Review

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The Role of Biomarkers in the Pathogenesis, Clinical Manifestations, and Therapeutic Approach in Systemic Sclerosis

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Abstract: Systemic sclerosis (SSc) is a progressive autoimmune disorder that mainly affects the skin. There are other clinical manifestations as renal, pulmonary, cardiovascular, and gastrointestinal tract involvements. Based on the skin involvement there are two subtypes of SSc, as limited cutaneous SSc (lSSc) which involves the acral part of the body and diffuse cutaneous SSc (dSSc) resulting in significant skin thickening of the body. The lSSc has usually moderate internal organ manifestations, except for the late-onset pulmonary arterial hypertension (PAH) mainly appearing in this form. In dSSc the severity of the organ manifestations or failure is more intensive such as scleroderma renal crisis (RSC), interstitial lung disease (ILD), myocardial fibrosis, and chronic gastrointestinal dysmotility with consequent malabsorption [1].

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Pathogenesis

Several environmental and genetic interactions predispose to the appearance of the disease. Besides the cellular and humoral immune abnormalities, inflammatory cytokines, the distortion in the balance of the growth factors and the autoantibodies - result to the fibroproliferative vasculopathy and finally, the cutaneous and visceral fibrosis.

The importance of the genetic and epigenetic background is continously increasing. The HLA genes in the SSc patomechanism are proved to have the strongest association with the antibodies and predisposing factors. There are some differences between the African-American (HLA-DRB1*08.04, HLA-DRB1*11.02) and European-American (DPB1*13.01, HLA-DRB1*07.01) cohorts. While the HLA-DRB1*08.04, HLA-DRB1*11.02 alleles are associated with the development of SSc, the HLA-DRB1*11.02 alleles are related to the anti-fibrillarin antibody onset. In the European-American cohort the DPB1*13.01, HLA-DRB1*07.01 refers to anti-topoisomerase-1 (ATA) and anti-centromere (ACA) antibodies (1). In the Ssc myofibroblast and non-myofiboblasts, the neuroblastoma breakpoint family (NBPF) genes are highly expressed. The mutations of potassium channel genes- KCNK5, ABCC- are related to the PAH in Ssc [2]. Moreover, there are many other tissue-specific transcription factors (ELF1, MGA) are overexpressed while KLF4 and ID4 are downregulated in Ssc blood cells [3]. Zou et al. have studied chromosome regions of SSc and their findings proved the number genetic loci were associated with high prevalence of Choctaw Indians [4].

The molecular mimicri hypothesis is also supported as homologous sequences of the autoantibodies of SSc and the viral proteins (Mimiviridae and Pycodnaviridae families) (1).

The other important molecular pathway is the epigenetic mechanisms, which leads to the pathognomic molecular alteration in the fibroblasts and drives the activation of profibrotic factors (HOTAIR/EZH2/NOTCH) by mi-RNA-34a. The abnormality of the chromatin tools of dendtritic cells has a prominent and accountable role in the epigenetic process in Ssc patients [5].

Both innate and adaptive immune system take a significant impact in the pathogenesis ~~in~~ of Ssc. Each genetic upregulations of the pathognomic factors, especially the type 1 interferon (T1 IFN), fibroblast growth factors (FGFs), and it receptors (FGFRs) contribute to the profibrotic process by the FGF9/FGFR3 abnormality.

The activation of the innate and adaptive immune system, the immune cells, the proinflammatory cytokines, the damaged and apoptotic cells, the adhesion molecules and damage-associated molecular patterns result the vascular damage and the fibrosis, also.

However, in Ssc the altered immune response could lead to fibrosis in short time, there are some upregulated prominent cytokines and signalling cascades in the early phase of the disease. Contrary, the anti-inflammatory responses are mainly downregulated and reduced in Ssc. The regulatory T-cells, regulatory B-cells, natural killer cells (NK-cells), and reduced IL-10 secretion are observed in Ssc pathway [6].

The molecular and cellular dysregulation leads to the endothelial cell activation, vascular occlusion, vasculogenesis, and tissue hypoxia by fibroblasts, T- and B-cells and endothelial cell activity. Immune cells are activated and characterise the progress of the disease. IL-4, IL-13 induce the B cell proliferation resulting production of immune globulin, adhesion molecules, and inflammatory cytokines. In the fibrosis, TGFß production and differentiation is overstimulated which results collagen synthesis, and fibroblast proliferation. On the other side, the inflammatory cascade direct inhibits the anti-inflammatory factors, as the synthesis of metalloproteinase 1 and 3 (MMP1, MMP3) [7].

Dendritic cells (DCs) also have a critical and pathognomic role in the Ssc pathophysiology. DCs contribute to antigen presentation by activating naïve T-cells. Interferon-α (IFN-α), chemokine ligand 4 (CXCL4) secretion is stimutaled by the Toll-like receptor-8 (TLR8). The TLR8 is placed on the surface of the plasmacytoid DCs (pDCs) and enhance the profibrotic processes in the skin [8].

The overstimulation of monocytes, M2 macrophages, mast cells and therefore the excessive TGFß, IL-4, IL-6, IL-13, PDGF, TNF-α production stimulate directly other profibrotic factors, chemoattractive and intercellular adhesion molecules. However, all types of pro-inflammatory cells could be detected in the inflammatory pathways. The Dcs, monocytes, M2 macrophages, mast cells, and Type 2 helper cells (Th2) contribute mostly in the early phase of the inflammation. IL-4 and IL-13 produced by Th2 activate macrophages and fibroblasts to produce TGFß, also [9,10].

Besides T-cell abnormalities, the wide scale of B-cells also interact in the progress of Ssc. B-cells secrete IL-6, which is one of the most relevant therapeutic target. The presence of specific autoantibodies - which can be present in most of the SSc patients – is also a strong evidence of the B-cells pathophysiologic role [11].

The obliterative vasculopathy and the fibroblast activation are connected strongly by the immune cells and cytokines mentioned above. However, in the fibroblast activation, resident fibroblasts, pre-adipocytes, endothelial cells, mesenchymal stem cells, fibrocytes overgo within trans-differentiation by activation of the TGFß. As a result of the transactivation, myofibroblasts are activated, and further pro-inflammatory cytokines are secreted rapidly or continuously [12]. Myofibroblasts are the source of the main extracellular matrix elements as elastin, collagens, fibronectin, proteoglycans etc. The presence of myofibroblasts are not specific but prognostic for connective tissue disease, especially for SSc. The loss of normal apoptosis of the immune cells is also a key process in the development of SSc. Therefore, the abnormal activity myofibroblast cells could survive to result prolonged fibrosis and increased rigidity of the tissues [13].

Clinical manifestations and screening tools

The skin involvement is still the hallmark manifestation of SSc. The cutan variablity usually wide, however the two forms, as limited or diffuse cutan scleroderm characterized by the spreading of the rigidity of the body. As the modified Rodnan skin scores (mRSS) are observed with high variability by the clinicians, the high-frequency ultrasound resulted a more specific and useful tool to detect the skin alterations [14].

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Musculoskeletal manifestations are strongly connected with the skin involvement. The progression of the disease is associated with the hand, foot, and further the elbow deformity, and one of the most progressive symptoms as acrosclerosis. SSc and rheumatoid arthritis overlap syndrome is studied in 25% in two French studies and the authors confirmed that the presence and co-existence of rheumatoid factor (RF), anti-citrullinated proteins (ACPA), anti-carbamylated protein (anti-CarP) antibodies predict a worse prognosis to vascular progression, synovitis, tenosynovitis, digital ulcers (DU) and interstitial lung diseases (ILD) [15].

The neurological manifestations are not rare in this disease. As a result of the direct damage of the immune mechanisms and spreading of the fibrosis both of the sensory and motor polyneuropathy are observed. Polyneuropathy, trigeminus neuralgia and mononeuritis multiplex were also reported in a wide range of SSc patients [16].

The vascular abnormalities are very significant symptoms in SSc and in the early phase of the disease. These abnormalities are very specific, as well. The Raynaud's phenomen could be the leading symptom in the early onset and during the progression of the disease, too. The worsening of the vasculopathy could manifest digital ulcers, internal organ involvements as PAH, or malabsorption. The calcinosis is also a specific clinical subset in SSc which usually reported on the extensor part of the extremities. While the anti-PM/Scl70 antibodies overlap refers a good prognosis, the male sex, DLCO<70%, cardiovascular manifestation, elevated CRP level (>5 mg/l) are all reported as worse outcome [17,18].

PAH and ILD are still the main two manifestations of the death in SSc. Based on vascular abnormalites the vascular, especially the arterial stiffness result hemodynamical changes in the main arterial brunches. Otherwise, PAH and ILD are characterized by both of micro-and macrovascular abnormalities. The DETECT algorithm, the echocardiography, cardiac MRI all a potential essential detecting tools in SSc to characterize the stage and phenotype of the cardio-pulmonary manifestation. The arrythmias, non-ischaemic cardiomyopathy, increased diastolic dysfunction, myocarditis could manifest as a cardiac onset [19].

The phenotype of the SSc-ILD has wide range of scale. Chest XR, HRCT imaging, as well as lung ultrasound, lung density on high resolution computer tomograpgy (HRCT) scan, bronchoalveolar lavage (BAL) could measure the radiological progression. In BAL fluid (BALF) – which is not routinely performed in Ssc - high level of biomarkers could be identified. The worsening of pulmonary fibrosis, the bronchiectasis, decreased lung diffusing capacity and neutrophils in the BAL are also negative prognostic factors [20].

In the gastrointestinal (GI) manifestations the oesophageal reflux disease, dilatation, dysmotility have a prominent impact in the prognosis. The transabdominal oesophageal ultrasound or manometry usually show a slower peristalsis or oesophageal dilatation [21]. The role of altered gut microbiome has a deep impact in the developing of Ssc and other immune-mediated disorders as psoriatic arthritis, inflammatory bowel disease (IBD) related spondyloarthritis, coeliakia etc. The dysregulation of the balance of gut bacteria - as increased number of Fusobacterium, Ruminococcus, Lactobacillus and decreased concentration of Faecalbacterium crosstalk - could result in damage of the gut permeability. Moreover, the changes of the gut permeability initiate further immune-mediated or autoimmune response in the joints and skin, as well. Behind the histopathological examination by intestinal biopsy which is often complicated to apply, the biomarkers could be potent tools to guide us in even the early phase of the disease [22].

The diversity of biomarkers

The diversity of biomarkers in systemic sclerosis is a continuously expanding field to monitor the pathomechanism, clinical course and therapeutic approaches. The biomarkers, as non-invasive and sensitive indicators reflect the physiologic, and pathological processes, disease prognosis and the response to therapy. In detail, specific biomarkers are needed for classification, early diagnosis, to distinct the subtype of the disease (lSSc and dSSc), the co-existence of the organ manifestation with the subtypes, clinical course as the prognosis and to evaluate the therapeutic response. In systemic sclerosis sensitive and specific, validated biomarkers are not confirmed yet, despite of the

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overwhelmed and extensive research, except of NT-proBNP in pulmonary arterial hypertension, the anti-Scl70 in dSSc and anti-centromere antibody in lSSc. However, the ANA positivity is one of the criterions in the early onset systemic sclerosis [23,24]. The modified Rodnan skin score (mRSS) is a functional biomarker and gold standard to measure the disease extension and activity, however, has numerous difficulties to assess the skin involvement. To differentiate the fibrotic skin from the borderline changes or the ~~o~~edema in the early phase are irrelevant by this assessment [14]. The initial and most critical process in the SSc pathogenesis, the vascular dysfunction which leads to the development of PAH and renal crisis. The endothelial cell abnormality is demonstrated by elevated von Willebrand factor level. The presence of adhesion molecules contributes to the development of early fibrosis and correlates the organ manifestation extension. The VEGF is a widely interested molecule for the progression of the disease and its level is significantly high in the early SSc, as well as in the worsening of the vital capacity (Fig 2) [25].

The endothelin-1 (ET-1) as a potential vasoconstrictor, stimulates the smooth muscle cells and has also an important role in the obliterative vasculopathy and in Raynaud's phenomenon. ET-1 correlates strongly with ~~the~~ von Willebrand factor and adhesion molecules. The elevated plasma levels of the endostatin are in positive correlation with the mega-capillaries, digital ulcers, and PAH [26].

Autoantibodies

To classify the biomarkers for the diagnostic and clinical classification, the endothelial dysfunction, fibrosis, immunological alteration, and organ manifestations are grouped as autoantibodies, growth factors, cytokines, chemokines, adhesive molecules.

The early diagnosis and identification the subtypes of systemic sclerosis provide the better outcome in this progressive disease. Therefore, the role of ANA positivity, besides the puffy fingers and Raynaud-phenomenon, is highly determined in the early onset systemic sclerosis. Most of times, in the early phase the phenotype of the two subtypes is common. Depending on the pattern confirmation of ANA – as centromere, nucleolar, RNA polymerase III, Scl-70, U3-RNP - is refer to the development of subtypes and clinical manifestation of SSc. The anti-Scl-70, anti-U3RNP, anti-Th/To and anti-Pm/Scl highly refer to interstitial pulmonary disease (ILD), however pulmonary arterial hypertension (PAH) appears often in the presence of anti-centromere, anti-U3RNP, anti-Th/To positivity (Table 1) [27].

Table 1. Systemic sclerosis specific antibodies.

Biomarker	Classification	Clinical association	Response to the therapy
Anti-Scl-70	Anti-DNA topoisomerase antibody	Diffuse cutan SSc, pulmonary fibrosis	No
anti-CENP-A (anti-centromere Ab (ACA))	Anti-kinetochore protein antibody	Limited cutan SSc, arterial pulmonary hypertension (10-20%)	No
Anti-Pm-Scl	110-120 kDA nuclear and nucleolar protein antibody	PM/SSc overlap	No
Antifibrillarin	Az U3-RNP 34 kDa nuclear protein component antibody	Diffuse cutan SSc	No

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Anti-Th/To	RNAase P ribonucleopotein antibody	Limited cutan SSc, pericarditis, ILD	No
Anti-RNA polymerase I and III	RNA polymerase antibody	Diffuse cutan SSc, renal involvement	No

Cytokines

Systemic sclerosis and its manifestations are mostly characterized by fibrosis during the disease duration. The IL1-like cytokines, as IL1α and β were detected in SSc patients versus in healthy controls and elevated ILα levels were observed patients with DU, higher concentrate of ILβ and IL-13 was elevated in PAH. IL-18-binding protein isoform (IL18BPa) was associated with the pulmonary arterial wedge pressure (sPAP). [28] IL-33 is correlated with the sPAP, DU, diastolic dysfunction, as well. Remarkable elevated levels of IL-13, IL-4, IL-6, IL-10 were detected patients with PAH and cardiac manifestation [29,30]. Overall, significant differences of IL-17 cytokines have not been observed in SSc patients verus controll, however IL-17A, IL-17B, IL-17E, IL-17F were significant elevated in SSc patients and IL-17E, IL-F have been correlated with DU [30,31].

There is a wide spectrum of the biomarkers reflecting fibrotic process and therapeutic approach. TGFβ stimulates the synthesis of extracellular molecules directly and decreases the matrix metalloproteinases. At the same time, TGFβ changes the phenotype of tissue fibroblast to initiate alteration into myofibroblast. CTGF is also a significant factor for reflecting fibrosis, however, is not clear if TGFβ or CTGF was the better biomarker of fibrosis process.

The PDGFα and β are also very informative and therapy sensitive indicators which show negative feedback to the efficacy of nintedanib therapy [32,33].

The mononuclear cell infiltration is a specific part of the immunological alterations both in the internal organs and skin, as well. The expansive T-cell population leads to the invasion of cytokines and growth factors. Changing of the physiologic phenotype of the cells usually results producing extensive collagen mass. In systemic sclerosis, the role of IL-6 is highly recommended being a target cytokine, as tocilizumab has been proved in ILD, PAH and musculoskeletal involvements [34]. Otherwise, lowel IL-6 levels have been detected in patients with DU [30].

The other prominent pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF) has been associated with PAH [35g. The IL-2 receptor has been detected a relevant biomarker of the disease progress and skin severity. The TNF-alpha is unquestionable one of the key markers in the pathophysiology of SSc and could show the progression of pulmonary function. However, it has not been clarified weather the TNF alpha or the receptor was the more informative biomarker in this disease [36]. Taken together, cytokines play a strong biomarker role in the pathomechanism in vascular abnormalities and PAH in SSc.

Chemokines

Chemokines (CXCL4, CXCL10, CX3CL1) have also a significant impact in the progression of SSc. CXCL4 is a prohibitor of IFN-γ and could enhance the skin fibrosis. The CXCL10 is predictive in the early onset SSc. Digital ulceration and pulmonary fibrosis are reported by CX3CL1 through the migration and adhesion [37]. The interstitial pulmonary disease and pulmonary arterial hypertension are highly responsive for the mortality and morbidity in SSc. There are several molecules are confirmed to reflect to the ILD severity and most of them are expected to be potential useful biomarkers. The endothelial microparticles,–e.g. CD144+—play an important role in the cell-cell interactions and signalling. The serum concentration of these molecules is significantly elevated in PAH. The lung-epithelial surfactant proteins are relevant diagnostic markers in ILD. KL-6 shows the fibrosis severity in ILD. The ILD severity associated with CCL-2, CXC4 and PF-4 that are produced by immune cells. CCL-18 have a pivotal role in the collagen synthesis and is a strong prognostic factor in ILD severity [38]. The YKL-40 (Chitinase-3-Like Protein 1) -as a tissue activator - is also a very

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important biomarker of ILD prognosis. The faecal and serum calprotectin – however not to be a chemokine- is also a good biomarker not only of the gastrointestinal manifestation and ILD. Furthermore, calprotectin is therapy sensitive, therefore it could be validated for monitoring the symptoms in SSc in the future [39]

Chemokine alterations could demonstrate the pathological pathways, e.g., stable serum CCL-2 level and decreased CXCL-10 level refer to the Th1 shift to Th2 way. The anti-Ro52 antibodies are biomarkers of infective pulmonary diseases but predictive for worse outcome in ILD. The OX40-OX40L axis correlates with the extension of fibrosis in the lung and skin, as well [40]. (Table 2.)

Table 2. ILD-associated biomarkers.

Biomarker	Function	Clinical association	Response to the therapy
KL-6 (Krebs von den Lungen-6)	II type pneumocyte mucinosous glycoprotein	Most informative biomarkers for ILD	Yes
SP- A and SP-D (Surfactant Protein-A and D)	Produced by II type pneumoncyte	Capillary and alveolar barrier distorsions	Not known
CCL2, CCL18 (Pulmonary and activation regulated Chemokine)	T-cell chemotaxis, migration	ILD progression and mortality	Not known
YKL-40 (Chitinase-3-Like Protein 1)	Tissue activator	Worse ILD prognosis and mortality	Not known
Calprotectin	60% soluble protein (neutrophil granulocyte, monocyte, macrophage, epithelial cells)	Gastrointestinal symptoms, ILD, more severe SSc form	Yes
CXCL10	cell migration, inflammation	ILD, kidney	Not known
Anti-Ro 52/TRIM21 (tripartite motif-21)	Mononuclear cells ubiquitin ligase	ILD, worse prognosis	Not known
OX40L	Direct effect on MMPs expression, fibrosis	dSSc-ILD, worse prognosis	Not known
MCP-1 (monocyte chemoattractant protein-1)	T-cell and monocyte migration, cell adhesion	ILD progression	Not known
Anti-Scl70	Anti-DNA topoisomerase antibody	ILD, FVC worsening	Not known

Circulating neurovascular guidance molecules

Many studies have been presented to understand the role of these molecules and to precise their functions. Several neural molecules have been discovered to regulate vascular remodelling, as ephrins, netrins, slits, and semaphorins. The balance of neurovascular communication is essential in the neurovascular stability. In SSc, the role of secreted class III semaphorin (Sema3s) is related to the angiogenesis. Sema3C have both pro- and anti-angiogenic factor function, Sema3E presented in the early vascular abnormalities [41].

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Increased level of NRPs has been showed in Ssc patients with PAH. In the Slit family (Slit1, Slit2, Slit3) the Slit2-SSc association has been proved in the early onset as a peripherial vascular biomarker. Among the sirtuins (SIRTs), SIRT1 and SIRT3 are decreased in SSc and being related to DU [42,43].

Metabolic properties

Adiponectin is a bifunctional hormone as having pro-and anti-inflammatory role in ~~the~~ different diseases. In SSc, decreased adiponectin concentrations has been found and there has been a significant increasing tendency of the concentration after prostaglandin analogue treatment [44]. Leptin activate the pro-inflammatory cytokines and enhance the angiogenesis. However, some studies have not reported significant differences in serum leptin levels between SSc patients and controls, some other researches have shown increased level of leptin in SSc patients with PAH [45]. Similarly, resistin was not alter in the two groups previously, but increased level of resistin was detected in patients with DU and PAH. Galectin 1 is associated with telangiectasias, galectin 3 refers to developing of DU. Contrary, the level of vaspin was decreased in SSc patients with DU. Chemerin has pro and anti-inflammatory effect, depends on the circulating immune cells and micro-environmental background. Chemerin was significantly increased in Ssc-PAH, also [46].

Vascular biomarkers

The vascular biomarkers are presented in very early Ssc, as microangiopathy could appear rapidly. The small vessel damage and chronic hypoxia could be intensified by angiogenic and fibroproliferative factors, also. Endostatin is associated with giant capillarity abnormai~~l~~ities and strongly appear with onset of right ventricular systolic pressure [47]. The endoglin has a remarkable role in angiogenesis and its level is significantly elevated in patients with DU, anti-centromer antibodies, ILD, PAH. The endoglin correlates positively with telangiactasia especially herediter hemorrhagic telangiactasia. Von Willebrand Factor (vWF) and ADAMTS-13 is also a positive biomarker for disease activity and severity in ILD and PAH [46,48].

Markers of pulmonary hypertension (PAH) and ILD

The r*ight heart catheterisation (RHC)* is the criteria of the diagnosis of pulmonary hypertension (PAH) in SSc, also. Although, RHC is an invasive method, it is suggested to use this procedure in cases of high-risk patients. Validated non-invasive and sensitive biomarkers are essential for detecting PAH. The NT-proBNP is a sensitive but not specific marker for PAH in SSc as elevated NT-proBNP level is also associated with left ventricle dysfunction and renal insufficiency. NT-proBNP is correlated with the skin fibrosis and its level is higher in dSSc. Two important biomarkers, as endothelin-1 and the A-type anti-endothelin (anti-ETaR) receptor antibody are representative for PAH, ILD and DU. Both markers reflect sensitively for bosentan. Receptor antibody (anti-AT1R) elevated in decreased DLCO and PAH. The anti-centromer antibody, anti-p4,2 and CD144+ EMP cadherin have a strong correlation with the DLCO<70 and PAH [49].

The FSTL3 expression is stimulated by heart failure and contributes to the activation of fibroblasts leading to increased cells adhesion and collagen synthesis. NA let-7-d is another promising biomarker in PAH. Selene has a potential role in the oxidative stress therefore the elevated Cu/Se rate, and the ceruloplasmin have a positive tendency towards in patients with PAH and fibrosis, also [50].

ILD and PAH, as cardiopulmonary manifestations of SSc, are the two major causes of morbidity and mortality in SSc. The mortality in patients with PAH and/or ILD is significantly higher than without it/them. Scleroderma renal crisis - as characterized by hypertension and renal failure - is a life-threating condition, however its prevalence declined after the preferable indication of angiotensin convertase inhibitor~~s~~ (ACE) therapies. The progressive phenotype of ILD could be identified and followed by FEV1, FVC and DLCO. The high-resolution computed tomography (HRCT) is frequently used to clarify and detect the patterns of the pulmonary involvements.

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However, we must take into consideration the frequented radiation exposure of the HRCT. LFT is also an inhibited diagnostic method during the COVID19 panendemia. Recently, the importance and role of the biomarkers in ILD/PAH is more emphasised in the clinical practise, as well [51]. In BALF, behind the autoantibodies (anti-Scl-70, anti-centromer antibodies), CCL18, macrophag 2-derived protein) are sensitive for progression of SSc-ILD. KL-6 (Krebs von den Lungen-6), MMP7, MMP12 are good prognostic factors in the early lung involvement or SSC-ILD, overall. CCL2 is related to ILD progression and poor prognosis. Some proteome-wide studies have shown that CXCL3 and CXCL4 levels were significantly higher in SSc-ILD patients, otherwise did not correlate with the severity of the disease [52].

Skin fibrosis markers

Besides of the modified Rodnan skin score further non-invasive but more objective biomarkers are needed to evaluate the skin involvement in SSc. The heat-shock protein, as a pro-inflammatory molecule, is higher in dSSc than in lSSc or healthy population. The IgG-Gal and IL-16 cytokine have a positive correlation with the mRSS and the skin severity and subtypes of SSc can be assessed by this molecule. Inverse correlation is based between the adiponectin and skin fibrosis or mRSS. The genetic analysis of the scleroderma skin has a promising candidate biomarker pattern. The THBS1, COMP, SIGLEC1 and IFI44 are correlated moderately with the mRSS and further analyses has confirmed that HOTTIP, SPRY4-IT1 is associated positively with mRSS, otherwise ANCR and SPRY4-IT1 are significant biomarkers for PAH [53].

Potential renal biomarkers

The renal manifestation is commonly appearing in SSc patients. The scleroderma renal crisis (RSC) could be a life-threatening episode in SSc. The exact role of anti-RNS polymerase III antibody is unknown. The pathogenetic role of GPATCH2L, CTNND2, ICAM-1 and VCAM-1 is confirmed. Additionally, there are some other molecules, e.g., C3b deposits and chemerin, are screened to be relevant biomarkers in several autoimmune disorders and in SSc, also [54].

Gastrointestinal biomarkers

There are some studies have emphasized that calprotectin level is a highly sensitive but not specific biomarker of GI manifestation [55]. The GI manifestation could be the early onset in Ssc, and the calprotectin (F-cal) is described to be presented in the early phase of the disease as well. Hamberg et al have described that, F-cal was not associated wth the ~~o~~esophagial radiological alterations. They emphasize the testing of the calprotectin at the time of the diagnosis or suspicion of Ssc onset [56]. In another cross-sectional study, Stec et al. have found that among the serum intestinal permeability markers as intestinal fatty acid binding protein, claudin-3, and lipopolysaccharides (LPS) the LPS were markedly different and elevated in~~n~~ Ssc patients with GI abnormalities. Higher levels of LPS and claudin-3 were associated in the patients with a shorter duration of the disease. Moreover, in this group the LPS concentration was related to the patients with ILD. Concomitant esophageal dysmotility was associated with a decrease in LPS in the patients with SSc. Both of the calprotectin and LPS are determined as early biomarkers in the gastrointestinal malformations [55].

Biomarkers of paraneoplastic SSc

Individuals with systemic sclerosis have a significant higher risk for developing cancer. Altough, cancers in SSc are depending on autoantibodies, several provoking and genetic factors. Chronic inflammation, tissue damage, immunsuppressive agents heightened the link between the cancer and Ssc. Contrary, the SSc could appear as a paraneoplastic syndrome, as cancer-induced autoimmunity [57]. Onishy et al. have found an increased tendency of haematological, lung, liver and bladder cancer in females and non-melanomatosous cancer in males. Anti-POLR3 positive patients with diffuse scleroderma have a higher risk for breast, prostate and tounge cancer [58]. Paraneoplastic syndrome could be a potential cause in the background development of SSc. The anti-NOR90 antibody is

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reported in lSSc and in myelodysplasia syndrome. In anti-NOR90 positive patients the IDH1 mutation and dysfunction therefore the elevated 2-hydroxyglutarate (2-HG) lead to the α-KG and the dimethyl- α-KG inhibition and elevated TGFβ and myofibroblast migration [59].

Summary

To summarise, systemic sclerosis is a complex, progressive, autoimmune disorder which has a considerable impact and challenge for the clinicians and patients as well. In Ssc, the wide range of vascular and fibrotic mechanisms lead to the relevant complications even in the early phase. The importance of biomarkers is emphasised in the differential diagnosis, to classify into the subgroups, to develop manifestations, in the activity, prognosis, response to the therapy, and to personalize the therapy, as well. The number of biomarkers is continously increasing. In the patomechanism, further efforts are needed to clear which immune cells, cytokines, co-stimulating process and factors could be a promising target.

Presently, the individualised biomarkers are not available, their sensitivity and specificity are different. Several potential biomarkers for the prognosis, vascular injuries, fibroproliferative process and organ damages are evaluated and proposed.

Further efforts for the evaluation of biomarker patterns by clinical and research centres are needed.

Conflicts of Interest: The authors declare no conflict of interest.

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