Review

Systemic sclerosis: a critical digest of the recent literature

C. Tani[1], S. Bellando Randone[2], S. Guiducci[2], A. Della Rossa[1]

[1]Rheumatology Unit, University of Pisa, Pisa; [2]Department of Biomedicine and Division of Rheumatology, AOUC, Department of Medicine and Department of Clinical Medicine, University of Florence, Italy.

Chiara Tani, MD
Silvia Bellando Randone, MD
Serena Guiducci, MD
Alessandra Della Rossa, MD

Please address correspondence to:
Dr Chiara Tani,
Rheumatology Unit,
University of Pisa,
Via Roma 67,
56126 Pisa, Italy.
E-mail: chiaratani78@gmail.com

Received on June 26, 2013; accepted in revised form on June 27, 2013.

Clin Exp Rheumatol 2013; 31 (Suppl. 76): S172-S179.

Key words: systemic sclerosis, diagnosis, pathogenesis, treatment

Competing interests: none declared.

ABSTRACT

Herewith we provide a critical digest of the recent literature on systemic sclerosis. The most relevant studies published over the last two years have been reviewed, with particular focus on the diagnosis, pathogenesis and treatment of the disease.

Introduction

Systemic sclerosis (SSc) is a disabling chronic disease characterised by autoantibody production, skin fibrosis and a peculiar microvascular involvement (1-2). Recently, a great deal of progress has been made in the understanding of the etiopathogenesis of this disorder, with consequent improvements in the early diagnosis and management. In this manuscript we will provide an overview of the recent advances in pathogenesis, diagnosis, classification and treatment of systemic sclerosis. A systemic Medline search was performed using the term "Systemic sclerosis" (MeSH terms and semantic search). The Medline search was focused on most relevant literature contributions published in 2011 and 2012.

Recent insights into the diagnosis and classification of systemic sclerosis

SSc is a complex disorder with skin and internal organ involvement. The first classification criteria, published by the American Rheumatism Association, lack sensitivity, particularly in early, limited and sine scleroderma subset (3). EUSTAR group, through Delphi rounds, promoted the search for items necessary for the very early diagnosis of systemic sclerosis (VEDOSS). The current validated or proposed criteria are indeed not appropriate to make a diagnosis of SSc at initial stages. This implies that the diagnosis of SSc and, consequently, an appropriate therapy, are delayed until microvascular remodelling, tissue fibrosis, or atrophy are already irreversible. In a recent Delphi exercise, 4 signs/symptoms have been identified as necessary for the very early diagnosis of SSc: Raynaud's phenomenon (RP), puffy swollen digits turning into sclerodactily, antinuclear antibodies and specific SSc antibodies (anticentromere and antitopoisomerase-I antibodies), and abnormal capillaroscopy with scleroderma pattern. Patients who fulfill these criteria are the target of the recently launched VEDOSS program, which has been designed to diagnose SSc at initial, potentially reversible stages and to examine whether this may change the disease outcome. Although patients with RP, autoantibodies, and SSc capillaroscopic pattern could be easily followed up, there is still debate on the predictors that may allow us to identify patients who will develop a full blown disease (4-5).

In the last two years, researchers have committed their efforts also to defining new classification criteria for established disease using consensus procedures, including the Delphi and nominal group techniques (6). In the first instance two independent consensus exercises were performed by the Scleroderma Clinical Trials Consortium and the European League Against Rheumatism Scleroderma Trials and Research Group to identify items for developing revised classification criteria in SSc. After three Delphi rounds a set of 23 items for the classification of SSc were collected. Presence of skin thickening, SSc-specific autoantibodies, abnormal nailfold capillary pattern and Raynaud's phenomenon ranked highest in the final list that also included parameters indicating internal organ involvement. The items selected demonstrated good face, discriminant and construct validity (6-7). Further item reduction and clustering is under way, based on internationally agreed guidelines (8).

Microvascular involvement is one of the distinguishing features of SSc. Capillaroscopy has a pivotal role for the differential diagnosis of Raynaud's phenomenon and the early diagnosis of the disease (9). Capillary microscopy abnormalities are also a useful tool for prognostication of vascular involvement (10).

Scores of microvascular involvement have been developed and validated and might be useful as an aid to identify patients at higher risk of digital ulcers (11) and/or internal organ involvement (12). Concomitantly, recent advances in non-invasive imaging modalities have added information on the dynamic of microcirculation in SSc subjects.

Thermography has a great value as an objective assessment tool both in clinical practice and in clinical trials for Raynaud's phenomenon (13).

Laser studies have shown an impairment of the thermoregulation of the finger skin, that can improve with treatment (14-15). The abnormalities of skin perfusion seem to involve different areas of the dorsum of the hand according to the stage of the microangiopathy (16).

Skin involvement is another frequent feature of SSc. Skin thickness progression rate has been identified as a useful and easy means to predict mortality and early internal organ involvement in diffuse systemic sclerosis (17). The non-invasive determination of skin biomechanical functions might be relevant in monitoring the progression of skin involvement in SSc (18).

Joint involvement, although deemed unimportant, is another frequent feature of SSc and compromises patients quality of life. Some pilot studies have underlined the potential usefulness of new imaging tools, such as ultrasonography and magnetic resonance. However, most of the tools used to assess arthritis in SSc patients have not been validated and additional work is needed to develop a "core set" of variables for assessment of arthritis in SSc and its response to treatment (19-22).

Although frequently overlooked, gastrointestinal (GI) involvement might be an important cause of disability and discomfort in SSc patients (23-24), moreover, overt gastro-esophageal reflux esofagitis have been correlated with a more severe vascular involvement (25). A questionnaire for GI involvement in SSc has been set up and validated by the Scleroderma Clinical Trial Consortium. (26-27). This instrument might prove useful both as a screening tool and in the follow-up of SSc patients.

Cardiopulmonary involvement is the leading cause of death in SSc. High-resolution computerised tomography (HRCT) scan is the gold standard for assessing the extension and severity of interstitial lung disease (ILD) (28). Data from the scleroderma lung study has outlined that the baseline HRCT fibrosis score is a predictor of a future decline in the FVC% predicted in the absence of effective treatment (29). Moreover, extensive disease (>20%) on HRCT at baseline, is associated with an increased risk of deterioration or mortality (30).

In most recent years growing interest has arisen in ultrasound as a non-invasive screening instrument for lung fibrosis in SSc (31-32).

The gold standard for the detection of pulmonary arterial hypertension (PAH) is right heart catheterisation, but in the clinical setting, simple and feasible tests, such as serial pulmonary function tests, NT pro-BNP and echocardiogram, could serve for the detection of patients to be closely monitored or candidate to right heart catheterism (33-34).

Pulmonary Hypertension Assessment and Recognition of Outcomes in Scleroderma (PHAROS) is the largest US and Canadian cohort of subjects with SSc at high risk for or with incident PAH. In this registry, 206 patients of a "high risk" cohort, defined on the basis of a set of rigorous clinical and instrumental criteria, underwent right heart catheterism (35).

Data from this study showed that patients in the borderline mean pulmonary arterial pressure (mPAP) group were more likely to have restrictive lung disease, fibrosis on high-resolution computed tomography (CT) and a higher estimated right ventricular systolic pressure on echocardiogram than patients with normal haemodynamics. Right heart catheterisation revealed higher pulmonary vascular resistance and more elevated mPAP on exercise in the borderline mPAP group. However, despite these disparities, no significant difference was outlined in the two groups in the appearance of overt resting PAH at follow-up. (36).

A systematic detection echocardiographic algorithm, regardless of the presence of signs or symptoms suggestive of PAH, could prove useful in the early identification of pulmonary hypertension in SSc patients and in improving long term outcome (37). Exercise induced pulmonary arterial hypertension is a frequent finding in SSc patients, however, further studies are warranted to assess the prognostic significance of this finding (38).

HRCT is mandatory in the assessment of cardiopulmonary involvement in SSc, regardless of the presence of ILD, since it allows the operator to detect signs of pulmonary venoocclusive disease, which are frequently observed in SSc patients with precapillary pulmonary hypertension (39).

Heart involvement, including primary myocardial involvement, is very common in systemic sclerosis. When clinically evident, it carries an ominous outcome. Risk factors for heart involvement include diffuse cutaneous subset, rapid progression of skin involvement (17) and older age at the onset of the disease. Given the poor prognosis associated with this kind of involvement, screening for subclinical cardiac disease is essential (40). Recent reports suggest that it is not uncommon for patient with SSc PAH to have concomitant left ventricular (LV) perfusion defects despite normal epicardial coronary arteries; this could explain the increased morbidity and mortality of SSc related PAH as compared with other types of PAH (41-42).

Several patients with SSc undergo routine annual screening with echocardiography, for the assessment of estimated pulmonary pressure, LV dysfunction and pericardial effusion. Newer echocardiographic techniques, such as tissue Doppler imaging and right ventricular quantification are recommended to better characterise SSc

heart involvement. The cornerstone for the assessment of myocardial fibrosis is represented by cardiac magnetic resonance (42).

Natriuretic peptides, although studied primarily as biomarkers of PAH in most studies, may be utilised as a screen for overall cardiac involvement in SSc (43).

Novel insights into the pathogenesis of systemic sclerosis

The pathogenesis of SSc is still largely unknown. In the last decade, considerable progress has been made in understanding some of fundamental aspects (endothelial damage, widespread microvascular alteration, fibrosis, immune imbalance with abnormal production of autoantibodies) so that, like pieces of a puzzle, the multiple facets of SSc are slowly emerging. The recent literature reports some interesting findings for many of the pathogenetic aspects, also highlighting potential new therapeutic targets. We herewith present a review of the literature on 3 pivotal aspect of the pathogenesis of the disease: vascular damage, immune system and fibrosis.

Disorganised microvasculature occurs very early, progresses during the disease course and seems to be strictly related to a dysregulated expression of a large array of proangiogenic and antiangiogenic factors that may be largely responsible for the impaired angiogenic response found in patients with SSc (44). Riccieri *et al*. have recently investigated in SSc patients molecules such as VEGF, PDGF-BB, HGF, Ang-2, leptin, PECAM-1 and IL-8 cytokine founding correlations with severe vascular manifestations, such as digital ulcers (PDGF-BB and PECAM-1), pulmonary hypertension (IL-8) and a "late" capillaroscopy pattern (Ang-2) or with the presence of an important marker of diffuse SSc form, such as anti-topoisomerase 1 antibody (VEGF and PDGF-BB). These findings may implicate a possible role for these factors as potential useful prognostic markers of SSc (45).

Bandinelli *et al*. reported that CCL2 monocyte chemotactic protein-1 (MCP-1/CCL2), CCL5 regulated upon activation, normal T expressed and secreted (RANTES/ CCL5) and CCL3 macrophage inflammatory protein 1 α (MIP1α/CCL3) are increased in SSc patients and down regulated after the infusions of the prostaglandin E1 (PGE1) analogue, alprostadil alpha-cyclodextrin used in the management of vascular features (46). In the light of these results PGE1 might be considered as a potential disease modifier treatment in SSc, acting not only as a vasodilatator but also interfering with cell recruitment and fibrosis process. Nowadays, particular interest is focused on VEGF: recent data provide evidence that switching from proangiogenic to antiangiogenic VEGF-A isoforms (called VEGF165b) may play an important role in the dysregulation of angiogenesis in patients with SSc. This evidence clarifies, in part, the SSc paradox: a disease with no angiogenesis with high levels of VEGF (47). Manetti *et al*. showed that the endogenous antiangiogenic VEGF165b splice variant is selectively upregulated in SSc skin and blood, providing evidence that profibrotic TGF-β1 and SRp55 splicing factor may contribute to the switching from proangiogenic to antiangiogenic VEGF isoforms. These results have direct implications for the development of novel potential antiangiogenic or proangiogenic therapies. In fact, administration of proangiogenic VEGF165 and other strategies aimed to fight the constitutive upregulation of antiangiogenic VEGF165b or molecular regulation of VEGF pre-RNA splicing might represent potential therapeutic approaches to promote effective angiogenesis and capillary regeneration in SSc (48). Some other innovation has been recently achieved in the genetics of SSc. It is known that genetic factors are crucial in the susceptibility and clinical features of autoimmune diseases such as systemic sclerosis, especially those related to the immune response (49). The availability of genome-wide association study (GWAS), which makes it possible to screen single-nucleotide polymorphisms SNPs across the entire genome, has made possible the discovery of new genetic susceptibility loci leading to the identification of new pathogenetic mechanisms. A recent literature review performed by Romano *et al*. confirms the role of genetics in SSc pathogenesis, including genes associated with autoimmunity, fibrosis, and vascular disease. Multiple genetic markers related to the innate and adaptive immune regulation, such as *HLA class II* gene region, *IRF5*, *BANK1*, *BLK*, *TNFSF4, STAT-4* and *CD247* genes, have been firmly established as SSc susceptibility genes through replication in independent studies (50-54). Besides *BANK1* and *BLK,* other B-cell genetic markers including CD19, CD20, CD22 and CD24 polymorphisms, that seem to play a role in B-cell activation, have been recently investigated as possible genetic susceptibility factors to SSc in European Caucasian and Japanese populations (55-57). Accumulating data have demonstrated shared autoimmunity pathways and susceptibility factors between various autoimmune diseases, indicating a common contribution to the pathogenetic mechanisms. However, there are still conflicting data in this regard (58). For example, the results recently obtained through a large cohort of European Caucasian SSc patients do not support the correlation between CCL21, CD244 and CDK6 genes and the pathogenesis of SSc, although these genes have been recently identified as RA susceptibility genes (58). PTPN22 C1858T (rs24 76601) polymorphism resulted also strongly associated with increased susceptibility to rheumatoid arthritis and SLE development, but not to SSc (60).

In a translational study, Avouac *et al*. reported interesting data on the well-known STAT-4 gene (one of the main shared genetic factors between autoimmune disease) and its role in tissue fibrosis using STAT-4–deficient (stat4$^{-/-}$) mice and their wild-type littermates (stat4 $^{+/+}$) treated with bleomycin or NaCl as animal models of SSc. It has been demonstrated that the transcription factor STAT-4 regulates fibroblast activation and collagen release indirectly by orchestrating leukocyte infiltration and regulating proinflammatory cytokines (IL-6 and TNF-alpha) production in the inflammatory early stages of SSc (61). It is well known that IL-6 is a key cytokine both in fi-

brosis and in the immunity process in SSc (62). Recently, Cènit *et al*. have analysed the influence of the IL6 gene in susceptibility to SSc through a large meta-analysis including a total of 2749 cases and 3189 controls from 6 white populations, finding that the *IL6**GGC allelic combination influences predisposition to SSc, conferring risk to the global disease (63). The focus on IL-6 is set to grow up as direct and indirect (blocking Th17 cells) potential therapeutic target (64). Furthermore, with regard to the role of T lymphocytes in the SSc pathogenesis, Carmona *et al*. have not observed any association with the presence of the functional variant *Delta32CCR5* of the chemokine receptor CCR5 in SSc, unlike what has been reported for other autoimmune diseases (65). Interesting novelties are also available in the literature on fibrosis, the most pathological hallmark of SSc. Several reports emphasise the importance of Wnt proteins that, if overexpressed, leads to accumulation of β-catenin in SSc fibroblasts with release of ECM and rapid development of dermal fibrosis (66-69).

This hypothesis was confirmed by Bergmann *et al*. who demonstrated that inhibition of glycogen synthase kinase-3β (GSK-3 β) results in increased release of collagen from cultured fibroblasts *in vitro*, and induces dermal fibrosis *in vivo* with accumulation of collagen, dermal thickening and differentiation of resting fibroblasts into myofibroblasts (70). Thus, even the blocking of the β-catenin/Wnt signalling may represent a future therapeutic target to reduce the progression of fibrosis in SSc. Another fibrogenic pathway has been recently analysed, showing the role of JAK-2 in the pathogenesis of SSc, with focus on the antifibrotic potential of JAK-2 inhibition as a novel treatment approach. Dees *et al*. demonstrated that JAK-2 is activated in SSc in a TGF-β -dependent manner, that it mediates the stimulatory effects of TGF β on fibroblasts and that its inhibition is sufficient to reverse the characteristic phenotype of cultured SSc fibroblasts (71). Over the years, more and more attention has been paid to the pathogenesis of SSc, discovering yet submerged aspects. The real breakthrough in the understanding of this disease will be the study of the very early SSc that will bring out the primitive aspects of the pathogenesis.

Recent insights into the therapy of systemic sclerosis

Treatment strategies in SSc are actually targeted to the main component of the disease pathogenesis: vasculopathy, inflammatory/autoimmune and fibrotic processes; in the 2011–2012 period, important steps toward new therapies and optimisation of available drugs have been made. (28)

Targeting fibrosis

Platelet-derived growth factor (PDGF) and transforming growth factor β (TGFβ) are actually considered the crucial players in the excessive fibrosis that characterises SSc; as they are produced by fibroblasts through tyrosine kinase signalling events, the inhibition of tyrosine kinase is considered as an important treatment target in SSc. Indeed, *in vitro* studies demonstrated that the small molecule imatinib mesylate (Gleevec), a tyrosine kinase inhibitor, significantly reduces the expression of fibrotic genes and blocks TGF-β signaling. Moreover, a five-clusters 'fibroblast imatinib response gene signature' including genes involved in fibrosis, cardiovascular disease, inflammation, and lipid and cholesterol metabolism, have been identified by genome-wide analysis (72).

From a clinical point of view, despite some encouraging results from isolated reports, the benefit/risk ratio of this therapeutic approach is still controversial. In a 6-month phase II double-blind trial, Imatinib failed to demonstrate efficacy to improve skin fibrosis, while in an open-label trial of longer duration (one year) a reduction in skin thickening and lung dysfunction were reported (73, 74). Moreover, in patients with active dcSSc poor tolerability and high rates of adverse events were also observed (75, 76).

Experimental data suggest a key role for B cells in regulating the fibrotic process, thus highlighting a strong rationale for the use of rituximab (RTX) in SSc. In a recent paper by Daoussis *et al*. skin biopsies obtained from eight patients with SSc prior and following RTX treatment were immunohistochemically assessed for the expression of PDGFR and their phosphorylated form; authors found a strong correlation between PDGFR expression on spindle-like cells and collagen deposition, and a significant decrease in the papillary dermis following RTX administration (77).

The beneficial role of RTX on fibrosis have also been suggested by observational data on patients with severe interstitial lung disease secondary to connective tissue diseases refractory to standard therapies; promising results have been reported by the authors which described a favourable treatment response to RTX in 7 out of 8 treated patients, thus suggesting the need for future research in this direction (78).

Among the traditional immunosuppressive drugs, in 25 patients with diffuse progressive cutaneous SSc of recent onset, mycophenolate mofetil (MMF) demonstrated marked improvement in skin involvement and stabilisation of pulmonary function (79). Moreover, bosentan is an endothelin-1 receptor antagonist largely used in SSc-related vascular complications, but it did not demonstrate efficacy on interstitial lung disease (ILD) in a 24-month prospective open-label study in 9 patients with SSc ineligible for cyclophosphamide therapy, thus not supporting their use in these patients (80).

Targeting pulmonary arterial hypertension and digital vasculopathy

PAH is a severe SSc complication and its management has significantly evolved over the last two decades with the introduction of agents that target different mechanisms in the pathogenic process: prostaglandin analogues, endothelin receptor antagonists (ERA) and Phosphodiesterase-5 (PDE-5) inhibitors which demonstrated efficacy in improving dyspnoea scores, subjective and objective measures of function, haemodynamic parameters and quality of life. Interestingly, a recent systematic review including 26 observational studies and 6 randomised

trials and evaluating the effect of any dual combination of ERA, PDE-5 inhibitors or prostaglandin analogues on a 6-min walk distance, functional class, haemodynamics, quality-of-life or time-to-clinical worsening in idiopathic PAH or SSc-PAH, has shown a beneficial effect of dual therapy, especially in patients with rapid deterioration during monotherapy (81).

There is a growing body of evidence supporting the notion that early recognition and treatment of PAH is of crucial importance in SSc and that a haemodynamic evaluation during exercise, which may allow for earlier diagnosis and initiation of appropriate therapy (82), actually represents a meaningful endpoint in clinical trials. Indeed, exercise-induced pulmonary hypertension (ePH) is considered an early, clinically relevant phase in the spectrum of SSc-related pulmonary vascular disease. A pilot open label study evaluated the effect of 24 weeks treatment with ambrisentan; interestingly the authors found a clinically meaningful improvement in exercise haemodynamics and exercise capacity in patients with SSc-associated ePH (83).

Similarly, in a pilot study on 10 SSc patients a 6-month treatment with bosentan demonstrated tolerability and beneficial effect on patients with borderline pulmonary arterial hypertension during the treatment period with respect to the observational period during which a significant deterioration in resting and exercise PAH was conserved (84).

The importance of an early treatment of PAH in SSc patients was also supported by the results of the SUPER (Sildenafil Use in Pulmonary Arterial Hypertension)-2 extension open-label trial which enrolled WHO-functional status II or III; the study demonstrated that the majority of patients (60%) improved or maintained their functional status and 6-minute walking distance over the 3-year study period (85).

Similarly, the impact of early therapeutic management was clearly demonstrated in the EARLY (Endothelial Antagonist Trial in Mildly Symptomatic Pulmonary Arterial Hypertension Patients) study and in their open-label extension study. The EARLY study showed that PAH patients in WHO-FC II rapidly deteriorated without treatment and suggested that drugs such as bosentan would delay disease progression. Indeed, by the end of the 4 years of the follow-up, WHO-FC was maintained in the majority of patients: 17.1% improved from WHO-FC II to WHO-FC I, 56.3% were maintained at WHO-FC II and 20.9% worsened to WHO-FC III/IV. Additionally, 6-minute walking distance remained stable in most patients throughout the duration of the study (86).

Among the new treatment strategies in PAH, ivabradine represents a promising option in SSc patients (87).

In 2011, the results of the RAPIDS-2 trial (RAndomised, doubleblind, Placebo-controlled study with bosentan on healing and prevention of ischaemic digital ulcers in patients with systemic sclerosis) have been published. The primary objectives were to evaluate the effect of bosentan on the reduction of new digital ulcers (DUs) and healing of DUs in patients with SSc over a period of 24 months. The study confirmed that bosentan treatment is associated with a 30% reduction in the number of new DUs while no difference between treatments in healing rate was observed (88).

A multicentre, non-interventional retrospective cohort study confirmed the safety and efficacy of bosentan therapy in SSc patients and DUs also in real life setting even after long-term follow-up (89).

Moreover, oral sildenafil has demonstrated to be an alternative option for microvascular involvement in SSc patients, as shown in patients with Raynaud's phenomenon and DUs secondary to SSc (90, 91).

Targeting the inflammatory/ autoimmune process

In 2011-2012 increasing attention has been directed to biological drugs in SSc with uncertain results; in particular, TNF inhibitors appear to be effective in the treatment of SSc-associated inflammatory arthritis in a small study on 10 patients, with no changes in skin or lung involvement, but a high frequency of malignancy is reported (92). Similarly, in a EUSTAR expert consensus report, the experience of 79 EUSTAR centres on anti-TNF-alpha use in SSc have been investigated with a Delphi approach; despite an extensive use is not recommended due to the absence of controlled studies in selected patients with severe arthritis, a treatment with anti-TNF-alpha may be considered (93).

The same conclusions were reached by a systematic literature review evaluating 5 studies on anti-TNF-alpha in SSc patients; indeed, literature data demonstrated efficacy in controlling joint symptoms and quality of life, while cutaneous effects and long-term safety data resulted still uncertain (94).

In the same paper, 6 reports on rituximab in a total of 40 SSc patients have been also included with no evidence of beneficial effect on skin or lung involvement. On the other hand, in the same year, promising results on treatment with rituximab have also been reported: in an uncontrolled study on 8 patients with SSc and interstitial lung disease, a linear improvement of lung function and skin thickening over 2 years of treatment was observed compared to baseline evaluations (95).

In 2011, the results of the second phase open–label randomised trial on autologous non-myeloablative haemopoietic stem-cell transplantation (ASSIST) trial have been reported. In comparison with pulse cyclophosphamide, 10 patients treated with HSCT showed sustained improvement in skin thickening and pulmonary function for up to 2 years (96). Encouraging results have also been reported in an open-label uncontrolled study in 26 SSc patients with severe organ manifestations; the authors observed a significant skin and lung function improvement and a progression-free survival of 74%, despite a transplant-related mortality of 4% and a treatment-related mortality of 11% (97).

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