostatic factors in systemic sclerosis: increased levels of vascular endothelial growth factor are a feature of the earliest disease stages and are associated with the absence of fingertip ulcers. Arthritis Res. 2002;4(6):R11. PubMed Pubmed Central PMCID: 153841.

51. Choi JJ, Min DJ, Cho ML, Min SY, Kim SJ, Lee SS, et al. Elevated vascular endothelial growth factor in systemic sclerosis. J Rheumatol. 2003;30(7):1529–33. PubMed.

52. Viac J, Schmitt D, Claudy A. Plasma vascular endothelial growth factor levels in scleroderma are not correlated with disease activity. Acta Derm Venereol. 2000;80(5):383. PubMed.

53. Kuryliszyn-Moskal A, Klimiuk PA, Sierakowski S. Soluble adhesion molecules (sVCAM-1, sE-selectin), vascular endothelial growth factor (VEGF) and endothelin-1 in patients with systemic sclerosis: relationship to organ systemic involvement. Clin Rheumatol. 2005;24(2):111–6. PubMed.

54. Papaioannou AI, Zakynthinos E, Kostikas K, Kiropoulos T, Koutsokera A, Ziogas A, et al. Serum VEGF levels are related to the presence of pulmonary arterial hypertension in systemic sclerosis. BMC Pulm Med. 2009;9:18. PubMed Pubmed Central PMCID: 2685370.

55. Distler O, Distler JH, Scheid A, Acker T, Hirth A, Rethage J, et al. Uncontrolled expression of vascular endothelial growth factor and its receptors leads to insufficient skin angiogenesis in patients with systemic sclerosis. Circ Res. 2004;95(1):109–16. PubMed.

56. Wipff J, Avouac J, Borderie D, Zerkak D, Lemarechal H, Kahan A, et al. Disturbed angiogenesis in systemic sclerosis: high levels of soluble endoglin. Rheumatology (Oxford). 2008;47(7):972–5.

57. Fujimoto M, Hasegawa M, Hamaguchi Y, Komura K, Matsushita T, Yanaba K, et al. A clue for telangiectasis in systemic sclerosis: elevated serum soluble endoglin levels in patients with the limited cutaneous form of the disease. Dermatology. 2006;213(2):88–92. PubMed.

58. Shovlin CL, Guttmacher AE, Buscarini E, Faughnan ME, Hyland RH, Westermann CJ, et al. Diagnostic criteria for hereditary hemorrhagic telangiectasia (Rendu-Osler-Weber syndrome). Am J Med Genet. 2000;91(1):66–7. PubMed.

59. Wipff J, Kahan A, Hachulla E, Sibilia J, Cabane J, Meyer O, et al. Association between an endoglin gene polymorphism and systemic sclerosis-related pulmonary arterial hypertension. Rheumatology (Oxford). 2007;46(4):622–5. PubMed.

60. Roumm AD, Whiteside TL, Medsger Jr TA, Rodnan GP. Lymphocytes in the skin of patients with progressive systemic sclerosis. Quantification, subtyping, and clinical correlations. Arthritis Rheum. 1984;27(6):645–53.

61. Gruschwitz M, Sepp N, Kofler H, Wick G. Expression of class II-MHC antigens in the dermis of patients with progressive systemic sclerosis. Immunobiology. 1991;182(3–4):234–55. PubMed.

62. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell. 1994;76(2):301–14. PubMed.

63. Abraham D, Lupoli S, McWhirter A, Plater-Zyberk C, Piela TH, Korn JH, et al. Expression and function of surface antigens on scleroderma fibroblasts. Arthritis Rheum. 1991;34(9):1164–72.

64. Sfikakis PP, Tesar J, Baraf H, Lipnick R, Klipple G, Tsokos GC. Circulating intercellular adhesion molecule-1 in patients with systemic sclerosis. Clin Immunol Immunopathol. 1993;68(1):88–92.

65. Gruschwitz MS, Hornstein OP, von den Driesch P. Correlation of soluble adhesion molecules in the peripheral blood of scleroderma patients with their in situ expression and with disease activity. Arthritis Rheum. 1995;38(2):184–9.

66. Ihn H, Sato S, Fujimoto M, Kikuchi K, Kadono T, Tamaki K, et al. Circulating intercellular adhesion molecule-1 in the sera of patients with systemic sclerosis: enhancement by inflammatory cytokines. Br J Rheumatol. 1997;36(12):1270–5.

67. Hasegawa M, Asano Y, Endo H, Fujimoto M, Goto D, Ihn H, et al. Serum adhesion molecule levels as prognostic markers in patients with early systemic sclerosis: a multicentre, prospective, observational study. PLoS One. 2014;9(2):e88150. PubMed Pubmed Central PMCID: 3916412.

68. Stratton RJ, Coghlan JG, Pearson JD, Burns A, Sweny P, Abraham DJ, et al. Different patterns of endothelial cell activation in renal and pulmonary vascular disease in scleroderma. Q J Med. 1998;91:561–6.

69. Mittag M, Beckheinrich P, Haustein UF. Systemic sclerosis-related Raynaud's phenomenon: effects of iloprost infusion therapy on serum cytokine, growth factor and soluble adhesion molecule levels. Acta Derm Venereol. 2001;81(4):294–7. PubMed.

70. Kohno N, Kyoizumi S, Awaya Y, Fukuhara H, Yamakido M, Akiyama M. New serum indicator of interstitial pneumonitis activity: sialylated carbohydrate antigen KL-6. Chest. 1989;96(1):68–73.

71. Hermans C, Bernard A. Lung epithelium-specific proteins: characteristics and potential applications as markers. Am J Respir Crit Care Med. 1999;159(2):646–78. PubMed.

72. Kobayashi J, Kitamura S. KL-6: a serum marker for interstitial pneumonia. Chest. 1995;108(2):311–5.

73. Asano Y, Ihn H, Yamane K, Yazawa N, Kubo M, Fujimoto M, et al. Clinical significance of surfactant protein D as a serum marker for evaluating pulmonary fibrosis in patients with systemic sclerosis. Arthritis Rheum. 2001;44(6):1363–9. PubMed.

74. Hant FN, Ludwicka-Bradley A, Wang HJ, Li N, Elashoff R, Tashkin DP, et al. Surfactant protein D and KL-6 as serum biomarkers of interstitial lung disease in patients with scleroderma. J Rheumatol. 2009;36(4):773–80. PubMed.

75. Yanaba K, Hasegawa M, Takehara K, Sato S. Comparative study of serum surfactant protein-D and KL-6 concentrations in patients with systemic sclerosis as markers for monitoring the activity of pulmonary fibrosis. J Rheumatol. 2004;31(6):1112–20. PubMed.

76. Hieshima K, Imai T, Baba M, Shoudai K, Ishizuka K, Nakagawa T, et al. A novel human CC chemokine PARC that is most homologous to macrophage-inflammatory protein-1 alpha/LD78 alpha and chemotactic for T lymphocytes, but not for monocytes. J Immunol. 1997;159(3):1140–9. PubMed.

77. Atamas SP, Luzina IG, Choi J, Tsymbalyuk N, Carbonetti NH, Singh IS, et al. Pulmonary and activation-regulated chemokine stimulates collagen production in lung fibroblasts. Am J Respir Cell Mol Biol. 2003;29(6):743–9. PubMed.

78. Kodera M, Hasegawa M, Komura K, Yanaba K, Takehara K, Sato S. Serum pulmonary and activation-regulated chemokine/CCL18 levels in patients with systemic sclerosis: a sensitive indicator of active pulmonary fibrosis. Arthritis Rheum. 2005;52(9):2889–96. PubMed.

79. Tiev KP, Hua-Huy T, Kettaneh A, Gain M, Duong-Quy S, Toledano C, et al. Serum CC chemokine ligand-18 predicts lung disease worsening in systemic sclerosis. Eur Respir J. 2011;38(6):1355–60. PubMed.

80. Elhaj M, Charles J, Pedroza C, Liu X, Zhou X, Estrada YMRM, et al. Can serum surfactant protein D or CC-chemokine ligand 18 predict outcome of interstitial lung disease in patients with early systemic sclerosis? J Rheumatol. 2013;40(7):1114–20. PubMed Pubmed Central PMCID: 3728890.

81. Prasse A, Pechkovsky DV, Toews GB, Schafer M, Eggeling S, Ludwig C, et al. CCL18 as an indicator of pulmonary fibrotic activity in idiopathic interstitial pneumonias and systemic sclerosis. Arthritis Rheum. 2007;56(5):1685–93. PubMed.

82. Steen V, Medsger Jr TA. Predictors of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. Arthritis Rheum. 2003;48(2):516–22. PubMed.

83. Cavagna L, Caporali R, Klersy C, Ghio S, Albertini R, Scelsi L, et al. Comparison of brain natriuretic peptide (BNP) and NT-proBNP in screening for pulmonary arterial hypertension in patients with systemic sclerosis. J Rheumatol. 2010;37(10):2064–70. PubMed.

84. Allanore Y, Borderie D, Meune C, Cabanes L, Weber S, Ekindjian OG, 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(12):3503–8. PubMed.

85. Williams MH, Handler CE, Akram R, Smith CJ, Das C, Smee J, et al. Role of N-terminal brain natriuretic peptide (N-TproBNP) in scleroderma-associated pulmonary arterial hypertension. Eur Heart J. 2006;27(12):1485–94. PubMed.

86. Allanore Y, Borderie D, Avouac J, Zerkak D, Meune C, Hachulla E, 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(1):284–91. PubMed.

87. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). Eur Heart J. 2009;30(20):2493–537. PubMed.

88. Braun-Moscovici Y, Nahir AM, Balbir-Gurman A. Endothelin and pulmonary arterial hypertension. Semin Arthritis Rheum. 2004;34(1):442–53. PubMed.

89. Yamane K, Kashiwagi H, Suzuki N, Miyauchi T, Yanagisawa M, Goto K, et al. Elevated plasma levels of endothelin-1 in systemic sclerosis. Arthritis Rheum. 1991;34(2):243–4. PubMed.

90. Morelli S, Ferri C, Di Francesco L, Baldoncini R, Carlesimo M, Bottoni U, et al. Plasma endothelin-1 levels in patients with systemic sclerosis: influence of pulmonary or systemic arterial hypertension. Ann Rheum Dis. 1995;54(9):730–4. PubMed Pubmed Central PMCID: 1009988.

91. Schmidt J, Launay D, Soudan B, Hachulla E, de Groote P, Lambert M, et al. Assessment of plasma endothelin level measurement in systemic sclerosis. Rev Med Interne. 2007;28(6):371–6. PubMed Interet du dosage plasmatique de l'endotheline au cours de la sclerodermie systemique.

92. Sulli A, Soldano S, Pizzorni C, Montagna P, Secchi ME, Villaggio B, et al. Raynaud's phenomenon and plasma endothelin: correlations with capillaroscopic patterns in systemic sclerosis. J Rheumatol. 2009;36(6):1235–9. PubMed.

93. Kim HS, Park MK, Kim HY, Park SH. Capillary dimension measured by computer-based digitalized image correlated with plasma endothelin-1 levels in patients with systemic sclerosis. Clin Rheumatol. 2010;29(3):247–54. PubMed.

94. Pendergrass SA, Hayes E, Farina G, Lemaire R, Farber HW, Whitfield ML, et al. Limited systemic sclerosis patients with pulmonary arterial hypertension show biomarkers of inflammation and vascular injury. PLoS One. 2010;5(8):e12106. PubMed Pubmed Central PMCID: 2923145.

95. Christmann RB, Hayes E, Pendergrass S, Padilla C, Farina G, Affandi AJ, et al. Interferon and alternative activation of monocyte/macrophages in systemic sclerosis-associated pulmonary arterial hypertension. Arthritis Rheum. 2011;63(6):1718–28. PubMed Pubmed Central PMCID: 4030759.

96. Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum. 1980;23(2):137–45. PubMed.

97. Steen VD, Medsger Jr TA. The value of the Health Assessment Questionnaire and special patient-generated scales to demonstrate change in systemic sclerosis patients over time. Arthritis Rheum. 1997;40(11):1984–91. PubMed.

98. Clements PJ, Roth MD, Elashoff R, Tashkin DP, Goldin J, Silver RM, et al. Scleroderma lung study (SLS): differences in the presentation and course of patients with limited versus diffuse systemic sclerosis. Ann Rheum Dis. 2007;66(12):1641–7. PubMed Pubmed Central PMCID: 2095310.

99. Valentini G, Matucci Cerinic M. Disease-specific quality indicators, guidelines and outcome measures in scleroderma. Clin Exp Rheumatol. 2007;25(6 Suppl 47):159–62. PubMed.

100. Medsger Jr TA, Bombardieri S, Czirjak L, Scorza R, Della Rossa A, Bencivelli W. Assessment of disease severity and prognosis. Clin Exp Rheumatol. 2003;21(3 Suppl 29):S42–6. PubMed.

101. Hasegawa M, Asano Y, Endo H, Fujimoto M, Goto D, Ihn H, et al. Serum chemokine levels as prognostic markers in patients with early systemic sclerosis: a multicenter, prospective, observational study. Mod Rheumatol ~~Jpn Rheum Ass~~. 2013;23(6):1076–84. PubMed.

102. Liu X, Mayes MD, Tan FK, Wu M, Reveille JD, Harper BE, et al. Correlation of interferon-inducible chemokine plasma levels with disease severity in systemic sclerosis. Arthritis Rheum. 2013;65(1):226–35. PubMed Pubmed Central PMCID: 3687352.

強皮症の皮膚潰瘍に エンドセリン受容体拮抗薬は有用か？

A 序論

全身性強皮症は，皮膚や内臓臓器の線維化と，血管障害を主病変とする膠原病である．レイノー現象がほとんどの症例で初発症状として認められ，指尖部に難治性の潰瘍を繰り返すことが少なくない．このような強皮症の末梢循環障害に対する治療薬として保険収載されているものはないが，ベラプロストナトリウムやCa拮抗薬の内服，プロスタグランジンE_1製剤の注射などが本邦では使用されることが多い．欧州では，全身性強皮症患者にみられる再発性の難治性指尖潰瘍に，肺動脈性肺高血圧症の薬であるエンドセリン受容体拮抗薬のボセンタンの使用が認可されている．

B 指針

本邦では，全身性強皮症診療ガイドラインの中で，“ボセンタンは血管病変に有用か？”という clinical question に対して，“ボセンタンは皮膚潰瘍新生予防に有用であるが，適応を慎重に考慮する必要がある”との推奨文が記載されており，Minds 推奨グレードはB（科学的根拠があり，行うように勧められる）となっている．

以下に示すように，ボセンタンは皮膚潰瘍の新生抑制を示すというエビデンスが高いが，肝機能障害などの副作用が少なくなく，薬価が高く，日本ではオーファンドラッグであり，中等度から重症の肺動脈性肺高血圧症にしか適応がないことから，適応を慎重に考慮すべきである．

C エビデンス

1 Digital ulcers in systemic sclerosis: prevention by treatment with bosentan, an oral endothelin receptor antagonist[1]. (RAPIDS-1 study)

目的 ボセンタン治療が，全身性強皮症患者にみられる新しい指尖潰瘍の新生を減少させるかどうか．

対照 二重盲検ランダム化試験．欧州と北米の17施設の全身性強皮症122例；ボセンタン79例 vs プラセボ43例．

治療 ボセンタン（62.5 mg）またはプラセボを1日2回，4週間内服．引き続いて，ボセンタン（125 mg）またはプラセボを1日2回，12週間内服．この16週の間に，指尖潰瘍がいくつ新生したかが主評価項目．副次評価項目としては，指尖潰瘍の治癒率と Scleroderma Health Assessment Questionnaire を用いた手の機能評価．

結果 ボセンタン内服群では，プラセボ内服群より平均で48%，指尖潰瘍の形成が減少した．

手の機能も，ボセンタン内服で有意に改善した．ただし，潰瘍の治癒には有意な影響がみられなかった．

2 Bosentan treatment of digital ulcers related to systemic sclerosis: results from the RAPIDS-2 randomised, double-blind, placebo-controlled trial[2)]

目的 上記の RAPIDS-1 study で報告されたようなボセンタン治療の全身性強皮症患者にみられる指尖潰瘍の治癒や予防に対する効果をさらに検証する．

対象 二重盲検ランダム化試験．欧州と北米の41施設の活動性のある皮膚潰瘍を有する全身性強皮症188例；ボセンタン98例 vs プラセボ90例．

治療 ボセンタン（62.5 mg）またはプラセボを1日2回，4週間内服．引き続いて，ボセンタン（125 mg）またはプラセボを1日2回，20週間内服．主要評価項目は，この24週間の間に，新生した指尖潰瘍の数と，活動性のある潰瘍が治癒するまでの期間．副次評価項目としては，疼痛，機能障害，安全性．

結果 ボセンタン内服群では，プラセボ内服群より平均で30%，新生した指尖潰瘍が少なかった．この効果は，試験開始前に皮膚潰瘍の多かった症例ほど大きかった．潰瘍の治癒率，疼痛，機能障害に有意な差はみられなかった．ボセンタン投与と関連して，末梢の浮腫とアミノトランスフェラーゼの上昇が認められた．

3 Long-term experience of bosentan for treating ulcers and healed ulcers in systemic sclerosis patients[3)]

目的 潰瘍または治癒した潰瘍を有する全身性強皮症患者におけるボセンタン内服の有効性と安全性を検証する．

対象 前向き非対照性観察試験．単一施設で皮膚潰瘍または治癒した潰瘍に対してボセンタンを投与した強皮症患者全例（15例）の経過を観察した．治療開始前と6カ月後に，皮膚と全身症状の評価を施行．

治療 ボセンタン（62.5 mg）を4週間内服．引き続いて，ボセンタン（125 mg）に増量して維持．治療期間は，中央値で24.7カ月（4～36カ月）．

結果 皮膚潰瘍や治癒潰瘍の数に関して，有意な減少がみられた．他の臨床症状には有意な効果がみられなかった．

D 根拠となった臨床研究の問題点と限界

1つの研究[3)]は，症例数も少なく，対照試験ではなく，観察試験である．ただし，試験開始までは潰瘍のほとんどが慢性で他の治療に反応しなかったことが強調されている．

他の2つの二重盲検ランダム化試験[1, 2)]においては，共通して参加している施設が多いのは確かだが，ほぼ同じ結果が得られていることから，データの信頼性は高いものと考えられる．両試験において，新生潰瘍の有意な抑制がみられ，特にもともとの潰瘍の数が多い症例で効果が顕著であった．一方で，どちらの試験においても，潰瘍治癒への有意な効果はみられなかっ

た．2つの研究で，肝機能検査の異常がみられたが，末梢の浮腫は2つ目の研究[2]でのみみられている．全体として，ボセンタンは安全性が十分であると結論されている．研究の限界としては，活動性のある指尖潰瘍の定義が，施設によって異なるかもしれない．また，潰瘍部の感染症や指の切断にいたる危険性を減少できるかどうかなどは，そのような症例が多くないために評価できていない．

E 本邦の患者に適応する際の注意点

本邦では，ボセンタンは肺動脈性肺高血圧症のWHO機能分類のクラスII，III，IVに保険収載されており，軽症のクラスIには使用できない．オーファンドラッグで薬価も高い．また，肝機能障害（文献1では11.4%，文献2では12.5%）や浮腫など（文献2では18.8%）の副作用が一定の割合でみられるため，現時点では安易に使用すべきではなく，慎重に適応を考慮すべきと考えられる．

F コメント

肺動脈性肺高血圧症に対して，ボセンタン以外にもいくつかのエンドセリン受容体拮抗薬が使用あるいは開発されてきている．それらの薬剤についても，ボセンタンと同様の効果が期待され，今後の検証が待たれる．

◘文献

1) Korn JH, Mayes M, Matucci Cerinic M, et al. Digital ulcers in systemic sclerosis: prevention by treatment with bosentan, an oral endothelin receptor antagonist. Arthritis Rheum. 2004; 50: 3985-93.
2) Matucci-Cerinic M, Denton CP, Furst DE, et al. Bosentan treatment of digital ulcers related to systemic sclerosis: results from the RAPIDS-2 randomised, double-blind, placebo-controlled trial. Ann Rheum Dis. 2011; 70: 32-8.
3) García de la Peña-Lefebvre P, Rodríguez Rubio S, Valero Expósito M, et al. Long-term experience of bosentan for treating ulcers and healed ulcers in systemic sclerosis patients. Rheumatology (Oxford). 2008; 47: 464-6.

〈長谷川　稔〉

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発行者　株式会社　中外医学社
代表取締役　青木　滋
〒162-0805 東京都新宿区矢来町62
電　話　(03) 3268-2701 (代)
振替口座　00190-1-98814番

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12. 膠原病検査法

1. どのようなときに，なぜ，膠原病の検査を考えるか？

どのようなときに自己抗体を調べるのか？

膠原病を疑う皮膚症状や他の臨床症状がみられる場合に，自己抗体を検索する．通常は，まず抗核抗体の蛍光抗体間接法をオーダーして，抗核抗体が陽性かどうかを確認する．抗核抗体が陽性の場合は，臨床症状や蛍光抗体間接法の染色パターンから存在の疑われる自己抗体の有無を個別に測定する．蛍光抗体間接法で**抗核抗体が陰性**の場合でも，抗体の力価が低い**自己抗体**や細胞質に対する**自己抗体が存在する可能性**があることには注意する．また，**抗好中球細胞質抗体（ANCA）は抗核抗体の検査**では検出できないので，血管炎が疑われる場合は，MPO-ANCA や PR3-ANCA を測定する．

なぜ自己抗体を調べるのか？

膠原病のほとんどの症例には自己抗体が検出され，しかもそれらの自己抗体は症状の出現に先行してみられることが知られている[1]．中でも疾患標識抗体と呼ばれる各膠原病に特異的な自己抗体は非常に特異性が高い．このため，自己抗体を調べることが，膠原病の正確な診断や早期診断に有用である．また，自己抗体の種類は，診断だけでなく，一つの疾患の中でのサブセット分類，重症度，臓器病変，予後などの予測に役立つ．さらに，一部の自己抗体の抗体価は病勢と相関する．

2. 準備・必要な物品

血清を採取して，抗核抗体の蛍光抗体間接法や各種自己抗体の測定を行う．

3. 膠原病検査にあたって留意すべきこと

自己抗体は，基本的に診断を目的とするため，一部の病勢と相関する抗体（SLE にみられる抗二本鎖 DNA 抗体や ANCA 関連血管炎にみられる MPO-ANCA や PR3-ANCA）を除くと，毎月のように測定した場合には保険が査定される．

4. 膠原病検査の実際，手技のポイント

一次スクリーニングとして，抗核抗体（蛍光抗体間接法）検査をオーダーする

Hep-2 細胞をスライドに固定したものに，患者血清中の自己抗体を反応させ，それら自己抗体に蛍光標識したものを鏡検することで，陽性かどうか，そして陽性の場合は染色パターンを判定する検査のことである．これにより，ある程度各種自己抗体の存在を推定することができる（表 1）．検出できる血清希釈が 40 倍，あるいは 80 倍くらいを陽性とすることが多いが，健常人の一部でもこの程度は陽性になること，抗核抗体陰性でも膠原病と関連した自己抗体が存在しうることには留意すべきである．

抗核抗体陽性の場合：discrete speckeled pattern（離散斑紋型）の場合は，抗セントロメア抗体と考えられる．homogeneous pattern（均質型）の場合は抗二本鎖 DNA 抗体や抗ヒストン抗体の存在が，peripheral pattern（辺縁型）の場合は抗二本鎖 DNA 抗体の存在が示唆される．nucleolar pattern の場合は，免疫沈降法により検出可能な抗 U3 RNP 抗体や抗 Th/To 抗体などの強皮症に特異的な抗体の存在が疑われる．なお，膠原病でみられる多くの抗体が，speckled pattern（斑紋型）を示す．

各種自己抗体の測定

上記の一次スクリーニングの結果や臨床症状から推定される疾患や抗体を中心に各種自己抗体を測定する．現在は，ELISA や CLEIA などで定量的に測定されることが多いが，時に基準値を少し上回る程度のときは偽陽性の場合があるので注意すべきである．SLE では，抗二本鎖 DNA 抗体や抗 Sm 抗体が特異的な自己抗体である（保険収載されていないが，抗リボソーム P

表 1　蛍光抗体間接法の染色パターンと対応する膠原病

染色パターン	存在が示唆される自己抗体	対応する膠原病
均質型 (homogeneous pattern)	抗二本鎖 DNA 抗体 抗ヒストン抗体	全身性エリテマトーデス，その他の膠原病
辺縁型 (peripheral pattern)	抗二本鎖 DNA 抗体	全身性エリテマトーデス
斑紋型 (speckled pattern)	抗 Sm 抗体，抗 U1 RNP 抗体，抗トポイソメラーゼ I 抗体，抗 SS-B 抗体など	全身性エリテマトーデス 全身性強皮症，混合性結合組織病，シェーグレン症候群など
離散斑紋型 (discrete speckled pattern)	抗セントロメア抗体	全身性強皮症
核小体型 (nucleolar pattern)	抗 U3 RNP 抗体 抗 Th/To 抗体	全身性強皮症
細胞質型 (cytoplasmic pattern)	抗 ARS 抗体（抗 Jo-1 抗体など），抗 SS-A 抗体，抗ミトコンドリア抗体	皮膚筋炎/多発性筋炎，原発性胆汁性肝硬変など

表 2　全身性エリテマトーデスでみられる自己抗体

自己抗体	頻度	疾患，症状との相関
抗二本鎖 DNA 抗体	50～70%	特異的 抗体価は（腎炎などの）活動性と相関
抗 Sm 抗体	10～30%	特異的 重症例にみられる
抗 U1 RNP 抗体	30～60%	混合性結合組織病では必ず陽性 SLE，強皮症，筋炎でもみられる
抗 SS-A 抗体	40%	シェーグレン症候群をはじめとするさまざまな膠原病にみられ，特異的ではない
抗リボソーム P 抗体*	10%	特異的 精神・神経症状を伴いやすい
抗リン脂質抗体	20～30%	血栓症を生じるリスクが高い

*外注検査で測定できるが保険適用なし