Systemic sclerosis: markers and targeted treatments

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ABSTRACT

Systemic sclerosis (SSc) is characterized by autoantibody production, progressive microvasculopathy, and aberrant extracellular matrix protein (ECM) synthesis in tissues. The disease presents two major clinical hallmarks: Raynaud's phenomenon (RP) and skin involvement, followed by varying prevalences of internal organ involvement. Despite significant advances in the management of certain organ-specific involvements and symptoms, the research for efficient markers and targets, to be used for an optimized treatment, is still ongoing. Therapies targeting the vasculature (i.e. ET-1 receptor antagonists, phosphodiesterase-5 (PDE-5) inhibitor, angiotensin-converting enzyme inhibition, prostacyclins), the immune system and/or the fibrotic process (i.e. traditional disease modifying anti-rheumatic drugs DMARDs such as methotrexate, cyclosporine or mycophenolate mofetil, biologicals like rituximab, tocilizumab or abatacept) have been or are being evaluated in SSc. Advanced approaches, reserved to unresponsive SSc patients, include autologous haematopoietic stem cell transplantation (HSTC) and intravenous immunoglobulins (IVIG). Interestingly, it is expected that new and future possible diagnostic and therapeutical approaches in SSc will come from epigenetic studies (MicroRNAs).

Ideally, combination therapy in SSc seems the best approach, together with the early intervention on the major hallmarks of the disease in "at risk" patients, that consists of the microvascular damage/altered function and the autoimmune reaction, followed by the progressive and systemic fibrotic process.

Keywords: Systemic sclerosis; Capillaroscopy; Raynaud phenomenon; Targeted therapies; Connective tissue diseases; Autoimmune diseases.

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INTRODUCTION

Systemic sclerosis (SSc) is a connective tissue disease (CTD) with autoimmune reactivity, chronic vascular and tissue stromal progressive alterations. These processes lead to autoantibody production, progressive microvasculopathy, and aberrant extracellular matrix protein (ECM) deposition with subsequent severe organ damage in skin, lungs, gastrointestinal tract and several other internal organs.

Research is still ongoing to define the initiating cause of the pathophysiological cascade and despite significant advances in the management of certain organ-specific involvements and symptoms, SSc remains the CTD with the higher morbidity and mortality[1,2].

Systemic sclerosis is an orphan disease with an annual incidence of 19 per million and prevalence of 19--75 per 100,000. The female:male ratio is 3:1, and 8:1 in mid to late childbearing years. Interestigly, juvenile SSc (children < 16 years old) is even more rare and one of the worst rheumatological conditions in children[3].

Based on the argument that the past SSc classification criteria did not meet current standards for clinimetric properties, recently, new classification criteria based on a collaboration between the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) have been introduced[4].

The sensitivity and specificity of the classification criteria have been significantly improved after this revision, and by introducing the analysis of the microvascular damage as detected by the presence of teleangectasia and capillarscopic features.

Additionally, to overcome the issue of properly classifying subjects with subclinical disease, Very Early Disease Onset Systemic Sclerosis (VEDOSS) criteria have been proposed[5].

SYSTEMIC SCLEROSIS HALLMARKS

Systemic sclerosis is clinically characterised by two ma-

jor hallmarks: Raynaud's phenomenon (RP) and skin involvement, followed by varying prevalences of internal organ involvement.

Based on the extension on skin involvement patients with SSc can be classified into limited cutaneous (lcSSc) or diffuse cutaneous (dcSSc) involvement. Patients with dcSSc with severe organ involvement may have the worst prognosis.

The vast majority of patients with SSc have RP (bi or tri-phasic colour changes of extremities upon exposure to cold or stress)[6]. It is noteworthy that the RP may occur in a healthy population (without any association with CTD) or in secondary Raynaud's phenomenon (SRP) (mostly due SSc). The key point as a clinical medical doctor or specialist is to be able to make this differential diagnosis. The combination of capillaroscopy and serology allows to do so.

Moreover, this combination also allows to pinpoint those patients who only have RP at baseline, without any sign of another CTD, who will progress to a secondary over long time follow up (13.6% of patients with isolated RP at baseline)[7].

Next to RP and skin involvement, other clinical complications are frequent in SSc.

Digital ulcers (DU) and gastrointestinal involvement occur frequently. DU occur in a major part of SSc patients in their disease course. They are associated with an important morbidity (pain, reduced quality of life, disability and disfigurement) and may evolve into tissue necrosis and amputation[8].

Gastrointestinal involvement is frequent in scleroderma (almost 90%) and in 10% of cases is the presenting feature of the disease[9]. Of note, several patients are asymptomatic, ranging from 20% for small bowel to half for esophageal involvement. Most frequent gastrointestinal organs affected in SSc are the oesophagus, followed by the anorectum and the small bowel, whereas severe gastrointestinal involvement luckily affects only 8% of the patients, but is associated with a high mortality, with a 9 year survival of only 15%. Also, the risk of malnutrition is high in SSc, with over 28% of patients being at medium or high risk of malnutrition.

Other complications are less frequent but linked with high mortality in SSc and should therefore be screened for. Among those pulmonary arterial hypertension (PAH), severe lung fibrosis, myocardial involvement and scleroderma renal crisis are associated with high mortality[10].

Screening for these afflictions is recommended. In this way screening for PAH is standardly incorporated into daily SSc management and screening according to highly performant the DETECT algorythm which encompasses echocardiography, pulmonary functional tests and optionally BNP or NT-proBNP.

Even though, at present, no therapies have attested to be able to stop the natural progression of the disease, still there are organ specific effective treatments. Both for PAH and digital trophic lesions therapies have attested treatment effect through randomised controlled trials.

Therapies targeting the vasculature (i.e. ET-1 receptor antagonists, phosphodiester-ase-5 (PDE-5) inhibitor, ACE inhibition, prostacyclins), the immune system and/or the fibrotic process (i.e. traditional DMARDs such as methotrexate, cyclosporine or mycophenolate mofetil, biologicals like rituximab or tocilizumab and abatacept) have been or are being evaluated in SSc.

Rather than waiting for the clinical overt disease to set in, eyes are geared to diagnose the disease "early" or "very early", with the aim to futurely be able to treat selected SSc patients before the clinical complications occur.

EARLY MARKERS OF SYSTEMIC SCLEROSIS

Key pathways and mediators involved in the SSc pathophysiology can be identified within the skin and blood vessels, and several new molecules are now being examined in early stage clinical trials.

However, early clinical symptoms (i.e. RP) and biomarkers (i.e. serum autoantobodies) seem to represent the best "early signals" of possible SSc to be considered.

Among them, RP may be present in 5% of healthy subjects, with a higher prevalence for females, whereas approximately 90% of SSc patients and 85% of mixed connective tissue disease (MCTD) patients, present RP as an early symptom.

As matter of fact, the best system for the early differential diagnosis between primary and secondary RP (PRP, SRP) and prediction of organ involvement in SSc is considered the nailfold videocapillaroscopy (NVC)[11-14]. Of course, the presence of defined and validated criteria for the definition of NVC SSc pattern should be considered in order to better distiguish specific from non-specific microvascular abnormatities.

Therefore, several authors stated that NVC abnormalities could be observed in healthy individuals as

well as in primary RP (PRP) subjects[15,16].

Indeed, NVC abnormalities in a subject with isolated RP are known to predict for evolution to a CTD within 2 years[17]. A prospective study on 3029 consecutive patients with RP, showed predictivity of scleroderma type capillary changes during follow-up of patients transitioned to scleroderma spectrum diseases[18].

A further prospective study on 412 patients followed up for 5 years, individuated the percentage of patients initially diagnosed with PRP and later transitioned to SRP associated to SSc[19]. The study offereded a hint at the increased probability of transition in RP patients into SRP presenting at baseline observation with capillary dilations and slight reduction of capillary number compared to those which did not present such alterations[19].

In a more recent study, a significant increased baseline frequency of average, arterial and venous capillary dilations, was found in SRP patients, and a threshold value with high negative predictive value for reassurance of patients that probably are not going to develop SSc[20]. Indeed, the very early diameter threshold value (over 30 μm) here observed, might represent the structural alteration preceding the uniform capillary loop dilation, that underlies the formation of the giant capillaries (over 50 μm), pathognomonic for the "Early" SSc pattern[21].

Together with the specific NVC alterations, specific autoantibodies (ANA, ACA and TOPO-I) seem to play an important role in the pathophysiology of SSc and in SRP patient they seem to represent another reliable early biomarker to monitor the evolution towards SSc[21]. More specifically, the combined presence of SSc-specific NVC patterns together with the presence of SSc--specific autoantibodies (anti-centromere, anti-topoisomerase I as well as anti-Th/To and anti-RNA polymerase III), in a patient group with isolated RP and no other sign of CTD, has a positive predictive value of 79% to predict which patients will develop SSc over time[22]. This study, as first, prospectively attested that the combination of a SSc pattern on capillaroscopy, in combination with an SSc-specific antibody, is highly predictive of a patient prone to develop SSc. On the other hand, the combination of a normal capillaroscopy together with the absence of one of the SSc-specific antibodies has a negative predictive value of 93%.

More recently, non-specific antibodies against the angiotensin receptor type-1 (AT1R) together with anti--endothelin-1 (ET) receptor type-1 (ETAR) autoantibodies have been studied in patients with SSc and about 85% of patients fulfilling the former ACR criteria showed autoantibodies against AT1R and ETAR[23,24].

The antibodies are agonistic for endothelin-1 (ET1) and therefore exhibit functional properties, which may contribute to SSc pathogenesis[25,26]. Both antibody levels seem to correlate with each other suggesting a strong interrelationship, which is possible since both the angiotensin as well as the endothelin system mediate similar effects[23].

In addition, high levels of anti-AT1R/ETAR antibodies were found to be associated with vascular and fibrotic SSc complications[23]. In particular, a recent study showed their capacity to predict the development of PAH and of PAH-related mortality and, as consequence, failure of response to PAH therapy[26]. Anti--AT1R antibodies below 15.8 units and anti-ETAR antibodies below 18.3 units revealed a negative predictive value of 76.9% and 77.1%, respectively.

TARGETED THERAPIES IN SYSTEMIC SCLEROSIS

Therefore, the early detection of microvascular damage (NVC pattern) together with RP and autoimmune biomarkers (autoantibodies) might be used to stratify patients for the risk of SSc development and subsequently start early-targeted therapies[27,28].

For example, combination treatment with immunosuppressive and vasoactive drugs, should represent an important early intervention with possible disease modifying activity[29,30]. Key question here is when to start early intervention. In order to be able to reply to this question indices are needed that evaluate which patients with early (scleroderma pattern and SSc-specific antibody) or very early (VEDOSS criteria) will develop a severe disease course[5].

Additionally, the concept of targeted therapy for SSc include at least two different approaches[31]. One approach to targeted therapy in SSc is the possible early treatment of individual disease mechanisms such as immune activation/inflammation, vascular disease or fibrosis. The other classical approach is to targeting specific organ based complications in overt disease, such pulmonary arterial hypertension, or specific symptoms such as RP, gastrooesophageal reflux or scleroderma renal crisis.

Interestinlgly, final result of the local SSc immune-inflammatory process is the activation of a sort of never ending "wound healing" mechanism (tissue repair),

that is finally characterized by the recruitment of circulating fibrocytes, resident fibroblasts and their precursors (pericytes) and activation into myofibroblasts, with high production of extra ECM proteins[32,33].

Furthermore, microvascular alterations and reduced capillary density, reduce blood flow, impair tissue oxygenation and the generated tissue hypoxia, further enhances the production of ECM proteins by SSc fibroblasts[34,35].

In relation to the "tissue repair", the endothelial-to-mesenchymal cell transition (EndoMT) process has been recently recognized a strongly implicated process and a potentially target for the early diseases modifying intervention in SSc[36].

In fact, the endothelial/microvascular injury and the myofibroblast activation, are crucial events that seem to contribute to the development of fibrosis in CTD such as SSc, and it seems mainly related to the local increased production and influence of several growth factor molecules including TGF-β and ET-1[33,37].

Interestingly, several fibrotizing conditions seem to involve the EndoMT process together with high ET-1 local concentrations, such as the cardiac fibrosis in diabetic hearts, the idiopathic pulmonary fibrosis or the scleroderma renal crisis in both glomeruli and arteriolar lesions[38-40].

Treatments targeting in SSc potential mediators of fibrosis and potential pathways (TGF-β, ET-1, VEGF, PDGF, FGF) have been tested or are under evaluation in clinical trials, with different results (Table I).

Very recently a further clinical trial has been described that blocks TGFbeta more effectively then earlier temptatives, and although this was an open label study, it does suggest that the skin score may be im-

TABLE I. POSSIBLE TARGETED THERAPEUTIC APPROACHES IN SYSTEMIC SCLEROSIS *(INCLUDING AGENTS UNDER EVALUATION)*

Immune-inflammatory target	**Agent to the target**
CD-20	Rituximab
TNF-α	Adalimumab, infliximab, etanercept
CD80/CD86	Abatacept
IL-6	Tocilizumab
IL-2Rα	Basiliximab
IL-1	Rilonacept
BLYS	Belimumab
LFS1/ICAM1	Efalizumab
CCR2	MLM-1202
αMSH, IL-10, CCL2	AIMSPRO (HCS)
LPA1	ACT12339
Ig	Immunoglobulin IV
Vascular target	**Agent to the target**
ETA/ETB receptor	Bosentan, Macitentan
ETA receptor	Ambrisentan, Zibotentan
cGMP agonist	Riociguat
IP receptor agonist	Selexipag
Pro-fibrotic molecule target	**Agent to the target**
c-Abl, c-Kit, PDGF	Imatinib, Dasatinib, Nilotinib
VEGF - PDGF - FGF	Nintedanib
TGFβ	Anti-αvβ6 integrin
TGFβ1	CAT-192, P144
TGFβ1, β2, β3	GC-1008
CCN2	FG-3019
TNF-α - L-1β - TGF-β	Pirfenidone

proved and that gene expression signatures may be attenuated with this approach[41]. The durability and clinical meaning of this therapeutic strategy remains to be determined in future studies, but this recently published clinical trial provides strong support to the rationale for targeting TGF-β in SSc, especially for skin disease[42].

In this perspective of targeted therapy in SSc, two very recent clinical studies have shown that long-term treatment with ET-1 receptor antagonist modifies the progression and morphology of the nailfold microvascular damage, as assessed by NVC, in patients with SSc, and respectively affected by pulmonary arterial hypertension and DU (in combination with iloprost), over a 1-3 year follow-up period[43,44].

Interestingly, both studies showed, at the nailfold capillaroscopy, at least after 1 year, a significant increased number of capillaries with reduced avascularity and increased neoangiogenesis, suggesting the possible achievement of a miscrovascular/tissue de-remodelling in SSc. The effects might be related to interferences exerted by ET-1 receptor antagonists on the already mentioned process of EndoMT and on the TGF-β activities in SSc[45-47].

An efficient system to target the autoantibody producing B cells in SSc is emerging from the successfull use of rituximab (RTX) in treatment of SSc patients[48].

As matter of fact, a recent clinical open-study by depleting B cells with RTX on a 2-treatment course (months 0/6), was found to be well tolerated and to have potential efficacy for skin disease and stabilization of internal organ status in early dcSSc[49]. Moreover, stabilisation of microcirculation (number of capillaries) has also been attested by this regimen[50].

In a more recent study, the comparison of RTX treated SSc patients versus untreated matched-controls showed improvement of skin fibrosis and prevention of worsening lung fibrosis, and further supports the therapeutic concept of B cell depletion in order to reduce autoantibody production/effects in SSc patients[51,52].

ADVANCED AND FUTURE THERAPIES FOR SYSTEMIC SCLEROSIS

A possible therapeutic option to be considered in SSc patients who are refractory to conventional treatments is the autologous haematopoietic stem cell transplantation (HSTC)[53].

The mechanisms responsible for the benefits of HSTC are not already fully understood, and possibly it induces a re-establishment of immunological tolerance together with non-specific immunosuppressive activities. However, HSTC seems to cause an improvement of vasculopahy, modified Rodnan skin thickness score (MRSS) and lung function, in patients with dcSSc and mild-moderate internal organ involvement (maximum disease duration of 4–5 years) or dcSSc patients with progressive internal organ involvement[54].

However, despite the potential benefits, HSTC is, dangerous therapeutic option with a high risk of death and a higher morbidity rate and new trials try to optimize this approach especially in dcSSc[55].

A further therapeutic option in SSc patients resistant to conventional therapies, seems be represented by intravenous immunoglobulins (IVIG), but their mechanisms of action remain unclear[56]. Recent clinical data about the effects of IVIG on SSc patients report that this approach may improve several clinical manifestations such as skin, joint and lung involvement although optimal dosages and timing of administration are not yet defined. A double- blind, randomised, placebo-controlled study is in progress to assess the safety and efficacy of IVIG in scleroderma patients[57].

Interestingly, new and future possible diagnostic and therapeutical approaches in SSc will come from epigenetic studies[58].

In fact, the interest about microRNAs (miRNAs) that are non-coding RNAs regulating a large variety of biological functions in plants and animals, is growing[59]. Each miRNA expressed in a cell may target about 100 to 200 messenger RNAs that it downregulates. It appears that about 60% of human protein coding genes are regulated by miRNAs, whilst many miRNAs are epigenetically regulated.

There is currently good evidence for the role of miRNAs in fibrotic diseases, either organ-specific or systemic fibrosis, such as in SSc[60]. Whereas the exact targets of these miRNAs are unknown, for some of them it is know, since they are regulating key downstream pathways in pathogenesis of diseases such as miRNA--29, which is a key mediator of fibrosis[61]. Gene therapy with the restoration of miRNA-29, at least in an animal model of fibrosis, appears to reduce fibrosis[62].

Therefore, pharma companies are searching for miRNA technologies for modulating various fibrotic conditions and recent data are emerging on the role of increasing miRNAs *in vivo*, especially miRNA-29a, to restore its function and thus suppress fibrosis without

the need for viral vectors[63].

As matter of fact, in complex diseases such as SSc, it may be that the clinical course of the disease is influenced by the expression of miRNAs, which themselves are epigenetically altered by body environmenal factors (i.e. estrogens).

CONCLUSIONS

The fast and progressive increase in knowledge about the mechanisms underlying the SSc pathogenesis should offer new possible targets for therapeutical approaches adressed to an efficient and early disease-modifying strategy (Table I).

Combination therapy in SSc seems the best approach, as well as the early intervention on the major hallmarks of the disease in "at risk" patients that consists of the microvascular damage/altered function and the autoimmune reaction, followed by the progressive and systemic fibrotic process.

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