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Review

Biomarkers in systemic sclerosis-associated interstitial lung disease: review of the literature

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Abstract

SSc is a rare disease of unknown origin associated with multiple organ involvement. One of the major complications that drives the mortality of SSc patients is interstitial lung disease. The course of SSc-interstitial lung disease progression has a wide spectrum. Since the treatment is based on aggressive immunosuppression it should not be given to stable or non-progressing disease. The correct identification of disease with high risk of progression remains a challenge for early therapeutic intervention, and biomarkers remain urgently needed. In fact, eight categories of biomarkers have been identified and classified according to the different biological pathways involved. The purpose of this article is to describe the main biomarkers thought to be of interest with clinical value in the diagnosis and prognosis of SSc-interstitial lung disease.

Key words: biomarkers, ILD, fibrosis, systemic sclerosis, pulmonary fibrosis, SSc-ILD

REVIEW

Rheumatology key messages

- Interstitial lung disease is a frequent complication of SSc and its main cause of death.
- The correct identification of SSc-associated interstitial lung disease with high risk of progression remains a challenge.
- In SSc-associated interstitial lung disease, biomarkers are needed to aid clinical decisions for therapeutic intervention and follow-up.

Introduction

SSc is a rare inflammatory disease of unknown origin associated with multi-organ involvement. One of the major complications that drives the mortality of SSc patients is interstitial lung disease (ILD) [1]. The typical physiopathological pattern associates vascular inflammation and fibrosing process [2]. The main clinical classification is based on skin fibrosis extension. lcSSc is characterized by a skin fibrosis restricted to distal areas to the elbows and knees. By contrast, dcSSc involves proximal areas, the face and the trunk, in addition to distal areas. Furthermore, internal organs (kidney, lungs, heart, etc.) are more frequently affected in this disease subtype. Like other connective tissue diseases, SSc is associated with autoantibody positivity (ANA). The implication of ANA in disease physiopathology is not yet fully understood, but they are associated with specific phenotypes. The course of SSc-associated interstitial lung disease (SSc-ILD) progression has a wide spectrum, ranging from slow-evolving disease to quick flare-up and deterioration. Since the treatment is based on aggressive immunosuppression it should not be given to patients with a stable and non-progressing disease. The problem, as in other ILDs [3–5], is to identify patients at high risk of progression for early therapeutic intervention [6, 7].

Biological markers, often referred to as biomarkers, are commonly defined as objectively measured elevated indicators of physiological/pathological processes or pharmacological response to therapeutic intervention [3, 8]. Biomarkers remain urgently needed as tools for differential diagnosis, prognosis and disease progression, and as therapeutic response predictors in SSc-ILD. Although ANA were the first biomarkers available in SSc, they still

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are unhelpful for the assessment of disease activity and treatment response [9, 10].

Ideally, for widespread utility, biomarkers should be easy to sample and analyse [3]. Therefore, we focused our review on blood molecules and circulating cells available in SSc-ILD to aid health care providers in clinical decisions and patients' follow-up.

Here we have performed a systematic research in PubMed by typing the words 'biomarkers' and 'systemic sclerosis', and selecting those measured in blood. Publications dates of selected papers range from 1993 up to 2019. Papers were selected according to their potential clinical utility.

We divided biomarkers into eight different categories (Table 1), according to their involvement in distinct biological process. Furthermore, we suggest the main clinically relevant biomarkers according to our literature review (Table 2).

Alveolar epithelial proteins

Alveolar epithelial biomarkers are easily identified because of their high turnover, but also due to the central role of epithelial cells. Nevertheless, the markers of alveolar epithelial cell lesions lack specificity and are modified in a broad range of diseases. The main biomarkers associated with alveolar epithelial cell damage (or dysfunction) are the Krebs von den Lungen-6 (KL-6) antigen and the surfactant proteins A and D (SP-A and SP-D).

SP-A and SP-D

Pulmonary surfactant proteins are lipoproteins secreted by alveolar epithelial and Clara cells. They are implicated in reducing the alveolar surface tension at the air–liquid interface to prevent small airway collapse. These proteins are pneumo-specific [3, 11] and the serum value is associated with the damage extent of the capillary–alveolar barrier (with a leak to the blood compartment).

SP-D is increased in patients with SSc-ILD compared with healthy subjects [12–15, 71]. Serum SP-D is higher in patients with SSc-ILD than in SSc patients without ILD [13, 16]. Due to the small sample size and population heterogeneity in previous studies, there were some discrepancies regarding the diagnostic and prognostic value of SP-D in SSc-ILD [12, 14, 15, 17–19, 72]. Recently, the largest prospective cohort evaluating SP-D in SSc was published ($n = 427$). In this large study, serum SP-D value combined with serum anti-topoisomerases I antibody could detect patients with SSc-ILD with 97% sensitivity, 69% specificity, an 80% positive predictive value and a 95% negative predictive value. Furthermore, with the association of these two biomarkers, three categories of patient could be identified according to their risk of ILD (mild risk 30–44%; moderate risk 45–70% and high risk >70%). Nevertheless, SP-D was correlated neither to the severity of the lung impairment, nor to its evolution or patient mortality [16]. Interestingly, a recent short retrospective study ($n = 32$) found that a rapid decrease in serum SP-D after CYC and prednisolone treatment (below 200 ng/ml) was predictive of good response to treatment [20]. This result needs further investigation in large scale prospective trials. According to these results, SP-D seems to be a good biomarker for SSc-ILD diagnosis, but not for severity or prognosis evaluation. Concerning the therapeutic response evaluation and prediction, large scale prospective studies are necessary.

Focusing on SP-A, which is known to be also associated with alveolar epithelial damage, many authors agreed that its sensitivity (around 45%) and specificity is hardly lower than SP-D in the context of SSc-ILD [11].

KL-6

KL-6 is a high molecular weight glycoprotein produced by type II pneumocytes and bronchial epithelial cells [73]. Its production is raised during epithelial lesions and cellular regeneration [22]. In normal lungs, this glycoprotein is involved in fibroblast stimulation and apoptosis inhibition [74]. In case of epithelial lesion, alveolo-capillary leak can occur and leads to an increase of serum KL-6 levels. Indeed, this modulation is not specific to SSc and can be found in numerous other diseases associated with alveolar epithelial cells lesions (autoimmune diseases, radiation-associated pneumonia, drug-associated pneumonia, etc.) [23, 71, 73, 75, 76]. In SSc-ILD, KL-6 serum levels could be of interest for diagnosis and prognosis, and for therapeutic response evaluation.

As already mentioned, KL-6 is not specific to SSc-ILD and is known to be increased in patients with active fibrosing lung disease [22, 24]. In SSc-ILD patients, former small studies identified a KL-6 serum value of 500 U/ml to diagnose SSc-ILD with a 93% diagnostic accuracy at sensitivity and specificity of 79 and 93%, respectively [25, 75]. However, a recent large prospective study ($n = 427$) in SSc patients found a sensitivity of only 44% and a specificity of 85% (with negative and positive predictive values of 75 and 54%, respectively) for a cut-off serum value of 923 U/ml [16]. Once the diagnosis is made, KL-6 serum level can help in disease severity evaluation since it is negatively correlated with lung function [assessed by forced vital capacity (FVC) and lung diffusion capacity for carbon monoxyde (DLCO)] and positively with radiological impairment or presence of extensive lung fibrosis [16, 20, 22–24]. These results were recently confirmed in the largest prospective SSc-ILD cohort evaluating KL-6 so far [16]. Otherwise, a serum level >1000 U/ml in patients suffering from ILD (various aetiologies considered) was associated with an increased mortality at 5 years [26], and Kuwana *et al.* identified that a serum level >1273 U/ml was found in patients with end-stage SSc-ILD [21]. Unfortunately, the recent large prospective cohort did not confirm the correlation of KL-6 with mortality or with the prognosis [16]. Finally, for some authors, serum level variations were associated with modification of the disease activity (flare-ups or improvements). Furthermore, a serum level >2000 U/ml in patients under treatment (corticoids and CYC) predicted poor therapeutic response in a small retrospective study [20, 24, 27].

Taken together, these results suggest that KL-6 could be a good biomarker to assess SSc-ILD severity, but not

Table 1 Main biomarkers associated with SSc-ILD

Category	Biomarker	Diagnosis	Prognosis	Therapeutic response prediction	Comments	References
Alveolar epithelial markers	SP-D and SP-A	+	–	+	Serum level (SP-D) associated with anti-topoisomerase I antibody predicts SSc-ILD with 97% ss, 69% sp, a 80% PPV and a 95% NPV (large prospective study) SP-D level <200 ng/ml during treatment predicts good response (small restrospective study) SP-A: low ss and few studies	[11–21]
	KL-6	–	–	+	High KL-6 serum value (>923 U/ml) associated with more severe pulmonary functional impairment (large prospective trial) KL-6 serum level remaining >2000 U/ml under treatment predicts poor TR (small retrospective trial)	[16, 20–28]
Chemotactic molecules	CCL18	–	+	NK	Serum value needs to be associated with patient gender and immunosuppressive drug use for a more precise evaluation of prognosis (large prospective study)	[16, 29–32]
	CCL2	+	+	NK	Higher serum levels (>0.66 ng/ml) = worse functional, clinical and mortality outcome (large prospective studies)	[33, 34]
	CXCL10	+	+/– (conflicting evidence)	NK	2 studies (n = 31 and 74) finding no association between CXCL10 and prognosis 1 retrospective study (n = 143) finding association between CXCL10 and worse pulmonary functional outcome	[35–37]
Extracellular matrix remodelling.	MMP-7	+	–	NK	Serum level >1.28 ng/ml ss 89.5%, sp 73.3% for ILD diagnosis in case of systemic sclerosis (small prospective study)	[19, 38]
	MMP-12	+	+	NK	High negative correlation with FVC (small prospective study)	[39]
	MMP-13	–	+	NK	Lower serum levels (<50.2 ng/ml) = worse pulmonary functional prognosis (Low level of evidence)	[40]
	TIMP-1 and -2	+	+	NK	Weak but significant correlation between TIMP-1, TIMP-2 and DLCO Low level of evidence	[41, 42]
Endothelial cell adhesion and activation	ECAA	Conflicting evidence	Conflicting evidence	NK		[43, 44]
	ET-1	+	–	NK	Studies of endothelin receptors antagonists for SSC-ILD treatment are inconclusive Low level of evidence	[45–47]

(continued)

Table 1 Continued

Category	Biomarker	Diagnosis	Prognosis	Therapeutic response prediction	Comments	References
	Selectins (E and P)	+	–	NK	Higher serum levels in SSc Low level of evidence	[28, 48, 49]
	sICAM-1	+	+	NK	Higher serum levels = worse functional prognosis Small prospective study	[28]
Fibrogenesis	TGF-β	–	NK	NK	Associated to extrapulmonary disease severity Small prospective study	[50]
	CTGF	+	+	NK	High serum levels correlated with lower pulmonary functional tests Low level of evidence	[51]
	GDF-15	+	+	NK	Higher serum levels (>370 pg/ml) = worse pulmonary functional outcome (prospective studies: n = 115 for the largest)	[52–54]
	YKL-40	+	+	NK	Higher serum levels (>275 μg/ml) associated with worse functional and clinical outcome and mortality (small prospective study)	[37, 55, 56]
Acute-phase reactant	CRP	+	+	+	Serum level >8 mg/l associated with more frequent ILD, worse pulmonary functional involvement and increased mortality (large prospective study) High baseline CRP serum level is associated with poor TR (small retrospective study)	[20, 57–59]
	IL-6	+	+	NK	Serum levels >7.67 pg/ml associated with more frequent and more severe ILD and increased mortality in early disease (large prospective cohort, n = 212)	[36, 60]
Circulating cells	Fibrocytes	+	NK	NK	Increase serum levels associated with more frequent ILD (small studies) In IPF, serum levels >5% associated with increased mortality	[61–63]
	Circulating endothelial progenitors	–	+	NK	Higher circulating levels associated with more severe ILD Low level of evidence	[64]

(continued)

Table 1 Continued

Category	Biomarker	Diagnosis	Prognosis	Therapeutic response prediction	Comments	References
	Anti-topoisomerase T lymphocyte	+	+	NK	Increased serum levels associated with more severe ILD Low level of evidence	[65]
	Th22 lymphocytes	+	–	NK	Increased serum levels associated with more frequent ILD	[66]
Low level of evidence						
miRNA	mIR-155	+	+	NK	Increased serum levels associated with more frequent and more severe ILD Low level of evidence	[67]
	miR-142-3p and mIR-92-a	+	–	NK	Lower serum levels associated with ILD Low level of evidence	[68, 69]
	mIR-200-c	+	+	NK	Increased serum levels associated with more frequent and more severe ILD Low level of evidence	[70]

Low level of evidence small study(ies): trial with <100 patients with SSc-ILD. Large studies: >100 SSc-ILD patients. CCL18, -2: chemokine ligand 18, 2; CXCL10: chemokine ligand 10; CTGF: connective tissue growth factor; DLCO: lung diffusion capacity for carbon monoxyde; ECAA: endothelial cell autoantibody; ET-1: endothelin-1; FVC: forced vital capacity; GDF-15: growth differentiation factor 15; ILD: interstitial lung disease; IPF: idiopathic pulmonary fibrosis; KL-6: Krebs Von Den Lungen 6; miR: microRNA; NPV: negative predictive value; NK: not known; PPV: positive predictive value; sICAM: soluble intercellular adhesion molecule; sp: specificity; SP-A and D: surfactant proteins A and D; ss: sensitivity; SSc-ILD: SSc-associated interstitial lung disease; Th: lymphocytes T helper; TIMP1–2: tissular inhibitor of metalloproteinase 1–2; TR: treatment response; YKL-40: chitinase 3 like protein 1.

Table 2 Main biomarkers with clinical interest for SSc-ILD according to current literature

Serum biomarkers	Main clinical interest	Suggested cut-off values	Level of evidence	Main references
KL-6	SSc-ILD severity evaluation	⩾923 UI/ml for: Restrictive lung disease (FVC <70%) *De novo* extensive lung fibrosis[a] DLCO <60% Fibrosis extent on CT scan	Multiple small prospective cohorts One very large prospective cohort (n = 423)	[16]
SP-D	SSc-ILD diagnosis	Serum value needs to be associated with anti-topoisomerase antibody status for a better diagnostic accuracy[b]	Large prospective cohort (n = 423) and small observational studies	[16]
CCL18	SSc-ILD prognosis	Serum value needs to be associated with patient sex and immunosuppressive drug use for a better prognosis evaluation[b]	Small prospective studies One large prospective cohort (n = 423)	[16, 32]
CRP	SSc-ILD prognosi Survival	>8 mg/ml	Multiple large prospective cohorts	[57, 59]

[a]Extensive lung disease defined as: fibrosis extent >20% on CT-scan or fibrosis extent 10–30% and FVC <70%. [b]According to Elhai *et al.* [16]. CCL18: chemokine ligand 18; FVC: forced vital capacity; KL-6: Krebs Von Den Lungen 6; SP-D: surfactant protein D; SSc-ILD: SSc-associated interstitial lung disease.

for diagnosis or prognosis. Concerning the therapeutic response prediction, more studies are necessary.

Chemokines and cytokines

CCL18

Chemokine ligand 18 (CCL18) is a constitutive chemokine produced by antigen-presenting cells (mainly dendritic cells and macrophages) in the lung. While the role of CCL18 is not yet fully understood, this molecule seems to be mainly involved in cellular trafficking and in immune response modulation [77]. Moreover, CCL18 is involved in multiple lung fibrosing diseases including SSc-ILD (hypersensitivity pneumonia, sarcoidosis and idiopathic lung fibrosis) [77].

In SSc, CCL18 acts by driving fibroblast proliferation and collagen production. As a positive feedback, native collagen increases CCL18 production, leading to a self-sustaining loop involved in pulmonary fibrosis [78]. In some studies, CCL18 has been found to be inversely correlated with pulmonary function [total lung capacity (TLC), FVC and DLCO] [77]. The first published of them, performed with 85 patients followed during 3.7 years, showed that elevated serum values (cut-off 187 ng/ml) are associated with worse pulmonary functional impairment and higher mortality [hazard ratio (HR) = 5.36] [29]. Similarly, a cut-off value of 140 ng/ml was discriminating on mortality and pulmonary functional impairment in another study [30]. In a third study, serum CCL18 >53 ng/ml was also associated with a more progressive lung disease based on pulmonary function tests and radiological extension of fibrosis [31]. These results were recently sustained by a large prospective trial (n = 427) showing that a high baseline CCL18 serum value (cut-off 84 pg/ml) (HR = 2.9) and male sex (HR = 2.48) were the best predictors of lung function decline during follow-up [16]. Unfortunately, this study did not confirm the correlation with mortality. Taken together, these results suggest that CCL18 is a really interesting biomarker with which to assess the prognosis of SSc-ILD [32], and maybe to predict disease severity and survival.

CCL2

CCL2 is a chemokine mostly involved in monocyte trafficking. Its inhibition stops leucocyte migration in the presence of scleroderma fibroblast in *in vitro* studies [79]. CCL2 is also involved in fibroblast stimulation, myofibroblasts differentiation, lymphocyte T trafficking and lymphocyte Th2 phenotype polarization. CCL2 is mainly produced by endothelial cells, monocytes and type II pneumocytes [35, 80, 81].

Serum CCL2 is increased in cases of SSc-ILD [81, 82]. Furthermore, there is a positive correlation between CCL2 and the severity of the pulmonary impairment (assessed by DLCO and FVC) in case of higher serum values [33, 83]. The correlation with the activity of the disease is not yet shown, but can be assumed because of its importance for fibrosis initiation and progression [84]. One study confirmed the prognostic value of CCL2 in two large independent SSc cohorts (n = 266 and 171) in terms of pulmonary functional decline and survival [33]. Those results have recently been confirmed in a prospective trial with 298 patients (only abstract available), showing similar results for patients with serum CCL2 >0.66 ng/ml [34].

CXCL10

CXCL10 is a strong chemokine for Th1, producers of IFN-γ, an important cytokine in scleroderma pathophysiology.

Higher serum values are found in SSc patients and are associated with more frequent lung and kidney involvement [82, 85]. Concerning prognosis, results are conflicting. Indeed, two trials ($n = 31$; $n = 74$) found no correlation between CXCL10 and the severity of lung involvement [35, 36]. On the other hand, another retrospective study performed with 143 patients showed that CXCL10 is a biomarker for lung disease occurrence (if absent) or worsening (if present) [37]. Those discrepancies may be explained by a major role of Th1 cytokine at early-stage disease and a lesser role in late-stage disease [82]. Therefore, CXCL10 should be assessed in a prospective study with regard to the disease duration.

YKL-40

YKL-40, a chitinase 3-like protein 1, regulates cell proliferation and survival and is produced by activated macrophages. It acts as a growth factor for connective tissue [86] with a promitogenic action on lung fibroblast as described in animal models [87, 88]. Some studies noticed higher serum values in case of SSc compared with healthy subjects [37, 55]. One prospective study on 88 patients showed higher serum levels in case of SSc-ILD (compared with SSc without ILD). A serum value $>275\,\mu g/l$ was associated with worse pulmonary involvement and higher mortality rate [56].

MMP and tissular inhibitors of MMP

MMPs are proteolytic enzymes that are involved, with their inhibitors, in extracellular matrix turnover [89, 90]. They are secreted by macrophages, fibroblasts and endothelial cells. MMPs are balanced in their activity by protease inhibitors called tissular inhibitors of MMPs, or TIMPS, to maintain homeostasis in extracellular matrix remodelling.

Serum concentrations of MMP-7 and -12 are higher in SSc patients than in healthy subjects [38, 39, 91]. They are found in greater serum concentrations in SSc-ILD than in SSc without ILD. Furthermore, there is a positive correlation between serum levels and the severity of the disease [38, 39].

Converse to what is seen for MMP-7 and -12, MMP-13 serum levels are lower in SSc patients than in healthy subjects, and are positively correlated to lung function assessed by FVC [40].

TIMPs have not been extensively studied in SSc-ILD. However, higher serum levels of TIMP-1 were found in case SSc-ILD compared with healthy subjects, with a weak correlation between TIMP-1 and the alveolo-capillar function (assessed by DLCO) [41]. Of note, comparable results are available for TIMP-2 [41, 42].

Biomarkers of endothelial activation

Endothelial cells are highly involved in the pathophysiological process of SSc. Endothelial cell aggression leads to cell activation and inflammatory response. Chronic inflammation ends in a multi-organ fibrosis, which is a key feature of ILD.

Anti-endothelial cell antibodies are found in some SSc patients, with a variable prevalence (22–86%) depending on detection methods and the studied population [43]. Targeted antigens are variable. Concerning SSc-ILD, although some authors found more frequent anti-endothelial cell antibodies positivity, others did not confirm those results [44]. These antibodies could be the cause of endothelial cell activation.

Endothelin-1 (ET-1) is a peptide with vasoconstrictive properties produced by activated endothelial cells. ET-1 is involved in myofibroblasts differentiation and stimulates extracellular matrix production and deposition. Furthermore, ET-1 is involved in fibrogenesis mechanisms induced by TGF-β [92]. Interestingly, ET-1 is increased in SSc both in serum and broncho-alveolar lavage [45]. Histological studies have demonstrated that patients with SSc-ILD exhibit lung overexpression of ET-1 (alveolar epithelium, endothelial cells of micro vessels, macrophages) [93]. Furthermore, affected lungs showed an overexpression of type B endothelin receptor (the opposite of healthy lung) relative to type A receptor [93].

Clinically, serum ET-1 is not correlated to SSc-ILD severity [45, 94], and treatment with ET-1 receptor antagonist in SSc-ILD patients did not show any improvement of the disease [46, 47].

Selectins are adhesion molecules. They are involved in leucocyte trafficking and migration. Although these molecules seem to be important in SSc-ILD, only a few retrospective studies with discordant results are available. A more recent study by Hasegawa *et al.* showed higher E-selectins and P-selectins serum levels in SSc patients without any correlation with lung involvement [28].

Concerning cellular adhesion molecules, soluble intercellular adhesion molecule 1 (sICAM-1) showed higher serum levels in SSc patients than in healthy subjects. Furthermore, higher serum levels are correlated with ILD and with its severity (FVC) until 4 years after initial evaluation [28].

Soluble vascular cell adhesion molecule (sVCAM) is also increased in SSc patients compared with healthy people, whether an ILD is present or not [95–97].

Connective tissue growth factors

TGF-β is widely known as a keystone mediator in the fibrosing process. By binding to its receptor, TGF-β drives myofibroblasts differentiation, stimulates extracellular matrix production and inhibits metalloproteinase production [98]. Before a recent prospective trial on TGF-β in SSc, there were conflicting results about serum values of this cytokine in SSc [99–101]. This recent study by Dantas *et al.* showed higher serum values of TGF-β in SSc patients than in healthy subjects with a positive correlation with the disease severity (digital ulcers, more extensive skin fibrosis). Unfortunately, it does not correlate with lung involvement or its severity [50].

Connective tissue growth factor (CTGF) is a peptide involved in TGF-β-induced fibrosis. CTGF stimulates myofibroblasts to produce collagen 1 and fibronectin, two

major extracellular matrix components [102]. Saton *et al.* identified that SSc-ILD patients exhibit higher CTGF serum levels than other SSc patients, with a positive correlation between serum level and the severity of the disease (lower FVC and lower DLCO) [51]. Interestingly, it appeared that some *CTGF* gene polymorphisms were associated with more frequent SSc-ILD [103, 104].

Growth differentiation factor 15 (GDF-15) is a member of TGF-β superfamily. GDF-15 is involved in fibroblast stimulation and in immunomodulation. Like CTGF, higher serum levels of GDF-15 (370 pg/ml) are found in SSc-ILD, with a negative correlation to pulmonary function tests (DLCO and FVC), as shown in a prospective study ($n = 119$). Otherwise, higher serum values at baseline seemed to be associated with progressive lung impairment, highlighting the correlation between GDF-15 and SSc-ILD activity [52–54].

Acute-phase proteins

Acute-phase proteins refer to proteins whose plasma concentrations increase (positive acute-phase protein) or decrease (negative acute-phase protein) >25% during inflammation. These changes are thought to contribute to host defense and other adaptive capabilities [105, 106].

Patients with serum CRP levels >8 mg/l had more frequent SSc-ILD with worse pulmonary functional impairment (TLC, FVC, DLCO) and higher mortality than those with CRP <8 mg/l [57]. Otherwise, CRP was also associated with worse multi-organ impairment (pulmonary vascular impairment, kidney, skin, etc.) and is therefore not pneumo-specific. These results were confirmed in two others studies [58, 59]. Interestingly, high baseline CRP serum level was predictive of poor therapeutic response in a small retrospective study.

IL-6 is an acute-phase pleiotropic inflammatory cytokine [107]. IL-6 is produced by numerous cells (lymphocytes, fibroblasts, monocytes, etc.) and is involved in T cell activation, acute-phase reaction, and haematopoietic myeloid and megakaryocytic precursors stimulation [108]. IL-6 plays a role in SSc by increasing collagen production through fibroblast stimulation, myofibroblasts differentiation and inhibiting the secretion of metalloproteinase [107]. Interestingly, higher serum levels of IL-6 are associated with SSc-ILD [109–111].

The prognostic value of IL-6 has been assessed in 212 SSc-ILD patients [36]. This study identified that serum levels >7.67 pg/ml are associated with increased mortality (HR = 2.58) at 30 months and with a worse lung functional impairment [FVC decrease >10% (HR = 2.58) after 1 month; DLCO decrease >15% (HR = 3.2) at 1 month]. A *post hoc* analysis finally concluded that prognostic power is only present for mild or early SSc-ILD (FVC >70%) and not for patients with severe lung functional impairment. Those observations suggest an important physiopathological role of this cytokine early in the disease.

Circulating cells

Monocytes

In 1994, Bucala *et al.* found that some circulating leucocytes could display some fibroblast phenotypic characteristics, and once they had migrated to injured tissues, behaved as them. These circulating leucocytes were called fibrocytes [112]. From a general point of view, some circulating progenitor cells are able to migrate in different targeted tissues (inflammation, tissular remodelling or repair, etc.) and to differentiate into different cell types with phenotypic characteristics of fibroblasts, endothelial cells or macrophages with respect to the local cytokine background [113, 114]. Since Bucala *et al.*'s publication, several progenitor circulating cells have been discovered. They all stem from monocytic precursors CD14+. Although these cells actually have a well-known physiological role (scarring, tissue repair and remodelling, host defence, etc.), they can be involved in different pathological processes, such as pulmonary fibrosis [115].

In SSc-ILD, progenitors cells migrate into the lungs because of chemotactic activity. They overexpress CXCR4, a chemoreceptor for CXCL12, a chemokine produced by macrophage and pneumocytes in affected spots of the lung [61, 62]. In fact, cells of monocytic origin and producing collagen (CD14+/CD34+/col1+) are present in lungs of SSc-ILD patients, whereas they are undetectable in healthy subjects [61]. Once there, they take part in the pathologic process. Serum measurement of progenitor cells is available and can be used as a biomarker of diagnostic and prognostic importance. Furthermore, they are of therapeutic interest. However, there are only a few studies about this topic.

Concerning diagnosis, blood levels of fibrocytes (CD34+/CD45+/col1+) and circulating monocytes producing collagen are higher in SSc-ILD patients than in healthy subjects [61, 63].

Regarding prognosis, the blood level of circulating endothelial progenitor cells is positively correlated to the severity of SSc-ILD [64]. Furthermore, in idiopathic pulmonary fibrosis, patients with higher circulating levels of fibrocytes were at increased risk of worse lung impairment and death [62].

Currently, there is no doubt about the importance of circulating progenitor cells in SSc physiopathology and aetiopathogeny. However, more studies are necessary to establish their roles with precision. Their use as biomarkers needs to be further evaluated. In the future they could be used as a therapeutic target.

Lymphocytes

Recent data from the literature suggest an important physiopathological participation of Th in SSc-ILD. Interestingly, Th subpopulations are different between healthy subjects and SSc-ILD patients, but also among SSc patients, suggesting that those subpopulations are important for the disease physiopathology and for phenotypic determination. For example, patients with SSc-ILD

exhibit a higher Th1/Th2 ratio (usually low in SSc) compared with those with SSc without ILD (with a negative correlation compared with FVC). Furthermore, patients with a FVC decline >10% in 6 months have a higher Th1/Th2 ratio than those with stable disease, establishing a link between Th1/Th2 ratio and ILD activity [116]. Patients with anti-topoisomerase antibodies were recently identified as being at higher risk for ILD. They also frequently exhibit autoreactive T lymphocyte CD4+ against topoisomerase. SSc-ILD patients have higher blood levels of these autoreactive cells than SSc patients without ILD. Furthermore, there is a positive correlation between the blood level of these cells and ILD severity (FVC) and activity (rate of FVC decline). This study shows that this population of autoreactive Th is enriched in Th17 and impoverished in Th1 [65]. Furthermore, Th17-related cytokines (IL-17 and IL-23) are associated with ILD in SSc patients, and with its severity [117, 118]. Others have found Th22 to be increased in the blood of SSc patients (compared with healthy subjects). Furthermore, SSc-ILD patients have higher blood levels of Th22 than others [66].

miRNAs

miRNAs are small (about 20 nucleotides) non-coding RNA. By fixing mRNA, they mainly down-regulate protein translation and they act as post-transcriptional regulators of gene expression [119].

The physiopathological importance of miRNA is now well established in SSc [120]. Concerning this complex regulation, the TGF-β signalling pathway has been the most studied [121, 122]. Although miRNA expression is tissue-specific and cell-type-dependent, the circulating fraction of miRNA can be used as a biomarker [123]. Numerous studies have already assessed miRNA expression at the tissular level [121, 124–127], but only a few of them have explored the blood compartment.

In SSc-ILD, miR-155 is overexpressed by circulating mononuclear cells and shows a strong negative correlation with pulmonary functional impairment (assessed by FVC and DLCO) [67]. Interestingly, miR-29a is also reduced in patients with SSc and is assumed to be relevant as an actor in the early phase of the disease [128].

Blood levels of miR-142-3p and miR-92-a are significantly lower in SSc than in healthy subjects, and seem to be specific to SSc compared with other autoimmune diseases (dermatomyositis or lupus erythematosus). However, in SSc patients, miR-142-3p serum level is not associated with any clinical manifestation [68, 69].

Of note, miRNA-200c is elevated in connective tissue disease without any specificity for SSc [70].

To summarize, miRNAs seem to be involved in the pathophysiology of SSc and could serve as biomarkers, but also as new tools for the exploration of potential therapeutic targets.

Limitations

One of the limitations of our review is that we chose candidate biomarkers in SSc-ILD according to our thinking and their potential usefulness. Another limitation is the lack of longitudinal studies for some of the biomarkers, which reduces the clinical impact of the findings. We believe that further longitudinal multicentric studies are urgently needed, using single or multivariate analyses to evaluate the real clinical impact of most of the biomarkers as diagnostic and prognostic tools.

Conclusion

SSc is a rare inflammatory disease frequently complicated by ILD, which drives mortality of the patients. Recent advances in early diagnosis and treatment of SSc-ILD shed light on the urgent need for biomarkers to assess the overall risk of mortality, but also the probability of disease progression and treatment response. So far, there is no easy-to-use biomarker to evaluate the likelihood of ILD progression in the context of SSc. Nevertheless, the recently published article from Elhai *et al*. [16] raised the utility of dosing SP-D, KL-6 and CCL18 in SSc-ILD diagnosis and prognosis. Further prospective longitudinal trials are needed to identify biomarkers with clinical utility and potential new therapeutic targets in SSc-ILD.

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