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An Overview of Systemic Sclerosis

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Abstract: ***Systemic sclerosis (SSc, scleroderma) is an uncommon connective tissue disorder with complex and poorly understood pathogenesis. It is characterized by prominent vascular alterations with endothelial cell damage and proliferation of subendothelial connective tissue, skin fibrosis and involvement of the lungs, gastrointestinal tract, heart, kidneys and central nervous system. Immune disturbances and production of general and specific autoantibodies accompany these disorders. Systemic sclerosis affects women more than men and age of onset varies between 20 and 60 years. On the basis of skin involvement, SSc is divided into two major categories: limited cutaneous SSc, which is usually associated with mild to moderate, delayed organ fibrosis and diffuse cutaneous SSc, which is characterized by progressive skin indurations, starting in the fingers and ascending to proximal extremities. Patients with this disorder are at risk for early pulmonary fibrosis and acute renal involvement. Face might be involved in both diffuse and limited cutaneous SSc. The cause of SSc is not fully clarified but data analysis show that infectious agents, environmental toxins, drugs, epigenetic modifications and microchimerism, might be potential causal agents in a genetically susceptible individual. This article presents an overview of the SSc disorder; the many facets of a very complex, disabling disease, affecting patient's quality of life and life expectancy.***

Keywords: Scleroderma, autoimmunity, inflammation, vasculopathy, pulmonary hypertension.

1. Introduction

Systemic sclerosis (Ssc, scleroderma) is a rare connective tissue disorder of poorly understood and complex etiopathogenesis, characterized by involvement of various organs, presence of a wide array of clinical manifestations and a chronic and often progressive course.

Neither the exact cause of SSc nor the precise contribution of genetic susceptibility is fully elucidated but current views postulate that infectious agents, environmental toxins, drugs, epigenetic modifications, and microchimerism, might be potential causal agents in a genetically susceptible individual.

In SSc repeated and constant injury results in persistent immune responses and progressive vascular damage. Studies show that myointimal cells proliferation, thickening and augmentation the basement membrane, fibrosis of the adventitial layer and the resulting widespread obliterative vasculopathy of the peripheral arteries and microcirculation, constitute the hallmarks of SSc vascular damage. Both general and specific autoantibodies are found in the sera of affected patients and activation of fibroblasts leads to excessive accumulation of collagen and extracellular matrix (ECM) molecules[1,2].

Some of the cell types implicated in disease pathogenesis are: endothelial cells, platelets, connective tissue cells (pericytes, fibroblasts and myofibroblasts), vascular smooth muscle cells and immune cells (T cells, B cells, macrophages and dendritic cells). Mediators of cells activation include: transforming growth factor-β (TGFβ), platelet-derived growth factor (PDGF), IL-6 and IL-13, endothelin 1, angiotensin II, lipid mediators and autoantibodies, reactive oxygen species (ROS) and many other biologically active substances[20].

SSc clinical outcome is determined by the extent of lungs, gastrointestinal tract, kidneys and heart pathological disorders, even thought skin fibrosis is the prominent disease hallmark. Skin involvement tends to correlate with visceral organ lesions[18,19].

Sleroderma is an uncommon disorder, affecting women 3 times more than men[3]. Reported incidence rates (number of new cases/year) and prevalence estimates (number of total cases) show considerable variability and depend on geographic location and the methods used to determine SSc in patients. Some reported prevalence figures from literature for definite SSc are : 30 cases/million in New Zealand[4], 443 cases/ million, Canada[5], 200 cases/ million south Australia[6], 38 cases/million in Tokyo[7], 71 cases/million in Iceland[8], 154 cases/million in North West Greece[9] and 158 cases/million in France[10].

Accordingly, the annual incidence rates also vary widely. Figures range between: 1.96 cases/million from the time period 1950 to 1973 in New Zealand[11], 3.7 cases/ million in Finland[13] 11 cases/million in Greece[11], to 23 cases/million from the time period 1988 to 2006 in Spain[12].

Monaco et al. conducted a retrospective review of Italian SSc patients (in the region of Ferrara) based on the ACR 1980 classification criteria and the revised LeRoy and Medsger 2001 criteria. Incidence and prevalence rate were respectively 43 and 341 cases per million when the LeRoy–Medsger criteria were used and 32 and 254 cases per million, when the ACR classification criteria were applied[14].

Studies data determine that the general SSc incidence is approximately 18 to 20 cases per million population per year and prevalence 100 to 300 cases per million population. SSc appears to be more common in United States (276 cases per million adults)[15], than in Europe (8-15 cases per million adults[15,16]).

In most cases, systemic sclerosis develops in persons aged 20-60 years. It tends to be more severe in African-American and Hispanic patients than in Caucasian ones and disease in

African-American subjects begins at an earlier age[17]. Scleroderma is usually categorized into two forms: localized, which affects only the skin and subcutaneous tissues, and systemic sclerosis (scleroderma, SSc), which affects both skin and internal body organs.

There are several subsets of localized scleroderma, in a simplified way it can be grouped into : **1. linear scleroderma**, which occurs most commonly in children and consists of a line of thickened skin usually involving an extremity, **2. scleroderma en coup de sabre** ("cut of the saber") which involves the frontoparietal skin and is characterized by a deep furrowing of the scalp and forehead, usually just to one side of midline[21] and **3. plaque morphea** (localized and generalized) which comprises patchy and well-circumscribed areas of thickened skin that can be seen as a single lesion or as multiple lesions[22].

Systemic sclerosis can be further divided into two principal subsets specified largely by the pattern of skin involvement, clinical and laboratory manifestations[38] : diffuse cutaneous SSc and limitied cutaneus SSc. Diffuse cutaneous SSc (dcSSc) is characterized by progressive skin fibrosis, starting in the fingers and moving up from distal to proximal extremities (proximal to knees and elbows), the face, and the trunk; early pulmonary fibrosis and acute renal involvement are more common in these patients.

Patients with limited cutaneous SSc (lcSSc) usually have stable Raynaud's phenomenon before other manifestations of SSc appear. Skin fibrosis in lcSSc is not very progressive and involves mainly the fingers (sclerodactyly) and distal extremities (distally to elbows and knees), the trunk is usually not affected and the face involvement can be observed in both SSc forms[38].

Other disease types are : Systemic sclerosis *sine* scleroderma (ssSSc) - a rare occurrence where patients have the clinical and laboratory features of systemic sclerosis (fibrotic damage to internal organs) and no skin involvement[24] and overlap syndrome – in which individuals show signs of another connective tissue disease, such as rheumatoid arthritis or polymyositis, in association with systemic sclerosis. SSc sine sclerodermia has similar outcomes to lcSSc.

Some patients with lcSSc suffer from the CREST syndrome; a condition which comprises the following symptoms : calcinosis cutis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia. This condition can also occur in dcSSc[23,35,36].

Autoantibodies are commonly found in SSc patients. Antinuclear antibodies (ANAs) are present in the majority of patients (75%- 90%) and typically show speckled or nucleolar patterns. ANAs are also found in other connective tissue disorders, while specific antibodies only in SSc[33-34].

The major specific SSc autoantibodies are: anti centromere antibodies (ACA), anti- topoisomerase I antibodies (Scl-70) and anti RNA polymerase III antibodies[25,26] and their profiling is helpful for diagnosis, classification and prognosis determination[27,28].

Limited cutaneous SSc is commonly associated with anticentromere antibodies and only 5–7% of patients with dcSSc can have ACA[30,31]; dcSSc is more often associated with anti - topoisomerase I or anti-RNA polymerase III-specific antibodies[29]. Scl-70 antibodies can also be found in lcSSc[30] (in 31–36% of patients) and are relatively good predictors of severe interstitial lung disease[32]; nuclear and centromere proteins are found in ssSSc and U1 RNP, PM–Scl, Ro and La antibodies in overlap syndrome.

2. Classification Criteria for Scleroderma

In 1980, the American Rheumatism Association (now the American College of Rheumatology) drafted preliminary classification criteria for SSc, based on a multicenter prospective study of 264 patients with SSc and more than 400 comparison patients with other connective tissue diseases (LES, polymiositis/dermatomyositis and Raynauld's phenomenon). According to these criteria a patient has SSc if either one major criterion (scleroderma proximal to the metacarpophalangeal or metatarsophalangeal joints) or at least two or more minor criteria (sclerodactyly, digital ulcerations and/or pitting digital scars and bibasilary pulmonary fibrosis) are found[37], (table 1).

In 1988 LeRoy et al.[38] suggested classifying SSc into limited cutaneous and diffuse cutaneous subsets. Limited cutaneous SSc was characterized by the presence of skin thickening distal to the elbows and knees only, whereas diffuse cutaneous SSc involved thickening on the proximal extremities and/or the trunk. In 2001 LeRoy and Medsger proposed a classification system which comprised also nail-fold capillaroscopic changes and autoantibody results, based on which SSc is divided in : limited SSc, limited cutaneous SSc, and diffuse cutaneous SSc [39].

In 2013, the ACR and EULAR joint committee defined new classification criteria for SSc. The new criteria, whose specificity and sensitivity are 0.91 and 0.92 respectively, encompass a broader spectrum of SSc including patients with early disease as well as others; comprise vascular, immunological, fibrotic manifestations and concord with criteria used for diagnosis of SSc in clinical settings[40]. Patients with a combined score of 9 or more points are classified as having scleroderma, (table 2).

Table 1: ACR 1980 classification criteria

1980 PRELIMINARY CLASSIFICATION CRITERIA FOR SYSTEMIC SCLEROSIS[37]
A. MAJOR CRITERION Scleroderma (symmetric thickening) proximal to the metacarpophalangeal joints
B. MINOR CRITERIA 1. Sclerodactyly (symmetric skin thickening limited to the fingers) 2. Digital pitting scars or loss of finger pad substance 3. Bibasilar pulmonary fibrosis
Data from : Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-90 [37].

Table 2: ACR/Eular scleroderma classification criteria

ACR/EULAR SCLERODERMA CLASSIFICATION CRITERIA[40]		
Item	**Sub-item(s)**	**Weight***
Skin thickening of the fingers of both hands extending proximally to the metacarpophalangeal joints (this criteria alone is enough to make the diagnosis of systemic sclerosis)	-	9
Skin thickening of the fingers (count the higher score only)	Puffy fingers	2
	Sclerodactyly (distal to the metacarpophalangeal joints but proximal to the proximal interphalangeal joints)	4
Fingertip lesions (count the higher score only)	Digital tip ulcers	2
	Fingertip pitting scars	3
Telangiectasia	None	2
Abnormal nailfold capillaries	None	2
Pulmonary arterial hypertension and/or interstitial lung disease (maximum score is 2)	Pulmonary arterial hypertension	2
	Interstitial lung disease	2
Raynaud phenomenon	None	3
Systemic sclerosis–related autoantibodies (maximum score is 3)	Anticentromere	3
	Anti–topoisomerase I	3
	Anti–RNA polymerase III	3

*The total score is determined by adding the maximum score in each category. Patients with a total score equal to or greater than 9 are classified as having definite systemic sclerosis (data from : van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum. Nov 2013;65(11):2737-47 [40]

Scleroderma diagnosis is based on clinical and laboratory features. In order to facilitate early scleroderma diagnosis new criteria are proposed by Eustar (VEDOSS). The VEDOSS (Very Early Diagnosis of Sclerodermia) criteria include the three red flag symptoms: Raynaud phenomenon, puffy fingers and ANA positivity, plus the presence of systemic sclerosis-specific antibodies and characteristic nailfold capillaroscopy[41,81].

3. Etiological Elements

Systemic sclerosis exact cause is unknown, but many infectious agents and toxic substances have been implicated to serve as causal factors and like for all other autoimmune disorders genetic components play an important role in disease susceptibility [154,155]. Below are some of most important genetic and environmental risk factors, associated with SSc development.

Genetic and epigenetic risk factors

The risk of SSc is considerably increased in families with a positive history for SSc; one study, found that the relative risk of SSc development among first-degree relatives of persons with SSc was 13, while a twin study of SSc found a 4.7% disease concordance rate[42,43].

Gene study (CGS) approaches and genome-wide association studies (GWAS) have been used in the last years to identify genetic associations that present susceptibility to SSc. Below are some of the MHC – human leukocyte antigen (HLA) variants, and non-HLA genetic SNP-s associated with SSc.

Regarding HLA genes a study of 1300 patients with SSc found close disease association for DRB1*1104, DQA1*0501, DQB1*0301 haplotypes in whites and Hispanic subjects, while DRB1*0804, DQA1*0501, DQB1*0301 alleles were associated with SSc in black patients[44]. DPB1*1301 and DPB1*0901 subtypes were mostly found in Korean patients with SSc, while DPB1*03:01, DPB1*13:01, DQB1*03:03, DQB1*05:01, and DQB1*06:11 were significantly increased in the Chinese SSc patients[82,83]. HLA-DRB1*1104, DQA1*0501, and DQB1*0301 haplotypes are over expressed in Italian and Spanish SSc patients[84].

Studies have shown that the following single nucleotide polymorphisms (SNPs) of non HLA genes are associated with increased SSc susceptibility : IRF5 (interferon regulatory factor 5)[45], CTGF[46] , BANK1[47], STAT4 (signal transducer and activator of transcription 4), CD247, PTPN22 (protein tyrosine phosphatase non-receptor type 22)[48-50], TNFSF4 (T cell co-stimulation) NLRP1,(inflammasome component). STAT4 and IRF5 have also been identified as susceptibility genes for the development of SLE and RA[51--52]

STAT4 encodes the transcription factor signal transducer and activator of transcription 4, a member of the STAT family that adjusts the expression of many genes. The STAT-4 transcription factor plays a key role in type I IFN receptor signaling by being activated and translocated to the nucleus after binding to type I IFN receptor [86]. STAT4 is also important in the orientation of T helper cells towards the Th1 and Th17 proinflamatory phenotypes. An experimental study with STAT4 deficient mice showed that its inactivation decreased leukocyte activation and significantly ameliorated inflammation-driven fibrosis[85]. Studies have identified associations between variants of the STAT4 gene particularly for a SNP within the third intron (rs7574865) and SSc in different ethnicities[52-56]. A large French study showed an association between rs7574865 and pulmonary fibrosis[54].

The BANK1 gene is responsible for encoding a B cell-specific scaffold protein with ankyrin repeats, which serves as a substrate of the LYN tyrosine, and promotes phosphorylation of the inositol 1,4,5-triphosphate receptors; BANK1 single-nucleotide polymorphisms were strongly associated with diffuse cutaneous SSc susceptibility in a sample of French and German individuals[58] (increased frequency of rs10516487, rs17266594 and rs3733197 BANK1 variants were observed in SSc patients); and in a multicentre European study of 2,380 patients and 3,270 controls[57].

Informative genetic polymorphisms in SSc include also : copy number variations, rare allelic variants, and epigenetic changes, in addition to classic SNP.

Epigenetic modifications, can alter gene expression without changes in the genetic code and are often the result of environmental influences; three essential forms of epigenetic modifications are DNA methylation, histone modifications, and expression of noncoding (long and micro) RNAs. Some epigenetic alterations observed in studies are : - existence of a large number of differentially methylated cytosine-phosphatidyl-guanine (CpG) sites in SSc fibroblasts[59]; - presence of post-translational modifications of nucleosome histones including acetylation/deacetylation and methylation (levels of acetyltransferase p300 are elevated in SSc fibroblasts)[60]; - microRNAs (miRNAs) : short (~22 nucleotides) single-stranded non-coding RNAs, which predominantly bind to the 3'untranslated regions (3'UTRs) of mRNA of target genes and regulate their expression[61]. In SSc, several miRNAs are associated with TGF-β and collagen expression. Studies have shown that miR-21 which suppresses expression of antifibrotic Smad7, and induces expression of profibrotic genes is elevated in SSc fibroblasts, while miR-29 which is inhibitory for fibrotic genes, is reduced in fibroblasts and suppressed by fibrotic stimuli[62-64].

Environmental risk factors and microchimerism

Exposure to Epstein-Barr virus (EBV), human cytomegalovirus (hCMV), parvovirus B19, hepatitis B virus, toxoplasmosis certain environmental and occupational agents and drugs have long been implicated as potential trigger factors for SSc.

Serum antibodies directed against the UL83 and UL94 protein epitopes of human cytomegalovirus (hCMV) have been observed in some patients with SSc[65]. Anti–topoisomerase I antibodies can cross-react with hCMV-derived proteins and there have been reports of previous exposure of SSc patients to human parvovirus B19 infection.

Molecular mimicry may play a role in the initiation of antibody response, making infectious agents potential cofactors in the commencement of autoimmune response in individuals with susceptible genetic and hormonal background, especially in the light of their ability to target endothelium and monocyte– macrophage axis[66,67] ; however, the etiologic role of viruses in SSc remains unproven.

Studies have shown that exposure to the following environmental agents: silica/silica dust, vinyl chloride, benzene, toluene, epoxy resins, heavy metals mercury and drugs: bleomycin, cardiopa, pentazocine, cocaine, docetaxel, metaphenylenediamine increases risk of scleroderma occurrence.

Men with exposure to silica or working in construction-related occupations have higher incidence of SSc[68]. Large-scale epidemiologic surveys, have not confirmed an increased risk for creation of connective tissue diseases associated with use of silicone breast implants.

It is well established that during and after pregnancy stem cells from fetuses circulate in the tissues of healthy mothers (microchimerism)[69,70], and transmaternal passage of cells from elder siblings has been suggested as possible source of non-fetal microchimerism in nulliparous women[71]. In SSc patients these microchimeric cells could be involved in disease pathogenesis by initiating a graft-versus-host–like reaction caused by the fetal cells or through a maternal immune response against the fetal cells.

4. Pathogenesis

Vascular damage, immunologic disturbances, and fibroblast activation resulting in generalized tissue fibrosis are the pillars of SSc pathogenesis. Studies have found evidence for each of them in patients with SSc; however, their individual contribution to the disease manifestations is variable.

Vascular damage

Many studies through the years have documented the presence of vascular changes in scleroderma's small arteries[73,74,75]: histopathological postmortem studies of specimens from the digital vessels of 16 SSc patients showed the presence of intimal thickening and marked luminal narrowing[76]; autopsy examinations of 40 patients showed subendothelial mucoid edematous intimal hyperplasia in the smaller vessels in the renal cortex[75] while other reports found intimal fibrosis with myxomatous changes and luminal occlusion in patients' small and medium-sized pulmonary arteries.

Vascular injury and activation are the earliest and possibly primary events in SSc pathogenesis[71]; unidentified cytotoxic agents, T-cell derived proteolytic enzymes, anti endothelial cell-directed autoantibodies (AECAs), vasculotropic viruses, inflammatory cytokines, and environmental stresses have been implicated to induce initial vascular injury.

Vascular damage leads to endothelial cell activation and dysfunction. Some of the earliest changes detected in the vases of SSc patients comprise - presence of large gaps between endothelial cells, vacuolization of endothelial cell cytoplasm, and loss of membrane-bound storage vesicles[87-89]. Events that follow endothelial activation include:

- Expression of vascular endothelial cell adhesion molecule 1 (VCAM1), intercellular adhesion molecule (ICAM) and endothelial leukocyte adhesion molecule-1, (E-selectin) is increased and recruitment and infiltration of inflammatory cells occurs. Activated lymphocytes secrete cytokines and activation of platelets is also observed.

- Activated platelets release thromboxane A2, platelet-derived growth factor PDGF, and transforming growth factor beta TGF-β which causes vasoconstriction; TGF-β is a cytokine capable of inducing transition of a fibroblast into a myofibroblast phenotype by stimulating α-SM-actin expression and production of collagen[90-92].

Impaired fibrinolysis, increased levels of von Willebrand factor, and ongoing platelet aggregation is observed. A study reported increased Von Willebrand factor activity and factorVIII/von Willebrand factor (fVIII/vWf) antigen

concentrations in patients with scleroderma[93-96]; higher circulating levels of both are thought to reflect in vivo endothelial injury[93].

-Activated endothelial cells increase secretion of vasoconstrictor substances like endothelin-1 (ET-1) and production of vasodilators like oxide nitric (NO) and prostacyclines is decresed[72].

Endothelin-1 (ET-1), a potent endothelium derived vasoconstrictor substance has been implicated in the pathogenesis of multiple vascular diseases[97]. Its overexpression is connected with mitogenic, fibrotic and inflammatory activity[98]. Several studies have demonstrated elevated ET-1 concentrations in SSc patients [97,99], which correlate with severity of RP, digital ulcers, pulmonary arterial hypertension PAH, and renal failure.

-These alterations lead to irreversible and progressive vascular wall remodeling, with: intimal proliferation and accumulation of proteoglycans in the arterioles and small arteries [100,101], fibrosis of the media and adventitia, luminal occlusion, in situ thrombosis, and tissue hypoxia.

Intimal prolifation, the most frequent histopathologic finding of the small and medium sized vases of patients with SSc, a finding that SSc patients share with chronic allograft arteriopathy, is thought to be the result of proliferation and migration of myointimal cells and accumulation of collagen[102]. Studies show that vascular basement membranes are thickened[103,104], and changes are observed mainly in the in blood vessels of the heart, lungs, kidneys, and gastrointestinal tract.

Loss of blood vessels may be further exacerbated by insufficient vasculogenesis. SSc neoangiogenesis, is damaged despite elevated levels of vascular endothelial growth factor (VEGF) and its receptors[77]. Episodes of ischemia–reperfusion lead to oxidative stress that further increases vascular injury.

Together with endothelial cell apoptosis and thrombosis, the end-result is an obliterative vasculopathy of small and medium sized arteries with the characteristic scarcity of blood vessels seen by angiography in progressive SSc and reflected clinically with the Raynaud's phenomenon, digital ulcers, scleroderma renal crisis, and pulmonary hypertension.

Menagement of SSc vascular disorders includes application of : angiotensin-converting enzyme (ACE) inhibitors for scleroderma renal crisis, endothelin receptor antagonists for pulmonary arterial hypertension (PAH); calcium channel blockers for Raynaud's phenomenon.

Immunological dysregulations

Immune dysregulation (activation) is apparent at multiple levels in SSc patients: the presence of perivascular accumulation of mononuclear cells, macrophages, and p dentritic cells (DC) in affected tissues, activation and polarization of circulating T cells, B cells, monocytes, and DCs are some of the alterations observed. Both general and specific antibodies are found in patients with SSc.

Systemic Sclerosis patients might be genetically predisposed to develop dysregulated innate and adaptive immune responses and the characteristic "type I IFN signature" prominent in SLE and other autoimmune diseases is also observed in SSc[105,106].

Another indicator of immune involvement in disease pathogenesis is the fact that aggressive immunosuppressive strategies seem to positively impact fibrosis in SSc patients. Cyclophosphamide, which targets T and B cells and does not have a direct impact on fibroblasts proliferation and extracellular matrix (ECM) deposition, has been proven to be efficacious in reducing progression of SSc-associated interstitial lung disease (ILD) after daily oral administration or in reversing severe diffuse skin fibrosis when given at immuno-or myelo-ablative regimens with or without Autologous Hematopoietic Stem Cell transplantation[78-80], - suggesting in this way that T and B immune cells are involved in sustaining SSc fibrosis.

Below are listed some alterations of the innate and adaptive immune responses observed in patients with SSc.

Dendritic cells – The potent antigen presenting cells of the immune system, get activated after exposure to an injurious event via Toll-like receptors (TLRs) and produce type I interferon (IFN), which induces T helper (Th)2 T cell polarization, monocyte differentiation to an activated phenotype, and B cell activation and production of autoantibodies. Activated leucocytes produce profibrotic chemokines and cytokines, inducing fibroblast activation. Studies show that CD11c+ DC accumulate in the tissue of animal models of fibrosis and in patients with SSc, and that the levels of : HSP70, HMGB-1 (when released from damaged, necrotic, and apoptotic cells, HMGB-1 binds to receptor for advanced glycation end products (RAGE), toll-like receptor (TLR)2, TLR4, and also TLR9. HMGB-1 system induces the nuclear factor-κB (NFκB) phosphorylation and secretion of cytokines and chemokines), and hyaluronan are elevated in SSc sera or tissues[1, 107-109].

T Cells - T cell activation is evident in SSc lesional tissues. Studies show that in early-stage SSc skin, fibroblasts with prominent procollagen gene expression are situated next to the inflammatory cells, suggesting that they or their soluble products hold a direct role in fibroblast activation [110, 112, 113]. Tissue-infiltrating T cells are predominantly CD3+ and CD4+, express markers of activation (CD69, CD45, HLA-DR, and IL-2R). predominace of CD8+ and γ/δ T cells is observed in the lungs[111, 114].

Patients with SSc display an altered Th1/Th2 cytokine balance with a predominant Th2 profile. Th2 cells secrete plenty of IL-4, IL-5, and IL-13[115-117](which are profibrotic), and only low levels of the principal Th1 cytokine IFN-γ. IL-4 is important in polarizing Th2 response mediated through its receptor and intracellular signaling molecules, such as STAT6[118 119].

Studies have shown reduced numbers and impaired function of Treg cells in SSc. Immunohistochemical modalities have

also revealed that FoxP3+ Treg cells are decreased in the skin of patients with SSc. According to one hypothesis Treg cells may convert into Th17 cells in SSc; and it can be possible that Treg cell priming in SSc is skewed toward Th17 cells in the presence of incresead levels of IL-6 and TGF-β [120-124].

B cells - Reports show that B cells have an important role in the pathogenesis of SSc. B cells may not only be responsible for autoantibody production in but their chronic activation may also contribute directly to fibrosis, because activated cells secrete TGF-β and IL-6, which stimulate fibroblasts. Studies have reported presence of polyclonal B cell hyperactivity in SSc patients; circulating memory B cells were found to display a state of chronic activation, clonal expansion, and antibody production. Hyper-gama-glubilimemia was also observed [125].

Humoral immunity - Autoantibodies are present in >90 % of SSc patients and selectively correlate with disease-specific clinical manifestations Various hypotheses exist to explain the generation of autoantibodies in SSc : exposure of cryptic epitopes that break immune tolerance[126], molecular mimicry as a result of viral infection, chronic B cell hyper-reactivity, altered expression or subcellular localization of potential autoantigenic peptides and activation of B cells by endogenous TLR ligands are some of the proposed mechanisms.

Anyhow a direct pathogenic role of SSc autoantibodies in tissue damage has not been conclusively established; autoantibodies tend to be highly specific, mutually exclusive and are strongly associated with particular disease types.

Some of the most common specific autoantibodies observed in patients with SSc are : Anti-Scl-70 (anti-topoisomerase I), anticentromere Antibodies (ACA), anti-U3-RNP (antifibrillarin), and anti-RNA-polymerase antibodies.

Vascular and immunological processes are central to the pathogenesis of scleroderma, but it is unclear what the initial events are and how different processes activate, amplify, or assist the development of characteristic SSc fibrosis

Fibrosis

Connective tissue consists of cells and the extracellular matrix (ECM). Fibroblasts are the permanent cells of the connective tissue while macrophages, plasma cells, and mast cells originate from hematopoietic stem cells in bone marrow, circulate in the blood, and are transported into the connective tissue where they operate.

Under the effects of appropriate extracelluar clues fibroblasts start to produce ECM macromolecules, growth factors, cytokines and chemokines, adhere to the connective tissue, and differentiate into myofibroblasts, that play an important role in wound healing.

Myofibroblasts synthesize collagens, tissue inhibitor of metalloproteinases (TIMPs), and other components of the ECM and are responsible for TGF-β secretion during the fibrotic response. When repair process is terminated most of the myofibroblasts undergo apoptosis and disappear, resulting in a connective scar that has very few cellular elements[1, 127, 128].

Fibrosis, the principal process of SSc disorder, represents an abnormal form of wound healing and is characterized by uncontrolled fibroblast activation, myofibroblast accumulation and persistence, permanent structural changes and replacement of normal tissue with stiff scar tissue. Fibroblasts secrete collagen especially type 1 and 3 with type 1 being more abundant.

Fibroblasts overproduction comes as a response to autocrine and paracrine signals such as cytokines and chemokines; hypoxia and ROS; signals from the surrounding ECM or via cell-cell interactions. TGF -β, PDGF, VEGF, ET-1, IL-13, IL-6, CTGF/CCN2, IGF-1, Wnt ligands are some of the mediators implicated in SSc fibrosis.

- A pleiotropic member of a large cytokine superfamily, TGF-β is considered the principal regulator of both physiologic fibrogenesis (wound healing and repair) and pathologic fibrosis and a key therapeutic target.

TGF-β signals are transduced by transmembrane type I and type II serine/threonine kinase receptors. Canonical TGFβ signaling involves activation (phosphorilation) of the type I TGFβ receptor (TGFR1), that in turn phosphorylates cytosolic Smad2/Smad3 proteins. Phosphorylated Smad2/3 form heterocomplexes with Smad4 and translocate into the nucleus, where they bind to consensus Smad-binding elements (SBEs), found in many TGF-β–inducible genes. The SMAD pathway is regulated (both positively and negatively by various factors, including SMAD7 and NR4A1 and Smad proteins control profibrotic genes[130,131].

Non-canonical TGFβ signalling pathways, significant to fibrosis include : focal adhesion kinase (FAK), ABL1, phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase 1 - MAPK1 and MAPK3 (also known as ERK2 and ERK1, respectively), p38, endoglin and SMAD1 pathways[132]. The ABL1 pathway, regulates the profibrotic transcription factor early growth response 1 (EGR1)[133].

Studies have shown that TGFβ has also a dual role in tumorigenesis. It exerts antiproliferative effects and functions as a tumor suppressor in the early stages, but serves as a tumor promoter aiding in metastatic progression through an autocrine TGFβ loop at later stages. Transgenic mice expressing a dominant negative TβRII in the epidermis and mammary glands display aggressive tumor formation and metastatic progression[134,135].

Increased expression of type I and type II TGF beta receptors in fibroblasts cultivated from the skin of patients with diffuse SSc, was observed[129].

- Epithelial mesenchymal transition (EMT) - a process involving transformation of epithelial cells under certain conditions to fibroblasts is being studied and might represent an important element in SSc pathogenesis. EMT is important during vertebrate embryonic development while pathologic EMT is prominent in cancer and has also been implicated in renal fibrosis and idiopathic pulmonary fibrosis[136,137]. Other

cells (adipocites, endothelial and mesotelial cells) can be also be transformed in fibroblasts.

- Studies have found increased levels of angiotensin II in SSc patients, which stimulates TGF-β1 production, fibroblast proliferation, and their differentiation into myofibroblasts. Damaged ECM degradation and turnover, and increase in the number of ECM-producing mesenchymal cells, also play pathogenic roles. Microvascular pericytes are increased and when activated they can differentiate into collagen-producing fibroblasts and myofibroblasts.

Even though various disorders are observed in SSc fibroblasts it still remains unresolved whether the activated SSc fibroblast phenotype is an autonomous abnormality or is due to activation by exogenous stimuli in the fibrotic environment.

5. Clinical Manifestations

Raynauld phenomenon

Raynauld phenomenon (RP) is the earliest symptom in patients with SSc. It affects fingers, toes, earlobes and tip of the nose and consists of reversible vasospastic attacks. Three different colors are observed in the skin of patients with RP[138]: 1) pallor - which is due to vasospasm of the arterioles and collapse of digital arteries, 2) cyanosis - resulting from ischemia and 3) hyperemia - which reflects regulation of blood flow and ensues spontaneously or with rewarming of the digit. Typically Raynaud's attacks are symmetrical, involving both hands and usually resolve in about 15–20 min in a warm environment.

3−5% of the general population has primary Raynaud's Phenomenon. Primary RP involves absence of signs or symptoms of an underlying condition and represents an exaggerated physiologic response to cold [139].

Secondary Raynaud's phenomenon can be a complication of SSc and other connective tissue diseases, a consequence of ccupational trauma, use of certain drugs, presence of compressive or obstructive vascular disease and increased blood viscosity. Nail-fold capillaroscopy is generally used to distinguish patients with primary RP from those with scleroderma or another rheumatic diseases. Capillary loop dilation/enlargements, microhemorrhages and a variable loss of capillaries with or without avascular areas are some of the observed morphological changes in patients with secondary RP. Fingertip ulcers may arise as a complication of RP and chronic ischemia. Usually ulcers heal slowly, can become infected and may transform to gangrene[139,140].

If lifestyle modifications and cold avoidance are not sufficient to control patient's symptoms, vasodilator therapy should be initiated. Calcium chanel blockers (CCB) are the first line of therapy for RP. Studies have examined: nifedipine, amlodipine, nisoldipine, isradipine, felodipine and found them efficacious in patients with Raynauld's phenomenon. The calcium channel blockers appear to work better in primary than in secondary RP. Other effective vasodilators that could be used in RP include: prostacyclines (IV ileoprost), phosphodiesterase-5 inhibitors (e.g., sildenafil), endothelin receptor antagonists, Rho-kinase inhibitors, antioxidants, statins, nitrates and angiotensin receptor blockers A combination of these agents, can be used in refractory cases if tolerated[141-145].

Skin involvement

Skin thickening and hardening, in both diffused and limited SSc forms are due to increased collagen and extra-cellular matrix deposition in the dermis.

Skin involvement follows these phases: I) *edematous phase*, which reflects the presence of the clinical signs of inflammation, characterized by nonpitting edema of affected body areas. Edema can also cause local tissue compression, II) sclerotic *(fibrotic) phase*, where acute inflammation is clinically less evident, collagen and other extra-cellular material deposit in the dermis and make the skin thick and stiff, III) *atrophy* is present in the late stages of the disease; here fibrosis extends beyond the dermis, the skin becomes thin and atrophies and there are no signs of inflammation. There is little published data on the duration of each of these 3 phases[146-149].

Masked facies, small oral and orbital apertures, and vertical furrowing of the perioral skin are consequences of skin and soft tissue fibrosis.

Gastrointestinal involvement

Esophageal dysmotility, stomach, small bowel, colon and ano-rectal disorders are observed in patients with Systemic sclerosis. Esophageal disease is characterized by decreased functioning of the muscle of the lower part of the esophagus[150], which causes reduction of the peristalsis and of the strength of the lower esophageal sphincter. Dysphagia and reflux episodes follow and they are responsible for esophagitis of the lower part of the esophagus[151].

The small bowel can become dilated and often atonic, losing its propulsive function. Fat, protein, carbohydrate and vitamin malabsorption are present[152].

Pulmonary involvement

Interstitial lung disease (ILD) and pulmonary artery hypertension (PAH) are the principal SSc lung disorders.

Non specific interstitial pneumonia (NSPI) is the most common morphological and pathological pattern found in the lungs of patients with SSc; usual interstitial pneumonia (UIP) can also be present. NSPI is characterized by varying degrees of inflammation and fibrosis but does not display UIP's variability (fibroblast foci and honeycombing is absent). Patients with NSPI have better prognosis than those with UIP[153,156].

Dyspnoea on exertion, hypoxemia and non productive cough are the most common manifestations of pulmonary fibrosis even in patients without radiological evidence of pulmonary damage. Haemoptysis, airway and lung inflammation can also occur in advanced fibrosis.

Pulmonary function test (PFT) in SSc may show a restrictive pattern with decrease of forced vital capacity (FVC) and diffusion capacity for carbon monoxide (DLCO).Chest

HRCT is the non invasive gold standard technique for the diagnosis of SSc ILD. Traditional means of identifying CTD-ILD, including chest radiography and clinical examination, are insensitive when compared with HRCT. Ground glass opacification on HRCT correlates to the presence of air space inflammation in the lung (alveolitis) and decreased DLCO[157].

Pulmonary arterial hypertension (PAH) is a serious and potentially life-threatening condition associated dysregulation of blood flow. Lung vessels constrict and become stiffer and thicker because of the irreversible fibrosis. In the earliest stages of PAH, the patient is asymptomatic. Then dyspnea starts on exertion and later, during ordinary activity; specific symptoms such as chest pain, dizziness and fainting also may occur.

Kidney involvement

Kidney involvement in SSc is often clinically uneventful. It may progress slowly toward renal failure and influence to a great extent prognosis. In some cases, breakdown of the renal system may be abrupt, without any warning symptoms. Sudden onset of high blood pressure and kidney failure is known as scleroderma renal crisis (SRC) and patients with dcSSc are at increased risk for developing it[158]. Cardiac (fibrosis of the myocardium and conducting system, pericarditis etc[159-162]), joint and muscolosceletal involvement (synovitis, arthralgia, arthritis[162]) are also present in patients.

6. Conclusion

Systemic sclerosis is a highly complex systemic auto-immune disease, whose pathogenesis remains incompletely understood, but involves mainly vascular alterations, immune activation and fibrosis. The roles played by the cell types and mediators implicated in SSc pathogenesis are being studied and all new research insights might lead to the identification of novel biomarkers of diseases activity and subtype, and discovery of novel therapeutic targets. The disease is heterogeneous and a wide array of organs and systems are affected. Early and accurate diagnosis and timely recognition of life-threatening complications and initiation of targeted therapies to halt progression helps improve patient outcomes.

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