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Abstract

Although being classified as autoimmune connective tissue disease, dominant components of the pathophysiology of systemic sclerosis (SSc) consists of mechanisms of vascular damage, which can occur early in the course of the disease. Amongst them are abnormal vasoreactivity, hypoxia, insufficient neoangiogenesis and direct damage of vascular and perivascular cells. They result in a decreased capillary blood flow, and subsequently in clinically overt symptoms such as Raynaud's syndrome and fingertip ulcers. In addition, in active disease vascular pathology can affect various other organs, predominantly the lung, the kidney, the heart but also the gastrointestinal tract. Vascular pathology contributes also significantly to overall morbidity and mortality in SSc patients and reduces life expectancy by at least a decade. Fortunately, molecular biology has revealed a number of underlying pathways on the cellular and subcellular levels, including key factors of the aberrant function of (peri)vascular cells and autoimmune effector cells, the dysregulation of vasoconstrictive molecules and their receptors, the upregulation of intracellular signaling kinases and the altered balance of hypoxia-induced vascular growth factors. This increasing knowledge of vascular pathology in SSc has also resulted in novel therapeutic approaches ranging from endothelin antagonists to application of progenitor cells to counteract this aberrant vascular pathology and to support the repair of the dysfunctional vasculature.
Mechanisms of Vascular Damage in Systemic Sclerosis

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Running Title
Vascular damage in SSc
Key words
Systemic sclerosis, vasculature, endothelin, fibrosis, molecular, angiogenesis
Abstract

Although being classified as autoimmune connective tissue disease, dominant components of the pathophysiology of systemic sclerosis (SSc) consist of mechanisms of vascular damage, which can occur early in the course of the disease. Amongst them are abnormal vasoreactivity, hypoxia, insufficient neoangiogenesis and direct damage of vascular and perivascular cells. They result in a decreased capillary blood flow, and subsequently in clinically overt symptoms such as Raynauds syndrome and finger tip ulcers. In addition, in active disease vascular pathology can affect various other organs, predominantly the lung, the kidney, the heart but also the gastrointestinal tract. Vascular pathology contributes also significantly to overall morbidity and mortality in SSc patients and reduces life expectancy by at least a decade. Fortunately, molecular biology has revealed a number of underlying pathways on the cellular and subcellular level including key factors of the aberrant function of (peri)vascular cells and autoimmune effector cells, the dysregulation of vasoconstrictive molecules and their receptors, the upregulation of intracellular signaling kinases and the altered balance of hypoxia-induced vascular growth factors. This increasing knowledge of vascular pathology in SSc has also resulted in novel therapeutic approaches ranging from endothelin antagonists to application of progenitor cells to counteract this aberrant vascular pathology and to support the repair of the dysfunctional vasculature.
Introduction

One of the early hallmarks of SSc is Raynaud`s phenomenon and the reduced density of capillaries in the skin and their unique phenotype, especially the so-called megacapillaries. These phenomena indicate both an insufficient vasodilative capacity to vasoconstrictive stimuli, e.g. cold, hypoxia or vasoconstrictive molecules such as endothelin-1, as well as a dysfunction in angiogenesis and vascular repair. This dysregulation of angiogenesis is occurring early in the course of the disease (1,2) and is paralleled by other pathogenic processes including fibrosis as well as cellular and humoral autoimmunity, which amplifies this dysregulated system in a vicious circle. Some of these mechanisms are reflected by biomarkers, although they do not yet allow an exact prognosis (3).

Of note, although not yet being examined in detail, it is most likely that this overt vascular pathology is also taking place in other organs and compartments of the affected patient. In the era of high-resolution computed tomography, these subtle changes can already been observed in early stages of the disease. Therefore, it is of utmost interest to add more pieces to this puzzle to facilitate the development of improved therapeutic regimen, which will not only have an impact on vascular pathology but also on overall outcome of the SSc patient.
Clinical implications of vascular damage

In the largest recent clinical data collection of SSc patients (4,5), various aspects illustrating the clinical impact of SSc vascular disease could be revealed. Both patients with diffuse (d)SSc and limited (l)SSc had an identical mean age of onset of about 43 years of the primary sign of vasculopathy, Raynaud’s phenomenon. However, the age at the onset of first non-Raynaud’s manifestation differed between dSSc and lSSc, being 45 years in dSSc and 48 years in lSSc patients. There was also a longer lag period between the onset of Raynaud’s phenomenon and the next non-Raynaud’s clinical feature of disease in the lSSc (about 5 years) in contrast to the dSSc (about 2 years) patients. In both subsets of SSc, patients with an earlier onset of Raynaud’s phenomenon had more often digital ulcers than those with a late onset. The frequency of pulmonary vascular disease, specifically pulmonary hypertension (PH) was similar in the two subsets, i.e. in 22 % of the dSSc patients and in 21% of the lSSc patients. Pulmonary arterial hypertension (PAH, PH in the absence of lung fibrosis) was found in 26% of dSSc, and interestingly in 45% of lSSc PH patients, indicating a somewhat different pathophysiology in the latter.

However, right heart catheter, which is the gold standard to measure the actual pressure indicates a prevalence of lower than 10% of the population. Patients with an early onset had significantly less pulmonary fibrosis, pulmonary hypertension, diastolic dysfunction and arterial hypertension. Objective cardiac complications (conduction block, diastolic dysfunction and left ventricular
ejection failure) were reported with a similar frequency (20-30%) among the two scleroderma subsets, and renal vascular complications (hypertensive renal crisis and proteinuria) were more frequent in the dSSc subset.

**Genetic and epigenetic factors in vascular pathology**

Although not definitively proven by large scale analysis, there are numerous indications that genetic abnormalities within the gene sequence of key molecules contribute to vascular pathology in SSc. For example, mutational analysis on SNPs (single nucleotide polymorphisms) of the hypoxia-induced factor (HIF)1A gene has been performed in a cohort of patients including 659 Caucasian SSc patients and 511 healthy matched controls. Three SNPs (rs12434438 A/G, rs1957757 C/T and rs11549465 C/T) were genotyped and the results suggest an association of the HIF1A gene with SSc. However, the functional tests showed also that rs12434438 is not a functional SNP but it could be in disequilibrium linkage with another variant which carries the susceptibility to SSc (6). Polymorphisms of genes which regulate endothelial cell plasticity and functions have been previously shown to be associated with the main vascular manifestations of SSc, such as PAH and ischaemic ulcers (7,8).

In addition, similar to other rheumatic diseases, epigenetic modifications most likely contribute to the alteration in the posttranslational process of vasoactive molecules operative in the pathogenesis of SSc. The term epigenetics refers to
chromatin-based pathways important in the regulation of gene expression and includes three different but interfering mechanisms: DNA methylation, histone alteration, and RNA-dependent modifications. For example, in vascular pathophysiology, endothelial nitric oxide synthase has been attributed to specific DNA methylation and histone posttranslational modifications. Other groups have examined chromatin-based mechanisms in endothelium-restricted genes including von Willebrandt factor, Notch4 and EPHB4 (9). More recently, it could be shown that expression of methylation-related factors such as Dnmt1 are involved in the development of a distinct scleroderma phenotype (10).

In SSc, epigenetic mechanisms can modulate fibrosis. When examining the effects of DNA methyltransferase and histone deacetylase inhibitors on collagen expression and the level of epigenetic mediators in fibroblasts, it could be shown that the methylation status of the FLI1 promoter determined the collagen production. Vice versa, the addition of epigenetic inhibitors to cell cultures normalized collagen expression. In these experiments, collagen synthesis was specifically linked to the epigenetic repression and the methylation status of the collagen suppressor gene FLI1 and its promoter (11). At the past annual meeting of the American College of Rheumatology, the same group presented an additional epigenetic repression in the bone morphogenetic protein II in SSc endothelial cells (EC), which resulted in an increased sensitivity of the EC to apoptotic signals. In the affected cells, DNA methylation and the histone deacetylation was found to be the underlying cause for this epigenetic modification. When examining the profibrotic pathways in SSc fibroblasts
Histone deacetylase 7 (HDAC7) repression appears also to be involved in epigenetic modifications. It could also be shown that, in contrast to the negative effects of trichostatin A on the progression of SSc via up-regulation of CTGF/CCN2 and ICAM-1, silencing of HDAC7 had no influence on the expression of these genes, which resulted in the idea of HDAC7 being a target for epigenetic modulation in SSc patients (12). Of note, trichostatin A prevents the accumulation of extracellular matrix in a mouse model of bleomycin-induced skin fibrosis (13)

**Mechanisms of dysfunctional angio- and vasculogenesis**

As illustrated in Figure 1, the process of new blood vessel formation, angiogenesis, requires the adequate balance between endogenous stimulators and inhibitors, which induce and inhibit blood vessel growth. Angiogenesis consists of a sequence of evolutionary highly regulated events. In healthy individuals, proangiogenic stimuli activate EC, which release proteolytic enzymes that degrade the basement membrane and the perivascular extracellular matrix. Thereafter, EC proliferate and migrate into the perivascular area. After forming primary small new vessels, the following lumenation of these primary sprouts results in capillary loops, followed by synthesis of a new basement membrane and subsequently in blood vessel maturation. In SSc, a number of these sequential steps have revealed to been altered and contribute to its pathophysiology (14-16). In addition, the expression of the angiogenic
inhibitor endostatin has been suggested to be associated with the presence of
giant capillaries in nailfold capillaroscopy.

Although persisting hypoxia, should be a major stimulus for angiogenesis,
sufficient angiogenesis does not occur in SSc patients despite severe tissue
hypoxia (15,16). Most likely, this is based on an insufficient response to the
elevated levels of VEGF in serum and tissue. VEGF controls several steps of
angiogenesis, increases the vascular permeability, stimulates the migration and
proliferation of ECs and induces tube formation (17). Serum levels of VEGF
correlate also with the development of fingertip ulcers. However, data indicate
that VEGF still can exert protective effects in SSc patients, if the levels of VEGF
exceed an individual threshold. A reason for the ineffectiveness of VEGF could
be that the time being close to the active angiogenic compartment is too short,
resulting in instable vessels. Vice versa, an extended exposure to VEGF may
have also negative effects by fusing by immature microvessels in an
uncontrolled manner resulting in a chaotic vessel network with giant capillaries.

Moreover, in experimental settings, isolated microvascular EC from SSc
patients show an impaired response to VEGF and other growth factors (18,19).
Moreover, the CXC chemokine stromal cell-derived factor 1 (SDF-1/CXCL12)
and its receptor CXCR4 regulate specific steps in new vessel formation. It was
recently demonstrated an altered expression of both SDF-1 and CXCR4 in skin
and microvascular ECs from patients with SSc, suggesting an involvement of
the SDF-1/CXCR4 axis in the pathogenesis of microvascular abnormalities in this disease (20).

With respect to vascular matrix pathophysiology, experimental data support the idea that the impaired ability to form capillaries in the matrigel assay might be in part caused by a matrix metalloproteinase 12 (MMP-12)-mediated cleavage of the urokinase-type plasminogen activator receptor (uPAR) isolated microvascular ECs from dSSc patients (21), especially as this urokinase-type plasminogen activator (uPa) – uPAR system modulates extracellular matrix degradation and the adhesion of EC to the extracellular matrix during angiogenesis. Furthermore, cleaved uPAR in SSc microvascular ECs results in loss of an integrin-mediated uPAR connection with the actin cytoskeleton which accounts for loss of motility and angiogenesis of these cells (22).

In contrast to angiogenesis, vasculogenesis includes the formation of new vessels by circulating EPCs, which can occur without preexisting vessels. The process is initiated by a release of EPCs from the bone marrow. Initial triggers are cytokines and angiogenic growth factors, including again VEGF, but also other growth factors such as GM-CSF. Besides insufficient angiogenesis, defective vasculogenesis with altered numbers and functional defects of EPCs might also contribute to the vascular pathogenesis of SSc. The initial study suggested a profound decrease of circulating EPCs, whereas subsequent studies found increased numbers of EPCs in patients with SSc (14,23). Scleroderma serum-induced EPC apoptosis was demonstrated in a recent
study, and this may account, at least in part, for the decreased circulating EPC levels in scleroderma patients (24).

Furthermore, bone marrow–derived mesenchymal stem cells (MSCs) from SSc patients have been examined to determine whether endothelial differentiation of these cells is altered following vascular damage, which may explain the impaired vascular repair processes (25), and recent data indicate that transcriptional regulation of Bim by FOXO3a and Akt mediates scleroderma serum-induced apoptosis in endothelial progenitor cells (24).

Vasoactive Molecules

Amongst several others, specifically endothelin-1 (ET-1) plays a significant role in SSc vascular pathology. Endothelin has been shown to be an effector downstream molecule for several growth factors known to be involved in vascular and fibrotic diseases, e.g. transforming growth factor-β (TGF-β). TGF-β is not only able to induce the expression numerous profibrotic genes, including type I collagen, fibronectin, and CTGF/CCN2, but also to contract a collagen gel matrix, all these pathways depended strongly on ET-1 (26, 27).

Three isoforms of endothelin, ET-1, ET-2 and ET-3, have been identified so far. Their vasoconstrictive effect is more than 100-fold stronger than that of noradrenalin. ET-1 is synthesized by ECs as pre-pro-ET-1. This inactive precursor is cleaved in two steps, first into pre-ET-1 and then into active
endothelin. It triggers vasoconstriction, upregulation of VEGF as well as vascular remodelling in combination with other cytokines and growth factors, e.g. PDGF. ET-1 binds to the endothelin receptor type B (ETb), which, among other effects, also regulates ET-1 synthesis. The primary vasoconstrictive effect of ET-1 is mediated by the endothelin receptor type A (ETA) which is expressed on smooth muscle cells of the vascular wall. Therefore, its effects are primarily local, and only small amounts are released into the blood stream. From a therapeutic point of view, the interaction of ET-1 and its receptor ETA represents an attractive target for pharmacologic interventions in SSc (28).

Of note, ET-1 can also exert some proangiogenic effects – even independent of inflammation - as shown in the rat corneal bioassay (29), and the matrigel plug model (30). In combination with VEGF, ET-1 promotes capillary growth and activates the hypoxia response pathway, which also leads to VEGF expression and angiogenesis. Additionally, ET-1 may trigger angiogenesis by stimulating the production of nitric oxide and matrix metalloproteinase (MMP)-2. However, these potentially beneficial effects have not shown to be operative in SSc in an adequate manner and do not appear to outweigh its intensive vasoconstrictive effects (31).
Direct vasotoxic effects of the immune system – Pathogenic autoantibodies

The „traditional“ role of autoantibodies in the pathophysiology of autoimmune diseases is the aberrant cellular damage or complement activation by molecular mimicry. On the other hand, an increasing body of data indicates that autoantibodies can also play an active functional role in pathophysiology (32). One of the most important examples in SSc is the presence of agonistic profibrotic autoantibodies against the PDGF receptor (33), which not only appears to be relevant for matrix formation but also for vascular pathology. The direct stimulation with anti-PDGF antibodies results – similar to hypoxic effects – in a tyrosine phosphorylation and an upregulation of the Ha-Ras-ERK1/2 pathway and vasculotoxic reactive oxygen species. In addition, there are indications that the number of stimulatory autoantibodies in SSc is even larger as expected as it could be shown that not only anti-fibroblast antibodies exist that upregulate profibrotic chemokines but also antibodies directly involved in vascular pathology such as angiotensin II-receptor type 1 and the endothelin receptor type A, which contribute to pulmonary vascular pathology via intracellular ERK activation (34-36).

Direct effects on vascular cells – endothelial cell apoptosis

The attempt to support angiogenesis by enhanced -but still deficient- VEGF synthesis is paralleled by the finding of an increased activation but also
apoptosis of EC, which is illustrated on a molecular level by serum markers including endothelin-1, sICAM-1, sVCAM-1, thrombomodulin and von-Willebrand factor (37,38). In addition, nailfold capillary microscopy illustrates these alterations by a capillary network with reduced density of capillaries, microhemorrhages, giant capillaries and bushy capillaries, with the latter reflecting the insufficient attempts of angiogenesis. When examining on a cellular level, a multiplication of the basal membrane of microvascular ECs has been observed as a marker for EC damage (39). In addition, endothelial cell injury can also be triggered by granzymes, endothelial cell–specific autoantibodies, vasculotropic viruses, inflammatory cytokines, or reactive oxygen radicals generated during ischemia/reperfusion. In in vivo experimental approaches using a SSc-like animal model, it could be shown that ECs undergo apoptosis before inflammatory infiltrates or accumulation of extracellular matrix occur (40). Most likely, this process is further enhanced by anti-endothelial cell antibodies as outlined above.

**Perivascular alterations**

Adjacent to the different layers of the affected microvessel are cells of mesenchymal origin, the so called pericytes or perivascular fibroblasts, which most likely also contribute to vascular pathology. For example, VEGF appears to affect pericytes and is produced by these cells, especially in the more severe forms of SSc (41). Platelet growth factor (PDGF), also a potent inducer of
angiogenesis is found in the vessel wall and deposited in the perivascular matrix (42).

It is also known from other fields of research (43) that perivascular fibrosis can be triggered by pericytes. Using a transgenic reporter mouse model for renal disease expressing enhanced green fluorescent protein (GFP) under the regulation of a collagen type I promoter, it could be shown that these pericytes can generate collagen type I in the fibrotic kidney. In addition, circulating fibrocytes were also recruited to the fibrotic kidney, but these fibrocytes did not contribute to interstitial fibrosis. Instead, using kinetic modeling and time course microscopy, the pericytes were found to be the major source of interstitial myofibroblasts. The latter have been found to express the chemokine receptor CCL2/CCR2 in early SSc, which most likely contributes to the initial inflammation observed adjacent to the dysfunctional microvasculature (44). Thus, it can be speculated that the different components of a injured vessels or vascular factors can contribute to the observed perivascular fibrosis.

Along this line, interfering with myofibroblast-dependent vascular remodelling, it has been speculated that statins could have a beneficial effect on SSc vasculopathy (45). Although not confirmed in all patient cohorts (46), this idea is supported by the finding that endothelial dysfunction appears also to be a trigger for atherosclerosis in SSc (47). When endothelium-dependent, flow-mediated dilation was compared to endothelium-independent, nitroglycerin-mediated dilation of the brachial artery in SSc patients, an impairment of
endothelium-dependent vasodilatation indicated by low flow-mediated dilation in SSc could be found. This was accompanied by carotid atherosclerosis after longer disease duration and at higher ages of SSc patients.

Specific Vascular Organ Pathophysiology

Lung

Aside digital ischemia, pulmonary arterial hypertension (PAH) is the most prominent problem in SSc-associated pathology (4,5,48). In normal individuals, vasoactive molecules produced from endothelial cells adapt the pulmonary vascular smooth muscle cell tone to the actual needs of the organism and keep the smooth muscle in a state of relaxation. In PAH, the increased pulmonary vascular reactivity and abnormal vasoconstriction has been attributed to dysfunctional endothelial cell integrity. Similar to the pathophysiology observed in digital vessels in SSc, the dysfunctional endothelial cells in PAH are associated with an increased production of vasoconstrictor mediators such as thromboxane A2, decreased synthesis of prostacyclin and nitric oxide, and uncontrolled proliferation (49). This intimal proliferation can even progress to an almost complete occlusion of the pulmonary arterioles (50). Progression of PAH and remodelling of the (peri)vascular structures are accompanied by impaired EC metabolism characterized by a dominance of vasoconstrictive and proliferative mediators and a decrease in vasodilative and antiproliferative mediators (49). Aside the clinical marker of a decreased DLCO/VA ratio, the
extent of pulmonal involvement and the level of damage appears to be nicely reflected by the levels of NT-proBNP (51).

Of note, PAH and Raynauds syndrome share numerous similarities, not only on a clinical level with a substantial number of patients suffering both from Raynauds syndrome and PAH (4,5) but also on a molecular level as both entities are characterized by endothelial dysfunction with reduced NO release and increased plasma levels of endothelin. Some authors even regard the severity of Raynauds syndrome as risk factor for pulmonary hypertension. Along this line, recurrent contractions and vasospasms of the small pulmonary arteries are thought to result in a change in prostanoid metabolism and subsequent in the development of pulmonary arterial hypertension (52,53), although not all experimental settings support this idea (54,55). However, patients with systemic sclerosis and Raynaud- syndrome exhibit a concurrent pulmonary vascular dysfunction as demonstrated by an enhanced pulmonary vasoconstriction during exercise and stress (56).

**Kidney**

The pathogenic mechanisms underlying scleroderma renal crisis involve also various vascular alterations including intimal thickening of the renal interlobular and arcuate arteries, most likely a result of endothelial cell injury. In addition, epithelial-to-mesenchymal transdifferentiation and fibrosis in the glomerular and tubulointerstitial compartments, and dysregulation of endothelin-1 (ET-1)
receptor expression, contribute also to renal vascular disease. In detail, the decreased renal perfusion results from narrowing of arterial vessels, leading to hyperplasia of the juxtaglomerular apparatus and increased renin release. However, pathological changes can also be found in the renal vasculature of SSc patients without renal crisis, and neither biopsy findings nor plasma renin activity can accurately predict the occurrence of SRC but other events such as hyperreninaemia could also trigger the acute onset and rapid progression of renal failure (57).

**Gastrointestinal tract**

Mirroring the cutaneous teleangiectasias especially in ISSc, in the gastrointestinal tract similar abnormalities can be observed, which are termed water melon stomach. These small vessel are vulnerable to mechanical irritation and contribute to anemia in these patients. In addition, vasoreactive molecules such as endothelin can also be found in full-thickness surgical samples (58), accompanied by a generalised fibrosis in the intestinal gastric wall, perivascular and around proliferating smooth muscle cells (59).

The lymphocytes infiltrating SSc gastric specimens and therefore contributing to vascular pathology and fibrosis, strongly expressed the adhesion molecules VLA-4, LFA-1 and ICAM-1. Furthermore, ECs showed corresponding surface activation with strong expression of VCAM-1 and ICAM-1, suggesting a pro-adhesive phenotype favouring a massive strong transendothelial migration of
inflammatory cells. However, using immunohistology for the pan-endothelial marker CD31 that facilitates selective staining of blood vessels, no significant difference in microvascular density was observed between SSc and control gastric biopsy samples. Furthermore, weak or no VEGF expression was detected in both SSc and control specimens. These data suggest a distinct but somewhat different pathology of the microvasculature in the intestinal tract (60).

Clinical Implications – Counteracting Vasoconstriction

As summarized in Figure 2, the abovementioned complex underlying mechanisms of SSc vasculopathy provides numerous targets for therapeutic intervention, both on the vasodilative side as well as by inhibition of vasoconstriction and vascular remodeling. Based on the published data, these strategies have already been evaluated by a group of experts in a Delphi process/metaanalysis approach (61). To improve vascular perfusion, especially with respect to digital ischemia, the data indicate that nifedipine and i.v. iloprost reduce the frequency and severity of SSc-related Raynauds phenomenon (RP) attacks. Therefore, dihydropiridine-type calcium channel blockers, usually oral nifedipine, should be considered for first-line therapy for SSc-related RP, and intravenous iloprost, or other available i.v. prostanoids, should be considered for severe SSc-related RP.

In addition, two controlled clinical trials indicate that intravenous prostanoids (particularly i.v. iloprost) are efficacious in healing digital ulcers (DU) in patients
with SSc. Intravenous prostanoids (in particular iloprost) should therefore be considered in the treatment of active DU in patients with SSc. With respect to counteracting ET-1, which exerts its effects via the ETA and ETB receptor subtypes, clinical data have shown that the oral, dual ET-1 receptor antagonist, bosentan, has beneficial effects in SSc patients with respect to PAH and DUs. However, bosentan has no proven efficacy in the treatment of active DU in SSc patients, but in protecting from the development of new ulcers. Thus, bosentan should be considered in diffuse SSc (dSSc) with multiple DU, after failure of calcium channel blockers and, usually, prostanoid therapy (61) in a preventive strategy.

Moreover, as two high quality RCTs indicate that bosentan improves exercise capacity, functional class and some hemodynamic measures in pulmonary arterial hypertension (PAH), bosentan should be strongly considered to treat SSc-related PAH (SSc-PAH). Numerous trials indicate also that sitaxentan and ambrisentan can improve pulmonary arterial hypertension - for the latter this has been shown in the ARIES studies 1 and 2 (62). Sildenafil also improves exercise capacity, functional class and some hemodynamic measures in PAH, supporting the recommendation that sitaxentan, ambrisentan and sildenafil may also be considered to treat SSc-PAH. However, several challenges for this dominating sequelae still remain to be solved in the future (63-65). With respect to the significant impact on morbidity and despite the lack of RCTs, experts are of the opinion that angiotensin converting enzyme inhibitors (ACEi) should be used in the treatment of scleroderma renal crisis.
Clinical Implications – Supporting Repair

Autologous stem cells and progenitor cells are theoretically an ideal tool to counteract and repair dysfunctional cells and tissues throughout the human body, given the fact that the underlying pathophysiology is known and/or the applied stem/progenitor cells home to the sites affected by the disease. In SSc, endothelial progenitor cells (EPC) could be such a therapeutic approach (23), as it has been shown that there is a lack of EPCs in SSc when compared to healthy individuals and lower EPC levels are also associated with a higher number of digital ulcers and a more severe course of the disease. One step further –similar to the therapeutic concept of implantation of hematopoietic stem cells in cardiovascular disease and in thrombangitis obliterans (66) - resulted the multilocular implantation of bone marrow-derived stem cells in the palms or the plantar region in an improvement of the symptoms in severe Raynauds syndrome within few weeks, which could also be visualized in arteriography (67). However, the mode of action of this approach in this study still needs to be evaluated in detail, as only in one patient neoangiogenesis could be confirmed. However, when comparing stem cell transplantation to conventional cyclophosphamide therapy, the effect on angiogenesis appears to be significant. A recent study showed that after hematopoietic stem cell transplantation, in beforehand avascular regions of the nailbed, a new formation of vessels, e.g. giant capillaries, could be observed (68).
Summary

Both high resolution molecular analysis as well as extensive data collection in nation-to-worldwide patient cohorts has provided substantial advance in knowledge of vascular pathology in SSc patients. Based on this progress, several novel ideas have been generated that have already or will be implicated for the diagnosis, monitoring or treatment of the patients suffering from this debilitating disease in the near future (69).
**Figures**

**Figure 1**: Angiogenic and angiostatic factors in SSc vasculopathy (modified from 31).

<table>
<thead>
<tr>
<th>Angiogenic Factors</th>
<th>Angiostatic factors</th>
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<tr>
<td>Ang-1, Ang-2, Tie-1, Tie-2, angioigenin</td>
<td>Endostatin</td>
</tr>
<tr>
<td>aFGF, bFGF, PDGF-B</td>
<td>Angiostatin</td>
</tr>
<tr>
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<td>Thrombospondin-1, thrombospondin-2, thrombospondin-3, thrombospondin-4</td>
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<td>PF-4, IP-10, MIG</td>
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<td>Plasminogen activator inhibitor type I (PAI-1), PAI-2</td>
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<td>(u-PAR)</td>
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<tr>
<td>MCP-1, ENA-78, Gro-α, SDF-1/CXCR4</td>
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Figure 2: Potential therapeutic options in SSc vascular disease

**Endothelial protection**
- Endothelin antagonists
- Antioxidants
- Termination of smoking
- Prostaglandins
- ACE inhibitors

**Antifibrotics**
- Endothelin antagonists
- Imatinib

**Antithrombotics**
- ASS, clopidogrel
- Heparin, cumarin

**Vasodilation**
- Prostaglandins
- L-Arginine
- NO/Rho-kinase
- CO₂
- CGRP
- Calcium antagonists
- Nitro-derivatives
- PDE-5 inhibitors

**Reduction of vasoconstriction**
- Endothelin antagonists
- ACE inhibitors
- Serotonin-reuptake inhibitors
- Selective α₂-adrenoreceptorblockers
References


