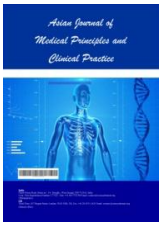

Asian Journal of Medical Principles and Clinical Practice

4(3): 1-13, 2021; Article no.AJMPCP.67250

Clinic - Diagnostic Aspects of Modern Biomarkers of Early Atherosclerosis and Fibrotic activity of Systemic Scleroderma

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Authors' contributions

This work was carried out in collaboration among all authors. Author GNA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript, authors RMZ and NDA managed the analyses of the study. Author ANA managed the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Erich Cosmi, University of Padua, Italy.
Reviewers:
(1) Barbara Ruaro, University Hospital of Trieste, Italy.
(2) Pablo Amair Miani, Sociedad Venezolana de Cardiología, Venezuela.
(3) Giovanni Pacini, University of Florence, Italy.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67250

Review Article

Received 04 February 2021
Accepted 09 April 2021
Published 13 April 2021

ABSTRACT

Systemic sclerosis (SS) is a systemic disease with heterogeneous clinical manifestations of the skin and internal organs. It is believed that the triggering mechanism of its development is initial vascular damage, which leads to inflammatory reactions and the development of the accumulation of collagen and other components of the extracellular matrix. It goes without saying what a disease treatment is. Diagnosis of SS is carried out by clinical observation and using methods such as the Rodnan skin counter (mRSS), durometry, cutometry, and ultrasound determination of skin thickness. These methods are quite of thickness of intima complex (TIC) consuming and subjective. In addition, these methods do not provide information about the activity of the fibrotic process. These disadvantages of the listed methods can be compensated for by studying biomarkers that reflect the activity of inflammatory and fibrotic processes, but can be used to assess the prognosis and effectiveness of treatment. The aim of the review focuses on cardiac and

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fibrotic biomarkers of patients with scleroderma. These include growth factors, cytokines and proteases, their inhibitors, as well as proteins of the extracellular matrix, especially collagens, adapted to skin biopsies and in serum samples from patients with SS. Summarized information on non-invasive physical and laboratory studies is proposed, which provides a better understanding of cardiovascular disease and fibrotic activity, can be effectively used to assess the potential therapeutic response and help in choosing the opTICal treatment options for SS.

Keywords: Systemic sclerosis; atherosclerosis; heart disease; fibrosis; endothelial dysfunction.

1. INTRODUCTION

Scleroderma or Systemic Sclerosis (SS) is a chronic autoimmune disease of connective tissue belongs to the group of systemic connective tissue diseases and characterized by progressive damage to the skin, musculoskeletal system, and internal organs and generalized vascular lesions, which occupies key positions in the pathogenesis and clinical picture of SS and has prognostic value [1]. Three main factors play a leading role in the pathogenesis of the disease - autoimmune disorders, vasculopathy and fibrosis [2]. The main clinical signs of SS are due to widespread microcirculation disorders, fibrosis of the skin and internal organs. Endothelial dysfunction is considered as the central link in the pathogenesis of Raynaud's syndrome and other vascular disorders. In SS, the microcirculatory bed is the target of immune-inflammatory damage, leading to impaired vascular tone, vascular architectonics and blood flow in the affected organs. At the cellular level, endothelial dysfunction is characterized by a change in the phenotype of endothelial cells towards the pro-inflammatory and pro-constrictor components of their metabolism. Raynaud's syndrome, an obligate clinical sign of SS, is the result of this pathogenic mechanism. The endothelial hypothesis implies a decrease in the production of vasodilation mediators (prostacyclin, nitric oxide - NO) and an increase in the synthesis of vasoconstrictor agents (endothelin) in the pathogenesis of Raynaud's syndrome. An increase in the level of endothelin in patients with SS has been identified by many researchers, however, a decrease in the concentration of nitrates (which are metabolites of NO) is found infrequently. Along with the deficit, in some patients, its excessive concentrations were detected, which is comparable to the physiological effects of NO - both positive (vasodilation) and negative (tissue damaging) [3-4].

The main clinical equivalent of microcirculation disorder in SS is Raynaud's syndrome, characterized by vasospastic crises accompanied by discoloration (whitening, redness, cyanosis) and numbness of the fingers of the hands, less often of the feet, which occur spontaneously or when exposed to cold and excitement. About half of patients with SS suffer from trophic disorders at least once during the course of the illness, and in 17% they are constantly present - from minor ulcerations to severe necrosis and gangrene of the fingers [5]. Ischemization of tissues also underlies the development of osteolysis, mainly of the nail phalanges.

The fundamental importance of vascular therapy, which occupies one of the main places in the complex treatment of the disease, is obvious. However, despite the sufficient variety of drugs, sclerodermic angiopathy and its severe complications, such as ischemic necrosis, and some thickness of intima complex (TIC) gangrene, are not always treatable. Even the therapy of Raynaud's syndrome, especially generalized, given its complex pathogenesis and progressive nature, remains a difficult task for practical medicine.

2. STRUCTURAL CHANGES IN MICRO-CIRCULATORY VESSELS IN SYSTEMIC SCLERODERMA

Currently, changes in the size of capillaries are considered the most striking manifestation of damage to microcirculatory vessels in SS, and capillaroscopy is recognized as an objective method for visual examination of structural and functional changes in capillaries in vivo. In the early 70s, a modified microscopy method was developed and proposed for clinical use — wide-field capillaroscopy of the nail bed [6]. The essence of the modification consisted in the study of capillaries at a small, from 12 to 40 TIC, magnification of the microscope. The object of observation in wide-field capillaroscopy is the capillaries of the nail bed, or rather, the nutritive capillaries of the papillary dermis covering the

root of the nail plate. The choice of this localization is explained by the location of the papillae of the dermis, which is characteristic only for the nail bed, parallel to the skin surface, which makes it possible to visualize the capillaries in a longitudinal section. A slight increase significantly expands the field of view and makes it possible to study not only individual capillaries, but also to assess the morphological and functional changes in the microvasculature as a whole. The advantage of wide-field capillaroscopy is also: the convenience of the location of the finger under the microscope lens, the involvement of the fingers in the pathological process in many rheumatic diseases, the possibility of dynamic observation of changes in capillaries in a strictly defined area of the skin and, most importantly, the characteristic structure of the capillary network of the nail bed in healthy people, not depending on gender and age [7]. Normally, the capillaries of the nail bed are characterized by an ordered, parallel arrangement of capillaries of the same size and shape, which are especially clearly visible in the distal row. The main capillaroscopic signs of damage to microcirculatory vessels are changes in the size and number of capillaries [8]. Most often, changes in size are manifested in the form of an increase in the caliber of the capillaries and the diameter of the capillary loop of varying severity. The diameter of the capillaries most closely reflects the changes in size. Determining the length of the capillaries for comparative assessment can present some difficulties due to possible individual differences between individuals. The result of damage to the capillaries is the reduction of the capillary network, that is, a decrease in the number of capillaries in a certain area. A decrease in the number of capillaries can be expressed in varying degrees, up to the formation of capillaries, the so-called avascular areas. Another important sign of damage to microcirculatory vessels is changes in the shape of the capillary loop. Pathologically altered capillaries can take bushy, spiral or other forms. As a result of damage and violation of the integrity of the capillary wall, erythrocytes exit into the perivascular space. These extravastes during capillaroscopy are visible as a series of successive points between the capillary apex and the edge of the nail plate. Much less often, extravastes are represented by large confluent foci, which consist of several small hemorrhages. Quantitative measurement of capillary parameters is a laborious and TIC-consuming process. To simplify the study in clinical practice, the assessment of the detected changes is carried out by a semi-quantitative method, according to the generally accepted point scale for each type of change, which reliably reflects the quantitative parameters [9].

Separate capillaroscopic signs and their combinations are characteristic of certain nosological forms. Among systemic diseases of connective tissue, the greatest sensitivity and specificity of capillaroscopic changes is observed in SS. Capillaroscopic signs characteristic of SS are expansion of capillaries and loops of varying severity, a decrease in the number of capillaries with the formation of avascular fields, extravastes, often associated with megacapillaries. Depending on the predominance of certain capillaroscopic signs, two variants of the sclerodermic type of changes are distinguished - active and inactive [10]. The active scleroderma type of changes is characterized by a significant decrease in the number of capillaries with the formation of avascular fields, and the expansion of capillaries is moderately expressed. This picture is more common in patients with diffuse skin lesions and a rapidly progressive course of the disease. Inactive, or slow scleroderma type of changes is characterized by a large number of significantly dilated capillaries, avascular areas are absent or minimally expressed. Point extravastes with a characteristic location are often detected. The described changes occur mainly in patients with a chronic course of the disease. In recent years, there has been a tendency to revise views on the clinical interpretation of capillaroscopic changes in SS. Repeated capillaroscopy at certain TIC intervals showed that capillaries undergo a series of successive changes during the development of SS [11]. The early stage of the disease is characterized by expansion of the capillaries, while at the later stage of the disease, avascular areas predominate. As a reflection and part of the current pathological process, capillaroscopic changes correlate with clinical, morphological and laboratory characteristics of the disease. In the original study by von Bierbrauer A et al. [12] showed that capillaroscopic changes correspond to histological changes in the skin of the nail bed in the form of expansion and splitting of the basement membrane, an increase in the volume of connective tissue, perivascular edema with a large number of fibroblasts and mast cells. Interestingly, in patients with SLE, the frequency of morphological changes in the vessels, similar to capillaroscopic changes, was significantly lower than in SLE.

3. SEROLOGIC MARKERS OF ENDOTHELIAL DYSFUNCTION

Expanded capillaries were considered, the diameter of which was 2 or more TIC the diameter of unchanged capillaries. The degree of expansion of the capillaries was assessed using a 3-point system: 1 degree - the diameter of the capillary is 2-4 TIC the diameter of the normal capillary; Grade 2 - the diameter of the capillary is 5-7 TIC the diameter of a normal capillary; Grade 3 - the diameter of the capillary is 8 or more TIC the diameter of a normal capillary. Capillaries with a diameter of 10 or more TIC the diameter of normal capillaries were designated as megacapillaries. In cases where the diameter of the capillary appeared to be dilated, but did not reach more than a twofold increase in diameter, the term "borderline dilated" capillaries was used.

Areas of the distal row of capillaries, in which capillary loops were not visualized, were considered as avascular. Depending on the size, 3 degrees of severity of avascular areas were distinguished: 1 degree - avascular areas correspond to the loss of 2-4 capillaries; 2 degree - avascular areas correspond to the loss of 5-7 capillaries; Grade 3 - avascular areas correspond to the loss of 8 or more capillaries. To objectify the degree of capillary reduction, we used an indicator such as the total area of avascular areas, which represents the product of the number and severity of avascular areas. Bushy capillaries are several capillary loops connected at the base and resembling, in the terminology of English-speaking authors, a "bouquet of flowers". In some cases, bushy capillaries take the form of glomeruli, in which it is impossible to identify individual capillary loops. Isolation of bushy capillaries is due to the fact that they are not found in healthy people. In addition to dilated and bushy capillaries, we separately evaluated altered capillaries, which are characterized by shapes that differ from normal capillaries (crimped, spiral, expanded loops, etc.), but with a common diameter. Hemorrhages are accumulations of red blood cells located in the perivascular tissue and indicate a violation of the integrity of the capillary wall. Unlike hemorrhages of traumatic genesis, with SS, hemorrhages have a characteristic appearance of sequentially located point extravastes directed from the capillary loop apex to the edge of the nail bed and described by some authors as “pearl threads”. In the case of significant damage to the capillary wall, hemorrhages look like large focal conglomerates of several extravastes. Laser Doppler flowmetry (LDF) is a non-invasive method for examining cutaneous blood flow in the microcirculation system. The use of various standardized functional tests makes it possible to study local and central mechanisms of microcirculation regulation. The principle of the research method consists in changing the frequency of the light wave of a low-power (2 mW) helioneon laser, reflected from the flow of moving erythrocytes, and having a small penetrating ability to a depth of less than 1 mm with a coverage radius of 1-1.5 mm. The reflected beam with a changed frequency (Doppler effect) is transmitted through the light-receiving optical fibers through the transducer to the output of the device and is measured in volts, which makes it possible to quantify the blood flow. Thus, all parts of the microvasculature, including arteriovenous anastomoses, fall into the field of study [13]. The study used a laser Doppler flowmeter "Periflux" ("Perimed", Sweden) connected in series with a single-channel recorder "Air Force (Austria). Blood flow was measured at the tip of the third finger of the left hand, located at the level of the heart. We used a signal frequency of 12 MHz. The TIC constant of the measurement was 3 sec "1, which makes it possible to avoid the influence of pulse fluctuations of blood flow, respiration and other artifacts on the signal value. The amount of blood flow was assessed in parallel using the scale of the device and the readings of the recorder. The study was carried out at room temperature, in a sitting position, after a 30-minute rest. The sensor with a built-in thermostat was fixed on the fingertip and the initial (bASI) blood flow was recorded for 3-5 minutes, after which the following functional tests were applied: holding the breath at the inspiratory height for 15 seconds, hydrostatic test and local heating. The breath-holding test at the inspiratory height causes sympathetic TIC and, as a consequence, vasoconstriction and decreased blood flow, which makes it possible to assess the state of the central regulatory mechanisms. To conduct a hydrostatic test, the patient's hand was lowered 40-50 cm below the level of the heart. An increase in hydrostatic pressure normally causes a temporary decrease in blood flow and reflects the state of local regulation of microcirculation. The test with local heating was performed by heating the investigated skin area to + 40C for 3 minutes using a thermostat built into the sensor. The test makes it possible to assess the vasodilatory potential of microcirculatory vessels. Each functional test was performed after

restoration of bASI blood flow. To unify the results of functional tests, changes in blood flow were assessed as a percentage in relation to bASI blood flow.

4. RISK FACTORS FOR EARLY DEVELOPMENT OF ATHEROSCLEROSIS (AS) IN PATIENTS WITH SS

SS varies in severity and progression. Most patients develop visceral complications, which are usually the cause of death [14]. Primary heart damage, which develops as a direct consequence of SS, can be manifested by changes in the myocardium, pericardium, and valvular apparatus. In patients with SS, cardiac pathology can also be secondary in acute scleroderma kidney and pulmonary arterial hypertension. Vasculopathy in SS is characterized by progressive structural restructuring of the microvasculature, which can contribute to the development of a variety of cardiovascular changes. Endothelial dysfunction and hemorheological disorders characteristic of SS are also considered risk factors for the early development of AS. The general pathogenetic mechanisms of SS and AS suggest a high probability of atherosclerotic vascular lesions in SS patients [15]. G.S. Ngian et al. [16] suggested that AS in SS patients makes a certain contribution to macro- and microvascular myocardial damage. In patients with SS, there is a fourfold increase in mortality compared to the general population, with cardiovascular diseases (CVD) accounting for a third of the causes of mortality [17]. In SS, the leading cause of death unrelated to pine disease is CVD, which account for 20 to 30% of deaths [18]. AS is a complex pathological process, part of which is inflammation, which is fundamental for all stages of the evolution of atherosclerotic plaque (ASP) [19]. In autoimmune inflammatory rheumatic diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), primary antiphospholipid syndrome, systemic vasculitis, one of the main causes of disability and mortality is CVD associated with the accelerated development of AS. Compared to the general population, the development of AS in RA and SLE occurs at an earlier age and is often asymptomatic [20]. In addition to traditional risk factors (TRF), additional risk factors are important in systemic connective tissue diseases: chronic inflammation, duration and activity of an autoimmune disease, and immunosuppressive therapy. The role of chronic inflammation is indicated by the fact that inflammatory mediators such as C-reactive protein and heat shock proteins are also involved in the pathogenesis of AS [21]. In rheumatic diseases, chronic inflammation can accelerate the formation of ASP, both through a direct effect on the walls of the arteries, and indirectly, by affecting the lipid profile. Along with inflammation, antibodies produced in autoimmune diseases can also lead to changes in the lipid spectrum of the blood. The development of premature AS can also be facilitated by other specific factors (decrease in the number and function of endothelial progenitor cells, accelerated apoptosis of endothelial cells, epigenetic changes) [22]. It has not yet been established whether AS develops in SS patients earlier than in the population. A number of studies indicate an increase in the detection rate of AS in SS. It is known that in SS with a high frequency, both pronounced and subclinical manifestations of AS are found. Thus, damage to the carotid arteries was noted in 40%, endothelial dysfunction - in 76.4% of patients. With SS, the incidence of CVD and macrovascular lesions is increased in comparison with the general population, and the combination of SS with AS worsens the prognosis [23]. The pathogenesis of AS in SS remains unclear. It is believed that TRF contribute to its accelerated development [24]. However, the results of studies in which TRF was assessed in patients with SS are contradictory and do not yet confirm significant differences in the profile of TRF compared to control. Preliminary data indicate an increase in the incidence of arterial hypertension (AH), dyslipidemia and an increase in body mass index (BMI) in patients with SS, which may contribute to the formation of functional and structural changes in the heart, worsening the prognosis of the disease in general. At present, a comprehensive analysis of the TRF is considered one of the promising approaches to improving the forecast. It was found that patients with SS are characterized by an accelerated development of the atherosclerotic process, and a high cardiovascular risk in SS is associated with traditional risk factors (hypertension, dyslipidemia, hypertriglyceridemia, TIC, endothelial dysfunction) and factors caused by the activity of the disease itself. In a study conducted by A. Nordin et al. (2013) in Stockholm (Sweden), it was found that the highest risk of developing cardiovascular complications (CVC) and signs of subclinical atherosclerosis were noted in the group of SS patients with positive antibodies to centromere. In Australia, Ngian G.S. et al (2012) found that in

patients with SS, the development of IHD associated with pulmonary hypertension was significantly more frequent, and the incidence was as follows their cardiovascular risk factors such as obesity, hypercholesterolemia, diabetes mellitus did not differ from those in the control group. The latest data from ongoing cohort studies show a 3.5-fold increase in mortality from cardiovascular complications in SS (Nikpour M.M., 2014). In addition, recent studies show that patients with SS have higher hospital mortality from CVC associated with atherosclerosis compared with patients with systemic lupus erythematosus and rheumatoid arthritis (Dave, A.J., 2014).

5. INTERLEUKIN-6 (IL-6)

Interleukin-6 (IL-6) is a multifunctional cytokine produced by a variety of cell types such as monocytes, T cells, B cells, NK cells, and fibroblasts. An increase in the level of interleukin 6 (IL6) correlates with the risk of developing coronary heart disease, is a predictor of cardiovascular events in patients with clinically stable coronary disease, proven angiographically. In addition, with unstable angina pectoris and myocardial infarction (MI), an increased level of IL6 is associated with a poor prognosis (Gumanova N.G., 2019). With an increase in IL6 concentration by 1 pg / ml, the relative risk of recurrent MI or sudden death increases by 1.7 TIC. An increased level of IL6 before coronary artery bypass grafting is associated with occlusion of the graft in the early postoperative period, and also predicts the development of delayed cardiovascular events (Patrikeyeva D.A., 2015). Udachkina E.V. et al. (2013) found that in arteries affected by atherosclerosis, the levels of IL6 mRNA expression are 10–40 TIC higher than in arteries without signs of atherosclerosis. In the thickened in TIC of the atherosclerotic defect, the presence of the IL6 gene transcript was noted. In addition, IL6 was found to be an independent biomarker of carotid atherosclerosis in patients with moderate to severe coronary lesions. They also showed that IL6 concentrations, regardless of traditional risk factors, positively correlate with the presence of atherosclerotic plaques in the carotid and femoral arteries according to duplex scanning data. The level of IL6 is significantly higher in patients with type II- and II b dyslipidemia than in the control group of healthy people, and correlates with TIC (Udachkina E.V., 2013). Khripunova A.A. (2012) studied the incidence of macrovascular complications and the prognostic value in their development of traditional cardiovascular risk factors and immune-inflammatory mechanisms in SS. The literature data on the thickening of the in TIC-media of the carotid arteries in patients with SS and subclinical atherosclerosis are contradictory. Macedo R. et al (2012) revealed a thickening of the in TIC-media of the carotid arteries in patients with SS, however, no connection was found between the BMI of the carotid arteries and the severity of the disease. Thus, the analysis of such clinical situations substantiated the need for a more in-depth study of the state of the cardiovascular system in patients with SS. It was reported that the expression of IL-6 was increased in fibroblasts, mononuclear and endothelial cells of the skin in patients with early SS. Serum IL-6 levels were persistently elevated in patients with SS and were associated with the severity of scleroderma. The administration of anti-IL-6R antibodies to the Scl-cGVHD line reduced skin fibrosis in a model of bleomycin-induced scleroderma [25].

The literature describes a significant improvement in the condition and a decrease in the severity of skin fibrosis in 2 patients with SS during treatment with tocilizumab (a recombinant humanized monoclonal antibody to the human IL-6 receptor), and a clinical study of tocilizumab in phase II revealed a significant decrease in skin fibrosis in patients with SS [26]. An international, multicenter, randomized, double-blind, Phase III trial of the efficacy of tocilizumab in patients with SSc is ongoing. Thus, IL-6 is a possible target of SS treatment and, at the same TIC, a sensitive biomarker of the severity of skin fibrosis in these patients.

Currently, cardiovascular diseases (CVD) are the leading cause of death worldwide. According to WHO TIC, 17.3 million people died from CVD in 2008, which accounted for 30% of all deaths in the world [27]. Of this number, 7.3 million died from coronary heart disease (CHD) and 6.2 million from stroke [28]. Cardiovascular pathology and related complications (heart attack, stroke, sudden cardiac death) are one of the most common causes of death in rheumatic diseases. Numerous studies strongly indicate an increased risk of death from CVD in patients with rheumatoid arthritis, seronegative arthropathies (psoriatic arthritis, ankylosing spondylitis) and systemic lupus erythematosus compared with the population [29]. In the period from 1972 to 2001. at the University of Pittsburgh, Pennsylvania (USA), a study was conducted to determine the

causes of death in SS. Patient survival was determined at 5-year TIC periods. At the beginning of the study, it was established that the leading cause of mortality was kidney damage (scleroderma renal crisis); subsequently, mortality from SS decreased significantly, and mortality from lung damage (pulmonary fibrosis, pulmonary hypertension) increased significantly. Mortality from cardiovascular complications (CVC) remained invariably high for three decades and accounted for 10 to 15% of all deaths in SS (3rd place among all deaths) [30]. The results of a meta-analysis (7 centers in Europe, USA and Japan) carried out by the J.P. Ioannidis in 2005, showed that patients with SS are characterized by a high risk of mortality from vascular catastrophes against the background of progression of atherosclerosis [31].

6. ANTIBODIES TO TOPOISOMERASE-I

It has been revealed that lesions of internal organs (heart, lungs, kidneys) and the presence of antibodies to topoisomerase-I are important factors determining the lethal outcome of patients with SS [32]. In the study by Faccini A. et al. (2014) showed that heart damage develops more often and earlier in patients with diffuse SS [33]. Antinuclear antibodies (ANA) to extractable nuclear antigens are antibodies to soluble components of the cell nucleus (ribonucleoproteins). Normally, ANA is absent in the body. In autoimmune pathology, the immune system begins to produce specific immunoglobulins to its own cells and their components. Antibodies to the Scl-70 antigen (anti-Scl-70) are antibodies to the non-histone chromosomal protein Scl-70, which is an enzyme topoisomerase I with a molecular weight of 70 kDa. The detection of these antibodies in the blood is most typical for diffuse (up to 40%) and limited (up to 20%) forms of systemic scleroderma, while they, as a rule, are not detected in the focal form of the disease. Also, antibodies to the Scl-70 antigen are characteristic of CREST syndrome, which in fact can be attributed to a limited form of systemic scleroderma. The detection of anti-Scl-70 serves as an unfavorable prognostic sign in these autoimmune diseases due to the development of pulmonary fibrosis.

One of the factors determining the prognosis of patients with SS may be impaired renal function. The results of recent randomized trials show that even subclinical kidney damage is an independent risk factor for CVD development [34]. Similar studies have not been conducted in patients with SS, therefore, assessing renal function, determining early markers of kidney damage, analyzing the relationship of kidney damage with cardiovascular risk factors, duration, activity, and visceral manifestations of SS seems to be extremely relevant.

7. BIOMARKERS CORRELATED WITH SKIN FIBROSIS

The presence of specific autoantibodies – to topoisomerase-I antibodies to topoisomerase 1, antibodies to RNA proteinase III, and antibodies to ribonucleoproteins (U3RNP) - is associated with the development of SS. It is important to note that the appearance of these autoantibodies in the peripheral blood precedes the development of clinical symptoms of SS [35]. In an Australian cohort study of 451 patients with SS, the presence of anti-RNA polymerase III was associated with the development of renal scleroderma crisis, diffuse skin thickening and joint contractures within 5 years after the detection of these antibodies [36]. Thus, these antibodies are considered not only as diagnostic, but also as prognostic biomarkers of SS. According to modern understanding, the pathogenesis of SS is based on a sequence of certain pathogenetic events. Initial damage to the vessel wall results in an inflammatory response with the induction of an immune response. Cytokines and chemokines are released: TGF- (transforming growth factor), CTGF / CCN2 (connective tissue growth factor), PGF (platelet growth factor), endothelin-1, interleukins, MCP (monocytic chemoattractant protein) -1 and -3 and other substances that induce fibroblast activity. It is believed that cytokines and growth factors are also critical for the recruitment of circulating progenitor cells to the site of injury, as well as for the expansion and differentiation of populations of resident mesenchymal progenitor cells in tissues.

8. TRANSFORMING GROWTH FACTOR BETA

(TGF) is a well-known main biomarker, an increased level of which leads to tissue fibrosis in patients with SS [37]. Increased expression of TGF and its higher levels were found both in the affected skin and in the blood serum. Therefore, the creation of neutralizing anti-TGF antibodies, apparently, will be a promising discovery for the treatment of SS.

9. CONNECTIVE TISSUE GROWTH FACTOR

(CTGF / CCN2) promotes proliferation of fibrous regions and production of extracellular matrix. Sequential injections of TGF-and CTGF induced skin fibrosis in an animal model [38]. In this model, TGF transiently induced subcutaneous fibrosis, and subsequent CTGF injections induced persistent fibrosis of the lake. In a series of studies using a mouse model, the hypothesis of two stage development of fibrosis in patients with SS was proposed: TGF initiates fibrosis, while CTGF maintains a fibrotic state by altering the phenotype of fibroblasts. It has been reported that serum CTGF levels in these patients were significantly increased, reflecting the severity of skin sclerosis and the severity of interstitial lung disease [39]. A recent open clinical trial of fresolimumab (neutralizing antibodies targeting all 3 TGF isoforms) demonstrated a significant reduction in skin fibrosis in patients with early stage SS and a significant decrease in CTGF gene expression in the skin of patients. Thus, TGF-and CTGF are the main therapeutic targets for the treatment of SS and, at the same TIC, biomarkers of the progression of skin fibrosis.

10. CARTILAGE OLIGOMERIC PROTEIN 1 (COMP)

(COMP) - is a non-collagen glycoprotein synthesized by chondrocytes, osteoblasts, tenocytes, synovial and dermal fibroblasts. This protein, regulated by TGF-, is not found in the skin of healthy individuals, but it is overexpressed by fibroblasts of patients with SS, which is detected by skin biopsy [40]. In addition, it was found that the level of COMP also increases in the blood serum of patients with SS and correlates with the severity of skin lesions. To assess the latter, mRSS and ultrasound are used. A recent study confirmed a high level of COMP in the serum of patients with SS, and its level was higher in the DF-SS subgroup than in the LT-SS subgroup.

11. MATRIX METALLOPROTEINASES (MMPs)

MMPs are responsible for the degradation of collagens and other proteins of the extracellular matrix (ECM); they are also involved in the release and activation of many cytokines and growth factors by macrophages, synoviocytes, and fibroblasts [41]. The role of MMP-9, MMP-12 and their inhibitors in the pathogenesis of SS has been thoroughly studied. Based on the data obtained, it was established that both MMPs are potential markers of skin fibrosis.

MMP-9, whose substrates include type IV collagen in the basement membrane, are associated with chronic inflammatory autoimmune diseases, including RA and SLE [18]. Moreover, overexpression of MMP-9 has been reported in a variety of pathological conditions characterized by excessive fibrosis, including idiopathic pulmonary fibrosis [3] and chronic pancreatitis [22]. As for the patients with SS, they showed an increased expression of MMP-9 by fibroblasts compared to the group of healthy individuals. The serum level of MMP-9 also correlated with mRSS and was more significantly increased in patients with SS [20]. MMP-12, also known as macrophage metalloelastase, has a broad substrate specificity for matrix macromolecules, recognizing elastin, type IV collagen, fibronectin or vitronectin. MMP-12 is involved in the development of various pathological conditions, including atherosclerosis, cancer, and skin diseases [42]. In SSc patients, dermal fibroblasts express and release MMP-12; serum levels of MMP-12 are significantly increased in patients with SS and closely correlate with skin fibrosis, while patients with DF-SS have higher MMP-12 values than patients with LT-SS [30].

12. LYSILOXIDASE

Lysiloxidase (LOX) is an extracellular copper enzyme that forms an intermolecular bond between collagen and elastin, thereby stabilizing collagen fibrils. Due to its increased expression in the skin and fibroblasts, LOX levels were also increased in the serum of SSc patients compared to healthy controls [39]. An in-depth statistical analysis revealed a relationship between LOX concentration and skin count in patients without pulmonary fibrosis, which indicates its specific association with skin fibrosis. Moreover, LOX levels were higher in patients with DF-SS than in patients with RT-SS, reflecting a more common fibrotic process [24].

13. MCP-1 AND MCP-3 (MONOCYTIC CHEMOATTRACTANT POTEINS)

Proteins which are overexpressed on fibroblasts in SS, play an important role in the development of skin fibrosis and correlate with the severity of pulmonary fibroids. M. Hasegawa et al. (2011) reported that serum MCP-1 levels were

significantly associated with skin thickening and decreased lung capacity in patients with SS.

14. THE CONCENTRATION OF PENTRAXIN 3 (PTX3)

Concentration in the serum of patients with SS, especially with a diffuse form, was increased. An increase in serum PTX3 concentration positively correlated with mRSS and, in addition, was associated with pulmonary fibrosis, cardiac damage, and digital ulcers [16]. F. Ogawa et al. (2008) conducted a study investigating heat shock protein 70 (HSP70) as a marker of cellular stress in the serum of SSc patients. They reported an increase in the concentration of HSP70 in patients with pulmonary fibrosis, skin thickening, renal vascular injury, vasculopathy, as well as in more severe disease [35].

15. INTERLEUKIN-1

Interleukin-1 plays an important role in the regulation of the inflammatory response and procollagen production by fibroblasts. IL-1 mRNA and protein forms of IL-1 are produced by dermal fibroblasts of the affected skin of patients with SS, whereas normal fibroblasts do not produce IL-1 in a steady state [11]. IL-33 is a member of the IL-1 family and has a critical role in the regulation of cytokine release by mast and dendritic cells, macrophages and fibroblasts. Elevated levels of IL-1, IL-33, and ST2 (IL-33 receptor) were detected in the blood serum of patients with SS, their association with the early stage of SS, severity and severity of skin sclerosis was established [5]. In turn, the level of soluble ST2 in the blood serum was found to be elevated in patients with SS with a disease duration of more than 9 years [37]. Thus, members of the IL-1 family play an important role in the development of skin fibrosis, and serum levels of IL-33 and ST2 are biomarkers of the severity of fibrosis.

16. B-CELL ACTIVATING FACTOR (BCAF; ALSO KNOWN AS BLyS)

It belongs to the TNFSF13B family of tumor necrosis factors and is involved in the survival and maturation of B cells. Serum BCAF levels are elevated in patients with autoimmune diseases such as SLE, rheumatoid arthritis, and Sjogren's syndrome. In a study by V.N. Minhetal (2019) found a significant increase in serum BCAF levels in patients with SS, especially with severe cutaneous sclerosis; in addition, the level of this marker decreased during therapy [32].

17. CXCL13 CHEMOKIN

Chemokin which is responsible for the migration of B cells to the site of inflammation, has been increased [42]. It has also been reported that serum BCAF levels were increased upon depletion of B cells in SSc patients [4]. Serum BCAF levels were significantly increased in mice (model SS), similar to what was observed in patients with SS [17]. Moreover, administration of the BCAF antagonist inhibited the development of skin fibrosis and the production of autoantibodies in mice. Therefore, the serum BCAF level is a biomarker of the severity of skin fibrosis in patients with SS.

Chemokine CCL2 (monocyte chemoattract protein-1, MCP-1) is produced by various immune cells such as macrophages, fibroblasts, endothelial cells, and mediates the migration and activation of monocytes and T cells. CCL2 levels were persistently increased in patients with SS [8], and the increase in this indicator was significantly correlated with an increase in the modified Rodnan Skin Thickness (mRSS) and skin fibrosis activity in SS patients.

18. TUMOR NECROSIS FACTOR (TNF)

Tumor necrosis factor (TNF) -plays a critical role in inflammatory reactions; its dysregulation has been established in various diseases. The TNF signaling effect is mediated by two types of TNF receptors and depends on expression on the cell surface. Serum levels of soluble TNF-(sTNF-) receptor are increased in patients with SS and are associated with the severity of skin tightening [33]. In addition, an increase in serum levels of sTNF-correlates with the presence of clinical and laboratory signs of inflammation and the progression of SS [26]. According to the results of the conducted studies, sTNF- is another sensitive biomarker of the severity and activity of skin fibrosis in SS.

19. PLATELET GROWTH FACTOR (PGF)

Platelet Growth Factor (PGF) is a dimeric peptide that is produced and expressed by platelets, fibroblasts, and smooth muscle cells. PGF causes migration, differentiation and transformation of various cells and plays an important role in oncogenesis, development of pulmonary arterial hypertension, pulmonary

fibrosis. In SS, autoantibodies are activated against two structurally related PGF receptor tyrosine kinases, PGFR-A and -B [24]. Increased levels of PGF were found in patients with SSc with significant fibrosis of the skin and lungs; in addition, an increase in this indicator correlated with disease activity. Studies show that in scleroderma, the PGF-A / PGFR-B autocrine loop of fibroblasts sTICulates IL-1 and IL-6 [19].

20. PLATELET FACTOR 4 (PLF4)

Platelet factor 4 (PLF4) is generally regarded as a proinflammatory chemokine; in addition to its chemoattractive activity, it regulates many immune cells, including T cells, monocytes, dendritic cells, as well as non-immune cells, for example, endothelial cells. L. Van Bon et al. (2014) used a proteomic approach in their study and identified PLF4 as a potential biomarker associated with multiple organ damage in SS. The level of circulating PLF4 closely correlated with the severity of skin fibrosis to a greater extent in patients with DF-SSD and was a predictor of a more rapid progression of skin lesions [2]. PLF4, in addition to its antiangiogenic activity, TIC the production of profibrotic cytokines such as IL-4 and IL-13, while decreasing the production of antifibrotic cytokines such as interferon (IFN). Thus, PLF4 may be of prognostic value in SS, as it allows predicting the progression of skin fibrosis.

21. ADIPONECTIN

Adiponectin is a common adipokine and plays a major role in the development of insulin resistance and atherosclerosis. Adiponectin has an anti-fibrotic effect on the peroxisome proliferation-activating receptor (PPAR) [9]. It was found that serum adiponectin levels are negatively associated with mRSS and in longitudinal studies are inversely correlated with the severity of skin fibrosis [23]. Thus, adiponectin can also be classified as predictive biomarkers of severity and activity of skin fibrosis in SS.

22. OX40

OX40 and its binding partner OX40L are members of the TNF superfamily and are expressed on both activated T cells and other lymphoid and non-lymphoid cells. The OX40-OX40L signaling cascade is an important part of late co-TIC T cell signaling. TNF-SF4, encoding OX40L, has a gene for susceptibility to SS [14]. Serum soluble OX40 levels have been reported to be elevated in patients with SS and are associated with early disease onset. In addition, the serum concentration of OX40L was significantly increased in patients with DF-SSD [8]. A longitudinal study showed that high levels of OX40L in serum at baseline were predictors of severe fibrosis of the skin and lungs, which was confirmed by follow-up [2]. At the same TIC, blockade of the OX40-OX40L signal reduced the progression of skin and lung fibrosis in a mouse model of SS [6].

23. CXCL10

CXCL10 (IFN - inducible protein 10; IP-10) and CXCL11 (IFN - inducible T-cell chemoattractant I-TAC) are produced by several types of cells in response to the release of IFN-CXCL10 plays an important role in the chemoattraction of monocytes, T cells, NK cells, and dendritic cells. CXCL11 also acts as a chemoattractant for activated T cells. The total values of plasma levels of CXCL10 and CXCL11 were increased in patients with SS and correlated with IFN gene expression, severity of skin and lung fibrosis [28]. It should be noted that autoantibodies against IFI-16 and IFN -inducible gene were found in patients with LT-SS, but not with DF-SS [6]. Thus, CXCL10 levels can be biomarkers of the severity of SS, while the presence of anti-IFI16 autoantibodies is a biomarker that can be used to determine the subtype of the disease.

24. GENE EXPRESSION ANALYSIS

Analysis of the level of gene expression from skin biopsy data is another promising approach for determining biomarkers of skin fibrosis. A skin biopsy allows you to directly analyze the features of the fibrotic process. Moreover, using this research method, genes specific for various phenotypes of SS were found, which makes it possible to predict the development of more severe forms of the disease. For example, S. Assassi et al. (2015) reported on fibroinflammatory and keratin gene expression profiles associated with the severity of skin involvement in a subgroup of patients with SSD [2]. This identification not only allows a better understanding of the pathogenesis of the disease, but also provides important information for the selection of therapeutic targets for treatment.

25. CONCLUSION

In recent decades, along with the pathophysiological basis of SS, individual clinical

and laboratory parameters of its activity have been studied in the world, but the problem remains unresolved, mainly due to the pronounced heterogeneity and complex pathogenesis of the disease. The combined use of instrumental methods and semi-quantitative scales (indices) makes it possible to more correctly diagnose the stage of the disease, evaluate the effectiveness of therapeutic intervention (but not the degree of progression of fibrosis), predict the rate of development of fibrosis and the relationship with damage to internal organs, primarily the lungs and kidneys.

Laboratory indicators of inflammatory activity are not very informative, can be significantly increased in subacute course and less pronounced in acute progressive course of SS with a predominance of fibrosis. In this regard, the role of biomarkers, which can be easily detected, are sensitive and reproducible, and correlate with fibrotic activity, increases significantly. Determination of circulating collagen fragments, its precursors or degradation products is promising and well established. These studies reflect not only the features of the fibrotic process in the connective tissue, but also complex pathogenetic mechanisms that allow assessing fibrotic activity and predicting the disease. Thus, high levels of serum levels of metalloproteinases, cartilage oligomeric protein 1 (COMP1), various cytokines of the TNF-family and their ligands (OX40, OX40L), platelet factor 4 (PLF4), lysyl oxidase (LOX), BCAF clearly correlate with the diffuse nature of lesions. skin and can be predictors of the development of certain phenotypes of the disease, in particular DF-SSD. In contrast, CXCL10 and 11 are associated with the development of SS. The complex and consistent use of new serum biomarkers will allow at an early stage of SS to adequately assess the activity of inflammatory and fibrotic processes in the skin, as well as determine the phenotype of the disease, and predict the rate of vascular and heart damage.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/67250