Review

Endothelial Dysfunction in Systemic Sclerosis

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Abstract: Systemic sclerosis, commonly known as scleroderma, is an autoimmune disorder characterized by vascular abnormalities, autoimmunity, and multiorgan fibrosis. The exact etiology is not known but believed to be triggered by environmental agents in a genetically susceptible host. Vascular symptoms such as the Raynaud phenomenon often precede other fibrotic manifestations such as skin thickening indicating that vascular dysfunction is the primary event. Endothelial damage and activation occur early, possibly triggered by various infectious agents and autoantibodies. Endothelial dysfunction, along with defects in endothelial progenitor cells, leads to defective angiogenesis and vasculogenesis. Endothelial to mesenchymal cell transformation is another seminal event during pathogenesis that progresses to tissue fibrosis. The goal of the review is to discuss the molecular aspect of the endothelial dysfunction that leads to the development of systemic sclerosis.

Keywords: systemic sclerosis; endothelial cell; angiogenesis; vasculogenesis

1. Introduction

Systemic sclerosis (SSc) is an autoimmune connective tissue disease. The disease is characterized by vascular defects, progressive fibrosis, and skin thickening [1]. There are two known types of SSc: limited and diffuse. These two forms can be distinguished by variations in the degree of skin involvement, association with specific autoantibodies, and the pattern of organ involvement. While the exact cause of SSc is unknown, the disease is believed to be a result of environmental factors and genetic predisposition. Family history plays a role in the development of SSc, and it can amplify the risk of developing the disease in an individual [1]. Three distinct underlying mechanism drive the disease: (a) aberrant innate and adaptive immunity leading to production of autoantibodies and cellular autoimmunity; (b) vascular dysfunction, and (c); defective fibroblasts leading to excessive collagen and matrix component deposition in vessels and various organs including skin [1].

Impaired vascular tone and permeability are some of the earliest signs of vascular dysfunction [2]. Dysregulation of vascular tone leading to vasospasm and compromised blood flow is the underlying mechanism of vascular dysfunction. This dysregulation of vascular tone results from the imbalances of vasodilator mediators, e.g., nitric oxide (NO) and vasoconstrictor, e.g., endothelin. Besides this, cellular changes such as large gaps between endothelial cells, vacuolization of endothelial cell cytoplasm, loss of membrane bound storage vesicles are some of the earliest endothelial cell changes [3–5]. Along with the endothelial cell changes, capillary enlargement and capillary losses continue progressively and furthermore intimal proliferation and proteoglycans accumulate in arterioles and small arteries [6,7].

The vascular endothelium maintains vascular tone, regulates platelet function, and controls inflammation. It plays a significant role in the tissue remodeling through angiogenesis and vasculogenesis [8]. Early in the disease process, typical vascular symptoms, e.g., Raynaud's phenomenon precede tissue fibrosis suggesting that microvascular injuries

and dysfunctions involving the vascular endothelial cells (VECs) and capillaries are the initial event [9]. This clinical observation led to the conclusion, as early as 1975, that the primary cause of SSc involves endothelial damage and dysfunction [10]. Furthermore, the interaction of endothelial cells with other cells, e.g., smooth muscles, platelets, fibroblasts, and pathways, e.g., the coagulation system and immune system drive the disease process [9].

Four major distinct processes involving the endothelial cells are crucial in the pathogenesis of SSc: Endothelial cell injury, defective angiogenesis, defective vasculogenesis, and endothelial to mesenchymal transformation (Figure 1). These processes are discussed more in detail below.
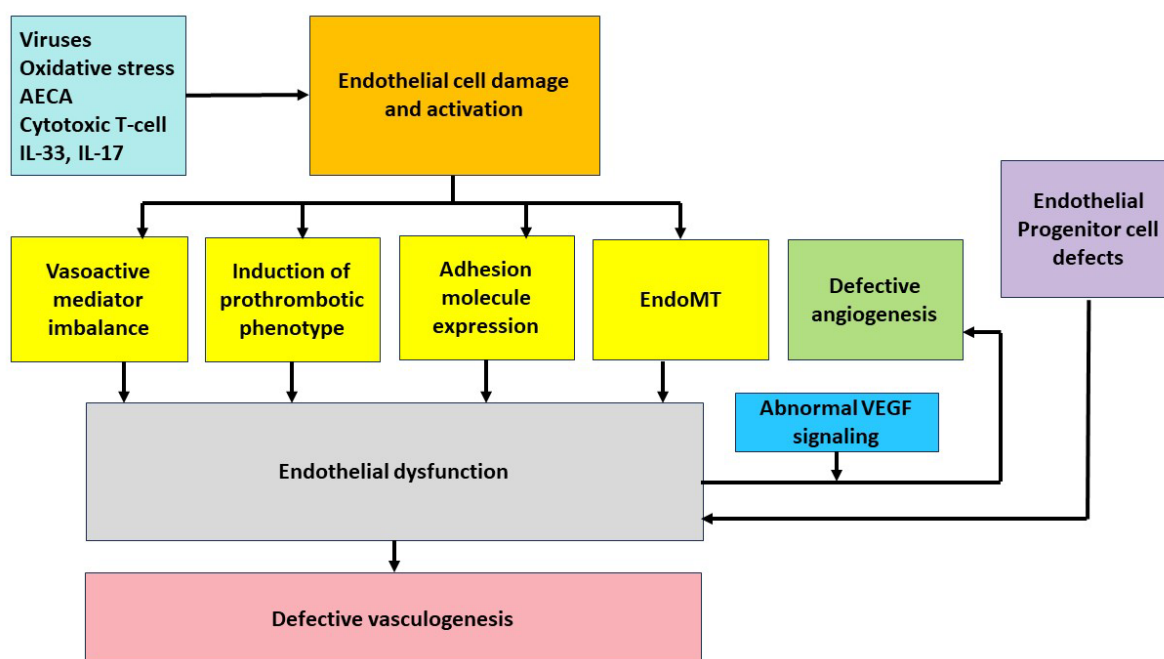
Figure 1. Endothelial dysfunction in systemic sclerosis; AECA: anti-endothelial cell antibodies, VEGF: vascular endothelial growth factor, EndoMT: endothelial to mesenchymal transition.

2. Endothelial Cell Injury

Initial vascular endothelial cell damage followed by vascular remodeling with arteriole intimal proliferation, capillary breakdown and vascular occlusion are some of the key events in the progression of SSc [11]. Endothelial cell injury is accompanied by lymphocytic and mononuclear lymphocytic perivascular infiltration in the affected tissues are few of the earliest changes seen in SSc [12]. Infectious agents, cytotoxic T cells, autoantibodies against the endothelial cells and NO-related free radicals have been cited for the endothelial cell damage but the exact role of each of these agents is not clear [2]. Evidence of endothelial cell injury, e.g., high serum levels of circulating von Willebrand (VW) factor, endothelin-1, circulating viable and dead endothelial cells, and soluble JAM-1 are present in the circulation [2,13–17]. There is increased expression of the genes encoding the von Willebrand factor (vWF), tissue factor (F3), ephrin A1, and ET-1 in stimulated endothelial cells suggestive of endothelial cell activation and damage [18]. Among the infectious agents, viral agents have been proposed as possible trigger of endothelial cell injury [19]. Cytomegalovirus has been implicated in the pathogenesis of SSc for its potential to infect endothelial cells and there is increased prevalence of anti-Hcmv antibodies in the sera of subjects with SSc [20,21]. There could also be activation of the autoreactive B cell clones through molecular mimicry as anti-topoisomerase I antibodies recognize a pentapeptide of the autoantigen-sharing homology with the hCMV-derived UL70 protein [22]. There is presence of anti-HCMV antibodies directed against an epitope (VTL GGAGIWLPP) con-

tained within UL94, a human cytomegalovirus-derived protein expressed in the infected endothelial cells. UL94 is localized in the nucleus of infected endothelial cells and may be responsible for the regulation of viral and/or cellular gene expression. The antibodies bind to the endothelial cell surface receptor NAG-2, bearing similarity to UL94 and cause endothelial apoptosis, which is a key event in SSc pathogenesis [18,23]. There is a significantly higher prevalence of HHV-6A/B DNA in both the blood and peripheral tissues of SSc patients compared to controls [24]. Even among the SSc patients one subgroup has higher viral load compared to another group suggesting that it may be responsible for a subgroup of patients. The authors were further able to demonstrate that human herpes virus A and B (HHV-6 A and B) interferes with normal function of the endothelial cells and causes pro-fibrotic molecule expression in the endothelial cells. Prior parvovirus B19 infection could result in increased expression of TNF-α in the endothelial cells, which may be of pathologic significance in SSc [25]. Epstein bar virus (EBV) has also been implicated in endothelial cell injury in SSc [26]. In an in vitro model, the investigators demonstrated that the human monocytes bound to the recombinant EBV act as a shuttle, EBV virus can infect the endothelial cells, and there is overexpression of the EBV early lytic genes. The EBV induces activation of the TLR9 innate immune response and type I IFN. Type I interferons (IFNs) are known to promote endothelial cell death, inhibit endothelial cell migration [27], and may play a role in endothelial cell injury in SSc [26]. It is possible that the expression of the EBV early lytic genes in the infected endothelial cells may cause vascular endothelial cell apoptosis [26]. Even though SARS-CoV-2 infection is a more acute process, it has a lot of similarity with early endothelial damage and vasculopathy seen in SSc [28]. CD4+ Cytotoxic T-cells and CD8+ cytotoxic T-cells are expanded in early SSc patients [29]. They are found near the endothelial cells undergoing apoptosis. Peripheral T-cell tolerance breakdown may lead these T-cells to interact with the endothelial cells presenting self-antigens in conjunction with HLA class II or HLA class I molecule resulting in the endothelial cell apoptosis. Apoptotic cells trigger TAM family kinases on macrophages and potentially could accelerate wound healing [30]. In another study, the authors found that CD226+CD8+ T cells produced a higher number of various cytokines than CD226$^-$ ones, and CD226^{high}CD8+ T cells from SSc patients showed upregulated IL-13 production and positive correlation with the cytotoxic capacity of CD8+ T cells against human umbilical vascular endothelial cells (HUVECs) [31]. However, neutralization of CD226 in CD8+ T cells impaired costimulation, cytokine production, and cytolysis against HUVECs. Ischemia reperfusion events, e.g., the Raynaud phenomenon, results in oxidative stress in SSc leading to the formation of reactive oxygen species, e.g., including superoxide anion radical ($O_2\bullet$), hydroxyl anion ($\bullet OH$), and hydrogen peroxide (H_2O_2) [32]. The source of these reactive oxygen species varies from the peripheral blood cells in the vessel lumen or monocytes, endothelial cells, fibroblasts in response to various noxious stimuli. These reactive oxygen species inhibit the release of NO, prostacyclin, tissue plasminogen activator, protein S and heparin sulphate from the endothelial cells leading to the alteration of the vascular tone [33]. Anti endothelial cell antibodies (AECA) are found in 22–68% of patients with SSc [34]. These antibodies cause endothelial cell apoptosis by both the fas-independent mechanism and the antibody-dependent cellular cytotoxicity mechanism [35,36]. In an invitro model, in human microvascular endothelial cells (HMVECs), when cultured with AECA, there is endothelial apoptosis and the HMVECs release endothelin 1 [37]. AECAs in SSc are linkedto higher levels of total and activated circulating endothelial cells suggestive of endothelial cell damage. Levels of AECAs are correlated with the number of apoptotic endothelial microparticles. Impairment of vascular repair by having fewer endothelial cell progenitors is seen in subjects with AECAs [38]. Immune complexes (ICs) containing SSc specific antibodies (anti-centromere, anti-topoisomerase I, and anti- RNA polymerase) induce the endothelial cells to a pro-inflammatory and pro-fibrotic phenotype [39]. Incubation of the endothelial cells with SSc-ICs increased the expression of several molecules which can cause vascular dysfunction (ET-1, IL-8), inflammation (IL-6 and ICAM-1), and fibrosis (TGF-β1).

3. Defective Angiogenesis

Angiogenesis is dysregulated in SSc indicating defective microvascular cell differentiation from the preexisting vessels to form new blood vessels [40,41]. The endothelial cells showed reduced angiogenic properties [42,43]. These cells have intrinsic defect in NO production due to the downregulation of the endothelial NO synthase [44]. There is overexpression of proangiogenic factors like VEGF-1 and endothelin-1 [45,46], but VEGF receptor signaling may be impaired [42]. There is also an impaired response to pro-angiogenic chemokines Gro-γ/CXCL3, GCP-2/CXCL6, or CXCL16. The endothelial cells do not migrate in response to the chemokines and vascular endothelial growth factor (VEGF) because of defective signaling pathways. Studies have found that the anti-angiogenic VEGF$_{165}$b isoform of VEGF is overexpressed compared to the proangiogenic VEGF$_{165}$. Binding of VEGF$_{165}$ to the tyrosine kinase receptor VEGFR-2 results in insufficient tyrosine kinase phosphorylation/activation and incomplete signaling, leading to incomplete angiogenic response. The authors have also demonstrated that the plasma level of VEGF$_{165}$b is associated with severity of microvascular damage suggesting that this isoform of VEGF is responsible for vasculopathy in SSc [47,48]. Vascular abnormality and reduced capillary density alter blood supply and tissue oxygenation in SSc. Chronic hypoxia leads to an increase in VEGF level and disturbs VEGF receptor signaling [49]. VEGF promotes angiogenesis by activating the endothelial cells and the endothelial progenitor cells [50]. VEGF further promotes angiogenesis by acting synergistically with the platelet derived growth factor (PDGF) and fibroblast growth factor [51]. Aberrant overexpression of the VEGF-like seen in SSc induces vascular malformations [52]. The vascular malformation is attributable to two specific endothelial cell activities: (i) neovascularization in normally avascular areas and (ii) the unregulated, excessive fusion of vessels. Hypoxia-inducible-factor-1-$\acute{\alpha}$, though, is expected to be high in SSc because of ongoing hypoxia but studies have shown otherwise, levels are low in the skin compared to the healthy controls [53]. Another mechanism of angiogenesis inhibition is through increased glycolytic metabolism of dermal fibroblasts leading to extracellular acidification [54]. The authors have demonstrated that acidosis in general and lactic acidosis impair in vitro endothelial cell capillary network/lumen formation and invasion without altering cell viability. The abnormality was corrected following PH adjustment. This response to acidic PH is secondary to MMP-12 upregulation by endothelial cells and expression of cleaved uPAR (urokinase-type plasminogen activator receptor). Endostatin, an inhibitor of angiogenesis has been found to be upregulated in SSc [55]. There is alteration of signal transduction, e.g.,: ang/Tie signaling pathways [56] and Upar truncation [57]. Friend leukemia virus integration 1 (FLI1) is a transcription factor and its expression is suppressed both genetically and epigenetically in SSc patients [58]. CCN1 expression was suppressed uniformly and remarkably in dermal blood vessels of Fli1(+/−) mice and partially in those of endothelial cell-specific Fli1 knockout mice [59]. Serum CCN1 levels were significantly decreased in SSc patients with previous and current histories of digital ulcers, as compared to those without the ulcerations. This suggests that epigenetic endothelial CCN1 downregulation is at least partially due to Fli1 deficiency and may contribute to the development of digital ulcers in SSc patients [59]. FLI1 deficiency suppresses the expression of CD31, VE-cadherin, S1P1, and PDGF-B in the endothelial cells, while upregulating matrix metalloproteinase-9 and CCR6 resulting in vascular destabilization and angiogenesis [60]. Desmoglein-2 belongs to the family of desmosomal cadherins and is involved in cell adhesion, morphogenesis, cytoskeletal organization, and cell sorting/migration [61]. There is reduced expression of desmoglein-2 (*DSG2*) in the endothelial cells of SSc patients shown by differential transcriptome profiling and by immunohistochemistry of the endothelial cells (EC) [62]. This has been linked to defective angiogenesis in SSc [63]. Circulating CXCL4 levels are elevated in SSc [64]. The proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVECs) are inhibited by CXCL4 or SSc derived plasma. This is reversed by the CXCL4 neutralizing antibody. CXCL4 downregulates Friend leukemia integration factor-1 (Fli-1) via c-able signaling in the endothelial cells and inhibits angiogenesis [64].

4. Defective Vasculogenesis

There has been a lot of interest in the role of endothelial progenitor cells which contribute to the postnatal vasculogenesis in SSc [65]. These cells are identified in flow cytometry as CD34+, CD133+, and/or CD309/VEGFR2+ [66]. There is correlation between digital ulcers and a reduced number of endothelial progenitor cells in SSc patients [66–68]. Low progenitor cell numbers are independent predictors of new digital ulcers on longitudinal follow up and associated with late pattern nail fold capillaroscopic changes in SSc [69,70]. In the Fra-2 transgenic mice model, the pulmonary vasculature remodeling was attenuated by blocking MMP-10 and there is increased expression of stromelysin 2 (MMP-10) in pulmonary vasculature and serum among subjects with SSc [71]. Multiple initial studies have been conflicting regarding the number of circulating endothelial progenitor cells in subjects with SSc [66–68,72,73]. However, these conflicting results, later found to be from different protocols used in different labs, and now using standardized protocols, it has been found that circulating progenitor endothelial cells are in fact reduced in numbers compared to the healthy controls [74]. Circulating lymphatic endothelial progenitor cells (CD34+CD133+VEGFR3+), are also found to be reduced in SSc subjects with active digital ulcers [75]. Besides that, there is inability of endothelial progenitor cells in SSc patients to differentiate into mature endothelial cells. This has been demonstrated in in vitro cultures using angiogenic growth factors [76] and using a murine tumor neovascularization model [77]. Several mechanisms have been proposed behind the qualitative and qualitative defects of the endothelial progenitor cells. An altered bone marrow microenvironment, as evidenced by increased fibrosis and reduced microvascular density, may alter progenitor endothelial capacity to differentiate into the endothelial cells [78]. Circulating pentraxin-3 potentially inhibits progenitor cell differentiation through FGF2 mediated progenitor cell differentiation. The authors have demonstrated that the number of progenitor cell counts varies inversely with circulating levels of Pentraxin-3 [79]. It has also been found that endothelial progenitor cells are defective before they are released from the bone marrow as they do not differentiate well in long-term culture with a proangiogenic growth factor [67]. Others have postulated that progenitor cells may undergo immune mediated apoptosis in circulation, which is mediated through the Akt-FOX03a-bim signaling pathway [72].

5. Endothelial to Mesenchymal Transition (EndoMT)

In SSc, there is subendothelial accumulation of activated fibroblasts or myofibroblasts in small arterioles in the lungs and kidneys which produce fibrotic tissue [80]. It has been demonstrated that some of these mesenchymal cells have originated from the endothelial cell transformation (EndoMT) [80]. Cipriani et al. showed endothelial cells showed reduced expression of vWF, CD31, and VE-cadherin (endothelial cell markers) and upregulation sSm22, α-SMA, and collagen (markers of fibrosis) suggesting the mesenchymal transformation of endothelial cells [81]. Von Willebrand factor/α-smooth muscle actin-positive endothelial cells are found in up to 5% of pulmonary vessels in subjects with SSc-associated pulmonary arterial hypertension and in the hypoxia/SU5416 mouse model [82]. The investigators demonstrated that transformed EndoMT cells on stimulation by inflammatory cytokines, e.g., IL-1 β, TNF-α, TGF β caused actin cytoskeleton reorganization, and the induction of a mesenchymal morphology. These cells showed up-regulation of mesenchymal markers, including collagen type I and α-smooth muscle actin, and a reduction in the endothelial cell and junctional proteins, including von Willebrand factor, CD31, occludin, and vascular endothelial-cadherin. Induced EndoMT monolayers failed to form viable biological barriers and induced enhanced leak in co-culture with pulmonary artery endothelial cells [82]. CD31+/CD102+EC isolated from SSc lungs expressed simultaneously mesenchymal and EC-specific transcripts and proteins [83,84]. This suggests occurrence of EndoMT in lung tissues from patients with SSc-associated ILD. Cells in intermediate stages of EndoMT were identified in dermal vessels of either patients with SSc or bleomycin-induced and urokinase-type plasminogen activator receptor (uPAR)-deficient mouse models [85]. Various cytokines and growth factors, e.g., TGF-β, PDGF, VEGF, and ET-1 activates the

vascular endothelial cell and modulates the expression of adhesion molecules on the endothelial cell surface leading to the recruitment of macrophages and B as well as T lymphocytes [86–88]. These inflammatory cells further release various growth factors, e.g., TGF-β and CTGF, in the tissue activating the endothelial cells to release endothelin-1, which then activates the endoMT cells [89,90]. Levels of leukotriene A4 hydrolase (LTA4 H), an enzyme for LTB4 synthesis, LTB4, and its receptor, BLT1 were increased in lesional areas of the skin and lungs of SSc patients, and were abundant in myofibroblasts and endothelial cells [91]. Fibroblast-myofibroblast and endothelial-mesenchymal transitions (EndoMT) were promoted via BLT1, and are dependent on activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mechanistic target of rapamycin (mTOR) pathway. Various cytokines and other factors are involved in the EndoMT process, including TGF-β, particularly the TGF-β 1 [92], Endothelin-1 [81], Wnt3a protein [93], IL-1β [94], TNF-α [95], IFN-γ [96], microRNAs [97], oxidative stress [98], hypoxia, and hypoxia inducible factor [99]. The growth factors, cytokines, and their mechanisms of actions are summarized in Table 1.

Table 1. Mediators of Endothelial to Mesenchymal Transition (EndoMT) [74].

Mediators	Mode of Action
TGF-β	Smad-dependent and independent pathways, e.g., c-abl kinase, protein kinase c-δ
Caveolin-1(CAV1)	Modulation of TGF-β signaling
Endothelin-1 (ET-1)	Synergic effect with TGF β, involving Smad pathway
Notch pathway	Activation of Snail and upregulate Smad
Wnt pathway	Smad-dependent autocrine TGF-β signaling
Hypoxia-inducible factor-1α (HIF-1α)	Interaction with TGF-β and VEGF, activation of Snail

6. Other Cytokines and Endothelial Dysfunction

A higher serum level of IL-33 and abnormal expression of IL-33 are found in the skin of subjects with early stage SSc [100–103]. The IL-33–ST2 axis increases proliferation, migration, and differentiation of endothelial cells with increased permeability and angiogenesis [103]. It is possible that IL-33 might mediate early events of SSc through recruitment and stimulation of ST-2 expressing cells such as immune cells, fibroblast, and myofibroblast [104]. IL-17 produced by endothelial induces expression of adhesion molecules and chemokines in HUVEC. It mediates vascular inflammation through ERK phosphorylation [105].

7. Endothelial Cell Interactions with Other Cells

Interaction between ECs and platelets is important for regulating vascular tone. Activated platelets induce thymic stromal lymphopoietin (TSLP) in human dermal microvascular ECs, inducing profibrotic and endothelial cell activating factors such as IL-13 [11]. Endothelial cells interact with the coagulation system through upregulation of multiple markers of coagulation, such as VEGF, leading to a prothrombotic state [106].

8. Clinical Manifestations of Endothelial Dysfunction

The Raynaud phenomenon, as mentioned earlier, is one of the earliest and most frequent manifestations of SSc affecting up to 95% of patients [107] It results from the altered vascular tone secondary to the imbalance between vasodilator mediators: Nitric oxide, prostaglandin I2 (PGI2), and vasoconstrictor endothelin 1. Progressive damage to the vasculature with alteration of the vessel wall by proliferative changes leads to obliterative vasculopathy and tissue damage. Frequent episodes of vasospasm, tissue hypoxia, superoxide radicals, and altered microvasculature contribute to developing digital ulcers in up to 30% of SSc patients each year [107,108]. Another form of severe vascular manifestations in SSc includes scleroderma renal crisis (SRC) seen in up to 10% of all SSc

patients [109]. It is characterized by an extreme rise in blood pressure accompanied by renal dysfunction, microangiopathic hemolytic anemia, and thrombocytopenia. Though the exact pathophysiology is poorly understood, it is believed that poor renal blood flow secondary to endothelial damage, intimal hyperplasia, and narrowing of renal arterioles play a role [109]. IL-6 has been implicated in the pathogenesis of SRC [110]. Simon et al. demonstrated that in subjects with SRC, autoantibodies against Protease-activated receptor-1 (PAR-1) induced endothelial cells to produce IL-6 with increased ERK 1/2, AKT, and p70S6K signaling, as well as increased activity of the c-FOS/AP-1 transcriptional factor [110]. The authors suggested that the blockade of the c-FOS/AP-1 pathway and endothelial PAR-1 receptor would mitigate endothelial IL-6 production. Penn et al. showed an increased level of Endothelin-1 and endothelin A and B receptor expression in renal biopsy of SRC patients, suggesting that endothelin blockade may be a therapeutic approach in SRC [111]. Another serious vascular complication in SSc is pulmonary arterial hypertension (PAH) seen in up to 15% of patients [112]. Endothelial dysfunction contributes to SSc-associated PAH, leading to the expansion and aggregation of collagen type-1 positive cells and α-SMA cells in pulmonary vasculature [82]. The authors have shown that EndoMT, though infrequent, contributes to vascular remodeling and inflammatory infiltration in pulmonary vasculature of SSc PAH patients. In SSc PAH patients, there is an increase in endothelin-1 production by endothelial cells and a reduction in the production of vasodilators such as nitric oxide and PGI2 [112]. In normal healthy subjects, endothelin-1 binds to the ET_A receptor which is expressed in endothelial cells causing vasoconstriction and results in vasodilation by binding to the ET_B receptor in smooth muscle cells. In SSc PAH patients, there is upregulation of ET_B receptors in muscles and downregulation in endothelial cells [112]. Endothelin 1, produced by endothelial cells, binds to the ET_B receptor and leads to unregulated cell proliferation, resulting in vascular occlusion and an increase in pulmonary vascular resistance [112]. Endothelial cells, present in the plexiform lesions of pulmonary vasculature of patients with idiopathic pulmonary hypertension, express IL-32, which is likely involved in the proliferation and activation of these abnormal endothelial cells [113]. IL-32 levels have been found to be significantly higher among SSc PAH subjects compared to SSc subjects without PAH and can be a marker of the presence of PAH [114].

9. Evaluation of Endothelial Dysfunction

9.1. Morphological Assessment

Nailfold capillaroscopy is a safe well-established method for assessing capillary microcirculation in SSc. It has been a widely used technique to investigate and monitor patients with Raynaud phenomenon. Subjects can be diagnosed as having SSc based on specific patterns of capillary architecture known as "scleroderma patterns", which can be further divided into three patterns (early, active, and late) based on the stage of disease [115]. The early pattern is recognized as having giant capillaries with normal capillary distribution, followed by the active pattern: giant capillaries, hemorrhage and disorganized distribution and loss of capillaries, and finally the late pattern is identified as loss of capillaries and capillaries with abnormal shapes [115]. These vascular patterns are reflective of the severity and progression of the disease, from mild to severe/progressive disease [116].

9.2. Functional Assessment

Various other methods of assessing the functional aspect of microcirculation have been adopted based on the principle of producing vasodilation in response to various stimuli, e.g., physical, mechanical, and chemical etc. [117]. The vascular response is determined by both the structural and functional condition of the vasculature. The various methods are as follows: peripheral arterial tonometry, laser doppler flowmetry, laser speckle contrast imaging, laser doppler imaging, laser speckle contrast analysis, and laser doppler imaging to assess small digital vessels. Flow-mediated vasodilation of the brachial artery is used to asses medium vessels [117]. Differential vascular response to NO donors (e.g., glycerol-trinitrate) or direct non–NO donors, such as adenosine, can be used to differentiate

endothelium dependent and endothelium independent response [118]. Alteration in the vascular structure and smooth muscle cell leads to impaired endothelial-independent function rather than changes in the endothelium [118].

10. Treatment Strategies for Endothelial Dysfunction

10.1. Calcium Channel Blockers (Dihydropyridines)

Dihydropyridines, e.g., amlodipine, nifedipine, and nicardipine bind to the L-type voltage-gated calcium channels on cells, reduce influx of extracellular calcium, and induce smooth muscle relaxation, resulting in vasodilation [119]. In vitro studies have shown the protective effect of calcium channel blockers on the endothelial cells from oxidative injury [120]. Calcium channel blockers decrease plasma markers of oxidative stress in SSc patients [121]. Calcium channel blockers have been widely used and are effective in uncomplicated Raynaud phenomenon.

10.2. Phosphodiaterase-5 (PDE-5) Inhibitors

PDE5 inhibitors such as sildenafil and tadalafil increase the intracellular cyclic guanosine monophosphate (c-GMP) level by inhibiting PDEs. C-GMP leads to vasodilation through vascular smooth muscle relaxation, increases apoptosis, and decreases proliferation of pulmonary artery smooth muscle cells [122]. PDE-5 inhibitors have been found to be beneficial in treatment of Raynaud phenomenon, digital ulcers, and pulmonary arterial hypertension [123].

10.3. Soluble Guanylate Cyclase Activators and Stimulators

These agents increase c-GMP production through binding to soluble guanyl cyclase (sGC). The sGC stimulators bind to reduced, heme-containing sGC independent of NO and sensitizes the sGC through stabilization of sGC–NO binding to low levels of NO. sGC activators bind to the oxidized heme-free and NO-unresponsive form of sGC [124]. These agents have an anti-inflammatory, antifibrotic, and antihypertensive effect [124]. In a in vitro model, a sGC stimulator, MK-2947, increased the migratory and proliferative effect of SSc-microvascular endothelial cells and inhibited the endoMT process [125]. Ricoguat, a sGC is an approved therapeutic agent for SSc PAH and has been found to be beneficial in Raynaud's phenomenon [125].

10.4. Endothelin-1 Receptors Inhibitors

Treatment of SSc PAH patients with bosentan led to reduction in the higher serum level of serum soluble PECAM-1, ICAM-1, VCAM-1, and P-selectin after 12 months of therapy, suggesting attenuation of endothelial cell activation [126]. It also reduced higher levels of CD3-LFA1 T cell, suggestive of restoring T-cell function. In an invitro EndoMT model, microvascular endothelial cells (MVECs), when incubated with bosentan and macitentan, had reduced expression of mesenchymal markers and restoration of CD31 expression and the imbalance between VEGF-A and VEGF-A165b [127]. This suggests the endothelin-1 receptor inhibitors prevent EndoMT. These agents have been effective in treatment of SSc associated PAH and preventing development of digital ulcers [123].

10.5. Prostacyclins

Vascular endothelial cells synthesize prostacyclins, which possess a vasodilatory effect and inhibit platelet aggregation and vascular smooth muscle proliferation [128]. Various prostacyclin analogs (epoprostenol, trepostinil, iloprost) and the prostacyclin receptor inhibitor, selixipag, promote vasodilation through induction of the prostacyclin pathway. Prostacyclin analogs have been shown to have beneficial effects in the treatment of Raynaud phenomenon, digital ulcers, and PAH in SSc subjects [123]. Tsou et al. showed IV iloprost has a long-lasting effect for weeks after infusion of iloprost, which has a short half-life otherwise [129]. Following the infusion of iloprost, there were increased endothelial cell junctional VE-cadherin clustering, VE-cadherin level, tubulogenesis and inhibition of

monolayers permeability, and EndoMT. The investigators suggested these endothelial effects may account for the long-lasting effect of IV iloprost following discontinuation of infusion.

10.6. Cyclophosphamide

Cyclophosphamide has been widely used to treat SSc-associated interstitial lung disease. It also has beneficial effect on peripheral microvasculature [130]. The investigators showed treatment with cyclophosphamide showed better proliferation of dermal microvascular endothelial cells (MVECs) and less apoptosis. This might be related to a reduction in pretreatment levels of antiangiogenic factors such as pentraxin-3, MMP-12, angiostatin, and endostatin. Cyclophosphamide increases the number of circulating endothelial progenitor cell numbers and reduces serum VEGF levels, E-selectin and thrombomodulin, suggestive of improvement of endothelial cell damage [131–133].

10.7. Statins

Statins have been studied in the treatment of SSc. In a small study of 14 patients with SSc using atorvastatin 10 mg/day for 12 weeks, the number of bone marrow–derived circulating endothelial precursors (CEPs) increased, and improvement of Raynaud symptoms occurred [134]. In another study of SSc patients using simvastatin 20 mg/day over 12 weeks, there was no increase in endothelial progenitor cells but there was a reduction in the number of circulating endothelial cells [135]. There was also a reduction in levels of endothelin-1, soluble E-selectin, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and interleukin-6, suggesting that statin inhibits endothelial cell activation.

10.8. Nitrate Therapy

Nitrates are metabolized into NO and they increase c-GMP concentration in vascular smooth muscle, leading to vasodilation [136]. Nitroglycerine tapes, MQX-503 (a novel nitroglycerine compound), and topical glyceryl trinitrate have been tried to improve NO levels in the peripheral circulation of patients with SSc suffering from Raynaud phenomenon and digital ulcers but met with variable levels of success [137–139].

10.9. Stem Cell Therapy

Stromal vascular fraction (SVF), isolated from the adipose tissue, contains mesenchymal stem cells, endothelial precursor cells, and T regulatory cells, etc. [140]. The mesenchymal stem cells, otherwise known as adipose-derived stem cells, can be further isolated from SVF. Local injection of these stem cells leads to the secretion of VEGF and fibroblast growth factor, which promote local angiogenesis [141], inhibit apoptosis, and promote endothelial cell proliferation [142]. A recent meta-analysis of the effectiveness of the adipose-derived stem cell injections showed improvement in digital ulcers, Raynaud symptoms, and an increase in the number of nail fold capillaries [143]. Autologous hematopoietic stem cell transplantation has a limited impact on vasculature. It only partially restored microvascular architecture [144] but had no effect on dermal vessel density [145]. Mesenchymal stromal cells (MSCs), isolated from adipose tissue or bone marrow, possess immunosuppressive, proangiogenic, and antifibrotic potential [146]. Allogenic and autologous IV infusion of MSCs have led to improvement in digital ulcers and peripheral circulation but the efficacy is limited to case reports and case series at the present time.

11. Conclusions

We have reviewed the critical role of endothelial cells in the pathogenesis of SSc. Endothelial cell injury is the initial event in scleroderma. Besides endothelial injury, defective angiogenesis and vasculogenesis involving the endothelial cells play key roles. Furthermore, endothelial to mesenchymal cell transformation leads to tissue fibrosis. Therapeutic advances targeting the various aspects of endothelial dysfunction would be useful.

References

1. Pattanaik, D.; Brown, M.; Postlethwaite, B.C.; Postlethwaite, A.E. Pathogenesis of Systemic Sclerosis. *Front. Immunol.* **2015**, *6*, 272. [CrossRef]
2. Kahaleh, B. Vascular Disease in Scleroderma: Mechanisms of Vascular Injury. *Rheum. Dis. Clin. N. Am.* **2008**, *34*, 57–71. [CrossRef]
3. Prescott, R.J.; Freemont, A.J.; Jones, C.J.P.; Hoyland, J.; Fielding, P. Sequential dermal microvascular and perivascular changes in the development of scleroderma. *J. Pathol.* **1992**, *166*, 255–263. [CrossRef] [PubMed]
4. Fleischmajer, R. Skin Capillary Changes in Early Systemic Scleroderma. *Arch. Dermatol.* **1976**, *112*, 1553. [CrossRef] [PubMed]
5. Freemont, A.J.; Jones, C.J.P.; Bromley, M.; Andrews, P. Changes in vascular endothelium related to lymphocyte collections in diseased synovia. *Arthritis Rheum.* **1983**, *26*, 1427–1433. [CrossRef]
6. Norton, W.L. Vascular Disease in Progressive Systemic Sclerosis (Scleroderma). *Ann. Intern. Med.* **1970**, *73*, 317. [CrossRef] [PubMed]
7. Fleischmajer, R.; Perlish, J.S. Capillary alterations in scleroderma. *J. Am. Acad. Dermatol.* **1980**, *2*, 161–170. [CrossRef] [PubMed]
8. Abraham, D.; Distler, O. How does endothelial cell injury start? The role of endothelin in systemic sclerosis. *Arthritis Res. Ther.* **2007**, *9* (Suppl. 2), S2. [CrossRef]
9. Matucci-Cerinic, M.; Kahaleh, B.; Wigley, F.M. Review: Evidence That Systemic Sclerosis Is a Vascular Disease. *Arthritis Rheum.* **2013**, *65*, 1953–1962. [CrossRef]
10. Campbell, P.M.; LeRoy, E.C. Pathogenesis of systemic sclerosis: A vascular hypothesis. *Semin. Arthritis Rheum.* **1975**, *4*, 351–368. [CrossRef]
11. Truchetet, M.E.; Brembilla, N.C.; Chizzolini, C. Current Concepts on the Pathogenesis of Systemic Sclerosis. *Clin. Rev. Allergy Immunol.* **2023**, *64*, 262–283. [CrossRef]
12. Roumm, A.D.; Whiteside, T.L.; Medsger, T.A.; Rodnan, G.P. Lymphocytes in the skin of patients with progressive systemic sclerosis. *Arthritis Rheum.* **1984**, *27*, 645–653. [CrossRef] [PubMed]
13. Manetti, M.; Guiducci, S.; Romano, E.; Rosa, I.; Ceccarelli, C.; Mello, T.; Milia, A.F.; Conforti, M.L.; Ibba-Manneschi, L.; Matucci-Cerinic, M. Differential expression of junctional adhesion molecules in different stages of systemic sclerosis. *Arthritis Rheum.* **2012**, *65*, 247–257. [CrossRef] [PubMed]
14. Blann, A.D.; Illingworth, K.; Jayson, M.I. Mechanisms of endothelial cell damage in systemic sclerosis and Raynaud's phenomenon. *J. Rheumatol.* **1993**, *20*, 1325–1330. [PubMed]
15. Schachna, L.; Wigley, F.M. Targeting mediators of vascular injury in scleroderma. *Curr. Opin. Rheumatol.* **2002**, *14*, 686–693. [CrossRef]
16. Del Papa, N.; Colombo, G.; Fracchiolla, N.; Moronetti, L.M.; Ingegnoli, F.; Maglione, W.; Comina, D.P.; Vitali, C.; Fantini, F.; Cortelezzi, A. Circulating endothelial cells as a marker of ongoing vascular disease in systemic sclerosis. *Arthritis Rheum.* **2004**, *50*, 1296–1304. [CrossRef]
17. Kuwana, M.; Okazaki, Y. Quantification of circulating endothelial progenitor cells in systemic sclerosis: A direct comparison of protocols. *Ann. Rheum. Dis.* **2012**, *71*, 617–620. [CrossRef]
18. Lunardi, C.; Dolcino, M.; Peterlana, D.; Bason, C.; Navone, R.; Tamassia, N.; Beri, R.; Corrocher, R.; Puccetti, A. Antibodies against human cytomegalovirus in the pathogenesis of systemic sclerosis: A gene array approach. *PLoS Med.* **2006**, *3*, e2. [CrossRef]
19. Moroncini, G.; Mori, S.; Tonnini, C.; Gabrielli, A. Role of viral infections in the etiopathogenesis of systemic sclerosis. *Clin. Exp. Rheumatol.* **2013**, *31* (Suppl. 76), 3–7.
20. Pandey, J.P.; LeRoy, E.C. Current Comment: Human cytomegalovirus and the vasculopathies of autoimmune diseases (especially scleroderma), allograft rejection, and coronary restenosis. *Arthritis Rheum.* **1998**, *41*, 10–15. [CrossRef]
21. Neidhart, M.; Kuchen, S.; Distler, O.; Brühlmann, P.; Michel, B.A.; Gay, R.E.; Gay, S. Increased serum levels of antibodies against human cytomegalovirus and prevalence of autoantibodies in systemic sclerosis. *Arthritis Rheum.* **1999**, *42*, 389–392. [CrossRef] [PubMed]
22. Muryoi, T.; Kasturi, K.N.; Kafina, M.J.; Cram, D.S.; Harrison, L.C.; Sasaki, T.; Bona, C.A. Antitopoisomerase I monoclonal autoantibodies from scleroderma patients and tight skin mouse interact with similar epitopes. *J. Exp. Med.* **1992**, *175*, 1103–1109. [CrossRef] [PubMed]
23. Lunardi, C.; Bason, C.; Navone, R.; Millo, E.; Damonte, G.; Corrocher, R.; Puccetti, A. Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. *Nat. Med.* **2000**, *6*, 1183–1186. [CrossRef]

24. Caselli, E.; Fabbri, C.; D'Accolti, M.; Soffritti, I.; Bassi, C.; Mazzacane, S.; Franchi, M. Defining the oral microbiome by whole-genome sequencing and resistome analysis: The complexity of the healthy picture. *BMC Microbiol.* **2020**, *20*, 120. [CrossRef] [PubMed]

25. Magro, C.M.; Nuovo, G.; Ferri, C.; Crowson, A.N.; Giuggioli, D.; Sebastiani, M. Parvoviral infection of endothelial cells and stromal fibroblasts: A possible pathogenetic role in scleroderma. *J. Cutan. Pathol.* **2003**, *31*, 43–50. [CrossRef]

26. Farina, A.; Rosato, E.; York, M.; Gewurz, B.E.; Trojanowska, M.; Farina, G.A. Innate Immune Modulation Induced by EBV Lytic Infection Promotes Endothelial Cell Inflammation and Vascular Injury in Scleroderma. *Front. Immunol.* **2021**, *12*, 651013. [CrossRef]

27. Lee, P.Y.; Li, Y.; Richards, H.B.; Chan, F.S.; Zhuang, H.; Narain, S.; Butfiloski, E.J.; Sobel, E.S.; Reeves, W.H.; Segal, M.S. Type I interferon as a novel risk factor for endothelial progenitor cell depletion and endothelial dysfunction in systemic lupus erythematosus. *Arthritis Rheum.* **2007**, *56*, 3759–3769. [CrossRef]

28. Matucci-Cerinic, M.; Hughes, M.; Taliani, G.; Kahaleh, B. Similarities between COVID-19 and systemic sclerosis early vasculopathy: A "viral" challenge for future research in scleroderma. *Autoimmun. Rev.* **2021**, *20*, 102899. [CrossRef]

29. Maehara, T.; Kaneko, N.; Perugino, C.A.; Mattoo, H.; Kers, J.; Allard-Chamard, H.; Mahajan, V.S.; Liu, H.; Murphy, S.J.; Ghebremichael, M.; et al. Cytotoxic CD4+ T lymphocytes may induce endothelial cell apoptosis in systemic sclerosis. *J. Clin. Investig.* **2020**, *130*, 2451–2464. [CrossRef]

30. Bosurgi, L.; Cao, Y.G.; Cabeza-Cabrerizo, M.; Tucci, A.; Hughes, L.D.; Kong, Y.; Weinstein, J.S.; Licona-Limon, P.; Schmid, E.T.; Pelorosso, F.; et al. Macrophage function in tissue repair and remodeling requires IL-4 or IL-13 with apoptotic cells. *Science* **2017**, *356*, 1072–1076. [CrossRef]

31. Ayano, M.; Tsukamoto, H.; Kohno, K.; Ueda, N.; Tanaka, A.; Mitoma, H.; Akahoshi, M.; Arinobu, Y.; Niiro, H.; Horiuchi, T.; et al. Increased CD226 Expression on CD8+ T Cells Is Associated with Upregulated Cytokine Production and Endothelial Cell Injury in Patients with Systemic Sclerosis. *J. Immunol.* **2015**, *195*, 892–900. [CrossRef]

32. Vona, R.; Giovannetti, A.; Gambardella, L.; Malorni, W.; Pietraforte, D.; Straface, E. Oxidative stress in the pathogenesis of systemic scleroderma: An overview. *J. Cell. Mol. Med.* **2018**, *22*, 3308–3314. [CrossRef] [PubMed]

33. Kahaleh, B. The microvascular endothelium in scleroderma. *Rheumatology* **2008**, *47* (Suppl. 5), v14–v15. [CrossRef] [PubMed]

34. Mihai, C.; Landewé, R.; van der Heijde, D.; Walker, U.A.; Constantin, P.I.; Gherghe, A.M.; Ionescu, R.; Rednic, S.; Allanore, Y.; Avouac, J.; et al. Digital ulcers predict a worse disease course in patients with systemic sclerosis. *Ann. Rheum. Dis.* **2015**, *75*, 681–686. [CrossRef] [PubMed]

35. Bordron, A.; Dueymes, M.; Levy, Y.; Jamin, C.; Leroy, J.P.; Piette, J.C.; Shoenfeld, Y.; Youinou, P.Y. The binding of some human antiendothelial cell antibodies induces endothelial cell apoptosis. *J. Clin. Investig.* **1998**, *101*, 2029–2035. [CrossRef] [PubMed]

36. Sgonc, R.; Gruschwitz, M.S.; Boeck, G.; Sepp, N.; Gruber, J.; Wick, G. Endothelial cell apoptosis in systemic sclerosis is induced by antibody-dependent cell-mediated cytotoxicity via CD95. *Arthritis Rheum.* **2000**, *43*, 2550–2562. [CrossRef]

37. Corallo, C.; Franci, B.; Lucani, B.; Montella, A.; Chirico, C.; Gonnelli, S.; Nuti, R.; Giordano, N. From microvasculature to fibroblasts: Contribution of anti-endothelial cell antibodies in systemic sclerosis. *Int. J. Immunopathol. Pharmacol.* **2015**, *28*, 93–103. [CrossRef]

38. Michalska-Jakubus, M.M.; Rusek, M.; Kowal, M.; Czop, M.; Kocki, J.; Krasowska, D. Anti-endothelial cell antibodies are associated with apoptotic endothelial microparticles, endothelial sloughing and decrease in angiogenic progenitors in systemic sclerosis. *Adv. Dermatol. Allergol.* **2020**, *37*, 725–735. [CrossRef]

39. Raschi, E.; Privitera, D.; Bodio, C.; Lonati, P.A.; Borghi, M.O.; Ingegnoli, F.; Meroni, P.L.; Chighizola, C.B. Scleroderma-specific autoantibodies embedded in immune complexes mediate endothelial damage: An early event in the pathogenesis of systemic sclerosis. *Arthritis Res. Ther.* **2020**, *22*, 265. [CrossRef]

40. Rabquer, B.J.; Koch, A.E. Angiogenesis and Vasculopathy in Systemic Sclerosis: Evolving Concepts. *Curr. Rheumatol. Rep.* **2011**, *14*, 56–63. [CrossRef]

41. Distler, J.H.W. Angiogenesis and vasculogenesis in systemic sclerosis. *Rheumatology* **2006**, *45* (Suppl. 3), iii26–iii27. [CrossRef] [PubMed]

42. Tsou, P.-S.; Rabquer, B.J.; Ohara, R.A.; Stinson, W.A.; Campbell, P.L.; Amin, M.A.; Balogh, B.; Zakhem, G.; Renauer, P.A.; Lozier, A.; et al. Scleroderma dermal microvascular endothelial cells exhibit defective response to pro-angiogenic chemokines. *Rheumatology* **2016**, *55*, 745–754. [CrossRef] [PubMed]

43. D'Alessio, S.; Fibbi, G.; Cinelli, M.; Guiducci, S.; Del Rosso, A.; Margheri, F.; Serratì, S.; Pucci, M.; Kahaleh, B.; Fan, P.; et al. Matrix metalloproteinase 12-dependent cleavage of urokinase receptor in systemic sclerosis microvascular endothelial cells results in impaired angiogenesis. *Arthritis Rheum.* **2004**, *50*, 3275–3285. [CrossRef] [PubMed]

44. Romero, L.I.; Zhang, D.-N.; Cooke, J.P.; Ho, H.-K.V.; Avalos, E.; Herrera, R.; Herron, G.S. Differential expression of nitric oxide by dermal microvascular endothelial cells from patients with scleroderma. *Vasc. Med.* **2000**, *5*, 147–158. [CrossRef]

45. Distler, O.; Del Rosso, A.; Giacomelli, R.; Cipriani, P.; Conforti, M.L.; Guiducci, S.; Gay, R.E.; Michel, B.A.; Brühlmann, P.; Müller-Ladner, U.; et al. Angiogenic and angiostatic factors in systemic sclerosis: Increased levels of vascular endothelial growth factor are a feature of the earliest disease stages and are associated with the absence of fingertip ulcers. *Arthritis Res. Ther.* **2002**, *4*, R11. [CrossRef]

46. Faller, D.V. Endothelial cell responses to hypoxic stress. *Clin. Exp. Pharmacol. Physiol.* **1999**, *26*, 74–84. [CrossRef]

47. Manetti, M.; Guiducci, S.; Ibba-Manneschi, L.; Matucci-Cerinic, M. Impaired Angiogenesis in Systemic Sclerosis: The Emerging Role of the Antiangiogenic VEGF$_{165}$b Splice Variant. *Trends Cardiovasc. Med.* **2011**, *21*, 204–210. [CrossRef]

48. Manetti, M.; Guiducci, S.; Romano, E.; Bellando-Randone, S.; Lepri, G.; Bruni, C.; Conforti, M.L.; Ibba-Manneschi, L.; Matucci-Cerinic, M. Increased plasma levels of the VEGF$_{165}$b splice variant are associated with the severity of nailfold capillary loss in systemic sclerosis. *Ann. Rheum. Dis.* **2013**, *72*, 1425–1427. [CrossRef]

49. Beyer, C.; Schett, G.; Gay, S.; Distler, O.; Distler, J.H.W. Hypoxia. Hypoxia in the pathogenesis of systemic sclerosis. *Arthritis Res. Ther.* **2009**, *11*, 220. [CrossRef]

50. Grunewald, M.; Avraham, I.; Dor, Y.; Bachar-Lustig, E.; Itin, A.; Yung, S.; Chimenti, S.; Landsman, L.; Abramovitch, R.; Keshet, E. VEGF-Induced Adult Neovascularization: Recruitment, Retention, and Role of Accessory Cells. *Cell* **2006**, *126*, 811. [CrossRef]

51. Makino, Y.; Cao, R.; Svensson, K.; Bertilsson, G.; Asman, M.; Tanaka, H.; Cao, Y.; Berkenstam, A.; Poellinger, L. Inhibitory PAS domain protein is a negative regulator of hypoxia-inducible gene expression. *Nature* **2001**, *414*, 550–554. [CrossRef]

52. Drake, C.J.; Little, C.D. Exogenous vascular endothelial growth factor induces malformed and hyperfused vessels during embryonic neovascularization. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7657–7661. [CrossRef] [PubMed]

53. Distler, O.; Distler, J.H.W.; Scheid, A.; Acker, T.; Hirth, A.; Rethage, J.; Michel, B.A.; Gay, R.E.; Müller-Ladner, U.; Matucci-Cerinic, M.; et al. Uncontrolled Expression of Vascular Endothelial Growth Factor and Its Receptors Leads to Insufficient Skin Angiogenesis in Patients With Systemic Sclerosis. *Circ. Res.* **2004**, *95*, 109–116. [CrossRef] [PubMed]

54. Andreucci, E.; Margheri, F.; Peppicelli, S.; Bianchini, F.; Ruzzolini, J.; Laurenzana, A.; Fibbi, G.; Bruni, C.; Bellando-Randone, S.; Guiducci, S.; et al. Glycolysis-derived acidic microenvironment as a driver of endothelial dysfunction in systemic sclerosis. *Rheumatology* **2021**, *60*, 4508–4519. [CrossRef] [PubMed]

55. Hebbar, M.; Peyrat, J.-P.; Hornez, L.; Hatron, P.-Y.; Hachulla, E.; Devulder, B. Increased concentrations of the circulating angiogenesis inhibitor endostatin in patients with systemic sclerosis. *Arthritis Rheum.* **2000**, *43*, 889. [CrossRef] [PubMed]

56. Noda, S.; Asano, Y.; Aozasa, N.; Akamata, K.; Yamada, D.; Masui, Y.; Tamaki, Z.; Kadono, T.; Sato, S. Serum Tie2 levels: Clinical association with microangiopathies in patients with systemic sclerosis. *J. Eur. Acad. Dermatol. Venereol.* **2011**, *25*, 1476–1479. [CrossRef]

57. Margheri, F.; Manetti, M.; Serratì, S.; Nosi, D.; Pucci, M.; Matucci-Cerinic, M.; Kahaleh, B.; Bazzichi, L.; Fibbi, G.; Ibba-Manneschi, L.; et al. Domain 1 of the urokinase-type plasminogen activator receptor is required for its morphologic and functional, β2 integrin–mediated connection with actin cytoskeleton in human microvascular endothelial cells: Failure of association in systemic sclerosis endothelial cells. *Arthritis Rheum.* **2006**, *54*, 3926–3938. [CrossRef]

58. Asano, Y. Epigenetic suppression of Fli1, a potential predisposing factor in the pathogenesis of systemic sclerosis. *Int. J. Biochem. Cell Biol.* **2015**, *67*, 86–91. [CrossRef]

59. Saigusa, R.; Asano, Y.; Taniguchi, T.; Yamashita, T.; Takahashi, T.; Ichimura, Y.; Toyama, T.; Tamaki, Z.; Tada, Y.; Sugaya, M.; et al. A possible contribution of endothelial CCN1 downregulation due to Fli1 deficiency to the development of digital ulcers in systemic sclerosis. *Exp. Dermatol.* **2015**, *24*, 127–132. [CrossRef]

60. Asano, Y.; Stawski, L.; Hant, F.; Highland, K.; Silver, R.; Szalai, G.; Watson, D.K.; Trojanowska, M. Endothelial Fli1 deficiency impairs vascular homeostasis: A role in scleroderma vasculopathy. *Am. J. Pathol.* **2010**, *176*, 1983–1998. [CrossRef]

61. Angst, B.D.; Marcozzi, C.; Magee, A.I. The cadherin superfamily. *J. Cell Sci.* **2001**, *114*, 625–626. [CrossRef]

62. Giusti, B.; Fibbi, G.; Margheri, F.; Serratì, S.; Rossi, L.; Poggi, F.; Lapini, I.; Magi, A.; Del Rosso, A.; Cinelli, M.; et al. A model of anti-angiogenesis: Differential transcriptosome profiling of microvascular endothelial cells from diffuse systemic sclerosis patients. *Arthritis Res. Ther.* **2006**, *8*, R115. [CrossRef] [PubMed]

63. Giusti, B.; Margheri, F.; Rossi, L.; Lapini, I.; Magi, A.; Serratì, S.; Chillà, A.; Laurenzana, A.; Magnelli, L.; Calorini, L.; et al. Desmoglein-2-integrin Beta-8 interaction regulates actin assembly in endothelial cells: Deregulation in systemic sclerosis. *PLoS ONE* **2013**, *8*, e68117. [CrossRef]

64. Jiang, Z.; Chen, C.; Yang, S.; He, H.; Zhu, X.; Liang, M. Contribution to the peripheral vasculopathy and endothelial cell dysfunction by CXCL4 in Systemic Sclerosis. *J. Dermatol. Sci.* **2021**, *104*, 63–73. [CrossRef]

65. Ferrante, A.; Guggino, G.; Di Liberto, D.; Ciccia, F.; Cipriani, P.; Balistreri, C.R.; Sireci, G.; Giacomelli, R.; Triolo, G. Endothelial progenitor cells: Are they displaying a function in autoimmune disorders? *Mech. Ageing Dev.* **2016**, *159*, 44–48. [CrossRef]

66. Avouac, J.; Juin, F.; Wipff, J.; Couraud, P.O.; Chiocchia, G.; Kahan, A.; Boileau, C.; Uzan, G.; Allanore, Y. Circulating endothelial progenitor cells in systemic sclerosis: Association with disease severity. *Ann. Rheum. Dis.* **2008**, *67*, 1455–1460. [CrossRef] [PubMed]

67. Papa, N.D.; Quirici, N.; Soligo, D.; Scavullo, C.; Cortiana, M.; Borsotti, C.; Maglione, W.; Comina, D.P.; Vitali, C.; Fraticelli, P.; et al. Bone marrow endothelial progenitors are defective in systemic sclerosis. *Arthritis Rheum.* **2006**, *54*, 2605–2615. [CrossRef]

68. Mok, M.Y.; Yiu, K.H.; Wong, C.Y.; Qiuwaxi, J.; Lai, W.H.; Wong, W.S.; Tse, H.F.; Lau, C.S. Low circulating level of CD133+KDR+cells in patients with systemic sclerosis. *Clin. Exp. Rheumatol.* **2010**, *28* (Suppl. 62), S19–S25.

69. Avouac, J.; Meune, C.; Ruiz, B.; Couraud, P.O.; Uzan, G.; Boileau, C.; Kahan, A.; Chiocchia, G.; Allanore, Y. Angiogenic biomarkers predict the occurrence of digital ulcers in systemic sclerosis. *Ann. Rheum. Dis.* **2011**, *71*, 394–399. [CrossRef]

70. Avouac, J.; Vallucci, M.; Smith, V.; Senet, P.; Ruiz, B.; Sulli, A.; Pizzorni, C.; Frances, C.; Chiocchia, G.; Cutolo, M.; et al. Correlations between angiogenic factors and capillaroscopic patterns in systemic sclerosis. *Arthritis Res. Ther.* **2013**, *15*, R55. [CrossRef]

71. Avouac, J.; Guignabert, C.; Hoffmann-Vold, A.M.; Ruiz, B.; Dorfmuller, P.; Pezet, S.; Amar, O.; Tu, L.; Van Wassenhove, J.; Sadoine, J.; et al. Role of Stromelysin 2 (Matrix Metalloproteinase 10) as a Novel Mediator of Vascular Remodeling Underlying Pulmonary Hypertension Associated With Systemic Sclerosis. *Arthritis Rheumatol.* **2017**, *69*, 2209–2221. [CrossRef] [PubMed]

72. Zhu, S.; Evans, S.; Yan, B.; Povsic, T.J.; Tapson, V.; Goldschmidt-Clermont, P.J.; Dong, C. Transcriptional regulation of Bim by FOXO3a and Akt mediates scleroderma serum-induced apoptosis in endothelial progenitor cells. *Circulation* **2008**, *118*, 2156–2165. [CrossRef] [PubMed]

73. Andrigueti, F.V.; Arismendi, M.I.; Ebbing, P.C.C.; Kayser, C. Decreased numbers of endothelial progenitor cells in patients in the early stages of systemic sclerosis. *Microvasc. Res.* **2015**, *98*, 82–87. [CrossRef] [PubMed]

74. Ota, Y.; Kuwana, M. Endothelial cells and endothelial progenitor cells in the pathogenesis of systemic sclerosis. *Eur. J. Rheumatol.* **2020**, *7* (Suppl. 3), S139–S146. [CrossRef] [PubMed]

75. Manetti, M.; Pratesi, S.; Romano, E.; Rosa, I.; Bruni, C.; Bellando-Randone, S.; Guiducci, S.; Maggi, E.; Ibba-Manneschi, L.; Matucci-Cerinic, M. Decreased circulating lymphatic endothelial progenitor cells in digital ulcer-complicated systemic sclerosis. *Ann. Rheum. Dis.* **2018**, *78*, 575–577. [CrossRef]

76. Kuwana, M.; Okazaki, Y.; Yasuoka, H.; Kawakami, Y.; Ikeda, Y. Defective vasculogenesis in systemic sclerosis. *Lancet* **2004**, *364*, 603–610. [CrossRef]

77. Kuwana, M.; Okazaki, Y. Brief Report: Impaired In Vivo Neovascularization Capacity of Endothelial Progenitor Cells in Patients With Systemic Sclerosis. *Arthritis Rheumatol.* **2014**, *66*, 1300–1305. [CrossRef]

78. Carrai, V.; Miniati, I.; Guiducci, S.; Capaccioli, G.; Alterini, R.; Saccardi, R.; Conforti, M.L.; Rigacci, L.; Rotunno, G.; Bosi, A.; et al. Evidence for reduced angiogenesis in bone marrow in SSc: Immunohistochemistry and multiparametric computerized imaging analysis. *Rheumatology* **2012**, *51*, 1042–1048. [CrossRef]

79. Shirai, Y.; Okazaki, Y.; Inoue, Y.; Tamura, Y.; Yasuoka, H.; Takeuchi, T.; Kuwana, M. Elevated Levels of Pentraxin 3 in Systemic Sclerosis: Associations With Vascular Manifestations and Defective Vasculogenesis. *Arthritis Rheumatol.* **2015**, *67*, 498–507. [CrossRef]

80. Jimenez, S.A. Role of endothelial to mesenchymal transition in the pathogenesis of the vascular alterations in systemic sclerosis. *ISRN Rheumatol.* **2013**, *2013*, 835948. [CrossRef]

81. Cipriani, P.; Di Benedetto, P.; Ruscitti, P.; Capece, D.; Zazzeroni, F.; Liakouli, V.; Pantano, I.; Berardicurti, O.; Carubbi, F.; Pecetti, G.; et al. The Endothelial-mesenchymal Transition in Systemic Sclerosis Is Induced by Endothelin-1 and Transforming Growth Factor-β and May Be Blocked by Macitentan, a Dual Endothelin-1 Receptor Antagonist. *J. Rheumatol.* **2015**, *42*, 1808–1816. [CrossRef] [PubMed]

82. Good, R.B.; Gilbane, A.J.; Trinder, S.L.; Denton, C.P.; Coghlan, G.; Abraham, D.J.; Holmes, A.M. Endothelial to Mesenchymal Transition Contributes to Endothelial Dysfunction in Pulmonary Arterial Hypertension. *Am. J. Pathol.* **2015**, *185*, 1850–1858. [CrossRef] [PubMed]

83. Mendoza, F.A.; Piera-Velazquez, S.; Farber, J.L.; Feghali-Bostwick, C.; Jiménez, S.A. Endothelial Cells Expressing Endothelial and Mesenchymal Cell Gene Products in Lung Tissue From Patients With Systemic Sclerosis-Associated Interstitial Lung Disease. *Arthritis Rheumatol.* **2016**, *68*, 210–217. [CrossRef] [PubMed]

84. Jimenez, S.A.; Piera-Velazquez, S. Endothelial to mesenchymal transition (EndoMT) in the pathogenesis of Systemic Sclerosis-associated pulmonary fibrosis and pulmonary arterial hypertension. Myth or reality? *Matrix Biol.* **2016**, *51*, 26–36. [CrossRef]

85. Manetti, M.; Romano, E.; Rosa, I.; Guiducci, S.; Bellando-Randone, S.; De Paulis, A.; Ibba-Manneschi, L.; Matucci-Cerinic, M. Endothelial-to-mesenchymal transition contributes to endothelial dysfunction and dermal fibrosis in systemic sclerosis. *Ann. Rheum. Dis.* **2017**, *76*, 924–934. [CrossRef]

86. Thuan, D.T.B.; Zayed, H.; Eid, A.H.; Abou-Saleh, H.; Nasrallah, G.K.; Mangoni, A.A.; Pintus, G. A Potential Link Between Oxidative Stress and Endothelial-to-Mesenchymal Transition in Systemic Sclerosis. *Front. Immunol.* **2018**, *9*, 1985. [CrossRef]

87. Neumann Andersen, G.; Caidahl, K.; Kazzam, E.; Petersson, A.-S.; Waldenström, A.; Mincheva-Nilsson, L.; Rantapää-Dahlqvist, S. Correlation between increased nitric oxide production and markers of endothelial activation in systemic sclerosis: Findings with the soluble adhesion molecules E-selectin, intercellular adhesion molecule 1, and vascular cell adhesion molecule 1. *Arthritis Rheum.* **2000**, *43*, 1085. [CrossRef]

88. Kuryliszyn-Moskal, A.; Klimiuk, P.A.; Sierakowski, S. Soluble adhesion molecules (sVCAM-1, sE-selectin), vascular endothelial growth factor (VEGF) and endothelin-1 in patients with systemic sclerosis: Relationship to organ systemic involvement. *Clin. Rheumatol.* **2004**, *24*, 111–116. [CrossRef]

89. Trojanowska, M. Cellular and molecular aspects of vascular dysfunction in systemic sclerosis. *Nat. Rev. Rheumatol.* **2010**, *6*, 453–460. [CrossRef]

90. Giordano, N.; Papakostas, P.; Pecetti, G.; Nuti, R. Cytokine modulation by endothelin-1 and possible therapeutic implications in systemic sclerosis. *J. Biol. Regul. Homeost. Agents* **2011**, *25*, 487–492.

91. Liang, M.; Lv, J.; Jiang, Z.; He, H.; Chen, C.; Xiong, Y.; Zhu, X.; Xue, Y.; Yu, Y.; Yang, S.; et al. Promotion of Myofibroblast Differentiation and Tissue Fibrosis by the Leukotriene B_4–Leukotriene B_4 Receptor Axis in Systemic Sclerosis. *Arthritis Rheumatol.* **2020**, *72*, 1013–1025. [CrossRef] [PubMed]

92. Sobierajska, K.; Wawro, M.E.; Ciszewski, W.M.; Niewiarowska, J. Transforming Growth Factor-β Receptor Internalization via Caveolae Is Regulated by Tubulin-β2 and Tubulin-β3 during Endothelial-Mesenchymal Transition. *Am. J. Pathol.* **2019**, *189*, 2531–2546. [CrossRef] [PubMed]

93. Lee, W.J.; Park, J.H.; Shin, J.U.; Noh, H.; Lew, D.H.; Yang, W.I.; Yun, C.O.; Lee, K.H.; Lee, J.H. Endothelial-to-mesenchymal transition induced by Wnt 3a in keloid pathogenesis. *Wound Repair. Regen.* **2015**, *23*, 435–442. [CrossRef] [PubMed]

94. Pérez, L.; Muñoz-Durango, N.; Riedel, C.A.; Echeverría, C.; Kalergis, A.M.; Cabello-Verrugio, C.; Simon, F. Endothelial-to-mesenchymal transition: Cytokine-mediated pathways that determine endothelial fibrosis under inflammatory conditions. *Cytokine Growth Factor Rev.* **2017**, *33*, 41–54. [CrossRef] [PubMed]

95. Yoshimatsu, Y.; Wakabayashi, I.; Kimuro, S.; Takahashi, N.; Takahashi, K.; Kobayashi, M.; Maishi, N.; Podyma-Inoue, K.A.; Hida, K.; Miyazono, K.; et al. TNF-α enhances TGF-β-induced endothelial-to-mesenchymal transition via TGF-β signal augmentation. *Cancer Sci.* **2020**, *111*, 2385–2399. [CrossRef]

96. Saigusa, R.; Asano, Y.; Taniguchi, T.; Yamashita, T.; Ichimura, Y.; Takahashi, T.; Toyama, T.; Yoshizaki, A.; Sugawara, K.; Tsuruta, D.; et al. Multifaceted contribution of the TLR4-activated IRF5 transcription factor in systemic sclerosis. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 15136–15141. [CrossRef]

97. Miscianinov, V.; Martello, A.; Rose, L.; Parish, E.; Cathcart, B.; Mitić, T.; Gray, G.A.; Meloni, M.; Al Haj Zen, A.; Caporali, A. MicroRNA-148b Targets the TGF-β Pathway to Regulate Angiogenesis and Endothelial-to-Mesenchymal Transition during Skin Wound Healing. *Mol. Ther.* **2018**, *26*, 1996–2007. [CrossRef]

98. Xu, H.; Zaidi, M.; Struve, J.; Jones, D.W.; Krolikowski, J.G.; Nandedkar, S.; Lohr, N.L.; Gadicherla, A.; Pagel, P.S.; Csuka, M.E.; et al. Abnormal fibrillin-1 expression and chronic oxidative stress mediate endothelial mesenchymal transition in a murine model of systemic sclerosis. *Am. J. Physiol. Cell Physiol.* **2011**, *300*, C550–C556. [CrossRef]

99. Tang, H.; Babicheva, A.; McDermott, K.M.; Gu, Y.; Ayon, R.J.; Song, S.; Wang, Z.; Gupta, A.; Zhou, T.; Sun, X.; et al. Endothelial HIF-2α contributes to severe pulmonary hypertension due to endothelial-to-mesenchymal transition. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2018**, *314*, L256–L275. [CrossRef]

100. Vettori, S.; Cuomo, G.; Iudici, M.; D'Abrosca, V.; Giacco, V.; Barra, G.; De Palma, R.; Valentini, G. Early Systemic Sclerosis: Serum Profiling of Factors Involved in Endothelial, T-cell, and Fibroblast Interplay is Marked by Elevated Interleukin-33 Levels. *J. Clin. Immunol.* **2014**, *34*, 663–668. [CrossRef]

101. Manetti, M.; Guiducci, S.; Ceccarelli, C.; Romano, E.; Bellando-Randone, S.; Conforti, M.L.; Ibba-Manneschi, L.; Matucci-Cerinic, M. Increased circulating levels of interleukin 33 in systemic sclerosis correlate with early disease stage and microvascular involvement. *Ann. Rheum. Dis.* **2011**, *70*, 1876–1878. [CrossRef] [PubMed]

102. Terras, S.; Opitz, E.; Moritz, R.K.C.; Höxtermann, S.; Gambichler, T.; Kreuter, A. Increased serum IL-33 levels may indicate vascular involvement in systemic sclerosis. *Ann. Rheum. Dis.* **2012**, *72*, 144–145. [CrossRef] [PubMed]

103. Choi, Y.-S.; Choi, H.-J.; Min, J.-K.; Pyun, B.-J.; Maeng, Y.-S.; Park, H.; Kim, J.; Kim, Y.-M.; Kwon, Y.-G. Interleukin-33 induces angiogenesis and vascular permeability through ST2/TRAF6-mediated endothelial nitric oxide production. *Blood* **2009**, *114*, 3117–3126. [CrossRef] [PubMed]

104. Mostmans, Y.; Cutolo, M.; Giddelo, C.; Decuman, S.; Melsens, K.; Declercq, H.; Vandecasteele, E.; De Keyser, F.; Distler, O.; Gutermuth, J.; et al. The role of endothelial cells in the vasculopathy of systemic sclerosis: A systematic review. *Autoimmun. Rev.* **2017**, *16*, 774–786. [CrossRef]

105. Xing, X.; Yang, J.; Yang, X.; Wei, Y.; Zhu, L.; Gao, D.; Li, M. IL-17A induces endothelial inflammation in systemic sclerosis via the ERK signaling pathway. *PLoS ONE* **2013**, *8*, e85032. [CrossRef]

106. Ames, P.R.; Lupoli, S.; Alves, J.; Atsumi, T.; Edwards, C.; Iannaccone, L.; Khamashta, M.A.; Hughes, G.R.; Brancaccio, V. The coagulation/fibrinolysis balance in systemic sclerosis: Evidence for a haematological stress syndrome. *Rheumatology* **1997**, *36*, 1045–1050. [CrossRef]

107. Steen, V.; Denton, C.P.; Pope, J.E.; Matucci-Cerinic, M. Digital ulcers: Overt vascular disease in systemic sclerosis. *Rheumatology* **2006**, *48* (Suppl. 3), iii19–iii24. [CrossRef]

108. Hughes, M.; Herrick, A.L. Digital ulcers in systemic sclerosis. *Rheumatology* **2016**, *56*, 14–25. [CrossRef]

109. Bose, N.; Chiesa-Vottero, A.; Chatterjee, S. Scleroderma renal crisis. *Semin. Arthritis Rheum.* **2015**, *44*, 687–694. [CrossRef]

110. Simon, M.; Lücht, C.; Hosp, I.; Zhao, H.; Wu, D.; Heidecke, H.; Witowski, J.; Budde, K.; Riemekasten, G.; Catar, R. Autoantibodies from Patients with Scleroderma Renal Crisis Promote PAR-1 Receptor Activation and IL-6 Production in Endothelial Cells. *Int. J. Mol. Sci.* **2021**, *22*, 11793. [CrossRef]

111. Penn, H.; Quillinan, N.; Khan, K.; Chakravarty, K.; Ong, V.H.; Burns, A.; Denton, C.P. Targeting the endothelin axis in scleroderma renal crisis: Rationale and feasibility. *QJM* **2013**, *106*, 839–848. [CrossRef] [PubMed]

112. Giordano, N.; Corallo, C.; Chirico, C.; Brazzi, A.; Marinetti, A.; Fioravanti, A.; Valenti, R.; Nuti, R.; Pecetti, G. Pulmonary arterial hypertension in systemic sclerosis: Diagnosis and treatment according to the European Society of Cardiology and European Respiratory Society 2015 guidelines. *J. Scleroderma Relat. Disord.* **2019**, *4*, 35–42. [CrossRef] [PubMed]

113. Nold-Petry, C.A.; Rudloff, I.; Baumer, Y.; Ruvo, M.; Marasco, D.; Botti, P.; Farkas, L.; Cho, S.X.; Zepp, J.A.; Azam, T.; et al. IL-32 promotes angiogenesis. *J. Immunol.* **2014**, *192*, 589–602. [CrossRef] [PubMed]

114. Di Benedetto, P.; Guggino, G.; Manzi, G.; Ruscitti, P.; Berardicurti, O.; Panzera, N.; Grazia, N.; Badagliacca, R.; Riccieri, V.; Vizza, C.D.; et al. Interleukin-32 in systemic sclerosis, a potential new biomarker for pulmonary arterial hypertension. *Arthritis Res. Ther.* **2020**, *22*, 127. [CrossRef]

115. Cutolo, M.; Sulli, A.; Pizzorni, C.; Accardo, S. Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis. *J. Rheumatol.* **2000**, *27*, 155–160.

116. Grassi, W.; Medico, P.D.; Izzo, F.; Cervini, C. Microvascular involvement in systemic sclerosis: Capillaroscopic findings. *Semin. Arthritis Rheum.* **2001**, *30*, 397–402. [CrossRef]

117. Moschetti, L.; Piantoni, S.; Vizzardi, E.; Sciatti, E.; Riccardi, M.; Franceschini, F.; Cavazzana, I. Endothelial Dysfunction in Systemic Lupus Erythematosus and Systemic Sclerosis: A Common Trigger for Different Microvascular Diseases. *Front. Med.* **2022**, *9*, 849086. [CrossRef]

118. Flammer, A.J.; Anderson, T.; Celermajer, D.S.; Creager, M.A.; Deanfield, J.; Ganz, P.; Hamburg, N.M.; Lüscher, T.F.; Shechter, M.; Taddei, S.; et al. The assessment of endothelial function: From research into clinical practice. *Circulation* **2012**, *126*, 753–767. [CrossRef]

119. Ennis, H.; Hughes, M.; Anderson, M.E.; Wilkinson, J.; Herrick, A.L. Calcium channel blockers for primary Raynaud's phenomenon. *Cochrane Database Syst. Rev.* **2016**, *2*, CD002069. [CrossRef]

120. Tong Mak, I.; Zhang, J.; Weglicki, W.B. Protective effects of dihydropyridine Ca-blockers against endothelial cell oxidative injury due to combined nitric oxide and superoxide. *Pharmacol. Res.* **2002**, *45*, 27–33. [CrossRef]

121. Allanore, Y.; Borderie, D.; Lemaréchal, H.; Ekindjian, O.G.; Kahan, A. Acute and sustained effects of dihydropyridine-type calcium channel antagonists on oxidative stress in systemic sclerosis. *Am. J. Med.* **2004**, *116*, 595–600. [CrossRef] [PubMed]

122. Lareo, A.; Nuche, J.; Cristo Ropero, M.J.; Arribas Ynsaurriaga, F.; Oliver, E.; Escribano-Subías, P. Recent advances in the pharmacotherapy of pulmonary hypertension: Practical considerations. *Kardiol. Pol.* **2021**, *79*, 386–392. [CrossRef] [PubMed]

123. Kowal-Bielecka, O.; Fransen, J.; Avouac, J.; Becker, M.; Kulak, A.; Allanore, Y.; Distler, O.; Clements, P.; Cutolo, M.; Czirjak, L.; et al. Update of EULAR recommendations for the treatment of systemic sclerosis. *Ann. Rheum. Dis.* **2017**, *76*, 1327–1339. [CrossRef] [PubMed]

124. Sandner, P. From molecules to patients: Exploring the therapeutic role of soluble guanylate cyclase stimulators. *Biol. Chem.* **2018**, *399*, 679–690. [CrossRef]

125. Romano, E.; Rosa, I.; Fioretto, B.S.; Giuggioli, D.; Manetti, M.; Matucci-Cerinic, M. Soluble guanylate cyclase stimulation fosters angiogenesis and blunts myofibroblast-like features of systemic sclerosis endothelial cells. *Rheumatology* **2022**, *62*, SI125–SI137. [CrossRef]

126. Iannone, F.; Riccardi, M.T.; Guiducci, S.; Bizzoca, R.; Cinelli, M.; Matucci-Cerinic, M.; Lapadula, G. Bosentan regulates the expression of adhesion molecules on circulating T cells and serum soluble adhesion molecules in systemic sclerosis-associated pulmonary arterial hypertension. *Ann. Rheum. Dis.* **2008**, *67*, 1121–1126. [CrossRef]

127. Corallo, C.; Cutolo, M.; Kahaleh, B.; Pecetti, G.; Montella, A.; Chirico, C.; Soldano, S.; Nuti, R.; Giordano, N. Bosentan and macitentan prevent the endothelial-to-mesenchymal transition (EndoMT) in systemic sclerosis: In vitro study. *Arthritis Res. Ther.* **2016**, *18*, 228. [CrossRef]

128. Proietti, M.; Aversa, A.; Letizia, C.; Rossi, C.; Menghi, G.; Bruzziches, R.; Merla, A.; Spera, G.; Salsano, F. Erectile dysfunction in systemic sclerosis: Effects of longterm inhibition of phosphodiesterase type-5 on erectile function and plasma endothelin-1 levels. *J. Rheumatol.* **2007**, *34*, 1712–1717.

129. Tsou, P.-S.; Palisoc, P.J.; Flavahan, N.A.; Khanna, D. Dissecting the Cellular Mechanism of Prostacyclin Analog Iloprost in Reversing Vascular Dysfunction in Scleroderma. *Arthritis Rheumatol.* **2021**, *73*, 520–529. [CrossRef]

130. Borghini, A.; Manetti, M.; Nacci, F.; Bellando-Randone, S.; Guiducci, S.; Matucci-Cerinic, M.; Ibba-Manneschi, L.; Weber, E. Systemic Sclerosis Sera Impair Angiogenic Performance of Dermal Microvascular Endothelial Cells: Therapeutic Implications of Cyclophosphamide. *PLoS ONE* **2015**, *10*, e0130166. [CrossRef]

131. Caramaschi, P.; Volpe, A.; Pieropan, S.; Tinazzi, I.; Mahamid, H.; Bambara, L.M.; Biasi, D. Cyclophosphamide treatment improves microvessel damage in systemic sclerosis. *Clin. Rheumatol.* **2008**, *28*, 391–395. [CrossRef] [PubMed]

132. Apras, S.; Ertenli, I.; Ozbalkan, Z.; Kiraz, S.; Ozturk, M.A.; Haznedaroglu, I.C.; Çobankara, V.; Pay, S.; Calguneri, M. Effects of oral cyclophosphamide and prednisolone therapy on the endothelial functions and clinical findings in patients with early diffuse systemic sclerosis. *Arthritis Rheum.* **2003**, *48*, 2256–2261. [CrossRef] [PubMed]

133. Furuya, Y.; Okazaki, Y.; Kaji, K.; Sato, S.; Takehara, K.; Kuwana, M. Mobilization of endothelial progenitor cells by intravenous cyclophosphamide in patients with systemic sclerosis. *Rheumatology* **2010**, *49*, 2375–2380. [CrossRef] [PubMed]

134. Kuwana, M.; Kaburaki, J.; Okazaki, Y.; Yasuoka, H.; Kawakami, Y.; Ikeda, Y. Increase in circulating endothelial precursors by atorvastatin in patients with systemic sclerosis. *Arthritis Rheum.* **2006**, *54*, 1946–1951. [CrossRef] [PubMed]

135. Del Papa, N.; Cortiana, M.; Vitali, C.; Silvestris, I.; Maglione, W.; Comina, D.P.; Lucchi, T.; Cortelezzi, A. Simvastatin reduces endothelial activation and damage but is partially ineffective in inducing endothelial repair in systemic sclerosis. *J. Rheumatol.* **2008**, *35*, 1323–1328. [PubMed]

136. Chen, K.; Pittman, R.N.; Popel, A.S. Nitric oxide in the vasculature: Where does it come from and where does it go? A quantitative perspective. *Antioxid. Redox Signal.* **2008**, *10*, 1185–1198. [CrossRef]

137. Kan, C.; Akimoto, S.; Abe, M.; Okada, K.; Ishikawa, O. Preliminary thermographic evaluation of new nitroglycerine tape on the peripheral circulatory disturbance in systemic sclerosis. *Ann. Rheum. Dis.* **2002**, *61*, 177–179. [CrossRef]

138. Hummers, L.K.; Dugowson, C.E.; Dechow, F.J.; Wise, R.A.; Gregory, J.; Michalek, J.; Yenokyan, G.; McGready, J.; Wigley, F.M. A multi-centre, blinded, randomised, placebo-controlled, laboratory-based study of MQX-503, a novel topical gel formulation of nitroglycerine, in patients with Raynaud phenomenon. *Ann. Rheum. Dis.* **2012**, *72*, 1962–1967. [CrossRef]

139. Hughes, M.; Moore, T.; Manning, J.; Wilkinson, J.; Dinsdale, G.; Roberts, C.; Murray, A.; Herrick, A.L. Reduced perfusion in systemic sclerosis digital ulcers (both fingertip and extensor) can be increased by topical application of glyceryl trinitrate. *Microvasc. Res.* **2017**, *111*, 32–36. [CrossRef]

140. Guo, J.; Nguyen, A.; Banyard, D.A.; Fadavi, D.; Toranto, J.D.; Wirth, G.A.; Paydar, K.Z.; Evans, G.R.D.; Widgerow, A.D. Stromal vascular fraction: A regenerative reality? Part 2: Mechanisms of regenerative action. *J. Plast. Reconstr. Aesthetic Surg.* **2016**, *69*, 180–188. [CrossRef]

141. Suga, H.; Glotzbach, J.P.; Sorkin, M.; Longaker, M.T.; Gurtner, G.C. Paracrine mechanism of angiogenesis in adipose-derived stem cell transplantation. *Ann. Plast. Surg.* **2014**, *72*, 234–241. [CrossRef] [PubMed]

142. Rehman, J.; Traktuev, D.; Li, J.; Merfeld-Clauss, S.; Temm-Grove, C.J.; Bovenkerk, J.E.; Pell, C.L.; Johnstone, B.H.; Considine, R.V.; March, K.L. Secretion of Angiogenic and Antiapoptotic Factors by Human Adipose Stromal Cells. *Circulation* **2004**, *109*, 1292–1298. [CrossRef] [PubMed]

143. Cao, Y.; Kan, H.; Ma, X.; Zhang, Y.; Huang, J.; Long, X. Autologous fat or adipose-derived stem cells grafting in systemic sclerosis treatment: A systematic review and meta-analysis. *Clin. Exp. Rheumatol.* **2023**, *41*, 1659–1669. [CrossRef] [PubMed]

144. Daikeler, T.; Kump, E.; Stern, M.; Hügle, T.; Hij, A.; Haeuserman, P.; Farge, D. Autologous hematopoietic stem cell transplantation reverses skin fibrosis but does not change skin vessel density in patients with systemic sclerosis. *Pathol. Biol.* **2015**, *63*, 164–168. [CrossRef] [PubMed]

145. Miniati, I.; Guiducci, S.; Conforti, M.L.; Rogai, V.; Fiori, G.; Cinelli, M.; Saccardi, R.; Guidi, S.; Bosi, A.; Tyndall, A.; et al. Autologous stem cell transplantation improves microcirculation in systemic sclerosis. *Ann. Rheum. Dis.* **2008**, *68*, 94–98. [CrossRef]

146. Farge, D.; Loisel, S.; Lansiaux, P.; Tarte, K. Mesenchymal stromal cells for systemic sclerosis treatment. *Autoimmun. Rev.* **2021**, *20*, 102755. [CrossRef]