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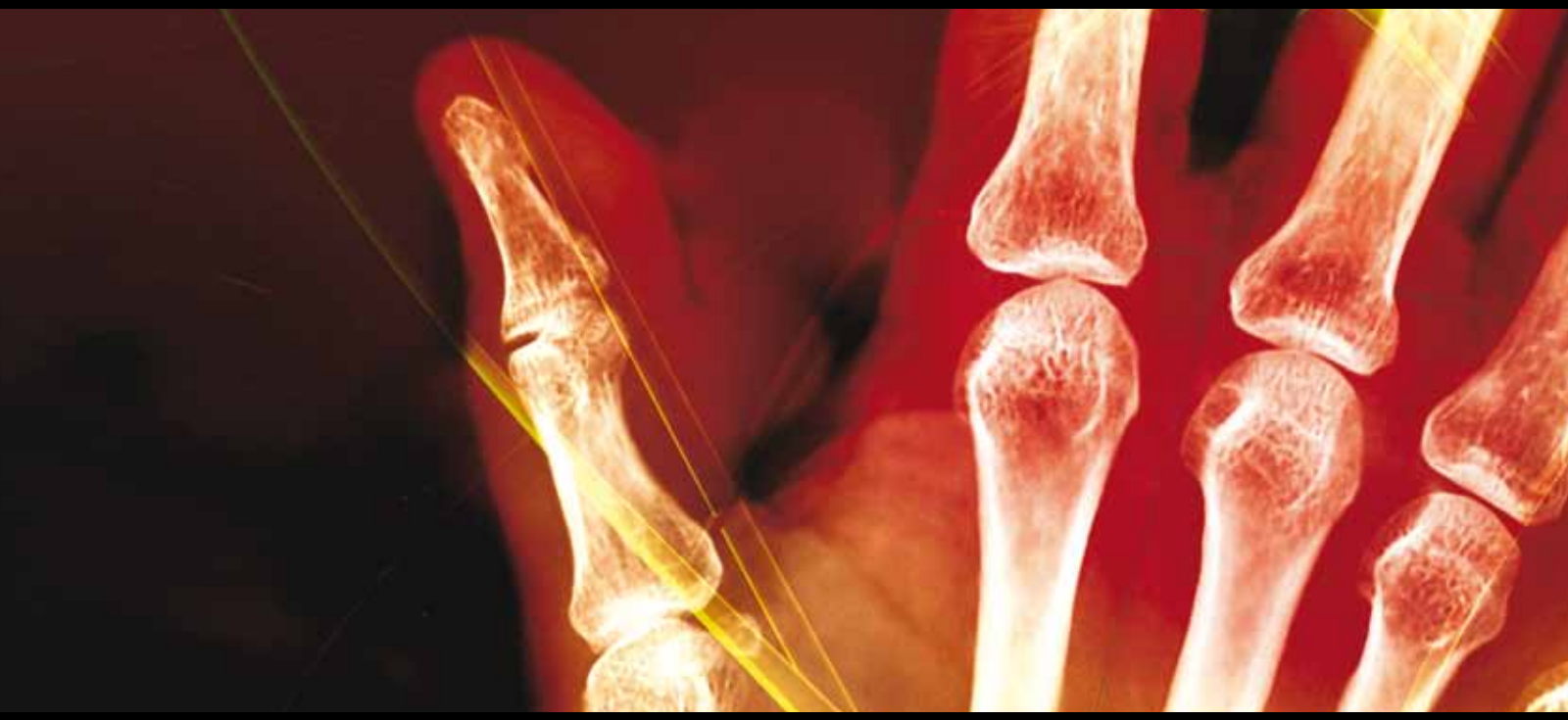
Vascular Disease in Systemic Sclerosis

Guest Editors: Lorinda Chung, Oliver Distler, Laura Hummers, Eswar Krishnan, and Virginia Steen



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Editorial Board

Contents

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Editorial

Vascular Disease in Systemic Sclerosis

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Systemic sclerosis (SSc) is an autoimmune disease characterized by widespread fibrosis affecting the skin, internal organs, and vasculature. Vascular disease with intimal proliferation and obliterative vasculopathy is extremely prevalent in SSc, most commonly manifesting as Raynaud's phenomenon and digital ulcerations. Other manifestations of vascular disease occur less frequently in patients with SSc, including ischemic digital loss, pulmonary arterial hypertension (PAH), and renal crisis. Vascular disease in SSc may also commonly affect pregnancy outcomes and sexual function. Finally, patients with SSc can rarely suffer from inflammatory vasculitis, and recognition of this has a significant impact on therapy. In this special issue on vascular disease in systemic sclerosis, we invited original research articles and review articles regarding the pathogenesis, epidemiology, natural history, evaluation, and/or management of vascular complications in patients with SSc.

The first paper of this special issue describes the vascular microenvironment that likely contributes to the pathogenesis of vasculopathy in SSc. This is followed by two papers of the current knowledge regarding the pathogenesis, evaluation, and management of digital ulcers and digital ischemic loss in patients with SSc. The fourth paper of the special issue is an original research article that describes the prevalence of and risk factors for nondigital lower extremity ulcers in SSc. The authors found that antiphospholipid antibodies and genetic prothrombotic mutations are highly prevalent in SSc patients with lower extremity ulcers. The next paper describes the utility of registries in understanding the natural history of digital ulcers and discusses the need for classification criteria for the assessment of digital ulcers. This is followed by a review article discussing the potential use of nailfold videocapillaroscopy as an outcome measure in clinical trials for patients with SSc and vascular complications, particularly digital ulcers and PAH.

The next section of the special issue relates to cardiopulmonary vascular complications in SSc, with a particular focus on PAH and right ventricular failure. The first paper in this section describes a case report of a patient with mixed connective tissue disease and severe, refractory PAH who experienced dramatic improvement in functional ability and hemodynamics in response to treatment with tocilizumab, a humanized monoclonal antibody to the human interleukin-6 receptor. This is followed by an original research article evaluating the relationship of serum endoglin levels in patients with and without elevated systolic pulmonary arterial pressures (sPAP) on echocardiography. This study found that SSc patients with and without elevated sPAP had much higher levels of serum endoglin compared with healthy controls, suggesting that endoglin may be a potential biomarker of vasculopathy that is not specific to the pulmonary vasculature. The next paper describes a histopathogic comparison of samples from the right ventricle of patients with SSc-associated PAH and idiopathic PAH (IPAH). The authors found that the right ventricular samples from patients with SSc-associated PAH showed

more inflammatory infiltrates than those from patients with IPAH, but the degree of interstitial fibrosis was similar in the two groups. The final paper in this section is a review describing available nuclear cardiology imaging modalities that may be useful in the assessment of early vascular disease and myocardial damage in patients with SSc.

The next section of the special issue is focused on renal manifestations of SSc. The first paper reviews the types of renal involvement seen in SSc and the potential utility of screening patients for subclinical renal disease. The next paper describes the histopathologic findings characteristic of scleroderma renal crisis (SRC) and the potential role of renal biopsies in predicting prognosis. Finally, an original research article describes the methodology and preliminary data of an international web-based prospective study designed to determine if the use of angiotensin converting enzyme inhibitors prior to the onset of SRC is associated with worse outcomes.

The subsequent section of the special issue addresses the effects of vascular disease on pregnancy and sexual function in patients with SSc. The first paper reviews the published literature regarding the effects of vascular complications, such as PAH and SRC, on pregnancy outcomes in patients with SSc. This is followed by review articles focusing on sexual dysfunction in patients with SSc, including male erectile dysfunction and female sexual arousal disorder. These papers also address the role of phosphodiesterase-5 inhibitors in the treatment of SSc patients with sexual dysfunction.

The final section includes two papers describing the known data on inflammatory vasculitis in patients with SSc. The first focuses on microscopic polyangiitis, while the latter article reviews the literature regarding small, medium, and large vessel vasculitis in SSc.

In summary, this special issue provides a comprehensive compilation of articles with novel information relevant to the topic of vascular disease in SSc. The papers included herein provide the framework for the current and future development of important biomarkers, outcome assessments for clinical trials, and novel therapeutic modalities for patients with SSc and vascular complications. We, the guest editors, hope that readers find this information useful in the care of patients with SSc.

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Review Article

The Vascular Microenvironment and Systemic Sclerosis

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The role of the vascular microenvironment in the pathogenesis Systemic Sclerosis (SSc) is appreciated clinically as Raynaud's syndrome with capillary nail bed change. This manifestation of vasculopathy is used diagnostically in both limited and diffuse cutaneous subsets of SSc, and is thought to precede fibrosis. The degree of subsequent fibrosis may also be determined by the vascular microenvironment. This paper describes why the vascular microenvironment might determine the degree of end-organ damage that occurs in SSc, with a focus on vascular cell senescence, endothelial progenitor cells (EPC) including multipotential mesenchymal stem cells (MSC), pericytes, and angiogenic monocytes. An explanation of the role of EPC, pericytes, and angiogenic monocytes is important to an understanding of SSc pathogenesis. An evolving understanding of the vascular microenvironment in SSc may allow directed treatment.

1. Introduction

Systemic sclerosis (SSc, scleroderma) is an autoimmune disease characterized early in the process by vasculopathy and subsequently by varying degrees of fibrosis in skin, lungs, and other tissues. The presence of vasculopathy is the hallmark of this condition, represented clinically as Raynaud's syndrome which occurs almost universally in both the limited and diffuse cutaneous subsets of this disease. Calcinosis and telangiectasias are also features of SSc vascular damage. Vasculopathy possibly results from abnormal vasoreactivity, hypoxia, and/or direct damage of vascular and perivascular cells [1]. Perivascular inflammatory infiltrates and neoangiogenesis ensues resulting in varying degrees of fibrosis in the skin and internal organs [2]. This paper describes why details of the vascular microenvironment might determine the degree of end-organ damage that occurs in SSc, with a focus on vascular cell senescence, endothelial progenitor cells (EPC) including mesenchymal stem cells (MSC), pericytes, and angiogenic monocytes. An explanation of the role of EPC, pericytes, and angiogenic monocytes is important to understanding SSc pathogenesis.

SSc is thought to be a genetically complex disease, influenced by multiple genes, with a substantial environmental component [3]. Nonetheless, SSc occurs significantly more frequently in families with scleroderma (1.6%) than in the general population (0.026%) [4]. Genome-wide association studies have found a strong association with the HLA II region on chromosome 6, and non-HLA candidate genes that regulate interferon production, such as interferon regulatory factor 5 (IRF 5) as well as genes that regulate immunological responses, such as signal transducer and activator 4 (STAT 4) [5, 6]. There are also multiple HLA class II associations with autoantibody markers and subphenotypes [7]. As such, systemic sclerosis is an autoimmune disease; however the inherited effects of vasculopathy and fibrosis remain to be determined. Our previous work showed that vasculopathy imparts a greater relative risk to family members than does autoimmune inflammatory conditions or fibrotic lung disease [8].

2. Vascular Senescence

The microvascular environment in SSc has a reduced density and disorganized structure [9]. Irrespective of the subset of SSc, perivesicular inflammatory infiltrates result in endothelial derangement in lesioned as well as perilesional tissue [10, 11]. These perivascular changes precede the excessive accumulation of extracellular matrix components, and fibrosis may represent a default pathway from vascular failure [12, 13]. The histopathological hallmark in SSc is a result of endothelium activation with cell adhesion molecule expression, inflammatory cell recruitment, intimal proliferation, and adventitial fibrosis, which results in apoptosis of endothelial cells [13, 14]. Despite the ensuing severe tissue hypoxia, proper adapted angiogenesis does not occur in SSc [2].

Vascular cells normally have a finite lifespan which is determined in part by telomere length and/or telomerase activity [15]. Telomerase is a reverse transcriptase which helps maintain telomere length, thereby preventing cell senescence and protecting chromosomes from aberration. Although telomerase activity is increased in many connective tissue diseases, it is decreased in systemic sclerosis (SSc), perhaps due to gene polymorphism [16, 17].

There have been contrasting reports of telomere length in SSc. Artlett and colleagues reported a decrease telomere length in a combined cohort of limited SSc (lSSc) and diffuse SSc (dSSc) whereas MacIntyre and colleagues reported increased telomere length and lack of age-related telomere erosion in lSSc [18, 19]. In a pilot study, we used a monochrome multiplex quantitative PCR (MMQPCR) method to evaluate the relative telomere lengths (*t/s* ratios) in DNA samples of 6 lSSc (1 male; 5 females) and 6 dSSc (3 males; 3 females) aging 40–60, and 50 healthy controls (HC) aging 37–60 [20]. Two factors were statistically associated (P value $< .001$) to *t/s*: age and diagnosis (Figure 1). Not correcting for age, the average length measure was 1.2 for normals, 1.15 for dSSc and 0.96 for lSSc patients (Figure 2). Gender was not statistically associated with *t/s*. Telomere length, which is shorter in SSc patients than in normal HC, is possibly a risk factor for vasculopathy. While the appearance of vasculopathy does not vary per subtype of SSc, the effect of telomere length on the fibrocyte or myofibroblast may be different in lSSc and dSSc, possibly contributing to differences in disease manifestations. The reduced telomere length in the endothelial cell likely results in chronic underperfusion and ischemia in the skin and internal organs in both lSSc and dSSc. However, if fibrosis is the default pathway of insufficient angiogenic response, the subsequent reduced lifespan of the fibrocyte (determined by telomere length) may be protective in the lSSc subtype.

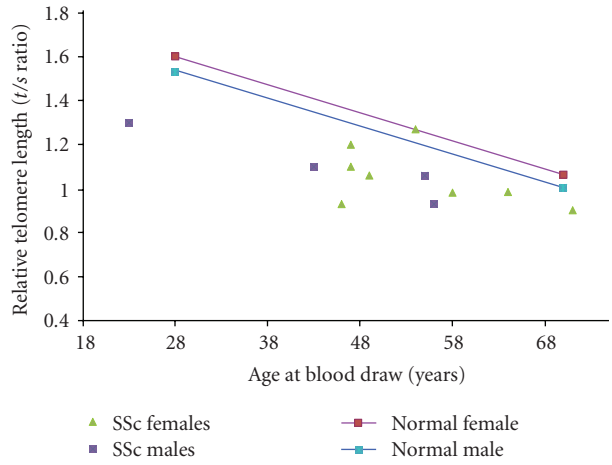

Figure 1: Telomere length of females and males with Systemic Sclerosis (SSc) compared to Healthy Controls.

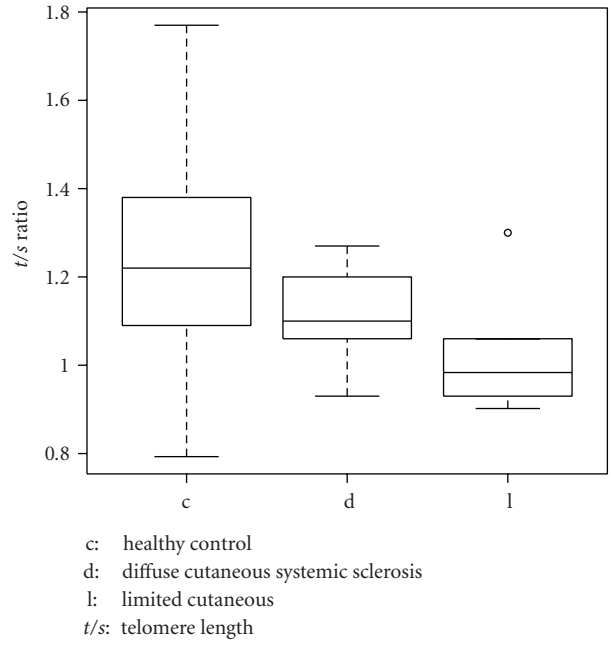

Figure 2: Telomere length of Healthy Controls, diffuse cutaneous Systemic Sclerosis, limited cutaneous Systemic Sclerosis.

3. Endothelial Progenitor Cells and Pericytes

The vascular network is a dynamic organ with an estimated surface area of $>1000\,m^2$ [21]. Neovascularization is a complex process that requires both the mobilization of cells derived from the bone marrow, named endothelial progenitor cells (EPCs), and proliferation and differentiation of resident cells, known as pericytes, to migrate to the correct location and assemble into vascular structures [22].

New vessels are produced by a combination of angiogenesis and vasculogenesis. In angiogenesis, fully differentiated endothelial cells arise from pre-existing vessels whereas vasculogenesis describes the formation of new vessels by circulating EPC which act to replenish damaged or senescent blood vessels [14]. This process requires dynamic and temporally regulated interactions between endothelial cells, soluble proangiogenic and antiangiogenic growth factors, and extracellular matrix molecules [23].

Primary contact between endothelial cells and mural cells (pericyte and vascular smooth muscle cells) is central

to the regulation of vascular formation in angiogenesis [24]. Recently formed endothelial tubes are initially unstable and become stabilized through the formation of a perivascular matrix and the connection with pericytes [25]. Pericytes are embedded within the endothelial basement membrane and are found primarily around blood capillaries, precapillary arterioles, postcapillary venules, and collecting venules [26]. They are arranged to facilitate and assimilate cell communication. With particular interest to SSc, pericytes may play a role in ectopic calcification and are able to transdifferentiate into fibroblast-like cells if they escape from the capillary basement membrane [27]. Furthermore, mural cell defects are reported in other diseases characterized by telangiectasias [28]. The pericyte role as a perivasicular mesenchymal stem cell with macrophage-like properties has not been welldefined in SSc, but is intriguing.

The pericyte is critical for maintenance of vascular stability. Its ability to perform this function is correlated with marker expression and the microenvironment of the endothelial-pericyte contact. Most likely, specific intercellular signals mediated by ligand-receptor systems are required for endothelial and pericyte vascular stability [24]. Numerous studies demonstrate the critical importance of transforming growth factor- (TGF)-beta signaling for vascular development and function [24]. TGF-beta has context-dependent effects on endothelial cells; proliferation is mediated by signaling through ALK/Smad1/5 and differentiation is mediated by ALK Smad2/3 [29]. TGF-beta/Smad signaling has been suggested to play a key role in the pathogenesis of SSc [30].

In postnatal vasculogenesis, pericytes develop from tissue-derived stem cells and/or peripheral EPC [31]. Identification and quantification of EPC population in SSc has been challenging and has resulted in consensus recommendations to help unify EPC research [14]. Research by Avouac and colleagues, using an accurate, reliable, and reproducible method of EPC quantification, supports that SSc is associated with EPC mobilization, but in active or severe stages, EPC may be recruited to injured sites and thus decrease in the circulation [9].

Multipotential mesenchymal stem cells (MSCs) might be a source of EPC in vasculogenesis [2]. MSCs show normal functional properties and a normal pattern of biological markers, but the angiogenic potential of these endothelial-like MSCs is reduced [32]. Cipriani and colleagues showed that when MSC from SSc patients are seeded on Matrigel, they have a reduced ability to form capillary-like structures and give rise to incomplete endothelial networks, even after vascular endothelial growth factor (VEGF) and stromal-derived factor (SDF-1) stimulation [23, 32].

VEGF is an important angiogenic peptide with specific proliferative, differentiation, and mobilization effects on EPCs, and is known to be upregulated in SSc, especially in advanced disease [33]. VEGF gene expression is also regulated by growth factors (such as TGF-beta) and other proinflammatory cytokines. The platelet-derived growth factor (PDGF) family is essential to vascular remodeling and maturation [34]. In a study of 62 SSc patients, EPCs were significantly increased in patients with early-stage SSc disease, but not in those with late disease irrespective of diagnosis subtype, and there was no correlation between the number of circulating EPCs and VEGF [24]. Bone marrow biopsy samples from 14 of these SSc patients (3 early limited SSc, 4 with late limited SSc, 4 with early diffuse SSC, and 3 with late SSc) showed fewer and functionally impaired EPCs in all patients [33]. Another study showed that the subset of SSc patients with digital lesions and high severity scores had low EPC counts [35]. It is possible that bone marrow from SSc patients cannot satisfy the continuous and prolonged demand for EPCs, despite the target organ increase in VEGF [33].

The role of target organ microvascular environment (pericytes and endothelial cells), which is producing TGF-beta, VEGF, and PDGF, on SSc pathogenesis is less clear. TGF-beta can be either pro- or antiangiogenic based on its concentration [36]. The elevated total number and activated state of circulating endothelial cells (CECs), suggest vascular damage and endothelial activation in SSc patients regardless of subtype correlates to disease activity [37]. Thus, vascular damage may drive the disease. It is also known that TGF-beta and PDGF from this microvasculature cooperate in inducing the activation of fibroblasts and their differentiation into myofibroblasts in SSc patients [38]. Thus, understanding the microvascular environment of target organs in SSc is of primary importance.

4. Angiogenic Monocytes

It is suggested that the major contribution of the bone marrow to angiogenic processes may come from progenitors of the periendothelial vascular mural cells [39]. Endothelial differentiation of monocyte-derived multipotential cells (MOMCs) can occur with angiogenic stimuli and result in the formation of mature endothelial cell tubules in Matrigel cultures [40]. Pericytes establish morphologic interactions with transmigrating leukocytes, mainly monocytes (macrophages) [31]. During angiogenesis, macrophages contribute to the dissociation and detachment of pericytes from the endothelial cell. Pericytes can act as antigen-presenting cells and can behave as macrophages; they also can show plasticity with potential to become myofibroblasts [31]. Thus, understanding the role of the interaction of circulating angiogenic monocyte and resident pericyte in SSc microvasculature has important implications. It is possible that this interaction is of primary importance for linking the inflammatory aspect of the disease to the vascular abnormalities.

Stromal cell-derived factor-1 (SDF-1) and its receptor (CXCR4) system is a component of the microvascular environment which is extremely important for new vessel formation. SDF-1 released by endothelial cells creates a gradient dictating directional response of endothelial cells expressing CXCR4 [41]. Skin biopsies in early disease of both SSc subtypes show a strong positive pattern of SDF-1 and its receptor CXCR4 in the endothelial cells and pericytes of microvessels, attesting to an attempted reparative process [42]. Of interest, in diffuse SSc, these skin biopsies

also showed dense mononuclear cells in the perivascular infiltrate, possibly suggesting a role of the monocytes in a more fibrotic phenotype. The staining for CXCR4 was weak in the late (sclerotic or atrophic) phases in both SSc subsets [42]. Another study of 40 SSc patients demonstrated higher serum levels of VEGF, PDGF, and increased concentration of SDF-1, particularly in the diffuse subset. In this same study population circulating CXCR4+ circulating progenitor cells coexpressing monocytic and endothelial cells positively correlated to the severity score, modified Rodnan skin score, and pulmonary involvement [43]. Taken in sum, these results suggest that overall disease activity correlates to the markers of activity in the microvascular environment.

It has recently been suggested that the actual angiogenic cell type recruited to the site of tissue injury and incorporated into a newly formed vessel is a monocyte [44]. Activated circulating monocytes have also been reported in SSc patients, supporting a potential role of these cells in disease pathogenesis [45]. Gene expression profiling of peripheral blood monocytes from SSc patients suggest that type I interferon may play a key role in the activation of monocytes in this disease [46]. If during the course of the disease, the mechanism of angiogenesis is impaired, the proangiogenic factors in the microvascular environment may serve to recruit proangiogenic monocytes which, with pericytes, result in overactivity of a myofibroblast phenotype. In a preliminary study, there were no significant differences in the expression of circulating monocyte surface molecules involved with cell transformation, function, or migration presumed to give rise to fibrocytes, in 8 patients with limited SSc [47]. It is possible that the role of the angiogenic monocyte may be greater in the diffuse subset of SSc and have prognostic implications.

5. Implications of the Vascular Microenvironment on Treatment

An evolving understanding of the vascular microenvironment in SSc may allow directed treatment. Therapeutics that modulate the phenotype of reparative cells can offer new opportunities for SSc treatment [48]. In particular, multipotential MSCs have attracted interest because of low acute toxicity and their availability [49]. The potential of human MOMCs which can proliferate and differentiate along the endothelial lineage in a specific permissive environment also may represent an autologous transplantable cell source for therapeutic neovasculogenesis [40]. In early SSc, prevention of vascular senescence may be most important. N-acetyl-cysteine (NAC), a chemopreventive antiangiogenic and antiapoptotic drug has been suggested to modulate parameters associated with endothelial cell aging [50]. Pilot data suggests that the statin class of medications may be beneficial in treating vascular manifestations of SSc, through an increase in angiogenic factors and reduction of vascular endothelial activation/injury markers ($P < .01$ for all comparisons) [51]. However, this treatment did not correct the defect in EPC recruitment. Cyclophosphamide, which remains the current gold standard for treatment of interstitial lung disease, is known to mobilize EPC [52]. Nutraceutical-based mobilization of EPC is an area of interest in the biomedical field, and has not yet been reported in SSc [53].

For the fibrotic aspect of SSc, the small molecule tyrosine kinase inhibitor imatinib and related drugs, such as dasatinib and nilotinib, which simultaneously target two of the major profibrotic pathways, TGF-beta- and PDGF-signaling are being studied [54]. The effect of these drugs on the microvascular environment, and their efficacy and tolerability in SSc patients are not yet known. Other anti-TGF-beta therapies are also in development and may have a major impact in systemic sclerosis. However, considerable concern regarding safety is needed given its pro- and antiangiogenic effects at different concentrations [55]. IFN inhibitors are also under investigation for treatment of SSc, though modulation of interferon may be most effective in the diffuse subset, in which there is a higher perivascular monocyte infiltrate [56]. Specifically, therapies that inhibit transdifferentiation of other cell types, such as pericytes and angiogenic monocytes into fibroblasts and myofibroblasts hold promise [57].

6. Conclusion

A predisposition to vascular senescence is probable in SSc and the pathogenesis may arise from a subsequent defect in vasculogenesis (possibly due to abnormal bone marrow function) and/or angiogenesis (perhaps due to pericyte and angiogenic monocytes) followed by overactivity of activated fibroblasts and myofibroblasts. Understanding the role of the vascular microenvironment will be critical to development of directed therapeutics.

Early SSc may be most amenable to treatments that decrease vascular senescence and increase EPC mobilization. Surprisingly, diffuse cutaneous SSc may be more responsive to therapeutics, which modulate pericyte and angiogenic monocyte differentiation into activated fibroblasts and myofibroblasts. The difficulty with therapeutics which modulate growth factor and chemokines is that locally varying levels of these substances are necessary for regulation of migration, proliferation, cell-cell interactions, differentiation, and extracellular matrix deposition [31]. Nonetheless, an improved understanding of the principle regulatory mechanisms of angiogenesis in SSc has profound potential therapeutic value. It is exciting to think that through understanding of the microvascular environment in SSc, that subsequent restoration of proper angiogenesis in SSc could limit fibrotic damage.

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Review Article

Digital Ischemia in Scleroderma Spectrum of Diseases

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Systemic Sclerosis (Scleroderma, SSc) is a disease of unknown etiology characterized by widespread vasculopathy and extracellular matrix deposition leading to fibrosis and autoimmune processes. Digital ischemia (digital ulcers (DUs)) is the hallmark of SSc-related vasculopathy and is characterized by endothelial dysfunction leading to intimal proliferation and thrombosis. It happens frequently (30% of the patients each year) and it is associated with significant morbidity. This paper summarizes the current information regarding pathogenesis, definitions, management, and exploratory therapies in DUs associated with SSc.

1. Introduction

The subject of digital ischemia in systemic sclerosis is complex and poses various challenges in terms of diagnosis, classification, risk factors, therapy, and morbidity. Raynaud's phenomenon (RP), characterized by exaggerated but reversible vasospasm in response to cold or stress, is the presenting symptom in over 90% of the patients with SSc [1]. RP is the most common manifestation of the SSc-related endothelial dysfunction and digital ulcers (DUs) are a clinical manifestation of SSc-related vasculopathy.

Digital ulcers in SSc are defined as necrotic lesions occurring at the distal aspects of fingers or toes. The underlying phenomenon is compromise of the arterial lumen which occurs as a combination of 2 major contributing factors:

(1) vascular wall structural (intimal proliferation) and functional (overproduction of vasoconstrictors) abnormalities,

(2) a variable degree of intraluminal thrombosis.

DUs are painful, heal slowly, and lead to a great deal of disability. Due to the inadequate blood supply and break in the skin, the ischemic lesions are prone to infection, loss of digital tissue, and progression to gangrene that requires amputation.

Currently, there is no official algorithm for diagnosis and therapy of digital ischemia in SSc. A conventional therapeutic approach to digital lesions should include vasoactive medications, antiplatelet agents, antibiotics as needed, and analgesia. The response to vasodilators in patients with SSc is variable and often disappointing. There is a visible need for strategies to facilitate healing of the DUs and to prevent occurrence of new ones.

2. Definition of Digital Ulcers in SSc

The correct diagnosis of DUs is instrumental both in clinical practice and in clinical trials focused on digital ischemia. Almost all SSc patients experience involvement of their hands: ischemic lesions, local infection, calcinosis, and traumatic ulcers occurring in areas affected by flexion contractions. Although the SSc-related vasculopathy affects the healing time of all the acral lesions, it is crucial to clinically define the true ischemic lesion.

A recent study tested the intra- and interobserver variability in defining DUs among clinicians with an interest in scleroderma [2]. 50 individuals (mostly rheumatologists) were shown pictures of various hand lesions and were asked to qualify the lesions ("ulcer" versus "no ulcer") and if "ulcer", to quantify it as "active" or "inactive". Although the intrarater reliability was high (average kappa value of 0.81), the interrater reliability was poor (kappa coefficient

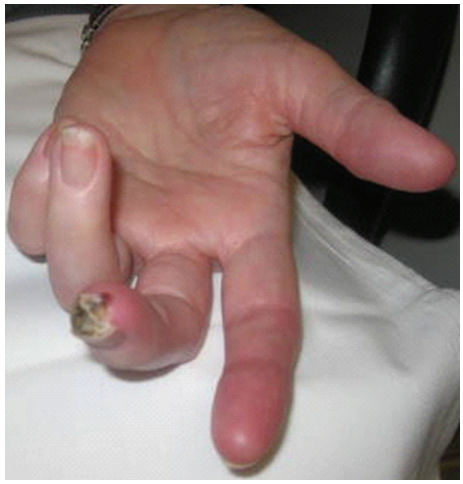

Figure 1: True digital ulcer.

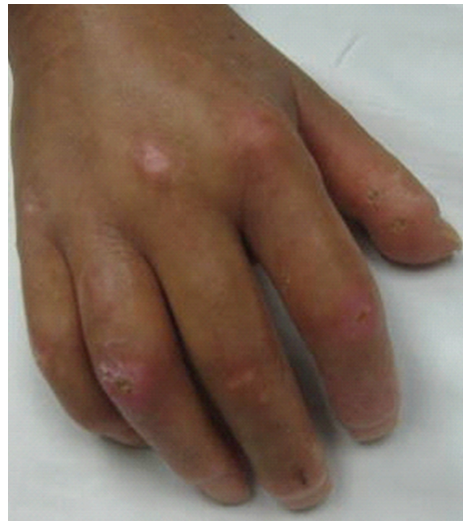

Figure 2: Traumatic ulcer.

of 0.46), so individual examiners were consistent with their assessment, while different examiners disagreed. This lack of agreement among rheumatologists who evaluate digital lesions on a regular basis may have an impact on interpretation of the results of clinical studies and more so on initiating and maintaining treatment of DUs in clinical practice.

One of the more precise definitions of SSc-DUs was described in the RAPIDS-1 clinical trial [3]. The definition was based on expert consensus and is currently used in the majority of trials focused on DUs. Digital ulcers are defined as a denuded area with well demarcated borders, involving loss of dermis and epidermis. They are located on the volar surface of the fingers, distal to the proximal interphalangeal joints (Figure 1). The DUs do not occur in the interphalangeal creases and should not be confused with paronychia, areas with underlining calcinosis, or traumatic lesions located on the dorsum of the hands (PIPs or MCPs) (Figure 2). A recent article focused on definitions and subclasses of SSc-DUs (1614 digital lesions were prospectively observed over 4 years) [4]. The digital lesions were classified as digital pitting scars (DPSs), DUs, calcinosis, and gangrene. Clinical characteristics, depth (superficial, intermediate, and deep) and time to healing of the lesions were recorded. The overwhelming majority of digital lesions, were DUs and DPSs (92.7%). The digital lesions were located more frequently on the second and third digit and mostly on the fingertip area. Presence of calcinosis, wet or dry necrosis, and infection significantly delayed the time to healing. In this study, the definition used for the "pure" DUs matched the one from the RAPIDS studies and the DUs had a distinct natural history. The authors concluded that a precise classification of the subtype of digital lesion is important when different therapies are entertained: DUs due to calcinosis may not be as responsive to vasodilators as a purely ischemic DUs would be.

3. Pathogenesis of Digital Ulcers in SSc

The initial trigger in SSc-related vasculopathy is unknown. It is believed that smooth muscle cells migrate into the intimal layer of the microvasculature and differentiate into myofibroblasts that secrete collagen and an other extracellular matrix. This process leads to a fixed narrowing of the intravascular lumen which hinders the blood flow and causes chronic tissue ischemia. Histological studies showed that 18 (79%) of the 23 evaluable biopsies of digital arteries of patients with SSc had greater than 75% luminal narrowing [5].

Aside from the structural change, the endothelial cells are perturbed, possibly through ischemia-reperfusion injury or an autoimmune insult [6] leading to an increased production of vasoconstrictors such as endothelin and an underproduction of vasodilators such as prostacyclin and nitric oxide. Another proposed mechanism of endothelial injury is the presence of antiendothelial cell antibodies [7]. One other possible consequence of the endothelial damage is platelet activation with release of thromboxane [8] which leads to intraluminal thrombosis.

4. Natural History of Digital Ulcers in SSc: Incidence, Risk Factors, and Clinical Impact

Various studies have revealed that 15%–25% of SSc patients have active DUs [9] and 35%–50% had a history of DUs [10]. DUs are painful, disabling, and associated with a variable rate of progression to gangrene or infection leading to amputation. A particularly emergent situation is the ischemically threatened digit (Figure 3) due to the high rate of need for surgical intervention.

Although a prospective registry of patients with SSc-DUs is lacking, the available data from retrospective analysis [11, 12] outline the following risk factors for developing DUs:

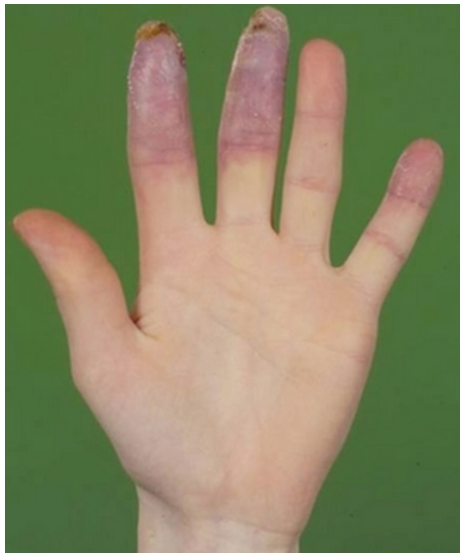

Figure 3: Ischemically threatened digit.

(1) male sex,

(2) presence of pulmonary hypertension and/or lower DLCO,

(3) diffuse subset of the disease,

(4) early onset of SSc,

(5) presence of antitopoisomerase I antibodies (anti-topo I),

(6) smoking.

Patients with DUs developed internal organ involvement 2-3 years earlier compared to patients without ulcers [12].

The use of nailfold videocapillaroscopy (NVC) may be a novel tool useful to predict development of SSc-DUs: the specificity and sensitivity were as high as 85.9% and 94.3%, respectively [13].

Episodes of DUs tend to reoccur, with 66% of patients having more than one episode and 50% having more than 2 episodes over a period of 7.26 years [14] despite the use of vasodilators. In the same cohort, the ulcers occurred more frequently in the second and third digits (II: 32.5%, III: 32.5%) and were equally distributed among both hands. Thirty patients (67%) had critical finger ischemia at least once, and 43% of patients received at least one course of intravenous iloprost; 7% of patients underwent surgical amputation.

The morbidity related to presence of DUs is significant. In the Pittsburgh database [15], the disability measured by the Scleroderma HAQ (SHAQ) was significantly greater in patients with persistent DUs. Patients also had more hospitalizations and more hospitalizations for antibiotics than patients without DUs (16 versus 9%). The incidence of gangrenous lesions was 11% and increased with the length of time since the first DUs, especially after 4 years. Data from the Randomized Placebo-Controlled Study on Prevention of DUs in SSc (RAPIDS-2) trial conducted in 188 patients with active DUs reported the incidence of amputation to be 11% (1-2%/patient-year of followup) [16].

A retrospective analysis of all the hospitalized cases due to ischemically threatened digits (ITDs) over a period of 10 years in a tertiary care center identified 79 patients, of which 22.8% had SSc [17]. In that particular cohort, the rate of amputation was 48.1%, and male sex, a history of previous ITD, or ITD developed in the hospital were associated with a higher rate of amputation.

5. Therapeutic Options for SSc-Digital Ulcers

The therapeutic approach in SSc-DUs poses multiple challenges. Ischemic DUs can be confused with DUs due to trauma or calcinosis. There is disagreement about the difference between active and nonactive ulcers. The pathogenesis of SSc-DUs is complex, and it involves multiple pathways which need to be addressed concomitantly. Pain and infection are common comorbidities that require supportive therapy. Response to the instituted medical therapy needs to be continuously assessed to detect the need for additional drug therapies or to consider surgical options.

The available clinical trials for treatment of active SSc-DUs, although largely negative, have contributed to our knowledge about outcome measures in this disease [18]. The crude measurement of the depth and length of DUs is not feasible due to the location of the ulcerations and the associated pain. The only direct parameter remains the absolute healing of the DUs, which includes an anatomical (re-epithelialization of the area) and a physiological (pain cessation) component. Other indirect parameters, like instruments focusing on quality of life (HAQ) and function of the hand (UKFS and the Michigan Hand Questionnaire) require further validation studies.

The DUs therapeutic approach includes: supportive measures, pharmacological interventions, and surgical options.

5.1. Supportive Therapies. Patients who develop SSc-DUs must be educated to keep their whole body warm (not just the hands) and to avoid direct trauma to the tips of the digits. It is paramount for smokers to quit. All other vasoconstrictors (cocaine, sympathomimetics) should be discontinued.

Pain related to SSc-DUs is exquisite and lasts as long as the DUs is active, which could be months. The intensity of the DUs-related pain is significant and could lead to anxiety which potentially worsens the Raynaud's symptoms which in turn could contribute to a lengthier healing process. Pain management should be instituted promptly and adequately escalated. Although the nonsteroidal anti-inflammatory drugs are very efficient, they should be avoided in favor of acetaminophen or opiates due to their vascular side effects. Since the cause of the pain is tissue ischemia, the real solution is improving the oxygen delivery to the affected area.

Infections are common in SSc-DUs and heal slowly because of the poor circulation. Clinicians should inspect each ulcer carefully at each visit. Clinical clues to DUs

infection are an increase in the amount of pain (and a change of character to throbbing) and presence of purulent discharge. Simple gram positive coverage is usually very efficient but broad coverage antibiotics might be used if the ulcers are more extensive. Patients may require more than one course of antibiotics.

If osteomyelitis is suspected, patients should be managed by a multidisciplinary team that includes infectious disease and orthopedic specialists. Intravenous antibiotics, hyperbaric therapy, and surgical amputation may be helpful.

5.2. Pharmacological Therapies. The purpose of the DUs treatment is to reduce their overall burden and impact on quality of life. Aside from controlling the pain and preventing infections, clinicians need to restore the hand function, improve the digital circulation, and promote healing of the existing DUs while preventing formation of new ulcers. Despite the substantial impact that SSc-DUs have on function and quality of life, there is currently no accepted therapy algorithm nor any FDA- approved therapies.

The treatment algorithm should mirror the pathogenesis of SSc-DUs and should include antiplatelet agents and vasoactive agents. It has become common practice that patients with a history of DUs or an active DUs should take at least a low dose of aspirin (81 mg) daily. Clopidrogrel is being used with or without aspirin for active DUs but there are no published data on safety and overall efficacy.

Vasoactive therapy includes the background therapy for Raynaud's Phenomenon (RP) and agents from a few available classes that were shown to improve SSc-DUs or are undergoing experimental trials. Potential therapies for SSc-DUs will be outlined below by class.

5.2.1. Calcium Channel Blockers. Calcium channel blockers (CCBs) are widely used for the treatment of primary and secondary RP, reducing the severity of attacks by 35% [19]. The effect of CCB on SSc-DUs was reported in a small 16-week randomized controlled trial that compared oral nifedipine with intravenous iloprost: the mean number of ulcers decreased (from 4.3 to 1.4) but there were no physiological changes in the microvasculatory blood flow or the temperature of the hands [20].

5.2.2. Phosphodiesterase-5 Inhibitors. Phosphodiesterase-5 inhibitors (PDE5 inhibitors) induce vasodilatation by increasing the levels of available endogenous nitric oxide (NO). The positive effect of sildenafil on RP was shown in a randomized controlled crossover study [21] and case reports and series indicate the benefit of sildenafil in SSc-DUs [22, 23]. An open label pilot study using a maximum dose of sildenafil in 19 patients with SSc-DUs showed a total reduction of ulcers from 49 to 17 ($P < .001$) after 6 months [24].

Shenoy et al. presented SSc-DUs healing results in a crossover trial of tadalafil [25]. The RP improvement was clinically significant, with a surprising absence of placebo effect. This is contradicted by another randomized crossover trial of tadalafil in SSc-RP which showed no benefit of the drug over placebo [26].

The role of PDE5 inhibitors in SSc-DUs needs to be further evaluated in prospective, randomized trials.

5.2.3. Endothelin Receptor Antagonists. The endothelial injury that is the hallmark of ischemic digital ulcers corresponds with an increase in levels of endothelin 1 (ET-1) [27, 28]. ET-1 is a potent vasoconstrictor that mediates its actions through two different receptors: endothelin type A (ET-A) receptors are found on vascular smooth muscle cells while endothelin type B (ET-B) receptors are found on both endothelial cells and vascular smooth muscle cells.

Bosentan is a receptor antagonist with activity against both types of receptors, ET-A and ET-B. Anecdotal reports and case series have suggested that bosentan may be helpful in reducing ulcer size or improving healing [29–31]. Other studies have demonstrated an improvement in flow-mediated dilatation of microvasculature with bosentan therapy in patients with systemic sclerosis [32].

The RAPIDS-1 trial analyzed the effect of Bosentan on the prevention and treatment of existing digital ulcers [3]. This randomized, placebo-controlled study of 122 patients with systemic sclerosis and preexisting digital ulcers evaluated a primary outcome of bosentan on number of new digital ulcers during a 16-week study period. Patients were treated with bosentan 62.5 mg twice daily for four weeks then 125 mg twice daily for 12 weeks. While no significant difference was seen in healing of existing ulcers, there were significant differences in hand function and number of new ulcers in the treatment group (1.4 versus 2.7).

In the open-label extension of the study 88 patients continued bosentan for 12 additional weeks (57 of these patients were previously treated with bosentan for 16 weeks and so received a total of 28 weeks of treatment) [33]. At the end of the extension period, 65% of patients did not develop any new digital ulcers while 8% (7 patients) developed more than two new ulcers. The mean number of new DUs after 12 weeks of open-label therapy was 0.7 (all subjects), 0.5 (subjects previously on placebo), and 0.8 (previously on bosentan). RAPIDS-2 was designed to confirm the positive effects of bosentan at reducing new DUs in 188 patients with at least an active SSc-DUs over a variable treatment course [34]. Patients received 62.5 mg bosentan twice daily for 4 weeks then 125 mg twice daily for 20 to 32 weeks. None of the measures of digital ulcer healing differed between the two groups (time to healing of a selected cardinal ulcer, time to healing of all digital ulcers, and percent of patients with complete healing). However, the total number of new ulcers during a 24-week followup period was 1.9 on bosentan and 2.7 on placebo ($P = .035$). Unlike what is known of RP, there was no clear influence of season on SSc-DUs, indicating that DUs may be more related to the severity of vasculopathy.

Overall, oral bosentan at a dose of 125 mg twice daily had no effect on ulcer healing in patients with digital ulcers and SSc. Although bosentan seemed to have lessened the ulcer burden in patients with more than four concomitant ulcers, it had no effect on RP.

There are also anecdotal reports and case reports of combination treatment with bosentan [35]. This case report of a 73-year-old patient with systemic sclerosis reveals that she was treated with both sildenafil and bosentan which resulted in complete healing of the digital ulcers.

Sitaxentan is a selective endothelin type A receptor antagonist that has been reported to help heal SSc-DUs in case reports [36], but no randomized clinical trials are ongoing.

5.2.4. Prostacyclin Analogues. Prostacyclins are potent vasodilators that also inhibit platelet aggregation and smooth muscle proliferation in the blood vessels walls. Various forms of prostacyclins are approved for use in idiopathic and connective tissue-related pulmonary arterial hypertension (PAH).

Epoprostenol is an intravenous prostanoid. In the pivotal randomized controlled trial of epoprostenol in patients with SSc-related PAH, the patients on epoprostenol had 50% fewer new DUs than those treated with conventional therapy, although no effect on healing of the existing DUs was reported [37].

Iloprost is the most studied prostacyclin analog and is available in intravenous (IV), oral, and inhaled formulations. Outside the USA, cyclic use of intravenous iloprost is the standard of care for treatment of ischemically threatened digits and severe SSc-DUs. The most important clinical trial supporting the use of iloprost was a 9-week, double-blind placebo-controlled multicenter trial in patients with SSc-RP [38]. In this trial, the intervention consisted of IV iloprost 6-hours infusion (dose of 0.5–2 ng/kg per minute) for 5 days. A significant proportion of the patients receiving the active drug had at least 50% reduction in the number of DUs. Interestingly, although the DUs healing was noted at earlier time points, the effect persisted at 9 weeks, suggesting a potential "reset" effect that iloprost might have on the endothelium. In this particular trial, a trend towards prevention of new DUs was noted. A randomized controlled trial of oral iloprost at a fixed dose for patients with SSc-RP had negative results [39], most likely because there was no titration to the maximum tolerated dose. Higher doses of iloprost may be more effective in SSc-RP at the expense of increased side effects [40].

Oral beraprost, another available prostacyclin analog, was evaluated in a randomized controlled trial for SSc-RP [41] and it showed a trend towards fewer new DUs in the treatment group but no effect of the RP.

Treprostinil is available in IV, subcutaneous, inhaled, and oral formulations. A pilot study of subcutaneous treprostinil in patients with SSc-DUs over a 12-week treatment period showed both healing and prevention of the DUs but only a few subjects were able to tolerate the injection site pain [42]. Treprostinil diethanolamine (TDE-SR) is an innovative salt form of treprostinil for oral delivery as a sustained release osmotic tablet for twice daily dosing. The pharmacokinetic profile of TDE-SR in patients with SSc-DUs is comparable to that of healthy controls [43] despite a variable degree of absorption related to SSc. An ongoing phase II randomized, double blinded, multicenter clinical trial of oral treprostinil in SSc-DUs is currently recruiting.

5.2.5. Statins. It is well recognized that statins (3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors) have beneficial effects on ischemic vascular events and consequences of vascular injury [44]. An open-label study of 14 SSc patients showed that 12 weeks of atorvastatin improved the Raynaud's severity and prevented new SSc-DUs [44]. A large randomized, double-blinded, placebo controlled study of 84 patients with SSc and history of DUs showed a significant difference in the number of new DUs in the treatment group compared to placebo over 4 months (1.6 versus 2.5) [45]. Significant improvement in the Scleroderma Health Assessment Questionnaire Disability Index (SHAQ-DI) score and endothelial markers from the baseline was noted.

5.2.6. Angiotensin-Converting Enzyme Inhibitors (ACEIs) and Angiotensin Receptor Blockers (ARBs). The role of ACEI in the management of scleroderma renal crisis has changed the primary cause of mortality in SSc from renal to pulmonary complications. ACE inhibition counteracts the renin-driven hypertension but it also improves endothelial function and promotes vascular remodeling in chronic hypertension.

A study of the long-acting ACEI, quinapril, used the rate of occurrence of new DUs in patients with SSc as primary outcome [46]. This was a multicenter, randomized, double-blind, placebo-controlled study of quinapril at 20 mg daily for at least 2 years but not more than 3 years. Of the 213 patients enrolled, 109 patients were followed for the full 3 years. At the end of the study there was no difference between the treatment and the placebo group in the number of new DUs, the total number of DUs, and measurements of RP (frequency and severity). As the authors commented, the lack of therapeutic effect was not attributable to insufficient statistical power.

In a randomized 12-week trial comparing nifedipine to losartan, an ARB, in patients with primary and secondary RP (total number of patients of 52), losartan significantly reduced the RP severity and frequency [47]. This study focused mainly on RPs and there was no mention of SSc-DUs.

5.2.7. N-Acetylcysteine. N-acetylcysteine (NAC) is the precursor of a major antioxidant, glutathione, and may have beneficial effects in SSc-DUs due to its vasodilating properties and impact on platelet aggregation. A pilot study of intravenous NAC in 20 patients with SSc-DUs showed that more than half of the DUs present at baseline completely healed after the 5-day infusion [48]. A prospective observational study of intravenous NAC dosed at 15 mg/kg/hr for 5 hours every 14 days was recently reported [49]. The median treatment was 3 years, and the mean of ulcers/patient/year decreased significantly from 4.5 to 0.81 with minimal reported side effects. Although promising, in order to better establish its use in SSc-DUs, this agent should be evaluated in a prospective, placebo controlled, randomized fashion.

5.2.8. Vitamin E Gel. Vitamin E has been used for chronic cutaneous lesion and ulceration treatment based on its antioxidant and anti-inflammatory effects. An open-label study of 27 patients with SSc-DUs randomly assigned 15 subjects to vitamin E gel. Patients treated with vitamin E experienced significantly reduced healing time (13.2 versus 20.0 weeks, $P < .0001$) when compared to controls and improved pain resolution ($P = .0022$).

5.3. Surgical Options. Surgical options are reserved for treatment of severe or recurrent DUs that are recalcitrant to medical therapy. Available procedures are microsurgical revascularization of the hand and digital sympathectomy [50]. Since the size of the blood vessels involved in the pathogenesis of SSc-DUs is usually small, revascularization is not readily applicable.

Sympathectomies are aiming to block the sympathetic nerve-mediated vasospasm which is thought to have an important role in digital ischemia. The long-term results of cervical sympathectomy have been discouraging. Local digital sympathectomy has the advantage of interrupting the sympathetic fiber more distally. During digital sympathectomy, the adventitia is excised, removing sympathetic fibers contained in the adventitia and most likely, the media. This procedure has been shown to help healing of SSc-DUs, improvement in pain, and prevention of new DUs for a mean of 31 months after the surgery [51]. The results of digital sympathectomies in patients with connective tissue disorders may not be as favorable as in patients with other diagnoses [52].

Although the microvascular involvement in SSc is well known, there are reports of macrovascular involvement as well. A small study of 8 patients with DUs or gangrene related to SSc found macrovascular involvement of the ulnar artery (3 patients) and radial artery (1 patient) by arteriography [53]. A larger retrospective study identified 12 (63.2%) of 19 patients with SSc who underwent brachial arteriography to have ulnar artery involvement (occlusion and/or stenosis) [54]. Potential for revascularization in selected SSc patients with arteriographic evidence of ulnar involvement was shown in a retrospective chart review [55]: 15 patients with SSc-DUs and ulnar artery occlusive disease confirmed by angiography were reviewed. Eight patients who underwent ulnar revascularization combined with digital sympathectomy had improvement in the healing of the ulcers.

The current evidence is limited but there is a role for brachial arteriography in selected patients with SSc-DUs to diagnose macrovascular involvement. More interventional trials are needed to assess the efficacy of ulnar revascularization in SSc-DUs prevention and treatment.

6. Conclusions

Digital ulcers affect patients with SSc with a frequency of 30% per year. A precise definition of the SSc-DU would be useful for the research community and also in clinical practice. The effect on function and quality of life of the SSc-DU is significant, so therapies focused on rapid healing and prevention of new ulcers are needed. Although patients note reduced pain and improved function when exposed to parenteral agents like iloprost, the more subtle agents that seem to be better at prevention (e.g., bosentan) offer very little benefit on pain and quality of life. Important clinical trials of oral or topical drugs with overall burden of the SSc-DUs as main outcome measure are ongoing.

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Review Article

Digital Ischemic Loss in Systemic Sclerosis

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Digital ischemic loss is a cause of significant morbidity in patients with systemic sclerosis (SSc). Microvascular disease with intimal proliferation and luminal narrowing of small digital arteries, as well as macrovascular disease with narrowing or occlusion of larger digital arteries, contribute to the perfusion defects involved in digital ischemic loss. Immediate clinical evaluation and treatment are mandatory at the onset of critical digital ischemia to prevent digital loss. Hospitalization for medical therapies including intravenous prostacyclin therapy should be considered for all SSc patients who present with critical digital ischemia. Surgical interventions are typically reserved for patients who fail medical therapies and for those with late stage, necrotic tissue. This paper summarizes the current knowledge regarding the risk factors, pathogenesis, evaluation, and treatment of digital ischemic loss in SSc.

1. Introduction

Systemic sclerosis (SSc) is a disease of unknown etiology characterized by immune activation, tissue fibrosis, and vasculopathy. Peripheral vascular involvement manifesting as Raynaud's phenomenon (RP) affects almost all patients. In a subset of SSc patients, episodes of progressive digital ischemia can result in a digital ulcer—a denuded area with a defined border with loss of epithelialization and loss of epidermis and dermis. Sustained reduction in digital perfusion with impaired tissue viability can lead to critical ischemia, in some cases resulting in gangrene necessitating amputation. On a microvascular scale, episodes of digital ischemia are thought to be due to neuroendothelial imbalance of vasoconstriction and vasodilatation, structural abnormalities of the vasculature, and intravascular factors such as platelet activation, procoagulants, and oxidative stress. Recent studies on macrovascular disease in SSc suggest that ischemic demarcation and loss of digits occurs secondary to narrowing or occlusion of larger digital arteries (vessels of the palmar arch, radial, or ulnar artery) or medium-sized and large arteries in the lower extremities. This paper will summarize the current knowledge regarding the risk factors, pathogenesis, evaluation, and treatment of digital ischemic loss in SSc.

2. Clinical Burden

SSc has a prevalence of 1–50 cases per 100,000 people worldwide [1]. A retrospective review of the clinical status of 98 patients with SSc, seen between 1985 and 1990, showed that amputation of 1 or more digits due to ischemia occurred in 20.4% of the patients while 9.2% had multiple digit loss [2]. A more recent review of a prospective cohort from 2001 found that 28 (16%) of 171 SSc patients attending a hospital in the UK had at least one digital amputation and 73 (43%) had experienced at least one episode of severe digital ischemia as defined by requirement for intravenous vasodilator therapy, surgical debridement, and/or amputation [3]. In another larger cohort of 1168 SSc patients in the UK followed over an 18 month period, 17.4% were found to have complications related to severe digital vasculopathy including digital ulcers, critical digital ischemia, gangrene, or the need for digital sympathectomy. One-third of these patients were on some immunosuppressive treatment, three-fourths were receiving at least one vasodilator, and one-fifth were on antiplatelet agents. Sixteen percent had at least one digital ulcer and 12% required at least one hospitalization for intravenous prostanoid therapy over the 18 month study period. Furthermore, 1.6% of the total cohort developed critical digital ischemia, 1.4% developed

Table 1: Potential risk factors for digital ischemic loss in patients with systemic sclerosis.

Likely	Possible
Diffuse cutaneous subtype	Anti-topoisomerase antibodies
Anti-centromere antibodies	
Current smoking	
Anti-beta2-glycoprotein I antibodies	
Anti-granzyme B antibodies	

digital gangrene, 1.1% underwent a sympathectomy, and 0.9% had an amputation during the 18 month period [4].

3. Risk Factors

Predictors of ischemic digital loss in patients with SSc have been identified from data collected from observational studies (Table 1). Patients with long-standing limited cutaneous SSc (lcSSc) have traditionally been thought to have more prominent vascular manifestations than patients with diffuse cutaneous disease (dcSSc) [5]. However, Denton and colleagues noted that in their analyses of a prospective cohort of 1168 patients with SSc, severe digital vasculopathy occurred in 27.5% of the patients with dcSSc versus 13% of the patients with lcSSc ($P < .0001$). There was no correlation between disease duration and severity of digital vasculopathy [4].

An initial observation by Herrick et al. found weakly positive anti-cardiolipin (aCL) antibodies in four of eight patients with SSc who had severe digital ischemia requiring amputation [6]. However, a follow-up retrospective analysis found that there was no difference in aCL positivity in SSc patients with severe ischemia (11/31) versus those without (16/37), or between those who had amputation (5/13) and those who had not (22/55). Instead, this study found an association between the presence of the anti-centromere antibody and severe peripheral ischemia. Seventeen of the 31 patients (55%) with severe ischemia were anti-centromere antibody positive compared with nine of 37 (24%) without ischemia ($P = .01$). There was also a trend for an association with the presence of the anti-topoisomerase antibody, in that six patients with severe ischemia had anti-topoisomerase antibodies compared with two patients without ischemia ($P = .08$). The authors concluded that complications from severe digital vasculopathy are more likely in patients with scleroderma specific antibodies [7].

Another review of a prospective cohort from the UK also found that anti-centromere antibody positivity is a predictor for amputation. Of 171 patients, 75% of whom had lcSSc, 37% were anti-centromere antibody positive and 16.4% were identified as having amputations. Of patients who had undergone amputations, 60.7% were anti-centromere antibody positive (OR 3.12). Smoking was also found to be an independent risk factor for amputation (OR 6.28 per pack per day, 95% CI 1.95 − 20.19) [3].

Other studies have also investigated whether smoking is a risk factor for poorer outcomes related to digital ischemia. Previously, Wigley et al. concluded that smoking was not a risk factor in 98 patients in their SSc cohort at a US tertiary care center [8]. However, an analysis from a database from the UK found that in their cohort of 101 SSc patients, current smokers were 3-4 times more likely than never-smokers to incur digital vascular complications. When adjusting for age, sex, and disease duration, current smokers were significantly more likely than never-smokers to have required debridement (OR 4.5, 95% CI 1.1–18.3) or admission for intravenous vasodilators (OR 3.8, 95% CI 1.1–12.9) [9].

A recent study investigated whether anti-beta2-glycoprotein I (anti-beta2-glycoprotein I antibodies) and aCL antibodies are correlated with macrovascular disease, including digital loss in SSc patients. Seventy five SSc patients with a history of ischemic digital loss were matched to 75 SSc patients without a history of digital loss. Anti-beta2-glycoprotein I antibodies was significantly more frequent in SSc patients with digital loss than in patients without digital loss: 27/75 (36%) in the digital loss group had these antibodies compared with 14/75 (19%) in the group without digital loss ($P = .017$). The IgA subtype showed the strongest association (OR 4.0, CI 1.1–14.2). However, there was no difference in aCL antibody frequency between the two groups, as has been previously reported. In addition, after adjusting for demographics, disease type, smoking status, and anti-centromere antibodies, anti-beta2-glycoprotein I antibodies positivity was significantly associated with active digital ischemia (OR 9.4, CI 3.5–25.4), elevated estimated right ventricular systolic pressure (RVSP) (OR 4.8, CI 1.0–11.4), and increased mortality (OR 2.9, CI 1.1–7.1). They also noted that patients with a history of ischemic digital loss have more severe pulmonary vascular disease with overall a higher RVSP, worse lung severity scores, and lower diffusing capacity of carbon monoxide (DLco) which might be contributing to the higher mortality. All in all, anti-beta2-glycoprotein I antibodies is likely important in scleroderma vascular disease; however, further research is necessary to determine whether these antibodies are directly involved in the pathogenesis of disease or represent an epiphenomenon [10].

In addition, antibodies against novel autoantigens expressed in the cytotoxic lymphocyte granule pathway have been associated with the clinical phenotype of ischemic digital loss in SSc. Specifically, autoantibodies to granzyme B, a serine protease found in the cytoplasmic granules of cytotoxic T cells and natural killer cells, with an important role in inducing apoptosis and clearance of intracellular pathogens, has been found to be highly associated with the phenotype of ischemic digital loss. Investigators at Johns Hopkins University found that the sera from 16/19 (84.2%) lcSSc patients with ischemic digital loss immunoblotted for autoantigens to granzyme B compared with 6/15 (40%) lcSSc patients without ischemic digital loss (OR 8.0, CI 1.6–40.0). The risk of anti-granzyme B antibodies persisted even when controlling for the presence of antiphospholipid antibodies [11]. This in vivo recognition of granzyme B-generated autoantigen fragments in lcSSc patients with ischemic digital loss identifies a distinct clinical subset and needs further investigation.

4. Pathogenesis

The pathogenesis of SSc is complex and incompletely understood. The current view of this disease includes the development of vasculopathy, activation of the cellular and humoral immune responses, and progressive fibrosis. The pathological hallmark of SSc is an obliterative vasculopathy combined with interstitial fibrosis in target organs. Raynaud's phenomenon, a reversible process in primary disease, is due to dysregulation of the peripheral and autonomic nervous systems leading to vasospasm. However, in SSc, ischemia can progress due to irreversible changes within the endothelium associated with decreased production and responsiveness of endothelial derived vasodilators (nitric oxide and prostacyclins) and increased production and responsiveness of vasoconstrictors (endothelin-1). In addition, microvessels have increased permeability, increased leukocyte extravasation, and activation of coagulation and fibrinolytic factors with platelet aggregation, eventually leading to thrombosis. This vasculopathy affects capillaries, arterioles, and even large vessels and progresses to luminal occlusion due to intimal/medial hypertrophy and adventitial fibrosis with persistent endothelial damage and apoptosis. The process of revascularization is also defective in SSc, affecting both angiogenesis, in which new vessels arise from preexisting vessels, and vasculogenesis, in which new vessels derive from endothelial progenitor cells to replace damaged or senescent blood vessels, despite elevated levels of angiogenic factors. Therefore, in SSc there is a widespread obliterative vasculopathy associated with the failure to repair and replace damaged vessels, thus resulting in poor digital perfusion and the potential for ischemic digital loss [12].

5. Vascular Disease Distribution

Evidence has shown that both proximal and distal arteries are affected in SSc-related vasculopathy resulting in digital ischemic loss. Stafford et al. evaluated Doppler studies of arteries in the limbs, neck, and abdomen of 20 SSc patients compared with controls who were non-SSc rheumatology patients. They found that the ulnar arteries in SSc patients were significantly narrower ($P = .002$) and smoothly thickened ($P < .0001$) than those of non-SSc controls, while other arterial beds were not significantly different [13]. Another retrospective observational study analyzing brachial angiography found that 12 of 19 patients with SSc who exhibited Raynaud's phenomenon and digital ulceration had ulnar artery occlusion/stenosis with only 2 patients with radial artery involvement. Ulnar artery involvement was associated with the dcSSc subtype ($P < .01$) [14].

Hasegawa et al. studied macrovascular disease in patients with SSc with digital ulceration or gangrene using catheter arteriography of the upper and/or lower extremities. Seven of eight patients in this study were found to have macrovascular occlusion in the upper extremities. Of these, three had occlusion limited to digital arteries, three had obliteration of the ulnar and superficial palmar arch, and one had a radial artery occlusion. Of five patients who underwent lower extremity angiography, one had limited digital artery occlusion, one had occlusion of the posterior tibial artery, one had dorsalis pedis and arcuate occlusion, and two had occlusion of the plantar arch [15].

6. Clinical Evaluation

The initial clinical evaluation for the signs of digital ischemia includes assessing for persistent discoloration (cyanosis or pallor), increased pain, digital ulceration, extreme tenderness, or frank gangrene. Nailfold capillaroscopic changes with dilatation, irregularity, megacapillaries, and drop-out are often present in SSc patients, and progression of these features may be predictive of the development of digital ischemia [16]. The differential diagnoses that should be considered in all connective tissue disease associated digital ischemia include proximal occlusive disease, vasculitis, thromboembolic disease, or severe distal microvascular disease. As such, all patients should be evaluated for peripheral pulses, dopplers if pulses are weak or nonpalpable, an Allen's test, and possibly ankle-brachial indices. Laboratory analysis for prothrombotic states including the antiphospholipid antibodies (lupus anticoagulant, anti-cardiolipin, and anti-beta2-glycoprotein I antibodies) should be performed in all patients. Prompt clinical evaluation and referral for treatment is critical to the prevention of progression to digital loss [17]; see Figure 1 for an evaluation and treatment algorithm.

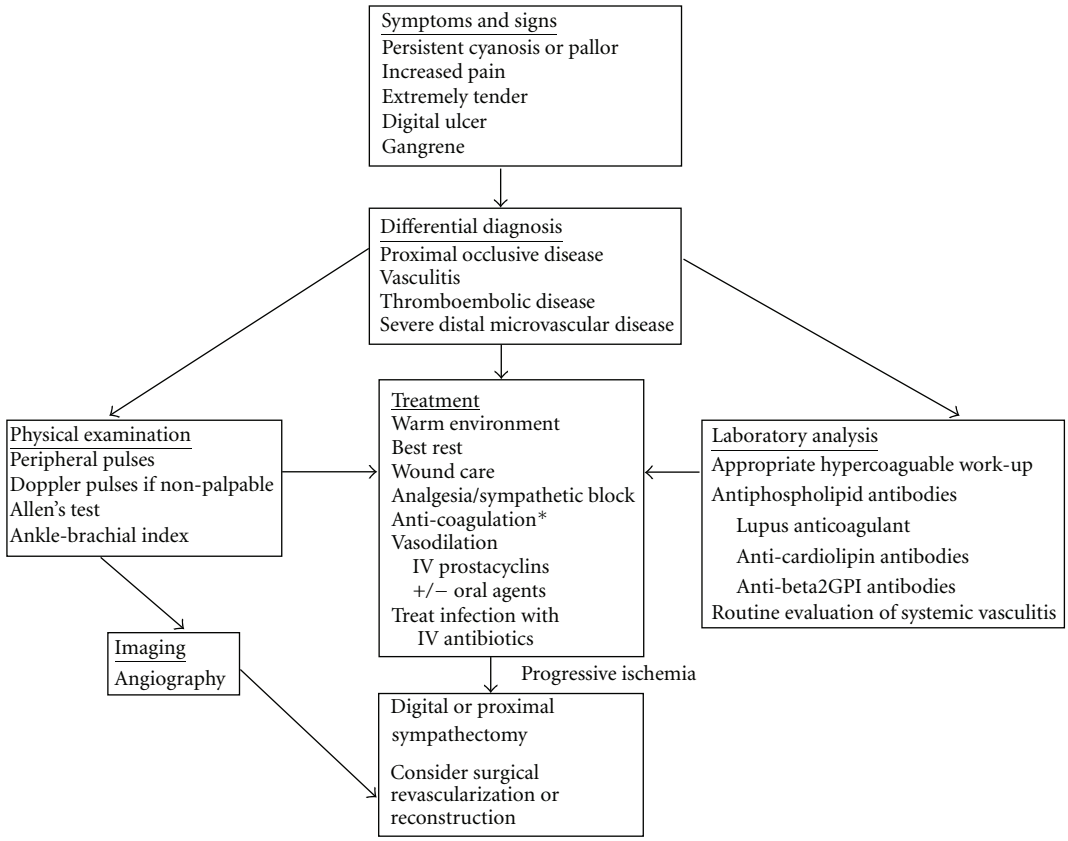
7. Imaging

Angiographic techniques to evaluate for digital occlusions include conventional angiography, magnetic resonance angiography (MRA) or computed tomography (CT) angiography (Table 2). Conventional angiography is extremely sensitive for identifying stenosis, occlusion, aneurysm, or other vascular irregularities, but is invasive, involves high contrast load, and radiation exposure. MRA is noninvasive, and can visualize the vessel wall in addition to the lumen and surrounding structures. Spiral CT angiography allows 3-D imaging with shorter scanning times and without intravenous contrast or ionizing radiation.

The main indication for imaging is to identify proximal lesions amenable to angioplasty or surgery in cases of severe digital ischemia. It should be noted that MR and CT angiography are still investigational techniques and should be reserved for patients who have contraindications to conventional angiography. Conventional angiography is still considered the gold standard to visualize more distal arteries, especially if arterial reconstruction is being considered [12].

8. Treatment

Digit-threatening ischemia warrants prompt and aggressive treatment to control symptoms and prevent digital loss. As this is considered a medical emergency, hospitalization should be considered for all patients to expedite interventions. Nonpharmacologic treatments such as a warm environment and bed rest to decrease activity or possible



*If no contraindications, and especially if suspect acute thrombosis or embolic disease or hypercoaguable state.

Figure 1: Algorithm for the evaluation and treatment of digital ischemic loss in systemic sclerosis.

injury to the affected limb are essential. A simple xeroform dressing with an antibiotic ointment can be used to prevent a superinfection and allow wound healing. Intravenous antibiotics should be used if overlying infection or osteomyelitis is suspected, especially in patients who are being considered for surgical debridement or if there is any collection of purulent material or necrotic tissue. Surgical debridement is generally reserved for patients with purulent drainage, necrotic/late stage ischemic tissue, or severe structural arterial disease who do not respond to medical therapies.

Analgesia with opioids is of utmost importance as pain due to critical digital ischemia is extremely intense. Local anesthetic blocks with lidocaine or bupivicaine without epinephrine may be helpful for pain control, but these interventions have not been studied in clinical trials. Temporary chemical sympathetic block should strongly be considered for patients with severe pain before surgical sympathectomy [12].

Anticoagulation is recommended for patients with rapidly advancing ischemic tissue who do not have contraindications. Intravenous heparin for 24–72 hours is typically used; however, no double-blind clinical trials have been performed [12]. Theoretically, this approach would be appropriate in cases where symptoms are suggestive of a new arterial occlusion thought to be due to an acute thrombosis or embolization. Further studies are needed to confirm the efficacy of this approach.

Aggressive vasodilatation is thought to improve blood-flow to ischemic areas. Firstly, oral calcium channel blocker doses should be titrated to maximum tolerated doses. However, intravenous prostacyclins, which not only vasodilate but also inhibit platelet aggregation, are considered the mainstay of management for acute digital ischemia. Intravenous epoprostenol or iloprost (0.5–2 ng/kg/min) daily infusions for 1–3 days, each infusion lasting 6 hours, is the recommended regimen. However, for patients with severe progressive ischemia, continuous prostacyclin infusion and higher doses may be required as tolerated. Studies have shown that intravenous iloprost was effective in reducing both the frequency and severity of ischemic attacks and in the healing of digital ulcerations [18, 19]. Epoprostenol was found to decrease the number of new digital ulcers in a double-blind trial in patients with SSc-associated pulmonary arterial hypertension [20]. Common side effects of these medications include hypotension, dizziness, headache, flushing, jaw pain, and gastrointestinal symptoms [21].

Table 2: Radiographic considerations for evaluation of digital ischemic loss in patients with systemic sclerosis.

Modality	Pros	Cons
Conventional Angiography	Extremely sensitive for identifying vascular abnormalities	Invasive
		High contrast load
	Standardized technique	Radiation exposure
		Risk of inducing vasospasm
Magnetic Resonance Angiography (MRA)	Standardized technique	Long scanning time
	Noninvasive	Resolution inferior to CT or conventional angiography in distal digital vessels
	No contrast load	
	No radiation exposure	
	Can visualize vessel wall in addition to lumen	
	Can visualize surrounding structures	
	Can visualized venous lesions	
Computed Tomography (CT) Angiography	Noninvasive	Less contrast load than conventional angiography
	Excellent bone and soft tissue spatial relationships	Shorter scanning time
		Resolution inferior to conventional angiograpy in distal digital vessels

If symptoms are persistent and medical therapy fails, proximal or digital sympathectomy, microsurgical revascularization of the hand, and digital arterial reconstruction have been reported to improve digital vascular perfusion, heal digital ulcers, and substantially relieve or eliminate pain from one to 46 months postoperatively [22].

A proximal sympathectomy involves resection or ablation of a section of the cervical or thoracic sympathetic chain. This treatment is now only rarely performed due to its significant associated risks such as permanent Horner's syndrome, persistent neuralgia, and decreased localized cutaneous sweating; however, an endoscopic thoracic approach may be safer. Sympathectomy is less effective in patients with secondary than primary Raynaud's phenomenon and the duration and degree of improvement is variable [12].

Digital sympathectomy was introduced in the 1980s as an alternative to proximal sympathectomy. A recent study in 20 patients primarily with SSc, who had 42 ulcerated digits due to ischemia, found that periarterial sympathectomy led to complete healing or decrease in ulcer number in 28 of 42 digits after a mean of 96 months of follow-up. In addition, the rate of amputation in the treated patients with underlying autoimmune disease was half of that for patients with underlying atherosclerotic disease, indicating that patients with autoimmune-induced ischemia may be more amenable to treatment with digital sympathectomy [23]. However, controlled studies have not yet been performed, and given that the risk for perioperative complications is reported at 37%, with amputations and recurrent ulcerations at 14% and 18%, respectively, these procedures should be reserved for patients with severe ischemia and a threatened digit only after failure of vasodilator therapy [24]. In addition, these procedures should be performed by experienced hand or vascular surgeons at specialty centers.

If there is larger vessel occlusive disease, especially at the level of the ulnar or radial artery, successful reconstruction can be performed with vein grafts [13]. Of 15 SSc patients with severe RP and digital ulceration and angiography-proven ulnar artery occlusion, no patients responded to conventional medical therapy; however, 8/15 patients underwent ulnar artery revascularization combined with digital sympathectomy and all subsequently experienced dramatic improvement in Raynaud's phenomenon and healing of digital ulcers [25].

A recent case report describes successful vascular reconstruction, with improved digital temperatures, pain and cold sensitivity, and health-related outcomes with peripheral artery bypass for vasoocclusive disease of the superficial palmer arch and tarsal arch in two SSc patients with severe digital ischemia [26]. Another case series noted that bypass surgery was successful in achieving early pain relief and ischemic wound healing for critical limb ischemia due to tibial artery occlusion in patients with SSc; however 4/8 of these patients subsequently experienced limb loss and 1 patient developed persistent recurrent ulcers [27]. Further studies particularly regarding the long-term outcomes of bypass surgery for the treatment of critical limb ischemia in SSc are warranted.

9. Conclusion

Systemic sclerosis is a complex autoimmune fibrosing disease with major vascular complications. Digital vasculopathy

with critical ischemia is one of the more challenging complications and carries significant morbidity in a substantial proportion of patients. Identified risk factors for ischemic digital loss include the diffuse subtype of SSc, SSc specific antibodies (anti-centromere antibodies and anti-topoisomerase antibodies), current smoking, anti-beta2-glycoprotein I antibodies, and anti-granzyme B antibodies. Ischemic digital loss is related to both macrovascular disease with involvement of proximal and larger digital arteries, as well as microvascular involvement caused by neuroendothelial, structural, platelet, and procoagulant effects. The medical treatment algorithm for critical digital ischemia includes urgent evaluation for reversible causes, analgesia, intravenous vasodilator therapies, angiography, and possibly anticoagulation. Surgical therapies such as sympathectomies and vascular reconstructions are considered in patients who fail medical therapy. Further research is necessary to identify specific biomarkers for digital ischemia in patients with SSc, and to develop more effective treatments.

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Clinical Study

Lower Extremity Ulcers in Systemic Sclerosis: Features and Response to Therapy

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Nondigital lower extremity ulcers are a difficult to treat complication of scleroderma, and a significant cause of morbidity. The purpose of this study was to evaluate the prevalence of nondigital lower extremity ulcers in scleroderma and describe the associations with autoantibodies and genetic prothrombotic states. A cohort of 249 consecutive scleroderma patients seen in the Georgetown University Hosptial Division of Rheumatology was evaluated, 10 of whom had active ulcers, giving a prevalence of 4.0%. Patients with diffuse scleroderma had shorter disease duration at the time of ulcer development (mean 4.05 years ± 0.05) compared to those with limited disease (mean 22.83 years ± 5.612, *P* value .0078). Ulcers were bilateral in 70%. In the 10 patients with ulcers, antiphospholipid antibodies were positive in 50%, and genetic prothrombotic screen was positive in 70% which is higher than expected based on prevalence reports from the general scleroderma population. Of patients with biopsy specimens available ($n = 5$), fibrin occlusive vasculopathy was seen in 100%, and all of these patients had either positive antiphospholipid antibody screen, or positive genetic prothrombotic profile. We recommend screening scleroderma patients with lower extremity ulcers for the presence of anti-phospholipid antibodies and genetic prothrombotic states.

1. Introduction

Non-digital lower extremity ulcers are a difficult to treat complication of scleroderma seen both in limited and diffuse scleroderma and also in scleroderma sine scleroderma. They contribute to the pain and disability of advanced disease. The etiology of these ulcers is unknown, but they may reflect chronic vasculopathy.

The prevalence of nonhealing lower extremity ulcers in scleroderma has not specifically been studied. Older data from the Pittsburgh Scleroderma Databank identified seven out of 1030 patients requiring amputation for refractory leg ulcers, giving an incidence of wounds requiring amputation of 0.67% [1]. However, this is likely to be an underestimate of total prevalence of leg ulcers, since most scleroderma patients with leg ulcers do not require amputation. More recently, Alivernini et al. evaluated 130 scleroderma patients over a 20-month period. They identified 26.15% with digital ulcers and 3.8% with "other" ulcers [2], and the latter may be a more accurate estimate of the true prevalence of non-digital lower extremity ulcers.

The impact of leg ulceration on health care costs and quality of life has not been studied in the scleroderma population. Extrapolating from other chronic diseases such as diabetes, it is known that leg ulcers result in significant morbidity and mortality, leading to recurrent hospitalizations, repeated surgeries, and significant costs to the health care system. A retrospective study of patients with diabetes and leg ulcers found that, in the first two years after diagnosis, the costs attributable to the ulcer were $27,987 [3].

Scleroderma is associated with delayed wound healing [2], and as with other chronic leg ulcers, the etiology of

delayed healing is likely to be multifactorial. Some have postulated a role for larger vessel venous and arterial disease [4], but many scleroderma ulcers remain refractory even after restoration of good blood flow and venous drainage.

Biopsy data from scleroderma wounds demonstrates fibrin plugging of the small vessels and persisting macrophage and fibroblast activation, suggesting that these wounds may be arrested in a chronic inflammatory phase. A similar fibrin occlusive vasculopathy is seen in biopsies of leg ulcers due to livedoid vasculopathy. Livedoid vasculopathy is associated with impaired fibrinolysis from a variety of genetic and acquired causes [5–7] and heparin an anticoagulant with profibrinolytic actions has been effective in some cases [8–10]. We postulate that dysregulation of the complement and coagulation cascades with inadequate fibrinolysis and angiogenesis may contribute to delayed healing in scleroderma-associated lower extremity ulcers.

In autoimmune diseases, antiphospholipid antibodies are recognized as activators of both coagulation and complement cascades [11, 12]. Preliminary data in our connective tissue disease population has suggested an association between autoimmune ulcers and both antiphospholipid antibodies and genetic prothrombotic states [13].

The primary aim of the current study was to evaluate the prevalence of lower extremity ulcers in our scleroderma population. The secondary aim of this study was to evaluate the presence of antiphospholipid antibodies and genetic prothrombotic states in patients with scleroderma-associated leg ulcers. The outcomes of empiric therapy in the small number of patients evaluated in this study are reported.

2. Methods

This study was approved by the Biomedical Institutional Review Board at Georgetown University Medical Center as part of the Connective Tissue Disease Leg Ulcer Etiology (CLUE) study.

2.1. Patient Selection for Prevalence Evaluation. All scleroderma patients followed in the Georgetown University Hospital Division of Rheumatology and Wound Healing Center between August 2007 and August 2009 were evaluated for the presence of non-digital lower extremity ulcers. Active leg ulceration was defined as presence of non-digital lower extremity wounds that have been refractory to standard wound care for more than 3 months.

2.2. Laboratory Studies. Patients with active ulcers underwent autoimmune testing including antinuclear antibody by immunofluorescence (ANA), anti-Scl70 antibody, anticentromere antibody, antidouble stranded DNA antibody (dsDNA), anti-Sm antibody (Sm), anti-U1-RNP antibody (RNP), anti-Ro antibody (SSA), anti-La antibody (SSB), rheumatoid factor, and anticyclic citrullinated peptide. Prothrombotic evaluation was also completed in all patients including prothrombin gene mutation, plasminogen activator inhibitor-I mutation (PAI-I), methyltetrahydrofolate reductase mutation (MTHFR C677T), Factor V Leiden (FVL), protein S functional activity, protein C functional activity, antithrombin III functional activity, anticardiolipin IgG, IgA, and IgM titers, anti-β2-glycoprotein I IgG, IgA and IgM titers, and lupus anticoagulant.

2.3. Venous and Arterial Testing. All patients had evaluation of ankle-brachial pressure index and venous Doppler ultrasound studies to identify any concomitant venous or arterial disease. If present, patients were referred to vascular surgery for therapy.

2.4. Biopsy. Surgical debridement of wounds was performed if clinically indicated, as determined by the wound healing attending physician (CEA). If debridement was performed, biopsies, including a small piece of normal skin at the edge of the lesion, were taken. This is standard procedure in our center when evaluating for the presence or absence of vasculitis and is thought to give the highest yield of detecting small vessel vasculitis in vessels away from the base of the ulcer. All specimens were evaluated by an attending pathologist and the primary investigator (VKS), both of whom are experienced at reviewing skin biopsy specimens for the presence of vasculitis. The specimens were graded as to the presence or absence of fibrin plugging, leukocytoclastic vasculitis, or vessel necrosis.

2.5. Evaluation for Infection. It is well known that many chronic wounds become colonized with bacteria, but not all colonized wounds are infected. Following standard procedure in the Center for Wound healing, all patients were evaluated at each visit for symptoms or signs of acute infection, based on presence of purulence, odor, ascending erythema, fever, or systemic symptoms. If infection was present, the wound was debrided, and antibiotics were initiated and tailored according to the results of deep cultures taken in the operating room [14].

2.6. Local Therapy. Patients were treated with aggressive local wound therapy as determined by standardized protocols used by the Center for Wound Healing [14]. Patients with associated macrovascular disease were referred for surgical intervention. Dressings were selected to promote wound healing based on accepted criteria of maintaining high humidity at the wound-dressing interface, removing excess exudate, promoting gaseous exchange, providing thermal insulation, being impermeable to bacteria and other contaminants, and being removable without causing trauma to the wound bed. At each follow-up visit ulcer planimetry was used to assess interval change in ulcer size. Reduction in size at a rate of 10% per week was consistent with healing.

2.7. Systemic Therapy. Based on case reports in the literature, open label low-dose, low-molecular-weight heparin (Enoxaparin, 40 mg subcutaneously once daily, Sanofi-Aventis) was used in four patients [9, 10], and darbopoetin alfa (Aranesp, 0.45 mcg/Kg subcutaneously once weekly, Amgen Inc.) was used in one patient [15]. The outcomes of these interventions are reported.

2.8. Pain and Quality of Life Assessment. At the initial visit and upon healing of the wound, patients were asked to complete visual analogue pain score and two quality of life assessments, the Short Form 36 (SF36) which has been validated in many populations including those with scleroderma [16], and diabetic leg ulcers [17] and the Cardiff Wound Impact Schedule (CWIS) which was specifically developed for assessing the impact of leg ulcers on quality-of-life and which has been validated in a population of patients with refractory leg ulcers [18]. Both the SF-36 and the Cardiff Wound Impact Schedule are validated quality-of-life instruments in which the patients' answers to specific questions are scored. The scores are computed using validated formulae to calculate a total score from 0 (worst quality of life) to 100 (best quality of life).

2.9. Statistical Analysis. Demographic data was analyzed using descriptive statistics. Interval change in wound percent surface area was calculated at each visit, and wounds were stratified as healed, healing (defined as a reduction of surface area >10% per week), or open (reduction in surface area <10% per week). Quality-of-life and pain scores were analyzed according to wound status (open or healed) at the time the data was recorded, and paired *t*-test was used to analyze these results. Due to the small number of patients being studied and the uncontrolled nature of the interventions in these patients, no statistical analysis was performed on the outcome data.

3. Results

3.1. Prevalence of Leg Ulcers in Scleroderma. Between August 2007 and August 2009, 10 of 249 scleroderma patients had active leg ulcers. The prevalence of leg ulcers in our scleroderma population was therefore 4.0%.

3.2. Demographic Features. Of the 10 patients with scleroderma associated leg ulcers, 2 had diffuse scleroderma; 6 had limited scleroderma, and 2 had scleroderma sine scleroderma (Table 1). Consistent with our scleroderma population, 70% were female, and 90% were Caucasian. The age at first ulcer was normally distributed with a mean age of 59.90 years (range, 42 to 76 years, median 59.50 years).

3.3. Disease Duration. The duration of scleroderma at the time of first ulcer development was also normally distributed with a mean age of 18.14 years (range from 4 to 46 years, median 18.5 years). The 2 patients with diffuse scleroderma had shorter disease duration prior to ulcer development (mean 4.05 years ± 0.05) compared to those with limited scleroderma (mean 22.83 years ± 5.612, *P*-value .0078).

3.4. Ulcer Distribution. Ulcers were bilateral in 7 of the 10 patients (70%). Notably, all three of the patients with unilateral lesions had underlying large vessel disease (two with arterial disease and one with venous insufficiency based on Doppler ultrasound measurements). Of the patients with bilateral ulcers, all had lesions in the perimalleolar or anterior ankle region, and two patients additionally had more distal lesions on the feet.

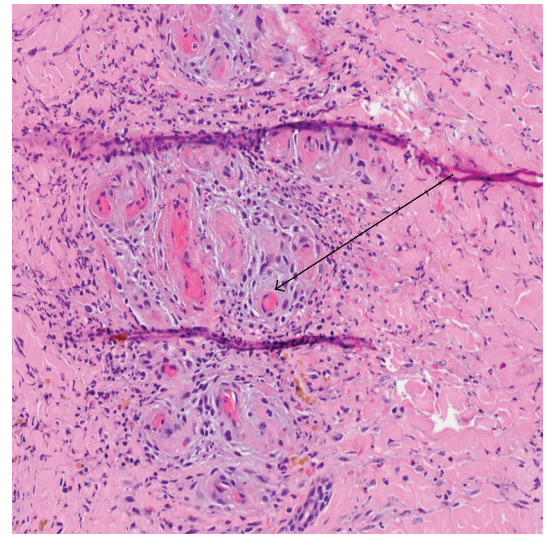

Figure 1: Biopsy of patient 7 showing fibrin occlusive vasculopathy (arrow).

3.5. Biopsy Findings. Biopsy specimens were available for review in 5 of the 10 patients (Table 1). All five biopsies showed fibrin plugging and vasculopathy changes as seen in Figure 1. None of the biopsy specimens had evidence of vasculitis.

3.6. Antibody Profile. The autoantibody profile is listed in Table 1. Anticentromere antibody was positive in four of the six patients with clinically limited disease. One patient had positive Scl70 antibody, and the other patient was a man with clinically limited scleroderma and positive SSA antibody. Two patients had clinically diffuse scleroderma. One had antitopoisomerase antibody (Scl-70), and another was positive for RNA polymerase III (pol 3). All patients had normal complement levels.

3.7. Antiphospholipid Profile. The results of the antiphospholipid profiles are shown in Table 1. All 10 patients had at least one antiphospholipid profile, and 9 patients had two phospholipid profiles 12 weeks apart, as it is recommended to confirm the presence of antiphospholipid antibodies [19]. Of the 10 patients, 5 had persistently positive antiphospholipid antibodies, giving a prevalence of antiphospholipid antibodies in this group of scleroderma patients with leg ulcers of 50%. Although phospholipid antibody data was not available on the cohort of scleroderma patients without leg ulcers in this study, these rates are higher than reported in the general scleroderma population. Of the 5 patients with persistently positive antibodies, 4 additionally had a history of pregnancy morbidity or vascular thrombosis although they were not on chronic anticoagulation.

3.8. Procoagulant Profile. The genetic procoagulant profile is also listed in Table 1. Homozygous or heterozygous mutation for methyltetrahydrofolate reductase (MTHFR) C677T mutation was seen in 7 of the 10 patients (70%). Plasminogen activator inhibitor gene (PAI-1) mutation was

Table 1: Features and outcomes of patients with scleroderma associated leg ulcers.

Pt			1	2	3	4	5	6	7	8	9	10
Sex			M	M	F	F	M	F	F	F	F	F
Race			C	C	C	C	C	H	C	C	C	C
SSc clinical subtype			Sine	Limited	Limited	Diffuse	Limited	Limited	Diffuse	Limited	Limited	Sine
Disease duration at time of ulcer development (years)			NA	20	17	4.1	4	46	4	26	24	NA
Scleroderma-specific antibody			U3RNP	Centromere	Centromere	RNA Pol3		Centromere	Scl70	Centromere	Scl70	Centromere
Other Antibodies			Nucleolar ANA; RF	Speckled ANA		Speckled ANA	SSA	Nucleolar ANA	Speckled ANA	Speckled ANA		
Other scleroderma features			GI dysmotility, GERD	GI dysmotility, GERD, SICCA, limited skin	GI dysmotility, SICCA, limited skin, joint contractures.	Diffuse skin, SICCA, arthritis, interstitial lung disease, GI dysmotility	SICCA, limited skin, joint contractures	GERD, Telangiectasias	Diffuse skin, interstitial lung disease, GI dysmotility, SICCA, calcinosis	Limited skin, joint contractures, SICCA, GERD, GI dysmotility, calcinosis	Limited skin, arthritis, pulmonary hypertension, GERD, SICCA, GI dysmotility	Calcinosis
Ulcer location			Left leg	Left medial malleolus	Bilateral malleoli and right posterior ankle	Bilateral malleoli	Bilateral Toes, dorsal foot and heel	Bilateral lateral calf	Right medial malleolus, left dorsal foot	Bilateral malleoli	Left lateral malleolus	Bilateral toes and bottom of feet
Venous insufficiency on doppler US			−	−	−	−	−	−	−	−	+	−
Arterial Disease on ABPI			+	+	−	−	−	−	−	−	−	−
SCREEN 1	LAC		+	−	−	+	−	−	+	−	−	−
	β-2GPI (normal <10 U/mL)	IgG	39	<10	<10	<10	<10	<10	41	<10	<10	<10
		IgA	<10	13	<10	<10	<10	100	23	<10	<10	<10
		IgM	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	ACL (normal <10 U/mL)	IgG	<10	<10	<10	45	<10	<10	23	<10	<10	<10
		IgA	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
		IgM	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
SCREEN 2	LAC		+	−	−	+	−	−	+	−	−	NT
	β-2GPI (normal <10 U/mL)	IgG	23	<10	<10	19	<10	<10	<10	<10	<10	NT
		IgA	<10	20	<10	<10	<10	100	<10	<10	<10	NT
		IgM	<10	<10	<10	<10	<10	<10	<10	<10	<10	NT
	ACL (normal <10 U/mL)	IgG	<10	<10	<10	20	<10	<10	<10	<10	<10	NT
		IgA	<10	<10	<10	14	<10	<10	<10	<10	<10	NT
		IgM	<10	<10	<10	<10	<10	<10	<10	<10	<10	NT

Table 1: Continued.

Pt		1	2	3	4	5	6	7	8	9	10
Summary APLprofile		+	+	−	+	−	+	+	−	−	−
Genetic procoagulant profile	MTHFR	0	1	2	1	1	1	0	1	1	NT
	PAI-1	0	1	1	0	2	0	1	0	0	NT
	Prothrombin Gene	0	0	0	0	0	0	0	0	0	NT
	FVL	0	0	0	0	0	0	0	0	0	NT
Summary Genetic procoagulant profile		−	+	+	+	−	+	+	+	+	NT
Biopsy		Fibrin occlusive vasculopathy	No biopsy	Fibrin occlusive vasculopathy	Fibrin occlusive vasculopathy	No biopsy	No biopsy	Fibrin occlusive vasculopathy	Fibrin occlusive vasculopathy	No biopsy	No biopsy
Treatment		Enoxaparin 40 mg daily, arterioplasty	Arterioplasty	Enoxaparin 1 mg/Kg twice daily	Enoxaparin stopped due to bleeding Darbepoetin alfa	Pentoxifylline 400 mg three times per day	Enoxaparin 40 mg daily	Enoxaparin 40 mg daily	None	Venous surgery pending	Healed with Nifedipine
Outcome		Healed	Healed	Not healed	Healed	Healed	Healed	50% healing in 3 months	Not healed	Not healed	Healed
Total duration of ulcer (months)		36	6	350	10	6	23	36	3	5	6
Time to healing after initiation of therapy		4	6	—	3	6	4	—	—	—	3

GERD: Gastroesophageal reflux disease; SICCA: dryness of the conjunctiva and cornea and dryness of the mouth; GI dysmotility: gastrointestinal dysmotility; ACL: anticardiolipin antibodies; β-2GP1 Ab: Beta-2 Glycoprotein I antibodies; LAC: lupus anticoagulant; MTHFR: Methyltetrahydrofolate reductase mutation; PAI-1: Plasminogen Activator Inhibitor-I mutation; FVL: Factor V Leiden mutation;
For gene mutation results 1: heterozygous mutation, 2: homozygous mutation; NT: not tested.

heterozygous positive in 3 patients and homozygous in 1 patient (40%). Factor V leiden and prothrombin gene mutations were not identified in any patient studied. All patients had normal protein C, S, and anti-thrombin III activity.

3.9. Pain and Quality of Life. Pain and quality-of-life data was available in 7 patients enrolled in the CLUE study.

Pain score was significantly lower in patients with healed wounds (0.6 ± 0.6) compared to those with open lesions (5.025 ± 1.007, P .0351), clearly demonstrating that wound healing correlates with a dramatic improvement in pain.

The CWIS well-being score was significantly better in the patients with healed wounds (58.84 ± 5.465) compared to those with open wounds (37.93 ± 3.757, P.0334). The CWIS physical score was also higher in the patients with healed wounds (86.97 ± 1.57) compared to those with open wounds (59.62 ± 6.295, P .0358). However, there was no difference in social functioning with wound healing. Analysis of the SF-36 data in this small population did not identify significant differences between the healed and unhealed wounds in any of the SF-36 domains.

3.10. Response to Therapy. Due to the association of ulcers with antiphospholipid antibodies and the successful outcomes seen in patients with livedoid vasculopathy, low-dose, low-molecular weight heparin (Enoxaparin, 40 mg subcutaneously once daily, Sanofi-Aventis) was used in 5 patients. Rapid and complete healing was seen in 2 of the patients (patient 1 and patient 6); both of whom had positive antiphospholipid antibodies. Patient 7 just recently commenced therapy with low-dose enoxaparin and to date has demonstrated 50% reduction in ulcer surface area in 3 months. Patient 4 developed bleeding with low dose enoxaparin and had to discontinue the medication. She was subsequently treated with darbopoetin alfa (Aranesp, 0.45 mcg/Kg subcutaneously once weekly, Amgen, Inc.) for anemia, and this resulted in complete healing of the ulcer. Patient 3 had negative antiphospholipid antibodies but homozygous mutation for MTHFR C677T and heterozygous mutation for PAI-1. To date, she has been refractory to low-molecular-weight heparin even at doses of 1 mg/kg twice daily. She remains unhealed after 350 months of follow-up. Pentoxifylline, a xanthine derivative with anti-TNF and fibrinolytic actions, was used in one patient (patient 5) with healing of the ulcers.

3.11. Prognosis of Leg Ulcers in Scleroderma. Of the 10 scleroderma patients with leg ulcers prospectively followed in this study, all had open lesions for ≥3 months. Complete healing has been seen in 6 patients (2 with LMWH, 1 with darbopoetin alfa, 1 with pentoxifylline, 1 with nifedipine, and 1 with arterioplasty), and one patient is responding to LMWH though not completely healed. Ulcers remain refractory to healing in three patients.

4. Discussion

The prevalence of non-healing lower extremity ulcers in our scleroderma population was 4%. In this study all scleroderma patients presenting to the Rheumatology Clinic were evaluated for scleroderma-associated leg ulcers. While there may be a perceived bias because of our special interest in scleroderma-associated lower extremity ulcers, only two patients were identified who were not previously known to have scleroderma both of whom had scleroderma sine scleroderma. Based on this finding, we think that the prevalence reported is likely a true estimate of prevalence of non-healing lower extremity wounds in scleroderma. Furthermore, this highlights the importance of evaluating patients with non-healing wounds for scleroderma even in the absence of overt skin changes. No other studies have specifically evaluated a cohort of scleroderma patients for non-digital ulcer prevalence, but our data are in line with that reported by Alivernini et al. [2]. The cumulative incidence of diabetic leg ulcers over a 5-year period has been reported at 5.8% [3], suggesting that the frequency of leg ulcers in scleroderma approaches that seen in diabetes.

Biopsy studies were available in 50% of patients in this study. We did not identify vasculitis in any of the biopsied wounds. While any biopsy always carries a risk of sampling error, we believe that biopsies which include the subcutaneous tissue and a perimeter of normal skin at the edge of the wound are usually sufficient to confirm the presence or absence of vasculitis [14].

Our study design had significant limitations since we do not have funding or IRB approval to screen our entire scleroderma population for prevalence of antiphospholipid antibodies and genetic prothrombotic states. This limits our ability to draw firm conclusions regarding the associations with lower extremity ulcers.

Although our study was limited due to the sample size and study design, we were able to demonstrate that scleroderma associated ulcers are refractory to usual wound care therapies. Additionally, while the quality of life questionnaires administered in this study work best in large population studies, our data do suggest that presence of open wounds in scleroderma adversely impact quality-of-life over and above the underlying scleroderma.

The prevalence of antiphospholipid antibodies in our cohort of patients with ulcers was higher than that reported in the general scleroderma population (50% compared to between 3.3 and 12%) [20–22]. Several other studies suggest an association between antiphospholipid antibodies and lower extremity ulcers in scleroderma. Lupus anticoagulant has been identified as a strong predictor of ulcer presence in scleroderma patients (OR 7.2) [2]. Furthermore, cutaneous ulcers are more frequent in scleroderma patients with antiphospholipid antibodies than those without (63% compared to 39%) [22]. Finally, a study reporting a series of eight patients with concomitant scleroderma and antiphospholipid syndrome identified three patients (37.5%) with associated leg ulcers [23], a much higher prevalence than we found in our more general scleroderma population.

MTHFR C677T heterozygous or homozygous mutation was also higher than expected in our cohort of scleroderma patients with leg ulcers. We found a prevalence of 60% for the heterozygous mutation and 10% for the homozygous mutation whereas in the general scleroderma population

49% expressed wild type (no mutation), 36% were heterozygous, and 15% were homozygous for the mutation [24]. Our population of patients with scleroderma associated leg ulcers had a prevalence of the plasminogen activator inhibitor gene (PAI-1) mutation of 40%. This is on a par with frequencies of this gene mutation in other populations, with the 4G allele being reported at a frequency of 62% in healthy pregnant women [25].

The outcome of the small number of patients treated with low-molecular-weight heparin therapy is promising. When tolerated, we found a 50% complete healing rate. Furthermore, response did not require full therapeutic doses of heparin, suggesting that, heparin may be acting via antiphospholipid-dependent pathways, such as complement activation and fibrinolysis, rather than purely through its anticoagulant effect as has been reported for antiphospholipid-associated pregnancy losses [12]. Only one patient in our study was treated with darbopoetin alfa but this resulted in complete healing of her ulcer. A similar response has been reported in one other case report [15]. The erythropoietin analogues are increasingly recognized as stimulators of angiogenesis pathways, and therefore these pathways may merit further investigation in scleroderma [26].

5. Conclusions

Lower extremity ulcers are seen in 4% of scleroderma patients and cause pain and morbidity over and above that of the scleroderma. In this small study we identified higher than expected frequency of antiphospholipid antibodies and MTHFR mutation. We recommend that scleroderma patients who develop leg ulcers should undergo prothrombotic evaluation. Clearly this small uncontrolled study is insufficient to draw clear conclusions as to etiology of delayed wound healing in scleroderma. However, lower extremity ulcers represent a challenging clinical problem in scleroderma and further studies into their pathogenesis and potential therapies may yield new insights into the vasculopathy of scleroderma at a cellular and molecular level.

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Review Article

Registry Evaluation of Digital Ulcers in Systemic Sclerosis

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Digital ulcers are a very frequent complication of systemic sclerosis affecting about half of the SSc patients, and about 75% of the affected patients have their first DU episode within 5 years from their first non-Raynaud symptom. The lack of adequate classification criteria as well as the lack of knowledge of the development of DU have contributed to the opening of specific registries to better understand the natural history of these lesions. For these reason, specific disease registries play a fundamental role in this field of research. Thanks to the systematic collection of data and their subsequent analysis and comparison between different cohorts, it is possible to improve understanding of the underlying trigger mechanisms of DU development and to determine temporal trends. In the future, the development of recommendations for the management of DU remains of pivotal importance to prevent DU development and obtain rapid healing as well as reduction of pain and disability.

1. Introduction

Digital ulcers (DUs) are a very frequent complication of systemic sclerosis (SSc) that affects almost half of the patients, either with limited (lSSc) or diffuse (dSSc) subset of the disease. About 75% of the affected patients have their first DU episode within 5 years from their first non-Raynaud symptom [1–3].

The aetiology of DU is complex and multifactorial. The principal mechanisms underlying the DU formation are ischemic, mechanic, and inflammatory, alone or in combination, acting over the SSc vasculopathy [4]. In fact, there are at least three types of DU: those localized in the acral parts of the body, as the fingertips, mainly resulting from an ischemic process, those localized on the dorsal aspect of the fingers where the skin retraction due to fibrosis over bony prominences seems to be the main cause and those evolved on a pitting scar or subcutaneous calcinosis due to a localized inflammatory irritative mechanism [1, 5].

In recent years, the increasing interest for the effects of drugs on healing and prevention of new ulcers and the awareness of the importance of DU for the Quality of life (QoL) of SSc patients, have led to the opening of specific registries to better understand the natural history and evolution of DU.

The present report provides an overview of the DU specific registries that are at the moment available and of the disease registries containing data on DU.

2. The Burden of DU

In SSc, DUs are a persistent and often recurrent complication, difficult to manage and slow to heal and can cause tissue loss [6]. Furthermore, DUs are frequently infected and may lead to osteomyelitis, gangrene, autoamputation, and in some cases to septicaemia [7].

DUs are also very painful with a disabling effect on patients, limiting hand function and daily activities, such as feeding, dressing, and hygiene [8]. The net effect is the heavy QoL impairment [9]. Indeed, the progressive scarring and tissue loss, that patients experience daily, can lead to severe social and self-esteem problems [10]. Moreover, severe cases of DU required frequent hospitalization with an elevated burden for the health care system and for the families due to the leave from work [8].

3. The Scleroderma Digital Ulcers Database (DAS-DU)

The DAS-DU was created in 2004, in the Scleroderma Ulcer Care Unit of the Division of Rheumatology of the University of Florence, to collect information on SSc DU and their management [5]. The DAS-DU includes basic clinical and demographic data that could be useful in clinical trials. Initially, the database was based on a system of paper datasheets that were then stored in chronological order. Despite being a valuable support to understanding the natural history of DU, in practice, this initiative has also identified a number of problems related to opening a long-term database. First, a larger amount of clinical data on DU required a continuous update of the database that was still insufficient to achieve the objective of this initiative. Secondly, the difficulty in data collection and statistical analysis with the paper-based system has led us to create a new electronic database to meet the needs of the progress of knowledge. The database is now maintained on an Access platform stored on a protected offline dedicated computer with anonymised data. All patients are associated with a unique ID, while all DU episodes in a single patient have a secondary unique ID.

Actually, the DAS-DU represents one of the largest and best characterized DU specific database of a single center SSc cohort. Good quality clinical information on more than 100 SSc patients with more than 2000 DU episodes has been recorded on the database. The dataset collected includes main DU characteristics such as localization (fingertips, nails area, dorsal, and palmar aspect of the finger), dimensions (area in mm^2), bed of the lesion (reepithelialisation, granulation tissue, fibrin, wet or dry necrosis, eschar, and gangrene), exudate (low, high or pus), borders of the lesions (regular or irregular), perilesional skin (normal or inflamed) and oedema, bone and tendons exposure, and autoamputation. Every DU was also staged in superficial (partial thickness skin loss involving epidermis), intermediate (full thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to, but not through underlying fascia), or deep (full thickness skin loss with extensive destruction or damage to muscle down over the fascia, supporting structures and bone). A pain scale (NRS 0–10) and the Cochin Hand function also known as the Duruöz hand functional disability scale [11, 12] were also included in the main dataset.

Essentially this database reflects the clinical practice in the *Scleroderma Ulcer Care Unit* of our centre which has generated a significant amount of specific consistent data on the main characteristics of DU integrated with clinical features. This has ensured quality and accuracy of the data collected.

The majority of SSc patients included in the DAS-DU (2004–2008) were limited SSc (70%), most were Caucasian women (86%), mean age of patients was 58 (57.7 ± 15.1 years) with a cumulative mean number of DU of $15.7 \pm 17,7$ over the four years followup [5]. The majority were observed in lSSc (72%) although the frequency of recurrent DU (61%) were higher in dSSc. DU were most frequently localized on fingertips (55%) with a mean dimension of 50 mm^2. Mean features of DU were irregular borders of the lesion (80%), and the presence of oedema and inflammation of perilesional skin (75%), and an intermediate stage (60%). Spontaneous moderate to severe pain was always present and is very frequently associated with infection. Despite the treatment, gangrene may occur and osteotendinous exposure (43%) and infection (40%) were frequently observed and required surgical amputation (14%). The database allowed also the record of the time to healing which was about 80 days [5] and also showed that DU, developed on a pre-existing calcinosis, had an increased time to healing to about 95 days and to over 280 days for those derived from gangrene.

4. The Digital Ulcers Outcome (DUO) Registry

The DUO Registry, meeting a postapproval commitment to the European Medicines Agency (EMEA), was started in April 2008 to collect information on DU associated with SSc and their management. In this registry, SSc patients with ongoing DU are enrolled to track key information of the clinical course and outcome of DU and to collect safety information on the use of bosentan, a dual endothelin receptor antagonist approved in Europe for reducing the number of new DU in SSc.

The registry is an European, multicenter, prospective, observational, noninterventional program for patients with DU/SSc. Participating patients undergo assessments and receive medical or surgical therapies according to their physician's judgment. Patients do not receive any experimental intervention or treatment as a result of their participation in the registry. Data are collected via a secure server based on the Internet where the access was guaranteed for registered users only. In order to protect privacy, a unique identifier was assigned to each patient.

The dataset includes demographics, past history and present status of the disease and DU, complications like soft tissue infection, gangrene or osteomyelitis, and care protocols including topical medications, drugs, and surgical interventions. From April 2008 to May 2009, 648 patients have been enrolled, with 47% lSSc, 41% dSSc, and 9% of overlap/mixed CTD. The mean age was higher for lSSc patient (56.2 years SD:13.4) than dSSc patients (50.8 years SD:14). Preliminary data analysis, still unpublished, shows that more than half of patients needed hospitalization for complications or intervention (59% of lSSc and 52% of dSSc). The most frequent complication observed was soft tissue infection requiring antibiotics (44.5% of lSSc and 40.5% of dSSc), followed by gangrene (34% of lSSc and 26.5% of dSSc) and finally by surgical amputation (17% of lSSc and 11% of dSSc) [13].

5. The German Network for Systemic Scleroderma (DNSS) Registry

The DNSS registry was founded in 2003 and includes demographic information of SSc patients and signs and symptoms of organ involvement (heart, lung, gastrointestinal tract,

kidney, musculoskeletal system, nervous system, and skin), characteristic laboratory data such as antinuclear antibodies, ESR and CK serum levels, as well as information on physical and on systemic therapies.

In 2008, a statistical study on more than 1880 SSc patients was performed to detect possible risk factors for the development of DU [14]. About 24% of the patients had active ulcers at the time of entering on the DNSS register. As expected, Raynaud phenomenon (RP) was the most prevalent symptom with a slightly higher prevalence in DU patients (98% versus 94%). Lung fibrosis was significantly higher in patients with DU (45% versus 33%) as well as pulmonary arterial hypertension (PAH: 24% versus 13%), heart (18% versus 13%), upper gastrointestinal (18% versus 13%), and oesophageal involvement (71% versus 57%). Also skin sclerosis (95%) and mouth involvement (36%) were associated with DU. The multivariate analysis of data obtained by comparing patients with or without DU showed that male sex, pulmonary arterial hypertension, oesophageal involvement, the extent of skin sclerosis assessed by the modified Rodnan Skin Score (mRSS), presence of anti-Scl-70 antibodies, young onset of Raynaud's phenomenon (RP), and an elevated ESR represent significant risk factors for the occurrence of DU in SSc. In addition, univariate analysis showed that the above factors and the presence of diffuse subset of disease (dSSc), pulmonary fibrosis and involvement of the upper gastrointestinal tract, and heart involvement are more common in patients with DU representing an elevated relative risk for DU.

Moreover, the combination of male gender, early onset of RP, an ESR >30 mm at the first hour, anti Scl-70 positivity and gastrointestinal and pulmonary arterial involvement showed the highest probability of developing DU (88%) [14].

After all, the DNSS registry showed a heterogeneity between centres for the DU management. In fact, only the 21% of patients with DU received prostacyclins and 40% of patients with DU did not receive a vasoactive therapy, and even in 27.8% of patients who already suffered from DU at the time of initial registration in the DNSS centres, calcium channel blockers was the only therapy. Bosentan (5.5%) and sildenafil (3.4%) are infrequently prescribed in these patients. It was remarkable that 56% of patients with DU performed physiotherapy with paraffin kneading or baths and lymph drainage [15].

6. Canadian Scleroderma Research Group (CSRG) Registry

The CSRG have collected annually data on presence, location, and number of DU with their complications and other organ involvement and skin score on their SSc cohort to determine possible associations of DU with other factors such as internal organ complications.

Out of the 938 patients enrolled, 86% were women with a mean age of 56 years, a disease duration of about 13 years and 53% with lSSc. Fifteen percent patient had currently a DU, 45% had a DU ever, and 53% had pitting scars. Digital necrosis were found on 1.8% of patients and amputation in about 7%.

A significant association were found with increased disease duration, younger age at the disease onset, Scl-70 autoantibodies, interstitial lung disease, reduced DLCO, increased mRSS, and hands and fingers skin score but no associations with smoking habits, gender, or other structural vasculopathy marker as PAH. It is to be noted that patients with diffuse SSc subset are nearly twice more likely to experience DU than patients with lSSc. Finally, DUs were associated with an increased burden of disease, as assessed by HAQ [16].

7. EULAR Scleroderma Trial and Research (EUSTAR) Group

EUSTAR has been founded in 2003 and the minimal essential data set (MEDS) has been created to allow all the members to store their data. In the first analysis of the database, which included a total of 3656 patients (1349 with dSSc and 2101 with lSSc) enrolled in over than 100 centres worldwide, DU resulted to be in 42.7% of patients with dSSc and about 33% of lSSc patients. In both subsets, patient with earlier onset of Raynaud's phenomenon had DU more often than those with a late onset (51% versus 35% in dSSc and 39% versus 28% in lSSc) [2]. Moreover, a difference of prevalence of DU in association with autoantibodies was demonstrated. Indeed, the prevalence was 36.7% with ANA positive, 44.8% with Scl70 positive, and 31.2% with ACA positive [2]. This has led EUSTAR to start a DU observational study with a retrospective and a prospective phase, opened to all member centres, with the aim to describe the natural history, treatment patterns, and outcomes of DU disease in a multinational cohort of incident DU patients. Actually, more than 120 patients have already been enrolled in retrospective phase and about 60 in perspective. Preliminary data will be available soon. Single center and national registries, as above mentioned, are very useful to understand the prevalence and incidence of DU in SSc but an international registry remains of invaluable utility to understand the overall behaviour of DU in SSc and its clinical features as well as being a solid base for large scale clinical and basic studies.

8. Assessment and Outcome Measure

The definition of outcome measure and indicators of DU severity is perhaps the main purpose of the DU registries. In recent years, several trials have studied the effects of drugs on healing or prevention of DU [10, 17–20] but, analyzing these studies, it is clear that DUs are *differently defined* and there is disagreement on the *outcome measures* used.

At the best of our knowledge, there are a considerable number of possible markers of DU *severity*: dimensions, depth, localization, origin, loss of tissue with bone and tendon exposure, inflammation or infection of perilesional tissues, pain, and loss of hand function [5]. A large and deep DU is associated with longer time to healing as well as the bone exposure might be associated with osteomyelitis that

may require surgical amputation [5]. Loss of hand function, hand disability, and QoL may also become markers of DU severity because they may have a substantial impact on patient well-being [9].

Localization may be helpful in determining the main trigger of DU. Usually, DU over the finger joints have been considered mechanical, mainly due to skin retraction and independent from ischemia [1] as the dorsal microvascular perfusion seems maintained in these area while the microvascular alterations are concentrated on the fingertips [21].

Pain is a pivotal symptom because it seems associated with severity of DU. Spontaneous moderate to severe pain is almost always present in large and deep DU, probably due to the extensive ischemia in the underlying tissues, but the presence of severe pain at DU presentation or the worsening of pain may be linked, in the majority of cases, with infection. Thus, the cause of pain must be carefully investigated in daily practice [5].

The past history of DU or DU as early manifestation of SSc may be a predictor of the future course and may help physicians in selecting a preventive aggressive treatment.

9. Conclusion

In SSc, a clinical classification of DU is still lacking, and today the treatment and prevention of DU represent a stimulating challenge for all rheumatologists.

The increasing number of clinical trials on DU highlights the need to increase knowledge of the mechanisms underlying the formation of DU in SSc. This has been the main reason for the creation of specific DU registries that have now a fundamental role in this field of research.

Thanks to the systematic collection of data and their subsequent analysis and comparison between different cohorts, it is possible to improve understanding of the different aspects of DU development and to determine therapeutic temporal trends [22–24].

The weak points of these registries, however, remain the lack of unambiguous criteria for the assessment of DU that are potential for selection bias, as well as the lack of data verification and probably patients are not monitored as rigorously as in randomized controlled studies (RCTs) thus leading to underestimation of the rate of some "events". Moreover, the lack of internationally accepted outcome measure has led to nonuniformity of the published. This evidence suggests to the community the urgent need to design a common strategy on DU mainly based on scientific evidence. Moreover, the DU classification still remains an unmet need that indeed may be helpful in identifying those lesions to be included or excluded from clinical trials [25]. The discrepancy of results in efficacy of some drugs seen in the trials published until today, may be probably due to the multifactorial pathogenesis of DU. In fact, DU caused by a predominantly ischemic process may be more responsive to vasoactive therapy than those caused by skin retraction or calcinosis.

There are some differences between disease registries and registries created for a specific feature of the disease. Data from a specific registry are more accurate than those derived from a general disease registry. In fact, some "event" might be underestimated, probably due to the amount of data included to the aim of the registry which is often to verify the natural course of disease and response to therapies. For this reason, it is fundamental to create specific registries, like EUSTAR and DUO registry, whose sole purpose is to study ongoing DU in SSc.

In the future, the development of recommendations for the management of DU remains of pivotal importance to obtain rapid healing as well as reduction of pain and disability.

Conflict of Interest

For all the authors no additional funding was received and there are no conflict of interest, both personal and/or institutional, that are relevant to the work conducted or reported in this manuscript.

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Review Article

Capillaroscopy as an Outcome Measure for Clinical Trials on the Peripheral Vasculopathy in SSc—Is It Useful?

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Peripheral microvascular impairment in systemic sclerosis (SSc) may be easily detected and scored in a safe noninvasive way by nailfold videocapillaroscopy (NVC). The paper highlights clinical conditions related to SSc in which NVC may represent an outcome measure of therapeutical interventions, by elaborating on their already assessed relationship with the NVC patterns and eventually scores. The 3 important biological/clinical conditions are: the positivity for SSc-specific serum autoantibodies, the presence of SSc skin digital ulcers (DUs) and of pulmonary arterial hypertension (PAH) SSc associated. In conclusion, to the question if capillaroscopy (NVC) may represent in SSc an outcome measure for clinical trials on the peripheral vasculopathy, based on the growing evidence and our detailed studies, the answer is positive. Recent therapeutic trials in SSc are confirming this role, and the experience is growing rapidly.

1. Introduction

Systemic sclerosis (SSc) is characterized by early and persistent microvascular impairment leading to functional Raynaud's phenomenon (RP) and clinical manifestations (i.e., digital ulcers, pulmonary arterial hypertension, etc.) (Figure 1) [1, 2].

Digital ulcers in SSc are considered to be related to tissue ischemia following several processes, including at the beginning persistent vasospasm (RP), but in the progression of the disease also to intimal fibroproliferation, tissue fibrosis, and thrombosis of digital arteries [3].

Progressive deficiency in vasodilatory capacity of the vessels and tissue fibrosis is proposed as a mechanism of the persistent vascular spasm; however, the mechanism of endothelial injury is still unclear [4].

The assessment of vascular involvement is still a matter of study, and several noninvasive techniques have been proposed. Peripheral microvascular impairment in SSc may be easily and safely detected by nailfold videocapillaroscopy (NVC). The morphological capillary abnormalities in SSc have been classified in 3 validated patterns (early, active, and late) of microangiopathy by NVC and scored (Figure 2) [5–7].

NVC may partially observe the column of red blood cells moving inside the capillary, but the technique does not allow measurement of the blood flow.

Laser Doppler flowmetry (LDF) is the best non invasive and safe technique to assess and to measure the blood perfusion at peripheral sites [8, 9].

Blood flow has been found to be reduced in patients with SSc, compared with healthy subjects and patients with primary RP. Patients with SSc showing the late NVC pattern of microangiopathy have a significantly lower finger blood perfusion (FBP) than patients with the active and early NVC patterns ($P < .05$) [10].

The question today is if capillaroscopy (and eventually LDF) may represent an outcome measure for clinical trials on the peripheral vasculopathy in SSc.

We will analyze clinical conditions related to SSc in which NVC may represent an outcome measure by considering their already assessed relationship with the NVC patterns

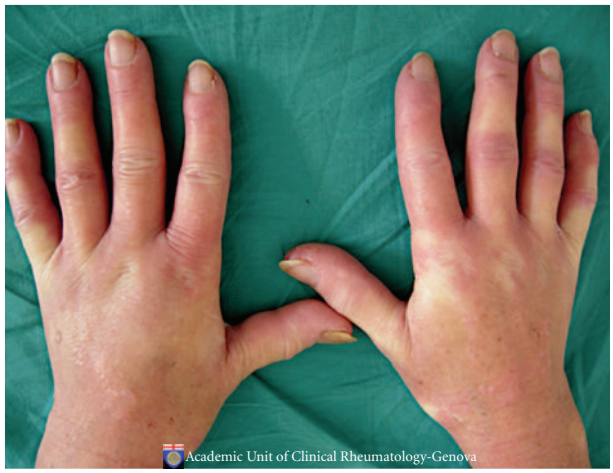

Figure 1: Hands of a patient with early systemic sclerosis suffering from secondary Raynaud's phenomenon.

and/or eventually scores. The 3 important biological/clinical conditions are: the SSc-specific serum autoantibodies, the SSc skin digital ulcers (DUs), and the pulmonary arterial hypertension (PAH) associated to SSc.

2. Serum Autoantibodies and NVC

SSc is characterized by serum autoantibodies, including anti-centromere (anti-CENP-B), anti-Th/To, antitopoisomerase I (anti-topo I), and anti-RNA polymerase I/III (anti RNAP III). Together, these markers account for almost 85% of autoantibodies specific for SSc and show a predictive value for clinical evaluation and prognosis [11, 12].

Anti-CENP-B and anti-topo I are known predictors of progression from isolated RP to SSc [13]. However, until recently, many of the studies on the significance of expression of these antibodies in SSc have been limited by small sample sizes, incorrect classification of patients with manifestations of connective tissue disorders as having primary RP, use of varying definitions of subsets of patients, lack of standardised methods for determining antinuclear antibodies, omission of tests for anti-Th/To and anti-RNAP III antibodies, and absence of multivariable analyses.

Antiendothelial cell antibodies (AECAs) are a heterogeneous class of antibodies whose role in the pathogenesis of autoimmune diseases with vascular involvement has been extensively studied and are present in the serum samples of many patients with SSc (22–86%) but are not SSc specific [14]. Even if, among the demonstrated clinical associations, lung and peripheral vascular involvement is the most common, further research on this topic, including longitudinal studies in patients with SSc, is mandatory for a better understanding of the clinical value of AECA.

However, for long time it has not been determined prospectively whether SSc autoantibodies are related to the course and type of microvascular damage detectable by nailfold capillaroscopy.

LeRoy and Medsger proposed that patients with RP who had abnormal findings on NVC and SSc-specific autoantibody should be classified as having early SSc [15]. This set of criteria had not been validated and considered for long time, until recently.

Finally, Koenig et al. prospectively studied a large cohort of Raynaud's patients who were referred to a single centre for evaluation of RP over a period of 20 years [16].

The objectives were to identify the strongest independent predictors of progression to definite SSc, to determine the type and time course of microvascular damage by nailfold capillaroscopy and its relationship to major SSc autoantibodies, and to validate the criteria for early SSc.

Of the 586 patients who were followed up for 3,197 person-years, 74 (12.6%) developed definite SSc.

In fact, this study validated the criteria of LeRoy by demonstrating that almost all patients who were to futurely develop SSc had "early" SSc (Raynaud's phenomenon plus a scleroderma pattern on capillaroscopy and/or SSc-specific antibodies) at the baseline visit.

Concerning the scleroderma pattern they reported a characteristic sequence of microvascular damage, starting with enlarged capillaries (giant capillaries) that identify the "early" SSc pattern, followed by capillary loss that indicates the "active" SSc pattern and then by capillary telangiectasias (neoangiogenesis) that might better characterize the "late" SSc pattern (Figure 2), from the stadium of "early" SSc until development of definite SSc.

Definite SSc was diagnosed in close temporal relationship to capillary loss.

Enlarged capillaries (giant capillaries), capillary loss, and SSc-specific autoantibodies independently predicted definite SSc.

Interestingly, anti-CENP-B and anti-Th/To antibodies predicted for the development of giant capillaries; these autoantibodies and anti RNAP III also predicted for capillary loss. Each autoantibody was associated with a distinct time course of microvascular damage.

At followup, 79.5% of patients with one of these autoantibodies and abnormal findings at the baseline nailfold capillaroscopy examination had developed definite SSc. Patients with both baseline predictors were 60 times more likely to develop definite SSc.

These data validated the proposed criteria for early SSc. In conclusion, in the presence of a secondary RP evolving to definite SSc, microvascular damage (as assessed by nailfold capillaroscopy) is dynamic and progressive, and SSc-specific autoantibodies are associated with the course and type of capillary abnormalities.

It was confirmed that the microvascular damage in secondary RP evolving to definite SSc is characteristically sequential, starting with enlarged capillaries (giant capillaries, "early" SSc pattern) followed by capillary loss ("active" SSc pattern), and then by capillary telangiectasias (neoangiogenesis, "late" SSc pattern).

Since new therapeutic agents are being evaluated in patients with SSc, awareness of this sequence of microvascular damage has potential implications for future trials. Of note, recently, it was established the evolution of

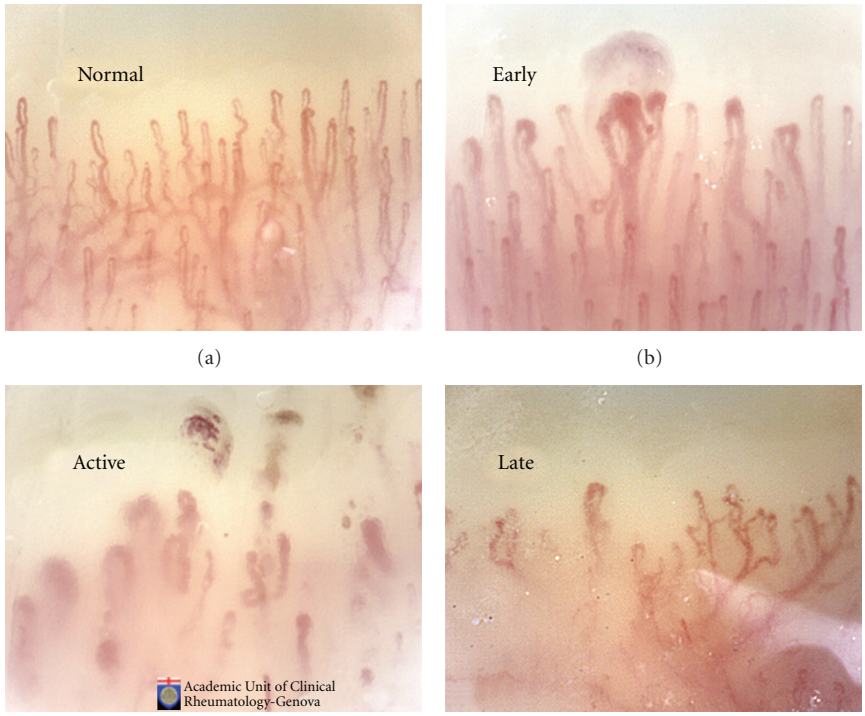

Figure 2: The morphological capillary abnormalities in SSc have been classified in 3 validated patterns (early, active, and late) of microangiopathy by NVC analysis.

microangiopathy in "established" SSc disease, by pointing out the progression of capillary loss and augmentation of ramifications in a microangiopathy score [7].

The final message is that abnormal findings on nailfold capillaroscopy at baseline together with a systemic sclerosis-specific autoantibody indicate a very high probability of developing definite systemic sclerosis whereas their absence excludes this outcome [17]. In SSc patients awareness of this sequence of microvascular damage and SS-associated autoantibodies has potential implications for future therapeutical trials.

For example, in trials of novel angiogenic, vasculogenic, or fibrosis-modulating agents, it would appear realistic to select SSc patients at a uniform stage of microvascular damage and with similar SSc-specific autoantibodies and eventually to evaluate such damage longitudinally by NVC to assess response to treatment.

3. Skin Digital Ulcers and NVC

Skin DUs represent one of the most frequent clinical manifestations of microangiopathy in patients with SSc (Figure 3) [18].

On the other hand, a decreased number of capillary loops should be considered highly specific for advanced RP, and it has been estimated that the number of normal capillaries may be reduced to just 20% in patients with active SSc. Skin DUs seem to be associated with the "late" NVC pattern, characterized by avascular areas (severe capillary loss).

Recently, using a semiquantitative score that highlights the importance of the number of capillaries, an association was reported between advanced stages of capillary loss (mean score class 2 and 3) and digital trophic lesions in 49% of patients with SSc [19]. Also, loss of capillaries may be relevant in determining tissue hypoxia, and, in patients with recent onset of RP, the appearance of rapidly progressive capillary loss may represent the first capillaroscopic evidence of severe SSc with destruction of microvessels [20].

The extensive disappearance of capillaries may generate large avascular areas giving a "desert-like" appearance to the nailfold bed, and progressive loss of capillaries has been associated with more extensive skin involvement (as well as diffuse SSc) and a poor prognosis.

As a consequence, the early detection of SSc patients who are at high risk of developing DU could allow preventive treatment of these complications with reduction of morbidity and social costs.

Very recently, it was found, in 130 SSc patients examined at entry and after 20 months of follow-up, that the diffuse cutaneous form of SSc with avascular areas on capillaroscopy represented, among other factors (e.g., increased interleukin-6) the major risk factor for DU development [21].

A previous study showed that, patients with late SSc pattern at NVC showed an increased risk to have an active disease (odds ratio (OR) 3.50; 95% confidence interval (CI) 1.31–9.39) and to present skin DU (OR 5.74; 95% CI 2.08–15.89) [2].

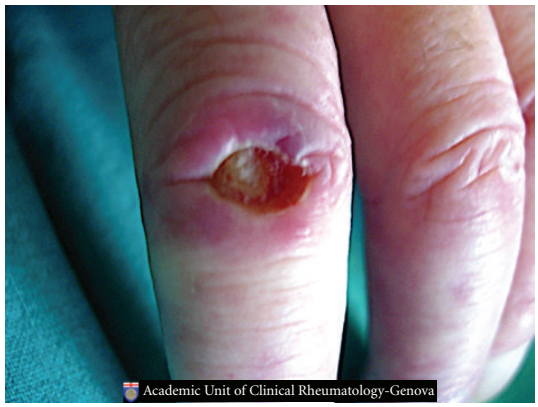

Figure 3: Classical skin digital ulcer in a patient affected by systemic sclerosis.

Another recent investigation showed that a quantitative capillaroscopic score was suggested highly predictive of the development of new skin DU within 3 months after NVC [22]. The predictive value of this index still needs to be confirmed in a validation study.

A clinical history of multiple skin DU is the most helpful predictor and indicator for preventive therapy, and the loss of capillary as assessed at NVC has been found the best possible NVC predictive marker to be considered.

However, the routine use of NVC now seems a possible predictive tool to enable the early detection of patients at a high risk of developing skin DU [1]. In this regard, at present, the cost/effectiveness ratio for the therapy of SSc skin DU is very unfavourable, and strategies for their treatment or prevention are under debate [2].

4. Pulmonary Arterial Hypertension and NVC

SSc is the main connective tissue disease associated with PAH and PAH is estimated to affect 12% of SSc patients, being the leading cause of death in this disease [23].

Structural changes in the systemic microcirculation, consisting of a reduction of capillary density and widening of the capillaries, are considered an hallmark of SSc.

It is now clear that the severity of this microvascular damage as assessed by NVC differs between patients with SSc and PAH (SSc-PAH) and those with SSc without PAH (SSc-non-PAH) and correlates with pulmonary haemodynamic parameters.

Interestingly, when compared to healthy controls, the same is true for patients with idiopathic PAH, a condition not known to be characterized by systemic microvascular changes [24].

A recent study suggests that capillary density reduction is a marker of the presence and severity of PAH [24].

A few studies have investigated nailfold capillary patterns in patients with SSc-PAH, with only one study including patients with idiopathic PAH. Two studies used echocardiography and/or right heart catheterisation to confirm the diagnosis of PAH. One of these, by Ong et al., found a significant reduction of capillary density in eight patients with SSc-PAH in comparison with 12 patients with SSc-non-PAH [25]. Pulmonary haemodynamic parameters were not reported in the study by Ong.

The other study, by Greidinger et al. using capillary density and qualitative scoring of nailfold patterns, found no differences in capillary patterns between eight patients with SSc-non-PAH and seven with SSc-PAH, but capillary density in these groups was not reported [26].

A third study, by Ohtsuka et al. using only right heart catheterisation to diagnose and exclude the diagnosis of PAH, showed a significant difference in semiquantitative scoring of nailfold patterns between SSc-non-PAH and SSc–PAH patients, but, again, capillary density was not assessed in this study [27].

Practically, only one of these studies included patients with idiopathic PAH and reported no differences in capillary density and capillary patterns between 13 healthy controls and 37 patients with idiopathic PAH.

There is, however, more recent evidence of a reduction of capillary density in both SSc-PAH and, albeit to a milder extent, in idiopathic PAH [24].

The explanation for a reduction in capillary density may not be the same for the two disorders. In SSc it is generally presumed that structural changes in the systemic (micro)circulation precede changes in the pulmonary circulation, as systemic microvascular changes may precede the development of SSc by many years.

Therefore, NVC abnormalities might also reflect what is going on in the pulmonary circulation. This may not be true for all capillary abnormalities, because most patients with SSc demonstrate nailfold capillary abnormalities whereas only a minority develop PAH. Recent data confirm that only capillary density is associated with the presence of PAH and is a marker of disease severity in SSc [24].

A further suggested explanation for the more pronounced capillary reduction in SSc-PAH could be that PAH itself amplifies the reduction of capillary density already present in SSc.

However, Houben et al. recently observed an increase rather than a decrease of nailfold capillary density in patients with heart failure [28].

The observation that circulating plasma levels of endothelin1 (ET-1) are raised in patients with PAH and that ET-1 production is increased in the pulmonary tissue of affected individuals makes this vasoconstrictor a particularly interesting target for therapeutic intervention in PAH [29].

Clinical trials with ET receptor antagonists have clearly shown that such antagonists provide symptomatic benefit in patients with PAH, thereby proving the clinical relevance of the endothelin system as a therapeutic target with optimised use of selective ETA or nonselective ETA/ETB blockade.

As matter of fact the highest serum ET-1 levels are found in SSc with late NVC pattern and visceral involvement [30].

Recent therapeutical examples of clinical trials with NVC as measure of outcome and conclusions are presented.

Very recent studies represent examples of clinical trials in which NVC has been successfully included as a measure of treatment outcome.

The objective of one study was to evaluate NVC pattern changes in SSc patients treated regularly on cyclic basis with iloprost and to find associations with clinical, serologic, and pharmacological variables [31].

Forty-nine patients affected by SSc underwent two NCV analyses at 3 years apart from each other. Six patients showed an amelioration of NVC abnormalities who changed from active to early pattern; five of these cases (83.3%) had been given cyclophosphamide therapy and the remaining case methotrexate plus azathioprine.

Cyclophosphamide administration was significantly associated with regression of the NVC pattern ($P < .001$). Interestingly, none of the SSc patients who received cyclophosphamide demonstrated worsening of the microvascular lesions; the progression of NVC pattern was inversely correlated to cyclophosphamide treatment ($P = .02$).

Therefore, cyclophosphamide treatment demonstrated to be effective in modulating the SSc microvascular damage as directly observed and monitorized by NVC.

In another study cyclophosphamide treatment showed to be effective for SSc microvascular damage as directly observed by rapid improvement of the NVC pattern [32].

Confirmation of the trend was obtained by a further study on autologous stem cell transplantation that confirmed a significant regression of the NVC pattern together with the improvement of the clinical conditions [33].

In conclusion, to the question if capillaroscopy (NVC) may represent in SSc an outcome measure for clinical trials on the peripheral vasculopathy, based on the growing evidences and our detailed studies, the answer is positive.

Early recent therapeutic trials in SSc are confirming this role, and the experience is growing rapidly [34].

To definitely establish its role as an outcome measure two requirements still need to be fulfilled. First, large multicentric and longitudinal and randomized controlled trials (certainly in establishing its role as an outcome measure in therapeutical trials) are needed. Second, multicentric reliability in scoring systems is warranted.

Recently, a first step in assessing reliability has been taken through demonstration of reliability both in qualitative and semiquantitative assessment of nailfold images of SSc patients in a bicentre setting [5].

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Review Article

Interleukin-6 as a Potential Therapeutic Target for Pulmonary Arterial Hypertension

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Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of biologic activities in immune regulation, hematopoiesis, inflammation, and oncogenesis. Recent accumulating evidence indicates a pathologic role for IL-6 in promoting proliferation of both smooth muscle and endothelial cells in the pulmonary arterioles, resulting in development of pulmonary arterial hypertension (PAH). Here, we describe a patient with mixed connective tissue disease and severe, refractory PAH. Her functional activity and hemodynamic parameters dramatically responded to tocilizumab, a humanized monoclonal antibody to human IL-6 receptor, which was aimed at treating multicentric Castleman's disease. It appears that IL-6 blockade may hold promise as an adjunct drug in treatment of PAH in idiopathic form as well as in association with connective tissue disease.

1. Introduction

Pulmonary arterial hypertension (PAH) is a cause of significant morbidity and mortality in patients with connective tissue disease (CTD), especially in those with systemic sclerosis (SSc) or mixed connective tissue disease (MCTD) [1]. In fact, a survival study over the past 30 years in consecutive patients evaluated at the University of Pittsburgh has demonstrated that PAH became the primary cause of SSc-related deaths today [2]. PAH is characterized by increased pulmonary vascular resistance due to remodeling of the pulmonary arterioles. Left untreated, PAH leads irremediably to right ventricular hypertrophy, pressure overload and dilation, and impaired cardiac output, resulting in death [3]. Until recently, there was no effective therapy for PAH, a disease with a median survival estimated to be approximately one year following the diagnosis in patients with SSc [4]. However, in the past two decades, novel therapies have been developed, focusing on vasoactive substances derived from the pulmonary vascular endothelium [5]. These substances, such as endothelin-1, nitric oxide, and prostacyclin regulate smooth muscle cell tone and proliferation and were shown to be central to the pathogenesis of PAH [6]. Therefore, current therapeutic agents target these 3 essential biological pathways: the endothelin-1/endothelin receptor, nitric oxide/cGMP, and prostacyclin/cAMP pathways. Improvement of symptoms, functional activity, and quality of life and even prolongation of survival have been partially achieved with currently available therapies, but mostly in patients with idiopathic PAH [5]. Indeed, it has become clearer in the past few years that SSc patients with PAH have a strikingly divergent response to current therapies and overall worse outcome compared with patients with idiopathic PAH in spite of seemingly milder hemodynamic impairment [7, 8]. In a recent multicentre longitudinal study to evaluate 3-year survival in SSc patients, 20 of 47 patients with PAH died during follow-up, giving a 3-year survival of only 56%, despite the fact that they were treated with modern PAH drugs [9]. Even in SSc patients with mildly symptomatic PAH in New York Heart Association (NYHA) functional class II, approximately two-thirds deteriorated to functional class III or IV, and some died during a 5-year period, although they were treated with one or more PAH drugs [10]. While there have been significant

advances in the treatment of PAH, survival of patients with PAH associated with CTD on modern PAH drugs remains unacceptably low. Therefore, novel therapeutic strategies targeting pathways beyond pulmonary vascular endothelium are required to further improve survival of CTD patients with PAH.

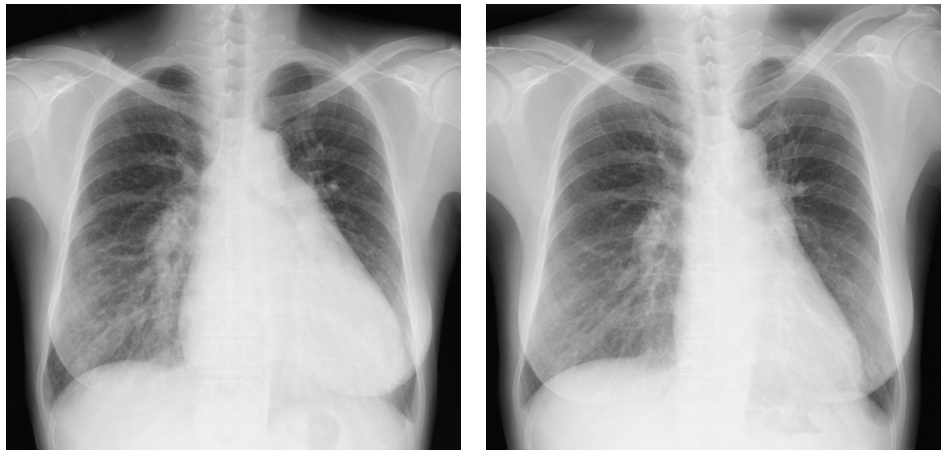
We have recently experienced a rare case of PAH-CTD complicated by multicentric Castleman's disease (MCD) during the course of the disease. MCD was successfully treated with tocilizumab, a humanized antihuman interleukin6 (IL-6) receptor monoclonal antibody, which dramatically improved functional activity and hemodynamic parameters of PAH as well.

2. Case Report

A 45-year-old woman first noticed polyarthralgia and puffy fingers in 1997 and developed slowly progressive dyspnea on exertion, which made her hospitalization in a regional hospital in 2001. Pulmonary hypertension was detected by transthoracic echocardiography, which showed mild right ventricular hypertrophy in conjunction with abnormal contour of the interventricular septum and increased systolic pulmonary arterial pressure (PAP) (100 mmHg) estimated by Doppler echocardiography. Interstitial lung disease (ILD) and pericardial effusion were also detected. Taken together with increased levels of C-reactive protein (CRP), positive antinuclear, and anti-U1RNP antibodies, she was diagnosed as having mixed connective tissue disease (MCTD) complicating pulmonary hypertension. She was treated with corticosteroid pulse therapy followed by high-dose prednisolone (1 mg/kg), resulting in improvement of exertional dyspnea and reduction in estimated systolic PAP to 60 mmHg. In November 2005, she visited a pulmonologist of the referring centre because of worsening dyspnea. She underwent a systematic cardiac evaluation, including right heart catheterization and ventilation-perfusion scan, and a diagnosis of PAH in NYHA functional class III was made based on mean PAP 58 mmHg, pulmonary capillary wedge pressure (PCWP) 10 mmHg, cardiac output 3.4 L/min, and pulmonary vascular resistance (PVR) 14.4 Wood units. The 6-minute walk distance (6MWD) was only 300 meters. Bosentan 250 mg was initiated with oxygen supplementation in January 2006, with subtle improvement of exertional dyspnea. After summer of 2007, her symptom gradually worsened again. In addition, she experienced low-grade fever, loss of appetite, and body weight loss (−5 kg/6 months) with cervical lymphoadenopathy and hepatosplenomegaly, which had worsened despite the use of low-dose prednisolone. She was referred to our hospital for additional evaluation into the etiology of PAH in April 2008.

She had marked limitation of physical activity (NYHA functional class III), and 6MWD was only 310 meters. Physical examination demonstrated jugular venous dilatation, lower extremity edema, and lymphoadenopathy on cervical, axillary, and inguinal lesions. Nailfold capillary changes were found, but sclerodactyly, muscle weakness, arthritis, and butterfly rash were absent. Laboratory data showed marked anemia (hemoglobin 8.1 g/dL), hypoalbuminemia (2.9 g/dL), polyclonal hypergammaglobulinemia with IgG 6451 mg/dL, CRP 9.1 mg/dL, brain natriuretic peptide (BNP) 181 pg/mL, a positive antinuclear antibody at a titer of 1 : 1,280 with pure speckled pattern, and a positive anti-U1 RNP antibody (86 Index; normal range <15). The protrusion of the main pulmonary artery, increased width of the descending branch of the right pulmonary artery, and an increase in the cardiothoracic ratio were noted on chest X-ray (Figure 1). Electrocardiogram showed signs of an increased right heart load. Hemodynamics assessed by right heart catheterization included man PAP 43 mmHg, PCWP 11 mmHg, right atrial pressure (RAP) 12 mmHg, cardiac output 5.5 L/min, and PVR 5.8 Wood units, indicating that 2-year treatment with bosentan partially improved these parameters. High-resolution computed tomography showed ground-glass opacities with minimal honeycomb cysts on bilateral lower lung field, dilatation of the right atrium, right ventricle, and central pulmonary arteries, and multiple mediastinal lymphadenopathy. Histological evaluation of the biopsied axillary lymph node demonstrated an intense plasmacytosis in the interfollicular areas with a prominent increase in capillaries and postcapillary venules, some of which were hyalinized. These findings were compatible with MCD in mixed histological features of plasma cell and hyaline vascular types [11]. Serology for human immunodeficiency virus (HIV) or human herpesvirus type 8 (HHV-8) was negative. Cytomegalovirus antigenemia was undetectable. Gene sequence for HHV-8 or Epstein-Barr virus was not found in the lymph node. A markedly elevated level of serum IL-6 (41.8 pg/mL; normal range <4) was consistent with the diagnosis of MCD [12]. Thus, we decided to first treat concomitant MCD with tocilizumab at a dose of 8 mg/kg every 2 weeks.

Serial functional, hemodynamic, and laboratory parameters before and after the tocilizumab treatment are summarized in Table 1. After 4 infusions of tocilizumab, low-grade fever and loss of appetite were completely gone, and fatigue was prominently improved with normalization of hemoglobin level. A prominent increase in circulating IL-6 concentration after introduction of tocilizumab indicated efficient IL-6 receptor blockade. Lymphadenopathy and hepatosplenomegaly were gradually resolved and were finally undetectable at 3 months after introduction of tocilizumab. Functional activity was gradually improved to NYHA functional class II and 434 meters at 6MWD. BNP was decreased to 48 pg/mL. Right heart catheterization at 3 months revealed that mean PAP was reduced to 31 mmHg and RAP was 5 mmHg. There was no improvement in cardiac output or PVR, but cardiac output was calculated with the Fick method by an equation containing a hemoglobin level, which rose up markedly after the tocilizumab treatment. In fact, systemic venous oxygen saturation had dramatically improved from 52.1% to 69.4%. Her dyspnea has continued to improve and was finally undetectable (NYHA functional class I) at 6 months when oxygen supplementation was discontinued. The increased right heart load findings on chest X-ray were remarkably improved (Figure 1). After 12 months of treatment with tocilizumab, she was in NYHA functional



Before tocilizumab (a)

After 6-month treatment with tocilizumab (b)

Figure 1: Chest X-ray before tocilizumab and after 6-month treatment with tocilizumab.

class I and was able to walk 663 meters at 6MWD without reduction of arterial oxygen saturation. The mean PAP was further decreased to 27 mmHg. The patient has been in NYHA functional class I and in remission of MCD on biweekly tocilizumab, as of May 2010. No side effect of the tocilizumab treatment was apparent.

3. IL-6 Overproduction and PAH

MCD is a rare lymphoproliferative disorder characterized by systemic lymphadenopathy and constitutional inflammatory symptoms [11]. Patients with MCD frequently have systemic manifestations, such as low-grade fever, fatigue, loss of appetite, and weight loss. Abnormal laboratory findings include anemia, hypoalbuminemia, hypergammaglobulinemia, and increased acute-phase proteins such as CRP. The etiology of the disease appears to be heterogeneous, but dysregulated overproduction of IL-6 is believed to be responsible for the clinical abnormalities [12]. In fact, IL-6 transgenic mice represented the disease phenotype resembling MCD, which was successfully treated with an anti-IL-6 receptor antibody [13]. In a multicenter prospective study to evaluate the efficacy of tocilizumab in patients with MCD, objective improvement was consistently observed in clinical symptoms, lymphadenopathy and other physical findings, and laboratory parameters [14]. In addition, HHV-8 is reported to be an etiologic agent of MCD, especially in patients infected with HIV [15], since HHV-8 encodes a human IL-6 homolog, which shares functional properties with human IL-6 [16].

PAH is a rare complication of MCD [11], and only 4 cases diagnosed with both of these conditions have been reported previously [17–19]. These case reports raise several hypotheses linking PAH and MCD. One hypothesis includes an association of PAH with HHV-8 infection rather than MCD itself. HHV-8 encodes genes homologous to human genes involved in cell proliferation, antiapoptosis, and angiogenesis [20, 21], and HHV-8 gene sequences have been found in plexiform lesions derived from some patients with idiopathic PAH [22], suggesting the possibility that HHV-8 could be involved in the misguided angiogenesis characteristic of PAH. However, HHV-8 infection was not detected in our case as well as in another 2 reported cases complicating MCD and PAH [17, 19]. One of the reported case infected with HIV and HHV-8 showed an unusual complete reversibility of both MCD and severe PAH, with an immunosuppressive treatment with cyclophosphamide, together with highly active antiretroviral therapy and epoprostenol [18]. In addition, tocilizumab induced partial remission of both MCD and PAH in our case as well as in the other HHV-8-uninfected case [19]. These case reports raise another hypothesis that IL-6 is a common pathogenic factor in both MCD and PAH.

Patients with idiopathic PAH are consistently found to have an increased level of IL-6 in circulation [23, 24] and in lung tissue [25]. In patients with lupus, MCTD, or SSc, a higher serum IL-6 level was reported in patients with PAH than in those without PAH [26–28]. In addition, elevated serum IL-6 was reported in patients with POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) syndrome, a rare variant of plasma cell dyscrasia, which sometimes complicates PAH [29]. Several animal models of PAH, including chronic hypoxia [30] and monocrotaline treatment [31], are also associated with increased production of IL-6. Moreover, daily subcutaneous injection of recombinant IL-6 in rats induced the medial thickness of small pulmonary arteries, leading to PAH [32]. These findings together suggest that PAH development is associated with IL-6 overproduction.

4. Roles of IL-6 in Pathogenesis of PAH

IL-6 is a pleiotropic cytokine with a wide range of biologic activities in immune regulation, hematopoiesis, inflammation, and oncogenesis [33]. Accumulating evidence indicates

Table 1: Serial functional, hemodynamic, and laboratory parameters before and after the tocilizumab treatment.

	Pretreatment	3 months	6 months	9 months	12 months
NYHA functional class	III	II	I	I	I
6MWD (m)	310	434	ND	ND	663
Mean PAP (mmHg)	43	31	ND	ND	27
PCWP (mmHg)	11	4	ND	ND	4
RAP (mmHg)	12	2	ND	ND	3
Systemic venous oxygen saturation (%)	52.1	69.4	ND	ND	75.3
Cardiac output (L/min)	5.5	4.5	ND	ND	4.4
PVR (wood unit)	5.8	5.6	ND	ND	5.3
Doppler systolic PAP (mmHg)	100	90	72	51	54
BNP (pg/mL)	181	48	44	46	37
CRP (mg/dL)	9.01	0.54	0.25	0.12	0.04
IgG (mg/dL)	6,451	3,266	2,679	2,433	2,238
Hemoglobin (g/dL)	8.0	12.9	13.9	13.0	12.8
IL-6 (pg/mL)	41.8	1,100	801	806	756

ND, 6MWD and hemodynamic assessment by right heart catheterization were not done at 6 and 9 months.

pathological roles for IL-6 in various disease conditions, such as inflammatory, autoimmune, and neoplastic diseases. Pathologic features in patients with PAH are characterized by muscularization of distal pulmonary arterioles, concentric intimal thickening, and obstruction of the vascular lumen by proliferating endothelial cells to form plexiform lesions [34]. It has been proposed that dysregulated cellular growth and apoptosis are responsible for a typical proliferative cellular phenotype, resulting in pulmonary vascular remodeling in PAH. On the other hand, perivascular infiltration of inflammatory cells, consisting of T cells, B cells, and macrophages, are often present within and around the affected pulmonary arteries of patients with PAH, suggesting that cytokines and growth factors secreted from these inflammatory cells may be involved in uncontrolled proliferation of pulmonary artery smooth muscle and endothelial cells [35].

In this regard, a lung-specific overexpression of IL-6 in mice resulted in increased PVR and pathological lesions similar to that seen in patients with PAH, including distal arteriolar muscularization, plexogenic arteriopathy, and periarteriolar infiltration of T cells [36]. These findings indicate that IL-6 directly or indirectly promotes proliferation of both smooth muscle and endothelial cells, which are potentially mediated through a number of proliferative, prosurvival, and anti-apoptotic processes (Figure 2). In this regard, IL-6 triggers vascular smooth muscle cell proliferation through upregulated expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR2 [36, 37], which was observed in the plexiform lesions of patients with PAH [38].

It has been known that transforming growth factor (TGF)-β/bone morphogenetic protein (BMP) signaling controls growth of vascular smooth muscle and endothelial cells by inhibiting excessive proliferation. Genetic mutations in the gene encoding the type II receptor of BMP (BMPR2) comprise a genetic hallmark of heritable PAH [39], and downregulated protein expression of BMPR2 has also been described in nonheritable PAH [40]. A recent study by Brock et al. demonstrated that IL-6 repressed protein expression of BMPR2 through overexpression of microRNA cluster 17/92 [41]. MicroRNAs regulate posttranslational mechanisms by binding to their target mRNAs and by altering mRNA stability or affect protein translation [42]. Interestingly, protein expression of TGFβR2, another receptor from the identical protein family, was also modulated by the same microRNA cluster [43]. This may explain the lack of TGFβR2 expression in plexiform lesions of patients with PAH [44]. The promoter region of the microRNA 17/92 gene C13 or f25 has a highly conserved binding site for STAT3, a major IL-6 signal transduction pathway [33]. In fact, persistent activation of STAT3 resulted in repressed protein expression of BMPR2 [41].

Angiopoietin-1 (Ang-1)-Tie2 pathway is essential for both embryonic and postnatal angiogenesis and involves in a protective action on endothelial cells by suppressing inflammation and apoptosis [45]. The role of this system in the pathogenesis of PAH has been poorly understood, but a recent study using Tie2-deficient mice found that endothelial survival signaling via the Ang-1-Tie2 pathway is protective in PAH [46]. Exposure of IL-6 decreased expression of Ang-1 in lung vascular smooth muscle cells, leading to reduction of Tie2 activity in endothelial cells and resultant excessive apoptosis.

On the other hand, a constitutive activation of STAT3 was described in vascular smooth muscle cells as well as in endothelial cells from the lung tissue of patients with PAH [47]. Upon stimulation with IL-6, endothelial cells produce CX3CL1/fractalkine, a potent chemokine that recruits monocytes and lymphocytes into the lung [48]. These mononuclear infiltrates are major source of IL-6, but vascular smooth muscle cells and endothelial cells also produce IL-6 upon stimulation with IL-6 [36, 49]. Taken together, IL-6 promotes the development and progression

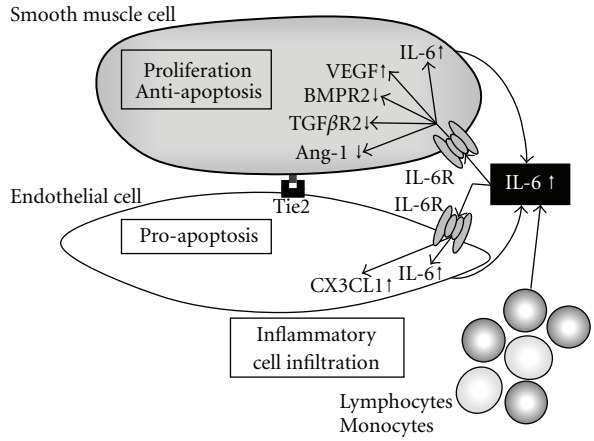


Figure 2: Hypothetical mechanism leading to pulmonary vascular remodeling via overexpression of IL-6. IL-6 induced proliferation and antiapoptosis in vascular smooth muscle cells through upregulation of VEGF, and downregulation of BMPR2 and TGFβR2. Upon IL-6 exposure, endothelial cells undergo apoptosis through repressed Tie2 signaling via downregulated Ang-1 expression in smooth muscle cells. Production of CX3CL1 results in recruitment of inflammatory cells, such as lymphocytes and monocytes, which produce enormous amount of IL-6, while vascular smooth muscle and endothelial cells also produce IL-6 upon stimulation with IL-6.

of pulmonary vascular remodeling, leading to PAH, via a variety of mechanisms.

5. The "Second-Hit" Process for Developing PAH

However, PAH observed in mice undergoing IL-6 overexpression was subtle, but was hemodynamically and histologically remarkable upon hypoxia exposure [36, 50]. In contrast, IL-6 knockout mice exposed to hypoxia were resistant to the development of increased PVR [51]. This is consistent with the "second-hit" theory for the pathogenesis of PAH, in which the response to an environmental or endogenous trigger is enhanced in susceptible individuals [52]. For example, exposure to serotonergic or inflammatory stressors produced an enhanced pulmonary hypertensive response in BMPR2 deficient mice [53]. Therefore, it is likely that overproduction of IL-6 alone has minimal impact on development of PAH, but the combination with other factors known to enhance susceptibility to pulmonary vascular remodeling, such as hypoxia and vasculopathy in CTD, appears to have synergistic effects resulting in the development of significant PAH.

The contribution of IL-6 to the pathogenesis of PAH may be different among associated conditions. In this regard, circulating IL-6 level was increased in patients with SSc, and the increased level was associated with the presence of PAH [28, 54]. In addition, the IL-6 level in bronchoalveolar lavage fluid was also increased in patients with SSc irrespective of the presence or absence of interstitial lung disease [55]. IL-6 is abundantly produced *in vitro* by affected skin fibroblasts and alveolar macrophages derived from patients with SSc [56, 57]. These findings together indicate that the lung tissue of SSc patients is always exposed to IL-6. This may explain why prevalence of PAH is the most frequent in patients with SSc among CTDs.

6. Summary and Future Perspectives

Current therapeutic strategies for PAH focus on the pulmonary vascular endothelium and its role in regulating smooth muscle cell tone and proliferation. By using these modern PAH drugs, treatment of PAH has undergone an extraordinary evolution even in patients with CTD, but PAH still remains a chronic intractable disease without a cure. A better understanding of the underlying pathophysiology of the pulmonary vasculature is needed for better therapy. In this regard, the findings of pathologically aberrant proliferation of smooth muscle and endothelial cells as well as increased expression of secreted growth factors, such as VEGF and platelet-derived growth factor (PDGF), in PAH have caused a shift in paradigm in treatment strategies for this disease [58]. The efficacy of imatinib, a prototypical PDGF receptor signaling inhibitor, was reported in patients with severe PAH [59–61], and a phase II multicenter clinical trial of imatinib in patients with PAH has been completed in the United States and Europe and the results are pending. On the other hand, our case report indicates that IL-6 is another potential therapeutic target for PAH.

In summary, this report describes our experience with the use of tocilizumab in a patient with MCTD and PAH. The rationale for such treatment derives from a numerous basic studies showing a critical role of IL-6 in the promotion of pulmonary vascular remodeling and consequent development of PAH. The concept underlying use of IL-6 blockade in PAH is prevention and reversal of lung vascular remodeling rather than prolonged vasodilation of pulmonary arteries. We recognize the limitations of a single case report, but we believe that blockade of IL-6 signaling may be a promising new therapy for PAH, especially in the context of CTD. Further studies of IL-6 blockade in PAH patients with and without CTD are warranted.

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Clinical Study

Serum Endoglin Levels in Patients Suffering from Systemic Sclerosis and Elevated Systolic Pulmonary Arterial Pressure

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Background. Pulmonary arterial hypertension (PAH) is the main cause of morbimortality in systemic sclerosis (SSc). Increased Eng expression has been demonstrated in SSc patients. *Objective*. Ascertaining serum levels of Eng in SSc patients with and without elevated systolic pulmonary arterial pressure (sPAP) and comparing them with that of healthy volunteers. *Methods*. A cross-sectional study was carried out. A commercial ELISA kit was used for measuring serum concentrations of Eng in 60 subjects: 40 patients with SSc with and without elevated sPAP, compared to 20 healthy control subjects. Elevated sPAP was detected by echocardiogram. *Results*. No association between positive Eng and elevated sPAP was found when compared to the SSc without elevated sPAP group (OR = 2.85; 0.65–12.88 95% CI; $P = .11$); however, an association was found between positive Eng and elevated sPAP compared to healthy controls (OR = 23.22; 2.46–1050.33 95% CI; $P = .001$), and weak association was found between the positive Eng with SSc without elevated sPAP group compared to healthy controls (OR = 8.14, 0.8–393.74 95% CI; $P = .046$). *Conclusion*. Raised serum levels of Eng in SSc patients compared to healthy controls were found, suggesting a role for Eng in SSc vasculopathy and not just in elevated sPAP. However, prospective studies are needed to verify such observations.

1. Introduction

Systemic sclerosis (SSc) is an autoimmune disease having an unknown etiology characterized by microvasculopathy, immunological abnormalities, and excessive collagen deposits [1, 2]. Endothelial dysfunction and microcirculation damage are cardinal features of systemic sclerosis (SSc); it is thought that vascular changes occur at an early stage and may include endothelial cell apoptosis, endothelium activation, inflammatory cell recruitment, intimal proliferation, and adventitial fibrosis, all of which may lead to vessel obliteration [3].

Although SSc pathogenesis remains uncertain, increasing evidence suggests that transforming growth factor-beta (TGF-β) plays a key role in tissue fibrosis development, a consequence of extracellular matrix accumulation in SSc pathogenesis. TGF-β regulates diverse biological activities including cell growth, apoptosis, differentiation, and extracellular matrix synthesis through interaction with TGF-β receptors [4, 5].

Endoglin (Eng) is a glycoprotein having antiangiogenic properties that acts as a TGF-β receptor complex component. Eng may act on fibroblasts to modulate TGF-β signaling by acting as a molecular sink regulating or reducing the total pool of TGF-β available for activating signal-transducing receptors.

Increased expression in fibroblasts and endothelial cells has been demonstrated in SSc patients, suggesting that deregulating Eng expression and/or function may be related to the vascular manifestation of SSc [6, 7].

Pulmonary arterial hypertension (PAH) is the main vascular complication in SSc, being an important cause of

morbidity and the main cause of mortality, having 50% survival rate at 12 months [8]. This vasculopathy is caused by a number of soluble factors and involves a complex interaction between endothelial cells, smooth muscle cells, extracellular matrix, coagulation factors, and circulating cells [9].

There are two types of PAH in SSc: PAH secondary to fibrosis and severe interstitial involvement and isolated PAH without fibrosis or interstitial lung disease, with the latter reflecting the illness' vascular pathology and resulting in a more indolent pulmonary process [8].

PAH prevalence in SSc varies depending on the methods used for diagnosis. Estimated PAH prevalence is 13.3% when using echocardiogram [10]. Cardiac catheterization is considered the gold standard for PAH diagnosis, but this technique is invasive, expensive, and not available in all centers. It can sometimes represent a high-risk factor for morbidity; this is why its use for detecting PAH and its followup are limited [11]. Markers should thus be sought to enable detecting patient subgroups having the highest risk of developing PAH as a vascular complication of SSc and allowing followup. This would enable PAH to be diagnosed as early as possible, thereby improving prognosis for PAH and SSc patients.

This study examined serum levels of Eng in patients with SSc and elevated sPAP (SSc-sPAP) and SSc without elevated sPAP (SSc-non sPAP) and these were compared with healthy volunteers for determining any association between Eng levels and elevated sPAP in SSc patients.

2. Materials and Methods

2.1. Patients. A cross-sectional study was carried out between June and October 2008 during which 60 subjects were analyzed; 40 patients met American College of Rheumatology (ACR) criteria [12] for SSc (20 patients had elevated sPAP by echocardiogram and 20 patients did not), and 20 control healthy subjects were also included. The patients were consecutively selected based on the available and willingness to participate in this study. Patients who presented any other connective tissue disease or a concomitant pulmonary illness from any other etiology, work, or environmental exposure for pulmonary disease were excluded.

Registration forms were completed which included demographic data, clinical characteristics, antibody levels, reports from diagnostic tools such as echocardiogram and high-resolution computed tomography of the thorax (HRCTT).

The patients were subdivided into two groups: limited SSc (lSSc) and diffuse SSc (dSSc) based on the limits proposed by LeRoy et al. [1]. Blood samples were drawn from the 40 SSc patients and from 20 healthy subjects matched by age and gender. No patient was receiving calcium channel blockers when being analyzed. All samples were stored at -20°C until being processed. The protocol was approved by the Universidad Nacional de Colombia's ethics committee and all patients signed the informed consent forms agreeing to take part in this study.

2.2. Echocardiogram. All echocardiograms were taken by an expert cardiologist using standard techniques for evaluating right ventricle dimensions and tricuspid gradients after a 20-minute rest. The tricuspid systolic pressure gradient was calculated by using Bernoulli's modified equation [13].

2.2.1. Estimating Elevated Systolic Pulmonary Arterial Pressure. The sPAP was calculated as being the sum of the tricuspid gradient and estimated right atrial pressure. Elevated sPAP was defined in Colombian patients living at 2,600 meters above sea level as being mean >35 mmHg sPAP, >3 m/second tricuspid regurgitation velocity, or 2.5 m/second in patients having unexplained dyspnea [14]. Elevated sPAP was defined as > 35 mmHg sPAP with HRCTT without evidence of interstitial lung disease (such as bibasilar pulmonary fibrosis or reticulonodular densities, being most pronounced in the lung bases), or the presence of heterogeneous opacities such as reticular opacities, ground-glass opacities, or honeycombing in HRCTT [14].

2.3. ELISA. A commercial ELISA kit (R&D Systems, Minneapolis, Minn, USA) was used for serum measurement for Eng from SSc patients and the control group, following the manufacturer's protocol. Each sample was done twice.

2.4. Statistical Analysis. STATA 9.0 software was used for statistical analysis. The Shapiro-Wilks test was used for evaluating data distribution. Clinical data, elevated sPAP and Eng serum values were compared by unpaired Student t-test or Mann-Whitney U test as appropriate. Fisher's exact test or Chi-^{2}test was used for determining association between categorical variables. Odds ratios (OR) with 95% confidence interval (95% CI) were also reported. The Kruskal-Wallis test or ANOVA was used for intergroup analysis, as appropriate. The Spearman test was used for calculating the correlation between sPAP and Eng levels. Two standard deviations (SD) above mean were taken for calculating positive Eng values. $P < .05$ was considered significant for all analysis.

3. Results

The study included 60 subjects, 40 having a diagnosis of SSc and 20 healthy control subjects (20 SSc-sPAP patients and 20 SSc-non sPAP patients). In the SSc group, 26 patients presented lSSc and 14 patients dSSc; 28 were female and 12 were men, having an average age of 44.3 ± 9.8 at disease onset.

Disease duration was 8.57 ± 5.2 years, measured from the point at which the first symptom appeared. The time from the onset of Raynaud's phenomenon was 9.5 ± 5.9 years and Rodnan's score [15] was 21.7 ± 10.2. All patients presented telangiectasias; 12 (30%) patients had calcinosis, 28 (70%) patients had gastrointestinal involvement, and mean sPAP value was 57.75 ± 14.5 mmHg in SSc-sPAP patients. Sixteen patients from the SSc-sPAP group (16/20) were diagnosed with > 35 mmHg sPAP and >3 m/second tricuspid regurgitation velocity; only four (4/20) had >3 m/second tricuspid regurgitation velocity. Sixteen SSc-sPAP patients (80%) had

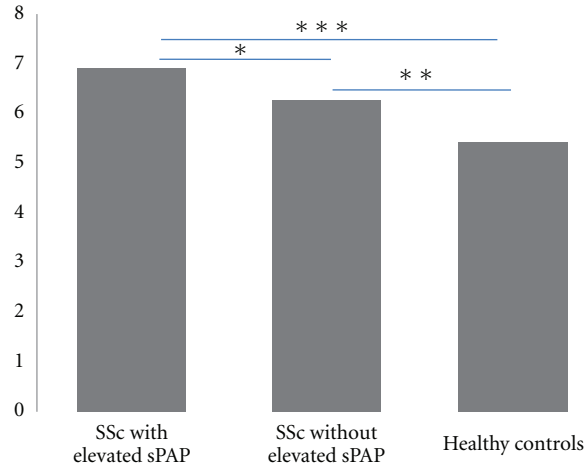

Figure 1: Eng levels in SSc with elevated sPAP, without elevated sPAP, and in healthy controls. Serum levels of Eng in patients with SSc and elevated sPAP, SSc without elevated sPAP, and healthy control subjects (mean ± SE). Mean serum levels were statistically higher in the SSc group compared to the control group (SSc group *cf* healthy controls $P = .0028$; SSc with elevated sPAP *cf* SSc without elevated sPAP $^{*}P = .2447$; SSc without elevated sPAP *cf* healthy controls $^{**}P = .057$; SSc with elevated sPAP *cf* healthy controls $^{***}P = .0006$).

>48 mmHg sPAP and four (20%) had <48 mmHg sPAP but more than 40 mmHg in sPAP and had no significant structural right atrial and/or ventricular damage. Forty-five percent had calcinosis in the SSc-sPAP group, whereas only 15% were present in SSc-non sPAP group ($P = .041$).

HRCTT revealed no ILD-related findings in any patient. No abnormality was found in 12 patients (60%) and PAH findings such as dilatation of proximal and segmental pulmonary arteries were found in 8 (40%). No patients had bilateral reticular linear or reticulonodular densities in the lung bases or opacities reticular, ground-glass opacities, or honeycombing in HRCTT.

Anticentromere antibodies (ACA) were reported to be positive in 27 patients (67.5%) and anti-Scl-70 in 9 patients (22.5%). Seventy-five percent had positive ACA in the SSc-sPAP group (5/7 having diffuse SSc and 10/13 having limited SSc), whereas antibodies anti-Scl-70 were positive in 10% of that group (2/7 SSc-sPAP patients and diffuse SSc). A speckled antinuclear antibody (ANA) pattern was present in 15% (3/13 SSc-sPAP patients and limited SSc). No patient presented renal crisis or ischemic cutaneous ulcers, but all presented telangiectasias. Table 1 gives the clinical characteristics for the SSc patients.

Serum levels of Eng in SSc-sPAP patients tended to be higher than in SSc-non sPAP patients, having no significant statistical difference ($P = .2447$) and were higher than healthy controls ($P = .0006$). Mean values were 6.89 ng/dL, 6.20 ng/dL, and 5.42 ng/dL and median values were 7.07 ng/dL, 6.01 ng/dL, and 5.42 ng/dL, respectively (Figure 1).

There was no difference between the SSc-non sPAP group and healthy controls ($P = .057$). Intergroup analysis revealed a difference between the three groups (Kruskal-Wallis test, $P = .0037$) which was mainly due to SSc-sPAP group compared to the healthy control group. Categorized analysis of serum levels from Eng reported a difference between all groups ($P = .003$); however, detailed analysis revealed interesting findings. The SSc-sPAP group consisted of eleven patients having positive Eng (OR = 2.85; 0.65–12.88 95% CI; $P = .11$, when was compared to the SSc-non sPAP group); the SSc-non sPAP group had six patients (OR = 8.14; 0.8–393.74 95% CI; $P = .046$, when compared to healthy controls) and one healthy control (OR = 23.22; 2.46–1050.33 95% CI; $P = .001$ when was compared to the SSc-PAH group). An association was reported for positive Eng (OR = 14.04; 1.79–617.05 95% CI; $P = .0028$) when SSc-sPAP and SSc-non sPAP groups were pooled and compared to healthy controls. There was no correlation between Eng levels and sPAP (Rho = 0.1384, $P = .39$).

Table 1: SSc patients' clinical characteristics.

Clinical characteristics	SSc- sPAP patients	SSc-non sPAP patients	*P*
	$n = 20$	$n = 20$	
Age (in years)	54.4 ± 11	51.1 ± 13.7	.4
Age at onset	44.75 ± 10	43.9 ± 9	.78
Male : female	2.3 : 1	2.3 : 1	1
Duration (means ± SD), years	9.65 ± 4	7.5 ± 6.3	.19
lSSc	13	13	1
dSSc	7	7	1
sPAP mmHg at rest	57.75 ± 14.5	19.4 ± 12	< .0001
Raynaud%	100	100	1
Time of onset of Raynaud	9.1 ± 4	9.95 ± 6	.81
Rodnan score	22.1 ± 9	21.3 ± 10	.21
Calcinosis%	45	15	.041
Telangiectasias%	100	100	1
Renal crisis%	0	0	
Ischemic cutaneous ulcers%	0	0	
Gastrointestinal involvement%	60	80	.15
normal HRCTT%	60	100	NS
HRCTT PAH%	40	0	NS
Anticentromere ab%	75	60	.21
Anti-Scl-70 ab%	10	20	NS
ANA%	15	20	NS
Methotrexate	11	11	1
Cyclophosphamide	6	7	.73

SD: standard deviation, lSSc: limited systemic sclerosis, dSSc: diffuse systemic sclerosis, sPAP: systolic pulmonary arterial pressure, HRCTT: high-resolution computed tomography of thorax, PAH: pulmonary arterial hypertension. Fisher's or Wilcoxon test was used for calculating the differences between groups.

4. Discussion

Two clinical hallmarks for SSc are its clinical heterogeneity and the wide range of vascular and fibrotic manifestations. Organ involvement, different patterns, and internal organ manifestation severity are the global outcome's most significant determinants [16].

PAH is the main cause of morbidity and mortality amongst vascular complications for SSc. A diagnosis of PAH has occurred late in the course of the disease until now and right heart catheterization has been the gold standard for its diagnosis. However, this diagnosis test is invasive and implies morbidity and mortality risks. The echocardiogram is a noninvasive technique and its limitations include it being operator-dependent and its false positive rate is close to 30% [16].

An imbalance in circulating angiogenic factors in SSc may be associated with vascular endothelial dysfunction. Eng is one of the factors supporting vascular integrity, with this being a 180 kDa homodimeric coreceptor for TGF-β superfamily members which is predominantly expressed on endothelial cell surfaces [17].

Eng may have roles in hematopoiesis, cardiovascular development, and angiogenesis and is highly expressed on vascular endothelial cells [18], chondrocytes [19], and term placenta syncytiotrophoblasts [20]. It is also found on monocytes [21], erythroid precursors [22], and a hematopoietic stem cell subpopulation [23]. Although its role remains elusive, circulating soluble Eng levels are raised in patients suffering from atherosclerosis [24] and certain cancers including breast [25], colon [26], and myeloid malignancies [27]. Eng is likely to be involved with angiogenesis in endothelial cells, since prominent Eng expression has been demonstrated in neovascular states, including the enhanced vascularity of psoriasis [28].

Previous reports have shown that the Eng gene is located at 9q34.1 and that mutations of this gene having reduced Eng expression are responsible for one of the two types of hereditary hemorrhagic telangiectasia, an autosomal dominant disorder characterized by multiple telangiectasia of the skin, mucous membranes, gastrointestinal tract, arteriovenous malformation, and pulmonary hypertension [29].

Leask et al. [6] found that the endothelial-enriched high-affinity TGF-β receptor endoglin was up-regulated in dermis fibroblasts cultured from involved areas of skin taken from SSc patients related to normal fibroblasts and that Eng expression increased with the disease's progression, suggesting that Eng might represent a potential marker for staging SSc. Another finding was that Eng overexpression in fibroblasts blocked the accumulation of activated nuclear Smads and suppressed TGF-β ability to induce connective tissue growth factor (CTGF) profibrotic cytokine target gene promoter. Such results suggest that SSc fibroblasts induce Eng expression to suppress TGF-β induction of gene expression in a negative feedback loop.

Fujimoto et al. [30] examined soluble Eng serum levels in SSc patients and found these levels to be higher in patients having lSSc, telangiectasias, and ACA. Furthermore, SPPA was positively correlated with Eng levels in patients having lcSSc, but only two of these patients presented PAH. There was no difference in heart, esophageal, or renal involvement between patients having higher Eng serum levels and those having normal levels.

Wipff et al. [17] demonstrated an association between Eng gene polymorphism and PAH, and another study by the same authors [31] found that Eng appeared to be increased in SSc and to be particularly associated with the vascular phenotype. They also showed a higher Eng concentration in SSc patients compared to healthy controls; they included 17 PAH patients and found that Eng serum levels in SSc patients with PAH were similar to those of patients without PAH. Higher Eng levels were found in SSc patients compared to healthy controls in a previous study by the present group [32], having a statistical difference between the two groups in favor of SSc and PAH patients. However, a correlation with sPAP value was not found, with a limitation of this study being the lack the comparison with patients with SSc without PAH.

Higher Eng levels were found in the two types of SSc in the current study, contrasting with the results of Fujimoto et al. [30]. The 20 patients in the current study presented elevated sPAP secondary to SSc, and Eng levels in these were more raised than in controls (SSc-non sPAP group and healthy controls). However, there was no statistical difference when the SSc-sPAP group was compared to the SSc-non sPAP group. These results differed from the recent ones reported by Wipff et al. [17] and suggest that Eng serum levels are elevated in SSc patients and not exclusively due to PAH. Endothelial activity in patients in the early stages of PAH may be a potential reason for finding elevated Eng serum levels. The difference in calcinosis percentage between the groups is striking, especially since calcinosis may develop in areas of poor perfusion and be the result of vascular disease [33].

Another interesting finding in this study was the presence of ACA in diffuse SSc. ACA have been associated with limited SSc and anti-topoisomerase I with diffuse progressive disease; however, 10 to 15 percent of patients with diffuse disease have these antibodies [34]. Coral et al. [35] reported positive ACA in 93% of patients studied in Colombia, independent of SSc subtype.

It thus seems that the higher presence of positive ACA could be related to PAH; in fact earlier reports have associated these antibodies with increased risk of pulmonary hypertension [36, 37]. Telangiectasias were present in all patients in the present study; it had already been reported in Colombian SSc patients [35].

This work had the following limitations. Right heart catheretization (RHC) is now considered the gold standard for PAH diagnosis due to the presence of high false positive results with echocardiogram and also because RHC allows therapeutic choices for vasoreactivity seen in PAH to be evaluated. Elevated sPAP was assessed and diagnosed with echocardiogram in this work due to the Colombian Health System's limitations regarding performing RHC. There was a high correlation between the measurements obtained using both techniques when the cardiologist operator had broad

experience in evaluating pulmonary arterial pressure and there was no change in right heart anatomical structural. Besides, >48 mmHg sPAP associated with >3.0 TRV was used for diagnosing moderate/severe PAH [38], thereby reducing the possibility of false positives occurring. Since our cohort had been continuously followed up, the cases were chosen during the early phases of PAH development to exclude even the smallest structural damage that could have altered echocardiographic evaluation.

A second limiting concerned the fact that a relative small population was included in each branch to include biomarkers having a potentially pathophysiologic role for generating elevated sPAP and because not enough scientific papers have assessed it, meaning that the current paper can be considered to be a pilot-study.

5. Conclusion

Raised Eng serum levels were found in SSc patients (SSc-sPAP patients and SSc-non sPAP) compared to healthy controls, suggesting a role for Eng in SSc vasculopathy and not just in PAH. However, prospective studies are needed which include a larger population to verify these observations. Serum biomarkers could detect an early stage of the disease in patients having a high risk of developing elevated sPAP which could afford a better outcome during these patients' follow up.

Conflict of Interests

None of this manuscript's authors have any financial interests which would have influenced the results or interpretation of this paper. The authors declare that they have no competing interests.

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Research Article

Characteristics of Interstitial Fibrosis and Inflammatory Cell Infiltration in Right Ventricles of Systemic Sclerosis-Associated Pulmonary Arterial Hypertension

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Objective. Systemic sclerosis-associated pulmonary arterial hypertension (SScPAH) has a disturbed function of the right ventricle (RV) when compared to idiopathic PAH (IPAH). Systemic sclerosis may also affect the heart. We hypothesize that RV differences may occur at the level of interstitial inflammation and—fibrosis and compared inflammatory cell infiltrate and fibrosis between the RV of SScPAH, IPAH, and healthy controls. *Methods*. Paraffin-embedded tissue samples of RV and left ventricle (LV) from SScPAH ($n = 5$) and IPAH ($n = 9$) patients and controls ($n = 4$) were picrosirius red stained for detection of interstitial fibrosis, which was quantified semiautomatically. Neutrophilic granulocytes (MPO), macrophages (CD68), and lymphocytes (CD45) were immunohistochemically stained and only interstitial leukocytes were counted. Presence of epi- or endocardial inflammation, and of perivascular or intimal fibrosis of coronary arteries was assessed semiquantitatively (0–3: absent to extensive). *Results*. RV's of SScPAH showed significantly more inflammatory cells than of IPAH (cells/mm^2, mean ± sd MPO 11 ± 3 versus 6 ± 1; CD68 11 ± 3 versus 6 ± 1; CD45 11 ± 1 versus 5 ± 1 , $P < .05$) and than of controls. RV interstitial fibrosis was similar in SScPAH and IPAH (4 ± 1 versus 5 ± 1%, $P = .9$), and did not differ from controls (5 ± 1%, $P = .8$). In 4 SScPAH and 5 IPAH RV's foci of replacement fibrosis were found. No differences were found on epi- or endocardial inflammation or on perivascular or intimal fibrosis of coronary arteries. *Conclusion*. SScPAH RVs display denser inflammatory infiltrates than IPAH, while they do not differ with respect to interstitial fibrosis. Whether increased inflammatory status is a contributor to altered RV function in SScPAH warrants further research.

1. Introduction

Systemic sclerosis (SSc) is a disease with a multifaceted pathology which is characterized by an enhanced inflammatory status, vasculopathy, and excessive fibrosis in skin and internal organs [1]. Pulmonary involvement, either lung fibrosis or pulmonary arterial hypertension (PAH), is the leading cause of death in SSc [1]. SSc complicated by PAH (SScPAH) carries a mortality of 50% at 3 years, which is higher when compared to idiopathic PAH (IPAH) [2–4].

These differences might be explained by comorbidity due to the systemic nature of SSc, a higher age of onset of disease,

Table 1: General patient characteristics.

	SScPAH **N = 5**	IPAH *N* = 9	Control *N* = 4
Age, yrs	47 ± 4	47 ± 4	31 ± 4
Male/Female no.	1/4	2/7	—
Survival	1.1 ± 0.5	3.7 ± 0.9	—
Heart rate	85 ± 3	78 ± 5	
mPpa, mmHg	46 ± 7	62 ± 4	
PCWP, mmHg	6 ± 2	5 ± 2	
PVR, dynes·s·cm^{-5}	1221 ± 691	1157 ± 144	
CI, l/min·m^2	2.3 ± 0.9	2.3 ± 0.5	
Systolic blood pressure	105 ± 2	118 ± 8	
Diastolic blood pressure	69 ± 6	73 ± 5	
TLC, %	89 ± 5	92 ± 5	
DLCO, %	42 ± 6	64 ± 6	
Therapy at time of death			
Prostacycline (*n*)	4	7	
ERA (*n*)	1	2	
PDE-5 inhibitor (*n*)	1	0	
ABS		1	

Values expressed as mean ± SE or otherwise as stated. Abbreviations: ABS: atrial balloon septostomy; CI: cardiac index; DLCO%: percentage of predicted of the diffusion capacity of the lung for carbon monoxide; ERA: endothelin receptor antagonist; mPpa: mean pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; PDE-5: phosphodiesterase 5; PVR: pulmonary vascular resistance; IPAH: idiopathic pulmonary arterial hypertension; SScPAH: systemic sclerosis-associated pulmonary arterial hypertension; TLC%: percentage of predicted total long capacity.

and differences in pulmonary vasculopathy [5, 6]. There are also indications that the RV in SScPAH adapts differently as compared with IPAH patients: several studies have shown lower pulmonary arterial pressures in SScPAH than in IPAH, while cardiac index and pulmonary vascular resistance were similar [6–10]. Additionally, it has been demonstrated that RV pump function is different in SScPAH when compared to IPAH [10]. Furthermore, a different response to cardiac load has been suggested by the disproportionate levels of N-terminal probrain natriuretic peptide found in SScPAH compared with IPAH, despite the less severe hemodynamic abnormalities [7, 11].

There is no knowledge concerning the etiology of altered RV function and adaptation in SScPAH. Here, for the first time, histolopathologic characteristics of the RV in a well-defined SScPAH group will be explored, focusing on inflammation and fibrosis, two main pathologic features of SSc. Comparison is made with RVs from IPAH patients and healthy controls. It is hypothesized that inflammatory status and fibrosis are quantitatively different in the RV interstitial myocardium of SScPAH patients as compared with that of IPAH patients. To test this hypothesis, we determined the numbers of macrophages, lymphocytes, and neutrophilic granulocytes and quantified fibrosis in the myocardial interstitium of RV's of SScPAH and IPAH patients. To evaluate an effect of pressure overload from the lesser circulation in PAH, RV interstitial fibrosis and interstitial inflammation were compared to the left ventricle (LV). Hearts from healthy individuals that died acutely of traumatic causes served as controls.

2. Methods

2.1. Patient Characteristics. The cases examined in this study were retrieved from the departments of pulmonary diseases of the VU University Medical Center, Amsterdam, the Netherlands. The study was approved by the Institutional Review Board on Research Involving Human Subjects of the VU University Medical Center.

Patients who had been treated for PAH between 1998 and 2007, of whom cardiac tissue obtained at autopsy was available, were deemed eligible for the study. General patient characteristics are described in Table 1. The diagnosis of SSc (as established by a reumathologist), SScPAH, and IPAH was verified by reviewing the medical records, including lung function data at baseline as well as HRCT studies. Patients with restrictive disease, as indicated by total lung capacity as percentage of predicted (TLC%) <70%, vital capacity (VC%) <70% and/or severe fibrosis on HRCT scan, were classified as pulmonary hypertension due to restrictive disease and therefore excluded from this study. SSc classification, SSc disease duration, and antibody profile were recorded (Table 2) [12, 13]. Of the SScPAH group, 3 patients had died of RV failure, one had died of hypovolumic shock due to iatrogenic intra-abdominal bleeding after ascites drainage which was caused by right heart failure, and one died postoperatively within 2 days after lung transplantation. Eight IPAH patients had died of RV failure and one of hemorrhagia from the arteria pulmonalis.

The hearts from four patients who had acutely died from traumatic, noncardiopulmonary, noncerebral causes

Table 2: Characteristics of SScPAH patients.

	Antibody- profile	Cause of death	SSc disease duration (yr)[§]	Survival after PAH diagnosis (yr)	Medication at time of death
1 LcSSc*	Anticentromere	RV failure	4	0,5	prostacyclin
2 LcSSc	Anticentromere	RV failure	12	0,75	prostacyclin
3 LcSSc	Anticentromere	RV failure	1	0,08	prostacyclin
4 LcSSc	Anticentromere	Iatrogenic abdominal bleeding due to ascites punction	1	3	ERA, PDE-5 inhibitor
5 LcSSc	ANA	Post- LTX	13	0,42	Prostacyclin

Abbreviations: ANA: antinucleolar antibody; ERA: endothelin receptor antagonist; LcSSc: Limited cutaneous SSc; LTX: lung transplantation; PDE-5: phosphodiesterase 5; RV: right ventricle; SScPAH: Systemic sclerosis-associated pulmonary arterial hypertension. *According to [13]. [§]Since first non-Raynaud symptom, at time of diagnosis of pulmonary arterial hypertension. Ascites caused by RV failure.

and who did not have a cardiopulmonary medical history, served as healthy controls.

2.2. Tissue Preparation and Immunohistochemistry

2.2.1. Inflammation. Serial adjacent sections of myocardial tissue (4 μm thick) were deparaffinised for 10 minutes in xylene at room temperature and dehydrated through ascending concentrations of ethanol. Endogenous peroxidase activity was blocked by incubation in 0.3% (v/v) H_2O_2 in methanol for 30 minutes. Tissue sections were subjected to antigen retrieval by boiling in 10 mM sodium citrate buffer, pH 6.0 for 10 minutes in a microwave oven. All antibodies and normal serum were diluted in PBS containing 1% (w/v) bovine serum albumin (BSA). Tissue sections were preincubated for 10 minutes with normal rabbit and normal swine serum (1 : 50), followed by incubation for 1 hr with either polyclonal rabbit antihuman myeloperoxidase (MPO) (Dako, A0398, Denmark, 1 : 50 dilution), or mouse monoclonal antibody CD68 (KP1) (Dako, M0814, Denmark, 1 : 400 dilution) and mouse monoclonal anti-human CD45 (Dako, M0701, Denmark, 1 : 50 dilution) antibodies. After washing in PBS, tissue slides were incubated for 30 minutes with a biotin-conjugated secondary antibody rabbit anti-mouse biotin (1 : 500 dilutions) and swine anti-rabbit biotin (1 : 300 dilution) (Dako, A0063, Denmark), followed again by washing in PBS. Then, slides were incubated with streptavidin-biotin complex (sABC; 1 : 1000 dilutions) for 1 hour. Finally, primary antibodies were visualized with 3,3′-diaminobenzidine (DAB; 0.1 mg/ml, 0.02% H_2O_2). Slides were counterstained with hematoxylin and mounted with Depex and a coverslip.

As a negative control, the primary antibody was replaced by phosphate-buffered saline or an irrelevant antibody; these heart tissue slides were found to be negative.

2.2.2. Fibrosis. In order to determine the amount of interstitial fibrosis, paraffin-embedded sections of myocardial tissue (4 μm thick) were stained with Picrosiriu-red. Sections were incubated for 10 minutes in xylene at room temperature to remove paraffin and transferred to water through descending concentrations of ethanol (100%, 96%, 80%, and 70%, all 10 s). Staining was performed using 0.1% solution of Sirius red F3BA in saturated aqueous solution of picric acid for one hour at 25°C [14, 15]. Subsequently, sections were differentiated in 0.01N HCl for 2 minutes. Sections were dehydrated in ascending concentrations of ethanol (70%, 80%, 96%, and 100%, each 10 seconds) and cleared in two stages in xylene, 10 minutes each. Sections were covered with Entellan mounting medium (Merck, Darmstadt, Germany) and a glass cover slip. In addition, to determine the presence of intimal fibrosis in coronary arteries and arterioles, adjacent sections were stained for Elastica von Gieson (EvG).

2.3. Morphometric Analyses

2.3.1. Inflammatory Cells. In each tissue slide of the RV and LV, intramyocardial areas were randomly chosen, with a minimum area of 15 mm^2. In these areas, the number of extravascular neutrophilic granulocytes (MPO$^+$), macrophages/histiocytes (CD68$^+$) and lymphocytes (CD45$^+$) was counted (Figure 2). Intravascular inflammatory cells were excluded. The average number of intramyocardial, extravascular inflammatory cells per mm^2 was then calculated as the total score for each specimen according to Begieneman et al. [16]. All cell counts and scorings were done by two investigators (MJO, KTBM) who were blinded to the clinical diagnosis. In order to minimize interobserver variability, both investigators counted a training set of 9 different histological samples (3 of each staining). The interobserver variation was <10% with a correlation of $r^2 = 0.98$. The presence of perivascular infiltrates and inflammatory infiltration of the endocardium and the epicardium was scored on a 4 point scale, ranging from 0 (absent) to 3 (extensive).

2.3.2. Fibrosis. Picrosirius-red stained sections of paraffin-embedded cardiac biopsies were scanned in total with Mirax scan system (Zeiss, AG Germany). Areas were randomly selected from the digitised slides at a 20x magnification, covering at least 15% of the total area (Figure 1). Care was taken not to include vessels into the selected areas to ensure only interstitial myocardial fibrosis was quantified. Interstitial myocardial fibrosis was assessed in the RV and LV, using a fully automated analysis according to Mouchaers [17].

In addition, the entire sections were, semiquantitatively, investigated for epicardial and endocardial fibrosis and

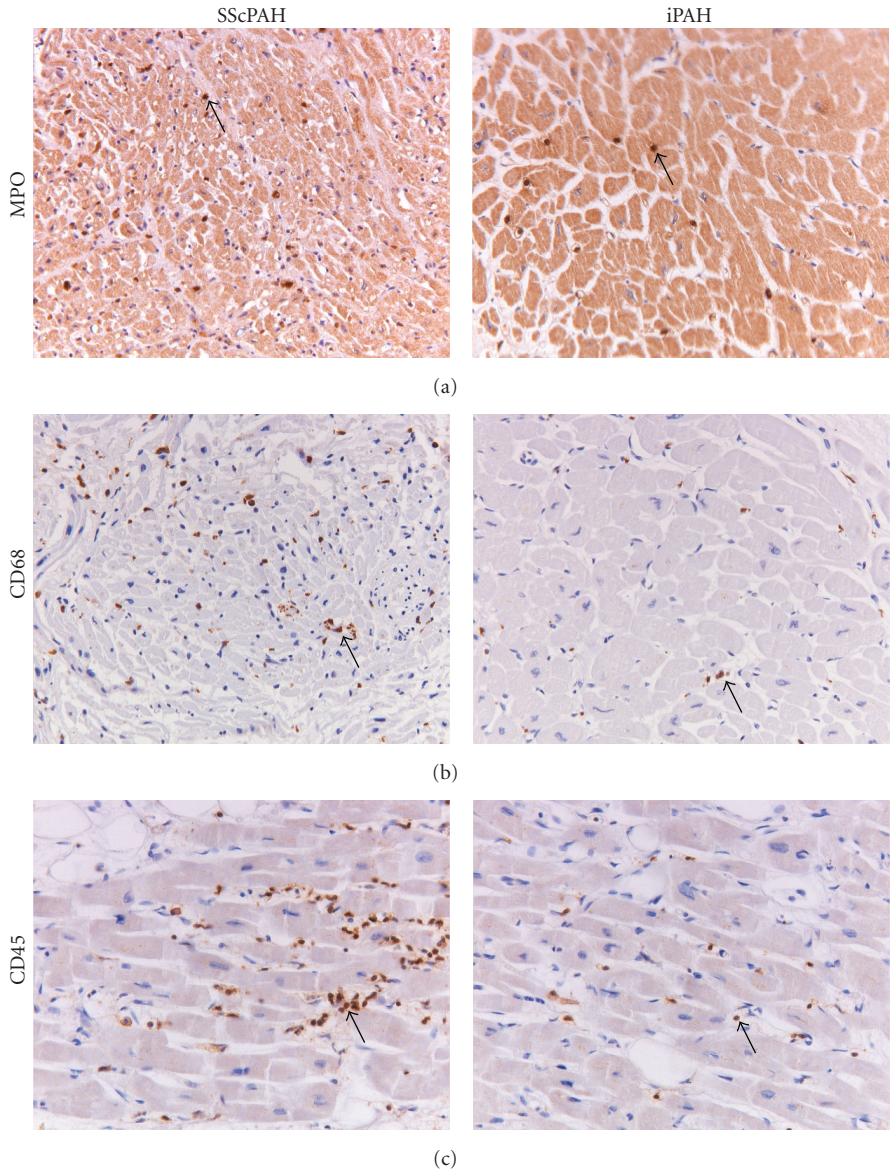

Figure 1: Sections of myocardial tissue stained with antibodies against (a) neutrophilic granulocytes (MPO positive), (b) macrophages (CD68 positive), or (c) lymphocytes (CD45 positive). Arrows indicate positive cells.

scored on a 4 point scale ranging from 0 (absent) to 3 (extensive). Likewise, EvG-stained sections were used to score the presence of replacement fibrosis, as defined by fibrotic areas within the myocardium coincident with a loss of cardiomyocytes as well as for perivascular and intima fibrosis of coronary arteries/arterioles.

2.4. Statistical Analysis. For the quantification of interstitial fibrosis and interstitial inflammatory cells, 5 samples of myocardial tissue from the RV and 4 from the LV of SScPAH patients were analyzed; 9 samples were available of both the RV and LV from IPAH patients and 4 samples from controls. Mann-Whitney U was used to determine differences between groups. All histochemical data are presented in graphs as median (range) and patient characteristics in tables as mean ± SEM. Fisher's exact test was used for comparison of infiltration of inflammatory cells of the endo- and epicardium between groups. $P < .05$ was considered statistically significant.

3. Results

3.1. Patient Characteristics and Haemodynamics. Patient characteristics are listed in Tables 1 and 2. SScPAH and IPAH groups did not differ with respect to mean age. Mean survival of the SScPAH patients was significantly shorter compared to IPAH patients. Haemodynamic parameters at diagnosis were not different between the groups. However, SScPAH patients tended to have a lower mean pulmonary artery pressure as compared with the IPAH patients. DLCO in the SScPAH

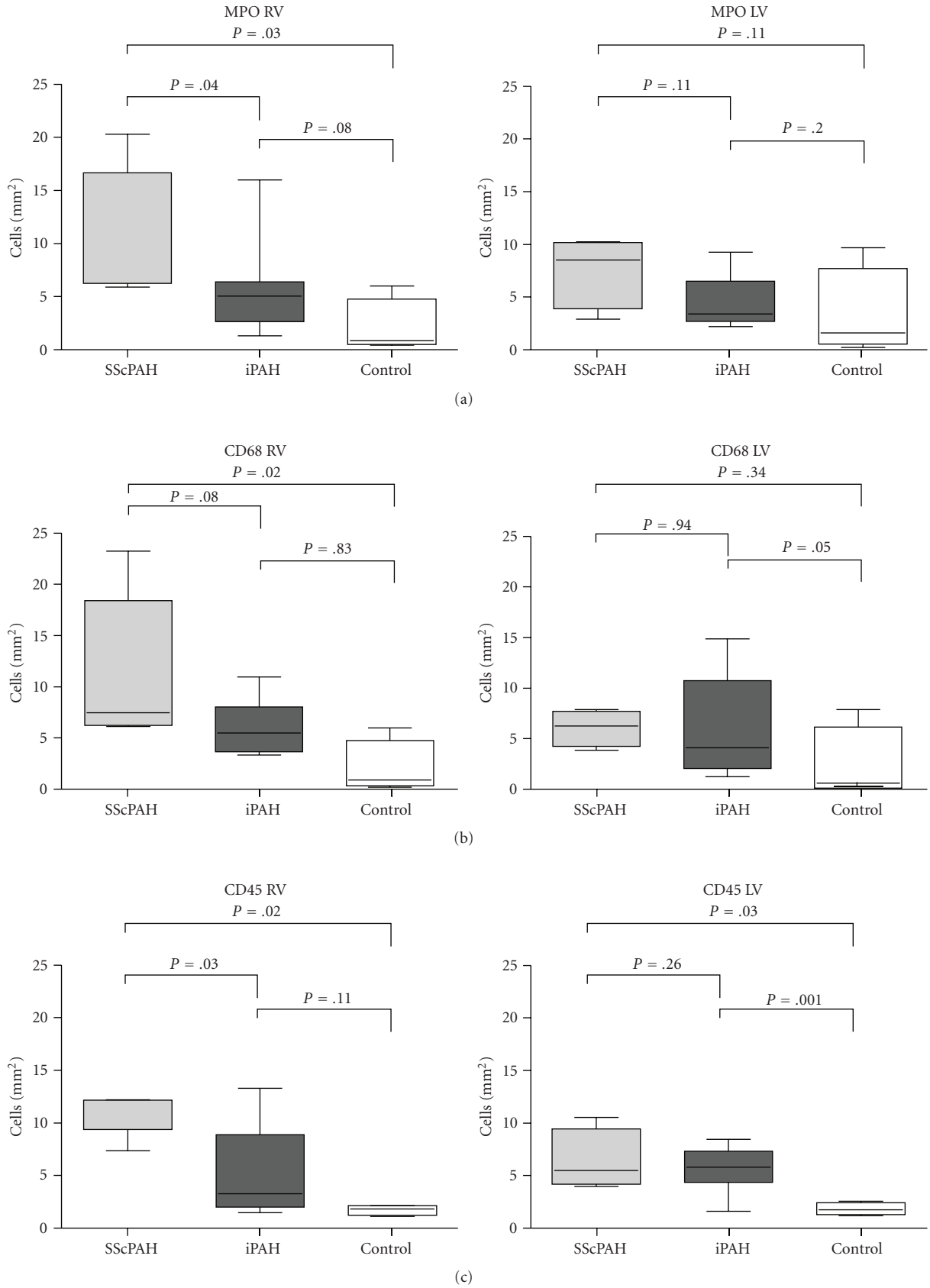

Figure 2: The number of (a) myeloperoxidase positive cells, (b) CD68 positive cells, and (c) CD45 positive cells was determined in the RV and the LV of systemic sclerosis-associated pulmonary arterial hypertension (SScPAH), idiopathic pulmonary arterial hypertension (IPAH) patients and in control subjects. Median and range are shown.

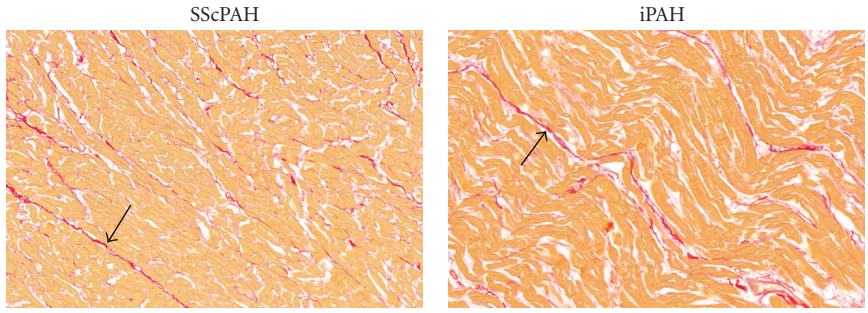

Figure 3: Representative samples of picrosirius red-stained myocardial sections of the RV of SScPAH and IPAH patients, used for quantification of interstitial fibrosis. Arrows indicate the red-coloured strains of fibrosis.

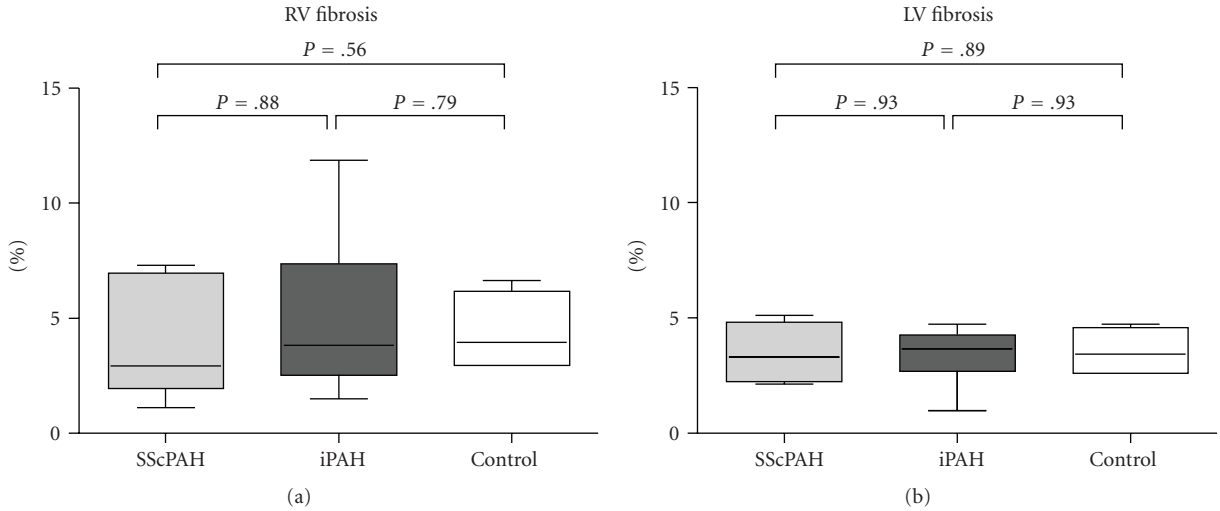

Figure 4: Quantification of picrosirius red staining in the RV of SScPAH, IPAH, and control subjects in (a) the RV and (b) the LV. Median and range are shown.

group was significantly lower as compared with the IPAH group. Two patients in the SScPAH group and 4 in the IPAH group had been treated with aldosteron antagonists or ACE-inhibitors. None of the patients had systemic hypertension.

3.2. Inflammation. The RV's of SScPAH showed significantly more interstitial MPO- and CD45-positive cells when compared to IPAH. The numbers of MPO-, CD68- and CD45-positive cells/area were also increased when the SScPAH RV's were compared to normal controls (Figures 2(a), 2(b), and 2(c), and examples of immunohistochemical stainings are shown in Figure 1). In the RV of IPAH versus normal controls, no significant differences observed. In the LVs of SScPAH and IPAH, there were no significant differences in the number of inflammatory cells either. In SScPAH LV's, significantly more CD45 positive cells were observed as compared to normal controls, but no such differences were found for MPO nor for CD68. IPAH LV's demonstrated significantly more CD68 and CD45 as compared with normal controls.

Infiltration of the endocardium and epicardium was not different between the SScPAH and IPAH, nor between RV or LV, for neither cell type (not shown). In all ventricles, a mild perivascular infiltration was observed, but no transmural infiltration of the vessel wall suggestive of vasculitis.

3.3. Fibrosis. Representative samples of picrosirius red-stained sections, used for quantification of interstitial fibrosis, are depicted in Figure 3. Interstitial fibrosis in the RV was not different between the SScPAH and IPAH groups (Figure 4). LV interstitial fibrosis did not differ between the three different groups either. Focal epi- and endocardial fibrosis was seen in all subjects.

On EvG-stained sections we analysed putative foci of replacement fibrosis. This was observed in 4 out of 5 RV's from SScPAH patients and in 5 out of 8 RV's from IPAH patients. The LV demonstrated replacement fibrosis in 2 out of 4 SScPAH patients and 5 out of 8 IPAH patients. In most cases, this fibrosis was patchy, showing microscopic foci, mostly localised subendocardially (Figure 5(a)). In few cases, a pattern of perivascular fibrosis of the microvasculature was observed, radiating from the epicardial coronary arteries to the subendocardial myocardium, ending in microscopic fibrotic foci (Figure 5(b)). In some cases, small infarcts (observed at gross pathology) were observed (1 SScPAH RV, 2 SScPAH LVs, and 2 IPAH RVs) (Figure 5(c)). This was not

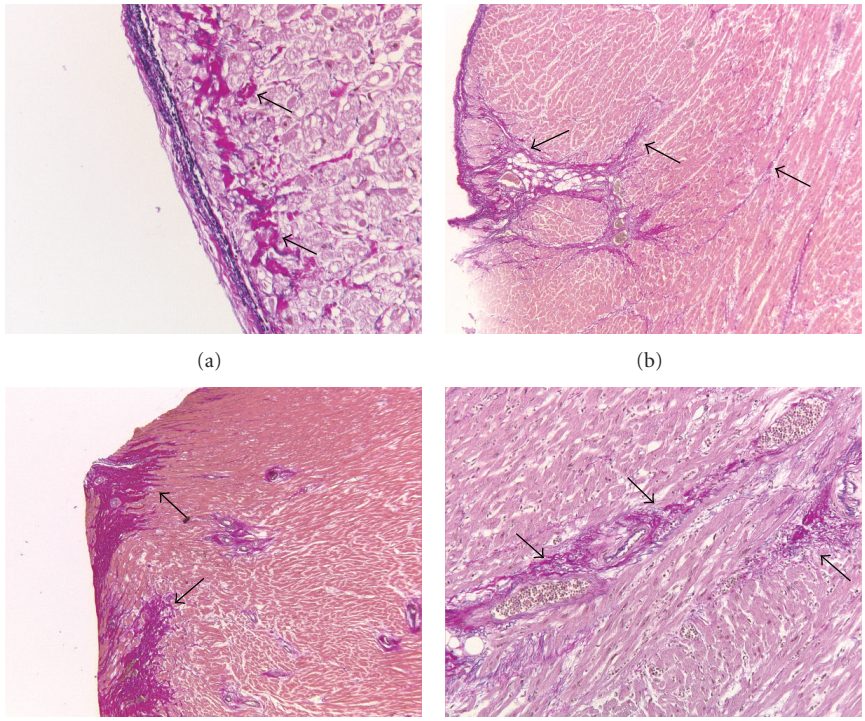

Figure 5: Representative samples of Elastica von Gieson stained myocardial sections of the RV of SScPAH patients, used for studying fibrosis in detail. (a) Some RV's of SScPAH patients revealed a pattern patchy replacement fibrosis, mostly localized subendocardially. This was also seen in some hearts of IPAH patients, but not observed in control. (b) In few cases, a pattern of strands of collagenous fibrous tissue surrounding microvasculature was observed, radiating from the epicardial coronary arteries to the subendocardial myocardium. (c) In some cases small infarcts in both SSc and IPAH hearts were observed. (d) An increase in perivascular fibrosis in some SScPAH hearts and in 1 IPAH heart was observed. All these observations were not made in control hearts.

observed in hearts of control subjects. Finally, we investigated the occurrence of intimal fibrosis in intramyocardial coronary arteries and arterioles. This was observed in SScPAH in 1 RV and 3 LVs and IPAH in 1 RV. Perivascular fibrosis and adventitial remodeling was equally observed in both SScPAH and IPAH right and left ventricles (Figure 5(d)). This was not different from controls.

4. Discussion

In this paper, for the first time, histopathologic features of fibrosis and inflammatory status are described in the interstitial myocardium of the RV in a well-documented SScPAH group. As RV's of SScPAH patients have worse function than IPAH RV's, comparison took place with IPAH RV's. We observed significantly more extravascular inflammatory cells in the myocardial interstitium of the RV of SScPAH as compared with the RV of IPAH and as compared with normal controls. No significant difference in this respect was found between IPAH RV's and control RV's, nor between the LV's of both PAH disease groups. Interstitial myocardial fibrosis in the RV did not significantly differ between SScPAH and IPAH, nor between the PAH disease groups and normal controls. No differences were found with respect to interstitial fibrosis for the LV either. Although PAH patients had more replacement, perivascular, and subendocardial fibrosis when compared to controls, no differences were found between SScPAH and IPAH patients.

The presence of inflammatory cells in (interstitial) myocardial tissue in SScPAH has not been described previously. Two SSc cases with clinical LV failure, but without signs of increased RV afterload, have been described, demonstrating an increase in T-cells and CD68 positive cells in endomyocardial biopsies of the RV. [18] In IPAH, interstitial inflammatory cell infiltration in the RV did not differ significantly from normal controls in a previous report [16], which is in agreement with the present findings.

Fibrosis in hearts of SSc patients has been shown in autopsy studies, and tended to be patchy and distributed throughout all levels of the myocardium of the RV and LV [19–23]. In endomyocardial biopsies of RV's of SSc patients, Fernandes et al. [24] found increased collagen deposition as compared with normal controls. None of the above described studies included patients with confirmed pulmonary (arterial) hypertension. A recent study on cardiac MRI features in 52 SSc patients described delayed contrast enhancement, indicating the presence of myocardial fibrosis, in 1 of 8 SScPAH patients [25]. In IPAH, fibrosis in

endomyocardial biopsies of the RV has been reported to be "mildly" increased, however, quantification nor specification concerning location was reported [26].

The study is limited by the small sample size. Despite this, the examined SScPAH RV histology is unique and has not been subject of study previously, set apart from the SSc group as a whole. Special care was taken to include only cases in which both the diagnosis of SSc and PAH was unequivocal, so as to optimize homogeneity, and thereby optimize statistical power. We therefore think that the exploratory data presented here provide relevant insight, warranting further study. An inherent limitation of this study is the use of archival autopsy material, which is shared by previous studies on this topic. As practically all patients died of PAH-related causes, the pathology in this series represents end-stage disease. Consequently, its features might differ from earlier and subclinical phases of the same disease. Also, sampling and processing of the RV and LV is often not done in a standardized way over the years. As little is known about uneven distribution of either fibrosis or inflammation, it is unknown as to whether this is a limitation to the study. For the immunohistochemical stainings, an internal positive control was present in all slides, and staining intensity as a possible consequence of differences in processing was irrelevant. The observation that even in end-stage disease significant differences were found in inflammatory cells suggests at least different pathogenic pathways. Our measurements included all intramyocardial areas (such as subepicardium, endocardium, or midwall area), and therefore the results depict the overall amount of interstitial fibrosis within the myocardium. Additionally, global scanning of the samples did not reveal differences between midwall and subepi-endocardial areas. This issue might be relevant as studies on cardiac MRI with delayed contrast enhancement suggest the mid-wall area as the predilection area in the majority of SSc patients who demonstrated DCE [25, 27, 28]. However, these studies did not describe DCE-uptake in the RV and results could not be histologically confirmed. One patient died within 2 days after lung transplantation. The short-term effects on RV of a sudden afterload normalization at histopathologic level are not known. It is known that the RV undergoes significant RV-remodelling within 3 to 6 months after lung transplantation as demonstrated by MRI [29]. We do not think that the RV in this patient morphologically underwent major changes.

How do we interpret the elevated numbers of interstitial inflammatory cells in the RV of SScPAH hearts compared to IPAH? Mechanical stress due to RV pressure overload could be responsible for this finding as such a difference was not found when the LV's of both disease groups were compared. Indeed, increased mechanical stress may induce cytokine expression (e.g., monocyte chemoattractant protein-1 (MCP) and interleukin (IL)-8) in several cell types such as endothelial cells and cardiomyocytes [30, 31] through production of reactive oxygen species (ROS) with secondary activation of NF-κB [32].

Increased susceptibility to ischemia, a known trigger to induce inflammatory cytokines, might also explain increased inflammatory cell infiltration in SScPAH, as structural and functional abnormalities of the small coronary arteries are known features of SSc [33–37]. However, in the present study we did not find differences in the presence of arteriolar intimal fibrosis between SScPAH and IPAH RV's.

It is unclear whether the inflammatory cells are innocent bystanders or whether they play an active role in altered RV function in SScPAH. Most links between inflammation and a reduced contractility in chronic heart diseases have been forged on the basis of cytokines and chemokines [38–43] but there are limited data on the effect of cardiac tissue injury by neutrophils. In addition, it is known that macrophages can directly impair contractility of individual cardiomyocytes [44–48].

A significantly higher CD45-positive cell infiltration was found in the LV's of both SScPAH and IPAH as compared with controls, which is difficult to to explain. Increased CD45 infiltration of the myocardium is seen in viral myocarditis [49], however, in none of the patients other signs of myocarditis, such as necrosis or degradation of myocytes along with lymphocytic infiltrates, was seen.

We found no quantitative differences of interstitial fibrosis between SScPAH and IPAH. This, however, does not exclude the possibility that the composition or structure of the extracellular matrix components is different in SScPAH, which may result in different effects on ventricular function [47, 50, 51]. In agreement with previous reports, we did observe patchy and moderate replacement fibrosis in several hearts of SScPAH patients, both in the RV and LV [22, 52–54]. Replacement fibrosis is the end-result of either inflammation mediated and/or ischemia-mediated damage [55]. It is not clear in this study which mechanism predominates in SScPAH. As replacement fibrosis was also observed in IPAH patients, but not in control hearts, the focal fibrosis may ultimately be the result of increased RV pressure overload in pulmonary hypertension, regardless of its cause.

In conclusion, the present paper shows an increased number of inflammatory cells in the RV myocardial interstitium in SScPAH as compared with IPAH. No differences in (interstitial) fibrosis between the groups were found. Further research is warranted to evaluate the significance of these findings for the RV function in SScPAH patients.

Key Messages

(1) RV's of SScPAH patients show more interstitial inflammatory cells than RV's of IPAH patients.

(2) No quantitative differences in interstitial fibrosis were found between SScPAH RV's and IPAH RV's.

Conflicts of Interest

The authors declare no conflicts of interest.

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Review Article

Identification of Myocardial Damage in Systemic Sclerosis: A Nuclear Cardiology Approach

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Myocardial involvement is an important prognostic factor in patients with systemic sclerosis, and early diagnosis and staging of the disease have been sought after. Since myocardial damage is characterized by connective tissue disease, including fibrosis and diffuse vascular lesions or microcirculation, nuclear myocardial perfusion imaging has been a promising option for evaluating myocardial damages in early stages. In addition to the conventional stress-rest perfusion imaging, the current use of quantitative electrocardiographic gated imaging has contributed to more precise evaluation of cardiac perfusion, ventricular wall motion, and diastolic function, all of which have enhanced diagnostic ability of evaluating myocardial dysfunction. Abnormal sympathetic imaging with Iodine-123 metaiodobenzylguanidine might be another option for identifying myocardial damage. This paper deals with approaches from nuclear cardiology to detect perfusion and functional abnormality as an early sign of myocardial involvement as well as possible prognostic values in patients with abnormal imaging results. The role of nuclear cardiology in the era of multiple imaging modalities is discussed.

1. Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by diffuse vascular lesions and fibrosis, and it systemically involves various organs such as skin (scleroderma), heart, lung, kidney and gastrointestinal tracts [1–6]. Of these organ involvements, cardiac complications include arrhythmias, pericarditis, angina pectoris, congestive heart failure and sudden death. Autopsy findings demonstrated that myocardial fibrosis in SSc has been a common occurrence [1, 7]. Thus, it has become evident that early diagnosis and accurate staging of visceral involvement are fundamental for appropriate management and therapeutic approaches for SSc [8]. These approaches may provide a significant prognostic value to systemic sclerosis. Although the precise mechanism for pathogenesis and etiology is not the aim of this article, nuclear medicine approaches to SSc patients are presented in this paper. The mechanisms of cardiac dysfunction and insight that can be gained from nuclear imaging are discussed.

2. Subsets of SSc and Organ Involvements

SSc is usually classified into two subsets of diffuse and limited cutaneous types (dcSSc and lcSSc) [3]. The major findings of skin sclerosis and organ involvement are summarized in Table 1. Common manifestations of organ involvement in dcSSc include interstitial lung disease, renal failure, diffuse gastrointestinal disease, and myocardial involvement. It has been found that cardiac involvement is more common in patients with dcSSc, and one of the least predictable of the visceral involvements during the clinical course of dcSSc. However, even in the lcSSc subset, ischemic response has been detected in 64% of the patients using thallium-201 (^{201}Tl) myocardial perfusion imaging [9]. A research group database from the EULAR scleroderma trials showed that scleroderma subsets (lcSSc and dcSSc types), autoantibody status and age at onset of Raynaud's phenomenon were found to be independently associated with the prevalence of organ manifestations [10]. It was also important to separate patients into two SSc subsets for the purpose of survival

analysis. Poorer prognosis was associated with the dcSSc type, positive antitopoisomerase I antibody and negative anticentromere antibody in the long-term followup [11–13].

3. Nuclear Cardiology Studies for Cardiac Involvement in SSc and Pathophysiological Bases

3.1. Myocardial Perfusion Imaging and Underlying Pathophysiology. In nuclear cardiology, myocardial perfusion imaging has been used extensively for evaluating coronary artery disease, which includes diagnosis of ischemic heart disease, physiological assessment of known coronary stenosis, viability assessment after acute coronary syndrome, reevaluation after coronary intervention, and risk stratification for future cardiac events [14]. The diagnostic sensitivity of coronary artery disease is approximately 80%–90%, and its specificity is around 70%–80%. The advent of electrocardiography (ECG) gated perfusion imaging has further enhanced diagnostic accuracy by simultaneously evaluating myocardial ischemia and functional abnormality [15].

In more than three decades of history of nuclear medicine in cardiology, an early finding of myocardial perfusion abnormality in SSc was documented in 1984 by planar ^{201}Tl perfusion imaging with circumferential profile analysis that added quantitative support [16, 17]. Coronary angiography was normal in those patients. A reduced coronary flow reserve has also been documented without coronary stenosis. A subsequent study using cold-stress showed transient myocardial perfusion defects as visualized by ^{201}Tl [18]. The authors suggested that cold exposure in SSc patients might elicit transient reflex coronary vasoconstriction resulting in reversible myocardial ischemia and dysfunction. Using cold stress and dipyridamole stress, half of the patients with long-standing Raynaud's phenomenon presented ischemic ^{201}Tl defects [19]. It is noteworthy that scleroderma patients with a normal dipyridamole test demonstrated cold-induced transient myocardial ischemia. Thus, primary involvement is not major coronary artery stenosis in SSc, but the target of perfusion abnormality is related to microcirculation. Despite the potential differences in imaging targets, nuclear medicine studies with ^{201}Tl and Technetium-99m (^{99m}Tc)-labeled radiopharmaceuticals have shown that either stress-induced ischemia or persistent perfusion defects occur in SSc patients [18–24]. After the advent of single-photon emission computed tomography (SPECT), the detectability of small perfusion defects was enhanced. A study with ^{201}Tl SPECT in patients with SSc and systemic lupus erythematosus showed a high incidence of (82%) of abnormal findings by ^{201}Tl SPECT [25]. The authors used quantitative analysis with a polar map and a 17-segment model, and found reverse redistribution finding in patients with collagen diseases.

Based on a pathophysiological viewpoint, abnormalities of microcirculation seemed to play an important role leading to myocardial damages [16]. Focal myocardial lesions ranging from contraction band necrosis to fibrosis and reversible vasospastic abnormality in small coronary arteries have been found to be a key mechanism in myocardial dysfunction. Patchy scarring and focal necrosis unassociated with coronary artery disease were observed, and pathologic changes were sometimes associated with fibrous pericarditis and a cardiac conduction system [26]. Myocardial perfusion abnormality may be related to scattered or diffuse fibrosis and contraction band necrosis due to vasospasm or repeated focal ischemia [7, 27, 28]. When the severity of disease advances, perfusion defects may become larger or might be detected by an exercise stress protocol. However, in the early stage, the abnormality might not be evident based on conventional exercise-stress protocol. Vasodilator imaging, currently using dipyridamole, adenosine triphosphate, and adenosine, might be useful for detecting ischemia. However, pharmacological vasodilator stress would be more appropriate for detecting ischemia by atherosclerotic coronary stenosis, rather than by vasospastic microcirculation abnormality. Conversely, dipyridamole significantly improved resting ^{201}Tl myocardial perfusion: the mean number of segments with perfusion defects decreased from resting condition after dipyridamole [29]. The same group also revealed short-term improvement in ^{201}Tl myocardial perfusion with nifedipine in patients with progressive systemic sclerosis [23]. In contrast, fixed perfusion defect, either scattered or segmental, reflects myocardial fibrosis, and stress-induced ischemia reflects vasospasm of small coronary arteries or coronary stenosis. Both ischemia and fibrosis coexist in an individual heart, and the induced ischemia may be reversible and potentially treated by medications.

Table 1: SSc subsets and organ involvements.

	Diffuse cutaneous SSc	Limited cutaneous SSc
Skin sclerosis	Truncal and acral skin involvement	Limited to hands, feet, face, and forearms, or absent
Organ involvement	Early and significant incidence of interstitial lung disease, oliguric renal failure, diffuse gastrointestinal disease, and myocardial involvement	Significant late incidence of pulmonary hypertension, trigeminal neuralgia, calcinosis, and teleangiectasia
Antibodies	Anti-DNA topoisomerase I antibodies	Anticentromere antibodies

3.2. Left Ventricular Dysfunction in SSc. Resting left ventricular ejection fraction (EF) and its response to exercise also appeared to be unrelated to the findings on ^{201}Tl scanning, except for subtle abnormality [17]. However, ^{201}Tl perfusion defects appeared to be related to left ventricular function, which showed that patients with perfusion defect had scores above the median value. In contrast, several patients with diffuse scleroderma had prominently abnormal EF at rest

Table 2: Comparison of low and high skin thickness score in patients and control subjects.

	Control	Low MRSS (<10)	High MRSS (≥10)	*P*
N	16	16	18	
Age (years)	50 ± 12	56 ± 10	55 ± 15	n.s.
Male : female	2 : 14	2 : 14	1 : 17	n.s.
MRSS	—	4.0 ± 2.5	19.2 ± 6.7	<.0001
Myocardial perfusion imaging				
Induced ischemia	0	2	1	n.s.
Resting hypoperfusion	0	2	3	n.s.
Gated SPECT				
Heart rate (/min)	65 ± 7	68 ± 11	71 ± 8	n.s.
EF (%)	68 ± 9	73 ± 9	71 ± 12	n.s.
EF <55%	0 (0%)	0 (0%)	2 (11%)	n.s.
PFR (/sec)	2.46 ± 0.45	2.76 ± 0.44	2.74 ± 0.53	n.s.
1/3 MFR (/sec)	1.52 ± 0.25	1.57 ± 0.31	1.25 ± 0.42	.017
TPFR (msec)	166 ± 22	168 ± 38	216 ± 82	.015
TPFR/RR	0.18 ± 0.02	0.19 ± 0.04	0.26 ± 0.09	.002

Adapted from the results of [30]
MRSS, modified Rodnan total skin thickness score;
EF, ejection fraction; PFR, peak filling rate; 1/3 MFR, one-third mean filling rate;
TPFR, time to PFR; TPFR/RR, TPFR divided by RR interval.

with further prominent deterioration during exercise [16]. Thus, a baseline decrease in left ventricular contractility may be related to resting perfusion defects that reflect myocardial fibrosis.

In a Japanese population, we performed a stress ^{99m}Tc methoxy-isobutyl-isonitrile (MIBI) study with ECG-gating of 16 frames per cardiac cycle in 34 patients with SSc [30]. Compared with Western studies as described above, in contrast, only slight segmental defect and/or stress-induced ischemia were observed by perfusion SPECT (Table 2). We found a significant relationship between diastolic abnormality and modified Rodnan total skin thickness score (MRSS) [5, 31]. A decreased resting EF of less than 55% was found in no patients in the low-MRSS group and in two patients in the high-MRSS group. However, diastolic dysfunction was observed in the high-MRSS group. The time to peak filling rate differed significantly among the control, low-MRSS and high-MRSS groups. Impaired relaxation and diastolic asynchrony in SSc were also reported using radionuclide angiography and echocardiography [32, 33].

Diastolic dysfunction as detected by either gated myocardial perfusion imaging or radionuclide ventriculography was not specific to the findings of SSc. Diastolic dysfunction may have occurred even without any systolic dysfunction in patients with ischemic heart disease, hypertrophic cardiomyopathy, hypertensive heart diseases, and secondary cardiomyopathies [34–37]. In patients with the clinical syndrome of heart failure, determination of the presence and severity of diastolic dysfunction is increasingly important [14]. Complicated factors such as myocardial stiffness, wall elasticity, compliance, incomplete relaxation, and ventricular pressure may be involved in diastolic dysfunction, and a similar mechanism may also be at work in SSc. We postulated that diastolic function was an early sign of cardiac involvement, since it appeared even in patients with neither perfusion abnormality nor systolic dysfunction.

Thus, we postulate that myocardial perfusion SPECT abnormality is correlated to the severity of cardiac dysfunction. In mild dysfunctional patients, even when left ventricular contractility is normal, diastolic dysfunction may be related in some cases. A stress myocardial perfusion scan might show reversible perfusion defect in patients with mild to moderate severity. In patients with more advanced severe dysfunction, fixed perfusion defects and regional wall motion abnormality would occur accompanied by global decrease in EF. However, whether or not the slight degree of perfusion abnormality and diastolic dysfunction would result in further deterioration of cardiac function should be investigated in a long-term follow-up study.

The reason for the relatively low incidence of perfusion abnormality in our study might be explained by the selection bias of our study population, which might not have included the severer type of SSc. As for another possible reason, it might have been caused by differences in the sex and race of the patients, since ethnic differences have also been demonstrated in a Japanese population [38, 39].

3.3. Additional Information by Quantitative Gated SPECT Analysis. Our experience with SSc patients has shown that large perfusion defects and induced ischemia do not seem to be so common as described previously. Instead, small regional abnormality has become evident by the help of quantitative analysis. Current nuclear cardiology technology uses sophisticated computer-aided diagnosis for quantification. As an example, a patient with SSc is shown in Figure 1.

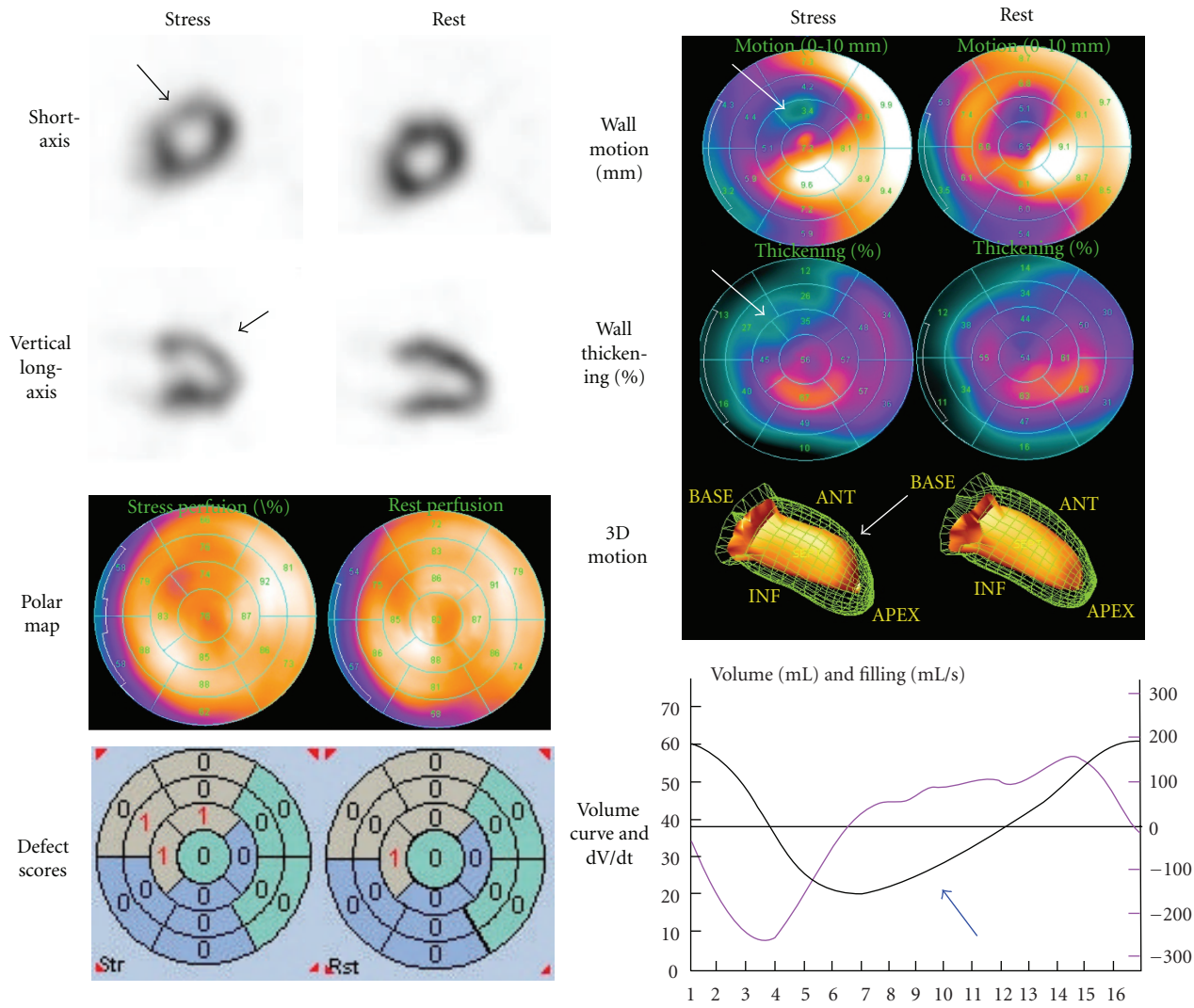

Figure 1: A patient with SSc showing slight anteroseptal ischemia. Quantitative analyses of perfusion, defect scores, wall motion and thickening showed significant abnormality, which supported abnormality in this region (arrows). Diastolic dysfunction was observed even at resting condition as shown by the blue arrow.

A 66-year old male patient diagnosed with dcSSc was referred to our department for nuclear cardiology for the purpose of evaluating possible cardiac involvement. His onset of disease was Raynaud's phenomenon 5 years ago prior to his visit. Scleroderma progressed since 2 years ago extending from hands, forearms, anterior chest to abdominal skin, and the MRSS indicated moderate severity (score 25 of 51). Significant pulmonary fibrosis was observed by X-ray computed tomography (CT). Myocardial perfusion SPECT was performed with ^{99m}Tc MIBI using stress-rest protocol. During exercise, only a slight decrease in perfusion was observed in the apical anteroseptal walls. A polar map display, with a 17-segment model overlaid, showed a localized decrease in perfusion in the anteroseptal region. The summed stress score and rest score namely, semi-quantitative defect scores during exercise and at rest were 3 and 1, which was judged as only a slight abnormality. In the polar map, wall motion and systolic thickening were apparently reduced in the anteroseptal region, particularly after exercise. A three-dimensional ventricular contour display showed apical anterior hypokinesis. A volume curve by gated SPECT showed characteristics of diastolic dysfunction. The time to peak filling rate (TPFR) as defined from the time points from end-systole to peak filling rate was prolonged to 352 msec (normal value for Japanese population: 159 ± 26 msec (SD)). The early diastolic parameter of 1/3 mean filling rate was 0.94 /sec (normal value: 1.68 ± 0.30 /sec)) [40]. Instead of simply judging positive or negative perfusion defects, we were able to definitely diagnose the abnormality in the SPECT study by integrating various quantitative parameters.

3.4. Possible Options of Radiopharmaceuticals. Myocardial perfusion imaging is fundamental for evaluating myocardial damage. In addition, patients with SSc have a high frequency of diastolic dysfunction and sympathetic abnormality as shown by ^{123}I-metaiodobenzylguanidine (MIBG) studies

[41, 42]. ^{123}I-MIBG is known as an analogue of norepinephrine and shares its metabolic pathways with catecholamine uptake and excretion. Sympathetic abnormality has been detected by decreased uptake and rapid clearance from the heart. Initial experiences of MIBG support its use in ischemic heart diseases, typically shown as regional denervation of the heart in acute coronary syndromes and cardiomyopathies. Currently, ^{123}I-MIBG has been used for patients with heart failure as well as neurological disorders involving Lewy-body diseases [43–45]. It has been understood that MIBG uptake reflects sympathetic neuronal activity or norepinephrine contents, and increased sympathetic activity drives results in rapid MIBG clearance. Figure 2 shows a patient with normal myocardial perfusion associated with severe decrease in MIBG activity. In this patient, early and delayed heart-to-mediastinum average count ratio (H/M) was 1.67 and 1.34, respectively (normal values: 2.39 ± 0.21 and 2.49 ± 0.25, resp., [40]). MIBG washout rate also increased to 33% (normal value was <20% [46]). In concordance with the MIBG abnormalities, some studies have indicated that autonomic dysfunction was extremely common in patients with SSc. It was characterized by parasympathetic impairment and marked sympathetic overactivity, particularly in the early stage [47, 48]. However, a cause and effect relationship between autonomic derangement and repeated myocardial damage has not been clearly identified.

The use of MIBG imaging has also been validated by a large prognostic multicenter trial in patients with heart failure of New York Hear Association (NYHA) functional class II/III and LVEF of 35% or less [49]. The two-year event rate was 15% for H/M $\geq$1.60 and 37% for H/M <1.60. The authors found that ^{123}I-MIBG provided additional discrimination in analyses of interactions between B-type natriuretic peptide, LVEF and H/M ratio. Although pathophysiological evidences of heart failure in the study could not be readily applicable to SSc patients, the MIBG imaging may provide new insights into the nature and prognosis of myocardial damage in SSc.

An article reported a case of systemic sclerosis with a subacute episode of myocardial disease assessed by ^{111}In-antimyosin antibody, which was a marker of the myocardial damage or necrosis [50]. However, antimyosin antibody was considered to accumulate in ongoing myocardial injury typically caused by ischemia or infarction, and thus it may not be applicable for an early stage of systemic sclerosis.

4. Prognostic Value of Nuclear Cardiology in Systemic Sclerosis

Large-scale prospective cohort studies have been performed extensively in the field of coronary artery disease, although such prognostic studies are still limited in patients with SSc. However, it has been recognized that cardiopulmonary involvement has been considered to be a poor prognostic factor since renal involvement is no longer a major cause of death in SSc, [7, 27, 51]. Steen and Medsger demonstrated that when natural history and timing of severe organ involvements were analyzed in the dcSSc, the 9-year cumulative survival rate of all patients with severe organ involvement was 38%, compared with 72% in patients without such involvement [52]. Long-term prognosis was found to be poor in patients with ^{201}Tl perfusion defect. A study followed up 48 patients who underwent a perfusion scan in the 1980s. Notably, the survival information over the last 10 years revealed that the size of the initial defect was the best predictor of later adverse events compared with other disease-related features. These kinds of long-term prognostic study, which has been accumulating in the field of coronary artery disease, should also be conducted in SSc patients both retrospectively and prospectively.

5. Practical Approaches to Cardiac Evaluation in SSc

Although we have focused on nuclear cardiology methods, echocardiography remains the mainstream tool for heart evaluation in SSc patients [6, 27, 53]. Diagnostic workups in patients with SSc are summarized in Figure 3 from the viewpoint of cardiac imaging. The first step for the diagnosis starts by careful evaluation of the history and symptoms, followed by the physical examination including skin sclerosis and possible organ involvements. The baseline 12-lead ECG and chest X-ray examination are standard procedures for screening. When considering the possibility of cardiac involvement, echocardiography, coupled with Doppler if possible, would be the first-line methodology for cardiac functional evaluation. This approach would show the presence of pericardial effusion, right ventricular involvement as well as left ventricular systolic and diastolic function.

When cardiac abnormalities are suspected by the initial screening tests, the next step would be diverse, depending on the clinical problems in the individual patient. In patients with pulmonary arterial hypertension (PAH), guidelines from both the American College of Chest Physicians (ACCP) and European Society for PAH recommended chest-X-ray, ECG and Doppler echocardiography as evidence-based approaches [54, 55]. The prevalence of pulmonary hypertension associated with scleroderma ranged from 4.9% to 38% as written in the ACCP guidelines. In addition, cardiac catheterization for the assessment of pulmonary hypertension might still be required in some patients who can benefit from intervention. The appropriate evaluation of PAH has practical values for guiding medical treatment, as four sets of recommendations were formulated for SSc-related PAH by EULAR scleroderma trials and their research group [56]. The ACCP also revised the treatment algorithm by taking into account recent developments in therapy [57]. Nuclear cardiology has no validated role for this purpose, although lung ventilation/perfusion may be evaluated in chronic thromboembolic pulmonary hypertension, and metabolism in the right ventricular overload [55, 58, 59]. Right ventricular function may also be evaluated by echocardiography or gated blood-pool study [60]. However,

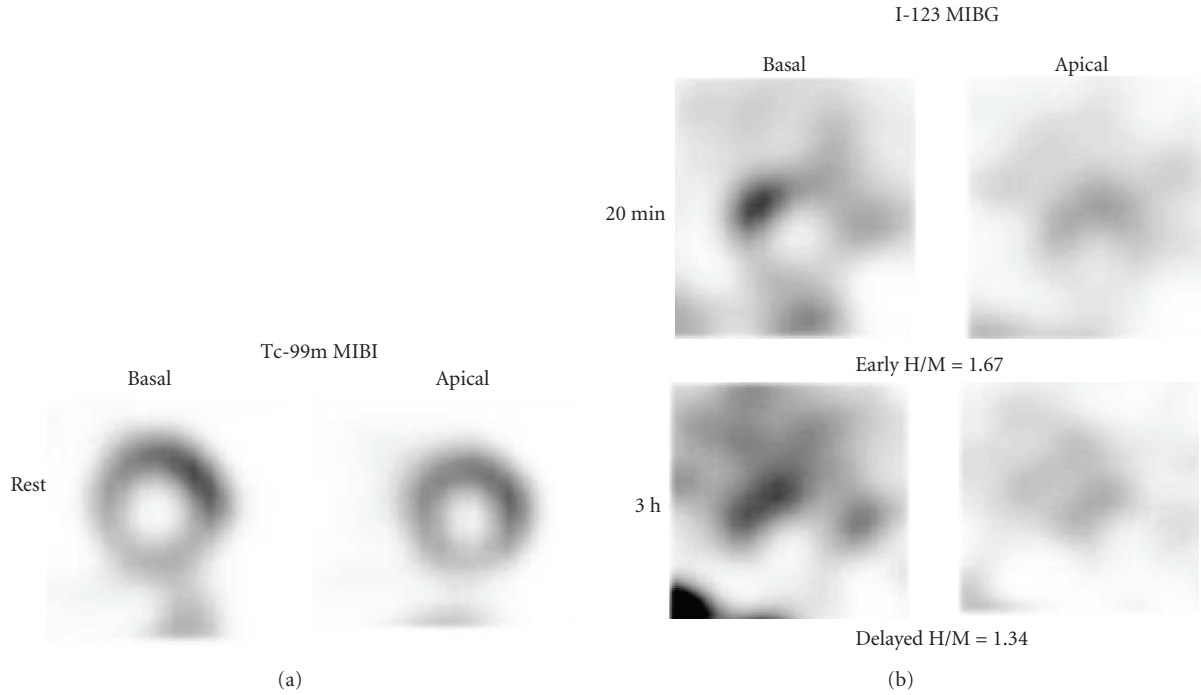

Figure 2: A patient with diffuse cutaneous type with MRSS 21, showing decreased MIBG activity and rapid washout rate (33%). ^{123}I-MIBG distribution showed marked heterogeneity in both early and delayed short-axis images. Resting perfusion was normal by ^{99m}Tc-MIBI SPECT. Adapted from [41].

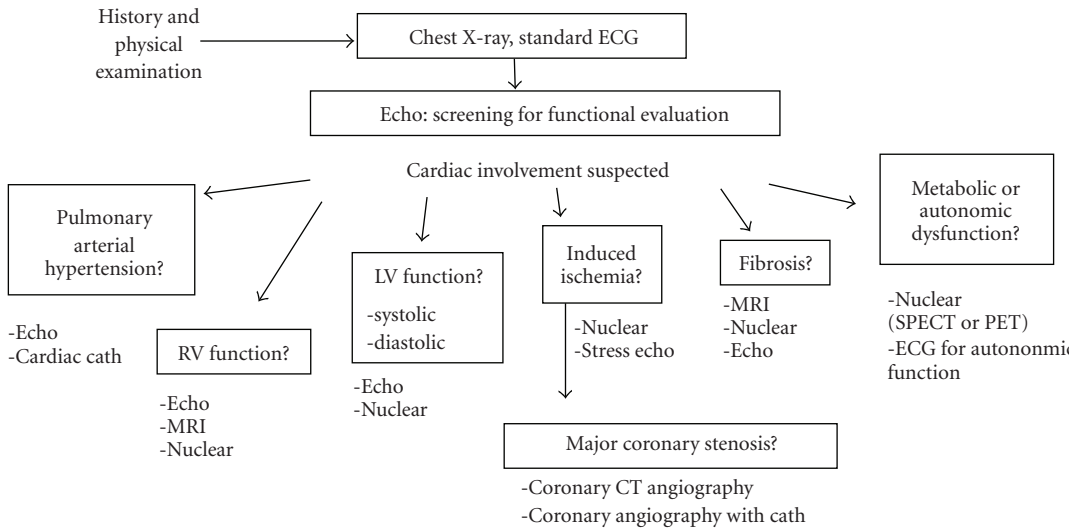

Figure 3: Possible roles of cardiac imaging modalities for diagnostic work-ups and followup in SSc. Abbreviations: ECG, electrocardiography; Cath., catheterization; MRI, magnetic resonance imaging; CT, computed tomography; SPECT, single-photon emission computed tomography; PET, positron emission tomography.

when a major concern of the patient problem involves induced myocardial ischemia, a stress nuclear study with vasodilator or vasoconstrictor stressors would be the best option for guiding management. If stress-induced large perfusion defects were found in the stress study, coronary work-ups with CT angiography using contrast media would be selected. Coronary evaluation may not be indicated when the perfusion defect is relatively small and microvascular origin is more likely. When myocardial fibrosis is suspected based on the ventricular contractility, regional wall motion or diastolic function, the severity might be evaluated by a nuclear study. Recently, using delayed enhanced magnetic resonance imaging (MRI), several studies successfully identified myocardial fibrosis in a significant percentage of patients with SSc [27, 61, 62]. The SPECT or positron emission tomography (PET) may identify larger defects

and/or associated ischemia. No comparative study for their effectiveness has been performed to date.

According to our study, it is apparent that all patients diagnosed with SSc are not indicated for a SPECT study. Based on our study population, it is noteworthy that patients with the lcSSc type or MRSS less than 10 showed neither significant perfusion defect nor ventricular dysfunction (Table 2). Therefore, we tentatively recommend a myocardial perfusion study in patients with dcSSc type and/or MRSS greater than 10, when the patient is suspected of having cardiac abnormality [30, 41]. Belloli et al. studied potential risk factors for microvascular involvement and found that perfusion defects were related to skin scores, digital ulcers, and esophageal involvement, and these patients might warrant screening for myocardial involvement [24]. Thus, patients with SSc who have a history of chest symptoms, conduction abnormality or arrhythmia on ECG, and wall motion abnormality and those who have findings of multiple organ complications would be potential candidates for myocardial SPECT imaging.

The best diagnostic approach or decision tree for cardiac evaluation has not been defined yet, although chest X-ray and echocardiography would be the first-step imaging method readily available in any hospital. While several possible diagnostic approaches have been proposed, some of them might have practical values for diagnosis, and others may provide pathophysiological insight or prognostic information. The determination of the appropriate role of imaging is of great concern in the era of multimodality cardiac imaging.

6. Conclusion

Myocardial involvement as part of diffuse organ fibrosis and vascular changes is commonly observed in SSc. Even when severe fibrosis as evidenced by myocardial perfusion defects is not observed, perfusion reserves or functional abnormality might be detected as an earlier sign of myocardial damage. The aid of computer-assisted diagnosis will enhance the diagnostic ability for identifying abnormalities. The relationship of nuclear imaging and prognosis should be evaluated to confirm the role of functional imaging for patients with SSc. Finally, the roles or effectiveness of various current imaging modalities remain to be defined.

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Review Article

Renal Manifestations in Scleroderma: Evidence for Subclinical Renal Disease as a Marker of Vasculopathy

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Scleroderma is a disease characterized by immune activation, vasculopathy, fibroblast stimulation, and connective tissue fibrosis. End-organ damage occurs due to progressive tissue fibrosis and vasculopathy. Markers of incipient vasculopathy have not been well studied in scleroderma. However, reduced renal functional reserve and proteinuria are common indicators of progressive vasculopathy in diabetic and hypertensive vasculopathy. Recent studies suggest a strong association between renal involvement and outcomes in scleroderma, with a threefold increased risk of mortality from pulmonary hypertension if renal insufficiency is present. We review the types of renal involvement seen in scleroderma and the data to support the use of renal parameters including proteinuria, glomerular filtration rate, and renal vascular dynamics measured with Doppler ultrasound to identify subclinical renal insufficiency. Further studies are warranted to investigate the use of renal parameters as prognostic indicators in scleroderma.

1. Introduction

Systemic sclerosis (SSc) is a chronic multisystem disorder with an annual incidence of 1 to 2 per 100,000 individuals in the United States. It has a peak age of onset of between 30 and 50 years and a strong female predominance [1]. The exact pathogenesis of SSc remains elusive but, autoantibody production, lymphocyte and fibroblast activation, vascular proliferation, obliterative microvascular disease, and connective tissue fibrosis all likely play a role [2]. In advanced disease, end-organ damage occurs as a result of progressive fibrosis and vasculopathy. Often by the time organ injury is identified, there is little that can be done to reverse vasculopathy, and therefore there is a strong impetus in the scleroderma community to identify potential markers of incipient vasculopathy before damage becomes clinically apparent and irreversible.

Numerous other diseases associated with vascular damage, such as diabetes and hypertension, use markers of renal impairment as preclinical indicators of vasculopathy. In this paper we review the types of renal involvement reported in scleroderma and discuss preclinical markers of renal pathology that might be helpful in identifying scleroderma patients at risk for progressive vasculopathy.

2. Clinical Subsets of Scleroderma

Two subsets of scleroderma, with different autoantibody profiles and internal organ involvement are recognized [3]: limited cutaneous scleroderma (lcSSc) in which cutaneous involvement is limited to the hands, face, feet, and forearms and diffuse cutaneous scleroderma (dcSSc) in which there is extensive skin involvement extending above the elbows and knees and involving the trunk. A further group, scleroderma sine scleroderma (sSSc) in which patients have manifestations of visceral disease without skin involvement, has an identical prognosis to lcSSc and is included in the lcSSc group [3–5]. Patients with scleroderma sine scleroderma are often unaware of the disease until end-organ damage becomes apparent. These subsets are well described elsewhere.

Table 1: Reported renal manifestations of scleroderma.

Reported renal manifestations of scleroderma
Scleroderma renal crisis
Normotensive scleroderma renal crisis
Myeloperoxidase-Antineutrophil Cytoplasmic Antibody (MPO-ANCA) associated glomerulonephritis and vasculitis
Penicillamine-associated renal disease
Antiphospholipid-associated nephropathy
Isolated reduced glomerular filtration rate
Reduced renal functional reserve
Microalbuminuria and proteinuria
Scleroderma-associated vasculopathy manifested by abnormal renal vascular resistance indices and endothelial markers

Patients with dcSSc develop rapidly progressive skin involvement with early organ involvement including interstitial lung disease, scleroderma renal crisis, and gastrointestinal involvement. One of the predictors of scleroderma renal crisis is the presence of the RNA polymerase III antibody (Pol3), and patients with this antibody are at high risk for early scleroderma renal crisis (SRC). In contrast, the anticentromere antibody is negatively associated with scleroderma renal crisis. Patients with limited scleroderma tend to have more indolent progression of skin disease but with time develop complications from vascular injury such as gastric antral vascular ectasia [6] and pulmonary hypertension.

3. Renal Disease in Scleroderma

Several forms of renal involvement are recognized in scleroderma. The most dramatic of these is scleroderma renal crisis which is seen in approximately 10% of the scleroderma population [7]. Autopsy studies, however, reveal occult renal pathology in 60% to 80% of patients with systemic sclerosis [8]. Others have found that up to 50% of asymptomatic patients have clinical markers suggesting renal disease such as proteinuria, elevation of creatinine, or hypertension [9]. Renal impairment from chronic renal vasculopathy, nephrotoxic medications (including cyclosporine and D-penicillamine), and glomerulonephritis have all been reported (Table 1) [10, 11].

3.1. Scleroderma Renal Crisis. Scleroderma renal crisis (SRC) is one of the most well-recognized complications of scleroderma. It is manifested by acute onset of moderate-to-severe hypertension with hyperreninemia, thrombotic microangiopathy, and progressive renal failure [7]. Biopsy reveals severe mucinoid hyperplasia and vascular fibrinoid necrosis of the interlobular and arcuate arteries and arterioles (Figure 1) with relative sparing of the glomeruli and absence of inflammatory or immune deposits. With advanced renal crisis secondary ischemic changes in the glomeruli may occur.

The pathologic findings seen in SRC suggest that it is an acute vascular manifestation of the disease. However, the renal vascular lesions may be present in SSc patients with normal renal function. Presence of these changes correlates with abnormal plasma rennin levels at baseline and in response to cold exposure but do not always correlate with development of SRC [12]. Risk factors for SRC include early (less than 5 years of disease) diffuse scleroderma, rapidly progressive skin thickening, presence of Pol3 antibody, and prior exposure to corticosteroids (>15 mg prednisone equivalent in the prior six months) [13]. Renal crisis is seen in approximately 10–20 percent of patients with dcSSc and only 1 percent of patients with lcSSc. However, of patients developing SRC, 78% had dcSSc [14]. Steroids are a precipitant in 60% of patients with SRC [14, 15]. Angiotensin converting enzyme inhibitors (ACE inhibitors) have been pivotal in the treatment of SRC, and since their introduction, mortality and renal morbidity has declined dramatically [16].

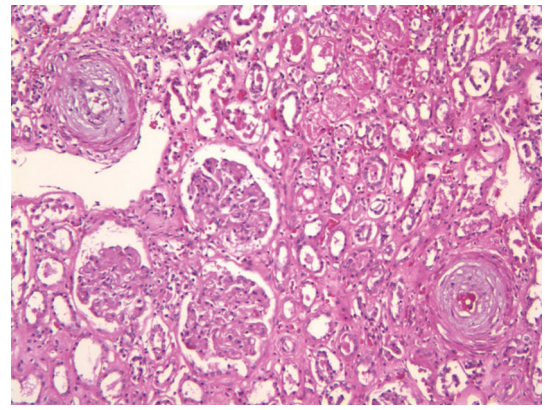

Figure 1: Hemotaxylin and Eosin stain of renal biopsy from a patient with scleroderma renal crisis, showing onion skinning concentric narrowing of arterioles with ischemia of glomeruli with flattening and degeneration of the tubular cells.

3.2. Normotensive Scleroderma Renal Crisis. A number of cases of acute, rapidly progressive renal failure with concomitant thrombotic microangiopathy but no malignant hypertension have been described, and this complication is termed "normotensive scleroderma renal crisis" [17, 18]. This manifestation is rare, accounting for only 11% of scleroderma renal crisis cases, and is associated with increased frequency of pulmonary hemorrhage and increased mortality. Anecdotal reports suggest that this presentation of renal crisis may be more common in patients receiving ACE inhibitors, but the mechanism by which this occurs is not well understood.

3.3. Coexistent MPO-ANCA-Associated Glomerulonephritis and Scleroderma. Several authors have reported myeloperoxidase antineutrophil cytoplasmic antibody- (MPO-ANCA-) associated glomerulonephritis resulting in renal failure in scleroderma [10, 19, 20]. In contrast to classical scleroderma renal crisis, these patients tend to have established lcSSc, and the process has a subacute presentation with progressive renal failure, mild hypertension, and proteinuria. This

diagnosis should be considered in any scleroderma patient with positive MPO antibodies and renal failure. It has been postulated that scleroderma vasculopathy exacerbates the interaction of ANCA with endothelium near the vascular pole and neutrophil activation in the glomerulus. This manifestation does not respond to ACE inhibitors but is steroid responsive. However, biopsy is often required to exclude scleroderma renal crisis.

3.4. Penicillamine-Induced Renal Injury in Scleroderma. D-Penicillamine is rarely used now but historically has been used in treatment of Wilson's disease, cystinuria, rheumatoid arthritis, primary biliary cirrhosis, lead poisoning, and systemic sclerosis. Up to 20% of patients treated with D-penicillamine develop membranous glomerulopathy with proteinuria which resolves with cessation of the medication. Drug-induced lupus syndrome, diffuse proliferative cresentic glomerulonephritis, and cases of pulmonary renal syndrome mimicking Goodpasture's Syndrome have also been reported [21]. Typically these manifestations improve with withdrawal of the D-penicillamine, but in severe cases steroids, plasmapheresis, and immunosuppression have been required. Based on cases reported in the literature, this complication has a roughly 40% mortality.

3.5. Antiphospholipid-Associated Nephropathy in Scleroderma. Unlike in other autoimmune diseases, the frequency of antiphospholipid antibodies in scleroderma is reported to be no greater than that seen in the general population ranging from 3.3% to 12% [22–24]. Based on a single aPL antibody measurement of anticardiolipin (ACL) or Beta-2 glycoprotein I (B2GPI) antibody of IgM or IGG class, Wielosz et al. found that 56% of SSc patients had positive aPL antibodies [25]. They correlated antibody positivity with renal function parameters and found that although there was no difference in serum creatinine between the aPL positive group and the aPL negative group, IgG ACL was associated with elevation of serum cystatin C (a marker of reduced glomerular filtration rate) and negatively associated with creatinine clearance. Furthermore, proteinuria (>0.5 g/24 hours) was found in 21% of the APL positive patients but only in 9% of the APL negative patients. aPL positivity was also associated with development of SRC, which occurred in 21% of the patients with positive aPL and none of the patients with negative aPL. Larger studies are needed to clarify these findings, but this suggests that aPL antibodies may play a role in renal injury in scleroderma.

3.6. Isolated Reduced Glomerular Filtration Rate in Scleroderma. Impairment of renal function can be present in SSc despite normal serum creatinine [26]. The principal determinants of creatinine are muscle mass and glomerular filtration rate (GFR), and it is well recognized that serum creatinine may not be elevated until the GFR is less than 50% of normal. Kingdon et al. evaluated patients followed in the Royal Free Hospital Scleroderma Clinic and found reduced GFR as measured by chromium-51-ethylenediaminetetraacetic acid (51Cr-EDTA) clearance in 95% of patients with serum creatinine within the normal range and all the patients with elevated creatinine. These patients also had abnormal calculated GFR. In patients with serum creatinine below the normal range (<60 μmol/L) the measured GFR was normal. The highest correlation between measured and calculated GFR was seen when the Modification of Diet in Renal Disease (MDRD) formula was used. This group advocates using MDRD to calculate GFR at baseline and followup visits.

More recently Scheja et al. have tried to investigate the occurrence of reduced GFR in a large cohort of 461 Swedish SSc patients followed over 10 years [27]. They measured GFR using either chromium-51-ethylenediaminetetraacetic acid (51Cr-EDTA) or by iohexol clearance. Patients with a history of SRC or who developed SRC during follow-up were excluded from analysis. Median follow up was 7.7 years (range 0.5–54 years). Decreased GFR was found in 11% of lcSSc and 8.6% of dcSSc, which accounts for approximately 10% of the total initial cohort. This is lower than reported in the Royal Free study but may simply reflect differing inclusion criteria. The Swedish study included 461 consecutive SSc patients; in contrast the Royal Free study included only 26 patients who were selected based on availability of measured and calculated GFR results on two occasions, an inclusion criteria which is likely to bias the results towards overrepresentation of patients with abnormal GFR. Of the patients with GFR <70% predicted in the Swedish cohort, 60% had hypertension, 52% had cardiac involvement, and 19% had other nephropathies identified on biopsy. Follow up data beyond 4 years was only available in 15 patients, but the majority (73%) had no progression of renal disease in that time.

Steen et al. examined records of 675 patients with dcSSc, and after excluding the 19.5% who developed SRC, they found 32% with abnormal renal function or proteinuria [28]. Most patients had proteinuria from penicillamine or other medical comorbidities. Only 2% had no explanation for elevated creatinine level. Over a mean follow-up of 10 years, none of these patients developed renal failure requiring dialysis, suggesting that although mild renal insufficiency is common in SSc, it often follows a more benign clinical course.

A smaller study from Cairo has evaluated 31 SSc patients and compared them to 31 healthy controls [29]. GFR was measured using technetium 99m DTPA (Tc99mDTPA) and calculated using MDRD and Cockcroft-Gault formulae. All patients had normal serum creatinine and normal renal ultrasound. Measured GFR was normal (>89 ml/min) in 45.1% but reduced in 54.9% as follows: Stage II CKD 60–89 ml/min 32.3%, Stage III (30–59 ml/min) in 22.6%. Renal impairment correlated to pulmonary vascular involvement but did not correlate with age, disease duration, lung fibrosis, gastrointestinal involvement, cardiac involvement, skin score, muscle involvement, antibody profile, or treatment exposures.

The association between renal dysfunction and pulmonary hypertension in SSc is increasingly becoming recognized. Campo et al. recently evaluated 76 consecutive SSc patients with pulmonary arterial hypertension (PAH) and found that 45.6% had renal dysfunction (eGFR <60 mL/min/1.73 m^2) at the time of diagnosis despite only

6.5% having had a prior episode of renal crisis [30]. Furthermore, eGFR was a strong predictor of survival in this cohort, with eGFR <60 mL/min/1.73 m^2 associated with a 3-fold risk of mortality. This strong association may be a reflection of pulmonary hypertension and right heart failure contributing to renal dysfunction through fluid retention and neuroendocrine activation. However, further studies are warranted to evaluate the role of renal dysfunction in SSc-associated pulmonary hypertension.

3.7. Reduced Renal Functional Reserve in Scleroderma. Renal functional reserve is a measure of the kidney's ability to increase GFR after stimulation with oral protein or IV amino acid load. Livi et al. studied renal functional reserve in 21 SSc patients (16 with lcSSc and 5 with dcSSc) with normal renal function and compared them to 10 control patients [31]. Effective renal plasma flow (ERPF) using para-aminohippurate clearance and calculated total renal vascular resistance (TRVR) were measured before and after an intravenous amino acid load. Creatinine clearance was similar at baseline in the two groups. However, the ERPF response was significantly lower, and TRVR was higher in patients than controls. In normal subjects, GFR should rise by 10% in response to stimulation, but only 28.6% of the SSc patients showed this response. Blunted renal functional reserve was seen in 80% of patients with dcSSc and 68.75% of patients with lcSSc. Additional studies are needed to establish if lack of renal functional reserve in SSc is a predictor of developing clinically evident renal involvement or vasculopathy in other organs.

3.8. Proteinuria in Scleroderma. Albuminuria is a useful marker of vasculopathy and is known to be an independent predictor of cardiovascular morbidity and mortality in patients with and without other vasculopathic diseases such as diabetes and hypertension [32–36]. Seiberlich et al. analyzed urine albumin, urine total protein, and urine electrophoresis to assess protein excretion in 80 SSc patients and 18 healthy age- and gender-matched controls [37]. All subjects had a normal GFR. Increased total protein excretion was seen in 17.5% of SSc patients, and albuminuria was identified in 25% (22.5% microalbuminuria and 2.5% macroalbuminuria). Albuminuria correlated with disease duration >4 years and elevation of systolic blood pressure, suggesting it may be reflective of chronic vascular injury. Dawnay et al. evaluated urine albumin in a cohort of scleroderma patients and found prevalence of microalbuminuria of 17.9% but this could not be correlated with clinical outcome due to the small sample size [38]. Larger studies are needed to evaluate the role of albuminuria as a predictor of morbidity, mortality, and outcome in scleroderma.

Urine electrophoresis has not been widely studied in the scleroderma population. In general, urine electrophoresis results are categorized into three patterns depending on the molecular weight of the protein detected. Low molecular weight proteinuria (LMWP) is usually due to diminished tubular resorptive capacity for example from interstitial nephritis and nephrotoxicity. In the Seiberlich study, LMWP was seen at a similar frequency in the SSc group as controls [37]. Intermediate weight proteinuria (IMWP) is a sensitive predictor of increased glomerular permeability and has been described in other forms of vasculopathy including diabetes and hypertension [39]. Urine electrophoresis in the Seiberlich study revealed IMWP in 31.3% of SSc patients, but none of the control subjects. IMWP was seen in 50% of patients with dcSSc compared to only 20% of lcSSc and correlated to the presence of gastrointestinal involvement. Francois et al. have also reported an association of glomerular proteinuria with scleroderma but did not correlate this to clinical parameters [40]. High molecular weight proteinuria (HMWP) is generally a reflection of glomerulonephritis and was not seen in any of the SSc patients or controls in the Seiberlich study.

Further studies are warranted to investigate the role of proteinuria and albuminuria detection in the scleroderma population. In diabetic vasculopathy it is recommended that initiation of angiotensin converting enzyme (ACE) inhibitors when microalbuminuria is detected is effective at delaying progression to advanced disease and improves cardiovascular outcomes [41]. While ACE inhibitors are known to exhibit antifibrotic effects [42] in addition to lowering systemic blood pressure, the use of prophylactic ACE inhibitors is generally not recommended in SSc. Use of prophylactic ACE inhibitors in SSc does not protect against SRC [15, 43], and their use prior to development of SRC may be associated with worse outcomes [14, 44] although this data was not adjusted for confounders, and the reported association did not reach statistical significance.

3.9. Renal Vascular Resistance Indices in Scleroderma. With the advent of new imaging techniques, several groups have investigated the use of non-invasive testing such as renal Doppler ultrasound to evaluate intrarenal vasculature in scleroderma patients.

In patients with lupus nephritis the renal vascular resistance index (RI) is a predictor of poor outcome, correlating with creatinine level and chronicity score on biopsy [45]. RI measures intrarenal elasticity and compliance and tends to be more sensitive to vascular and interstitial nephropathies because glomeruli are only responsible for 10% of the intraparenchymal flow resistances. Release of vasoconstrictive substances results in renal remodeling and elevation of RI as renal vascular disease progresses. In hypertension, both RI and albuminuria improve with angiotensin receptor blocking (ARB) agents [46], suggesting that RI may be measuring a potentially reversible component of vasculopathic damage.

Investigating SSc patients without clinical evidence of renal damage, Rivolta et al. measured RI on the main, interlobar, and cortical vessels in 25 SSc subjects and 25 normal volunteers. SSc patients had significantly elevated RI at every sampling site [47]. RI values correlated with disease duration but not creatinine clearance. These findings have since been replicated in a study evaluating 9 SSc patients with renal impairment, 13 SSc patients with normal renal function, and 20 age-matched controls. There was no significant difference in peak systolic flow velocities in any of the investigated arteries, between patients and control

groups. However, the mean end diastolic flow velocity (EDFV) in the interlobular artery (ILA) was lower in the SSc patients with renal disease compared to the SSc patients without renal involvement. SSc patients with renal disease had higher systolic to diastolic flow velocities (S/D ratio). The mean RI and pulsatile index (PI) were higher in SSc patients with renal disease than those without. RI, PI, and S/D ratios correlated with disease duration but were over and above those that might have been expected with age. Further studies are warranted to evaluate the use of Doppler ultrasound in predicting renal complications in scleroderma.

3.10. Renal Vascular Responsiveness to Iloprost in Scleroderma. Further understanding of the mechanisms of vascular involvement in SSc is obtained by evaluating the response of renal and other vascular beds to vasodilating agents.

In normal tissue, prostacyclin is released locally from platelets and vascular endothelium and inhibits production of endothelin, a potent vasoconstrictor. SSc patients have elevated levels of endothelin I and are known to be prone to toxicity from cyclosporine, an agent that causes vasoconstriction and elevation of endothelin I. Furthermore, there is some evidence that endothelin I may play a role in SRC [48].

Iloprost is a stable prostacyclin analogue with vasodilatory and platelet aggregating effects. It is used in Raynaud's syndrome and is effective in healing cutaneous scleroderma lesions. It has long lasting effects on peripheral blood flow and improves pulmonary artery resistance in PHT. In the kidney it increases renal plasma flow by dilating afferent and efferent arterioles, without changing GFR, and some centers recommend including intravenous iloprost during the hypertensive phase of SRC [44].

Scorza et al. investigated the effect of iloprost on RI in the renal vessels and compared it to the calcium channel antagonist nifedipine [49]. They found that acute iloprost infusion resulted in significant drop of RI in the interlobar and cortical vessels without causing systemic hypotension. Use of iloprost for 8 hours per day for 5 days had long-lasting effect on reducing the RI values in the interlobular and cortical arteries at 2 weeks. In contrast, 6 months of nifedipine treatment did not alter the RI of the renal vessels.

Long-term renal outcomes in response to iloprost therapy remain to be elucidated. Airò et al. evaluated the use of cyclic iloprost in a cohort of SSc patients with severe digital ulcers and Raynaud's and compared pulmonary and renal outcomes over 4 years with age, sex- and disease-matched controls [50]. There was no detectable difference in pulmonary, renal, or long-term outcomes in this study but selection bias and other confounding may have affected these results.

3.11. N-Acetylcysteine Infusion in Early Scleroderma. Renal hemodynamics in SSc patients have also been studied in response to N-Acetylcysteine (NAC) [51]. NAC is a sulfhydryl precursor of glutathione with potent antioxidant and cellular detoxifying actions. Via vasodilation and actions on oxygen free radical scavenging, NAC has been show to improve myocardial function after myocardial infarction [52]. In the kidney, NAC has been shown to enhance renal glutathione, ameliorate renal function, decrease arterial pressure and renal injury in salt sensitive hypertension, and improve endothelial dysfunction in dialysis patients by preventing flow-mediated dilatation [53, 54]. In radiocontrast exposure, it has an antioxidant function and causes vasodilatation and enhanced renal medullary flow [55].

In an open-label study [51], 40 SSc patients with either early or late capillaroscopic changes received NAC intravenously for 5 hours at 0.015 g/Kg/h, and renal hemodynamics were assessed using RI. In patients with early capillaroscopic changes, NAC reduced RI. In contrast, in the group with late capillaroscopic pattern, RI was increased. Based on these findings, it appears that NAC results in renal arterial vasodilation only if given early in the course of disease, prior to irreversible arteriopathy. Patients in this study with late capillaroscopic changes all had low diffusion capacity for carbon monoxide (DLCO), suggesting that they had a component of pulmonary vascular involvement. It seems that as vasculopathy becomes more extensive, NAC may have a detrimental effect, and the implications of this for the use of NAC clinically are unclear.

3.12. Vascular Endothelial Markers in Scleroderma Renal Disease. Cytokine activation of endothelial cells in states of inflammation induces expression of adhesion molecules that allow recruitment of leukocytes to the sites of inflammation [56, 57]. Activated, endothelial cells shed soluble forms of these adhesion molecules including intracellular adhesion molecule-1 (ICAM1), vascular cell adhesion molecule-1 (VCAM1), and E-selectin (E-selectin), and these molecules can be detected in the serum. It is thought that E-selectin has the greatest specificity for cytokine-activated cells. Prior studies have shown elevation of these molecules in diseases with endothelial injury including SLE, vasculitis, and sepsis [58, 59].

Stratton et al. evaluated levels of E-selectin, ICAM-1, and VCAM-1 in serum of SSc patients with and without SRC and SSc-associated pulmonary hypertension (PHT) and compared them to control patients with primary Raynaud's [60]. They found that E-selectin was elevated approximately 25% above normal in patients with lcSSc without SRC or PHT. In contrast, E-selectin levels were normal in patients with lcSSc and PHT, suggesting that the vascular lesions of PHT were not associated with activated endothelial cell phenotype. Although SRC is rare in lcSSc, E-selectin levels were two times normal in the lcSSc-SRC group, suggesting that there are different pathogenic mechanisms of vascular injury in SRC and PHT. Notably, all patients with dcSSc and PHT (all of whom had concomitant interstitial disease) did show elevation of E-selectin, suggesting that inflammatory lung disease leads to activation of endothelial cells in the pulmonary microcirculation with increased expression and shedding of E-selectin.

Serum VCAM-1 levels were elevated in both lcSSc and dcSSc patients with SRC. They were also increased in patients with lcSSc without SRC or PHT, suggesting they may be a reflection of the chronic vascular injury in this subgroup of patients. Soluble ICAM-1 levels were raised in all groups with the highest levels being seen in dcSSc with PHT.

Analysis of serial measurements did not show consistent trends or progressive increase in endothelial markers over time. Likewise, in patients with SRC, endothelial markers were not consistently elevated at the time of SRC.

4. Conclusions

Mild chronic renal insufficiency in scleroderma is probably under recognized and may be a manifestation of vasculopathy. Recent studies suggest a strong association between renal involvement and outcomes in scleroderma, with a threefold increased risk of mortality from pulmonary hypertension if renal insufficiency is present. Current data suggests that manifestations of renal insufficiency including proteinuria and altered renal vascular dynamics as measured with Doppler ultrasound may help identify early signs of renal involvement, and this may be a surrogate marker of vasculopathy. Further studies are warranted to investigate renal markers as prognostic indicators and targets for disease modifying therapy in scleroderma.

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Review Article

Scleroderma Renal Crisis: A Pathology Perspective

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Scleroderma renal crisis (SRC) is an infrequent but serious complication of systemic sclerosis (SSc). It is associated with increased vascular permeability, activation of coagulation cascade, and renin secretion, which may lead to the acute renal failure typically associated with accelerated hypertension. The histologic picture of SRC is that of a thrombotic microangiopathy process with prominent small vessel involvement manifesting as myxoid intimal changes, thrombi, onion skin lesions, and/or fibrointimal sclerosis. Renal biopsies play an important role in confirming the clinical diagnosis, excluding overlapping/superimposed diseases that might lead to acute renal failure in SSc patients, helping to predict the clinical outcome and optimizing patient management. Kidney transplantation may be the only treatment option available for a subset of SRC patients who develop end-stage renal failure despite aggressive angiotensin-converting enzyme inhibitor therapy. However, the posttransplant outcome for SSc patients is currently suboptimal compared to the general renal transplant population.

1. Introduction

Systemic sclerosis (SSc) is a multisystem autoimmune disorder that can manifest as either the diffuse cutaneous (dc) or the limited cutaneous (lc) variant, distinguished by the degree and the extent of cutaneous sclerosis [1]. Scleroderma renal crisis (SRC) can complicate the course of up to 10% of patients with SSc. Although most frequently seen in dcSSc, SRC can occur in patients with lcSSc [2, 3] and rarely in patients with no significant dermal sclerosis termed systemic sclerosis sine scleroderma (ssSSc) [4]. The etiology of SCR remains incompletely understood, with most models of pathogenesis suggesting an initial trigger of vascular endothelial injury. Alteration in cellular and/or humoral immunity may also play a role in SRC pathogenesis [1, 5, 6]. SSc has been associated with T helper lymphocyte type-2 (TH-2) activation, cytokine production (particularly Il-4, IL-13, and IL-17), and excess collagen accumulation, which could participate in the development of vasculopathy [7]. B cell activation has also been described in SSc patients [7]. The association between the presence of several specific autoantibodies and the development of SRC raises a potential contributing role of autoantibodies in the pathogenesis of SRC [8, 9]. In addition, antiendothelial cell antibodies, which are capable of inducing endothelial cell apoptosis [10] have been detected in up to 85% of SSc patients [11]. Overexpression of endothelin-1, a protein that plays a role in blood vessel constriction, and its receptor endothelin-B has been demonstrated in the small vessels of two SRC patients [12]. Furthermore, the C4d complement degradation product product is regarded as an immunologic marker of antibody-mediated rejection in renal allografts, has been detected in native renal biopsies from a subset of SRC patients [13].

Subsequent to the potential endothelial triggering injury, the proposed cascade of histologic alterations is initiated by rapid increase in endothelial permeability and intimal edema. This then places the subendothelial connective tissue in direct contact with circulating blood elements activating the coagulation cascade and vascular thrombosis. The underlying connective tissue reacts to this insult by promoting fibroblastic and nonfibroblastic stromal proliferation, which manifests as proliferative endarteropathy (onion skin type lesion). Decreased renal perfusion as a result of arterial narrowing can additionally lead to juxtaglomerular apparatus (JGA) hyperplasia and renin secretion, resulting

in accelerated hypertension and progressive renal injury. A milder form of vascular pathology, manifested usually as fibrointimal thickening can often be observed in SSc patients without SRC [14].

Adequate renal biopsy specimens are generally capable of reflecting the aforementioned pathophysiologic changes. A detailed histologic assessment can confirm the clinical diagnosis and help exclude potential overlapping or superimposed etiologies.

2. Clinical and Laboratory Features

SRC is typically characterized by a sudden and marked increase in systemic blood pressure (although normotensive SRC has been described [15]), and acute renal failure, with or without significant microangiopathic hemolytic anemia or thrombocytopenia. SRC is often accompanied by headache, blurring of vision, and dyspnea. These symptoms can be attributed to hypertensive encephalopathy, congestive heart failure, and/or pulmonary edema, respectively, as consequences of the rapid increase in blood pressure [16, 17]. Since SRC can present as acute renal failure, one would expect a significant elevation of serum creatinine and a considerable fall of glomerular filtration rate (GFR). In a relatively large study, the median serum creatinine value in SRC patients at presentation was 200 mmol/l (2.3 mg/dl) [18]. In our study, the median serum creatinine value at the time of biopsy was 362 mmol/l (4.1 mg/dl) [13].

SRC more frequently affects females than males [1]. This may reflect the overall increased prevalence of SSc in the female population. Rapid progression of skin thickening in patients with SSc [19] and high doses of corticosteroid therapy [20] are risk factors for the development of SRC. The latter is usually associated with systemic steroid administration [20]. However, rare cases of SRC have been described following topical steroid use [21]. In patients with SRC, presenting normal or mildly elevated blood pressures (normotensive SRC), older age and male sex have been suggested to be adverse prognostic factors [18]. The poor prognosis in normotensive SRC patients might reflect ongoing subclinical renal injury leading to severe irreversible destruction of renal parenchyma due to delayed diagnosis.

Virtually all SRC patients have detectable antinuclear antibodies (ANA). Anti-RNA polymerase antibodies (especially types I and III), which are the most frequent autoantibodies encountered in North American dcSSc patients, are significantly associated with the development of SRC [8, 9, 22]. An association with antitopoisomerase I (anti-Scl 70) antibodies in dcSSc patients has also been reported. In contrast, anticentromere antibodies, which are commonly detected in lcSSc, are rarely encountered [19]. Anti-U3 RNP antibodies were found to have an association with SRC in some [23] but not all [24] studies.

Microangiopathic hemolytic anemia occurs in up to half of SRC patients [25] and is characterized by abrupt onset of anemia, the presence of schistocytes in the peripheral blood smear, and thrombocytopenia. Thrombotic microangiopathy is often accompanied by elevated serum LDH and decreased haptoglobin.

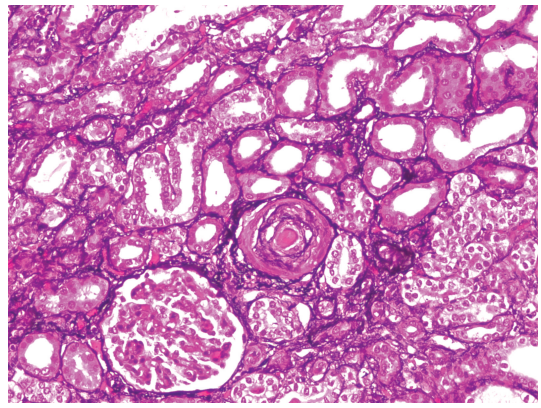


Figure 1: Arterial thrombosis associated with prominent glomerular ischemic collapse in a patient with scleroderma renal crisis (Methenamine silver stain; original magnification x100).

3. Gross Pathology

Multiple, small petechial hemorrhages are frequently present on the surface of the affected kidneys. The cut section may reveal tiny wedge shaped infarcts and foci of cortical necrosis [26]. These changes are nonspecific and can be observed in other thrombotic microangiopathic disorders, such as hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, and idiopathic malignant hypertension, or in association with some medications.

4. Microscopic Pathology

Renal biopsies, even though necessary to confirm the diagnosis, are not routinely warranted in SRC. Theoretically, unless the patient is suffering from typical clinical features and is associated with thrombotic microangiopathy picture on peripheral blood examination, the diagnosis cannot be confirmed with certainty without a renal biopsy. However, renal biopsy is an invasive procedure. Practically, such biopsies are recommended when doubt exists about the etiology of renal dysfunction, or, alternatively, to exclude the presence of other pathologic conditions.

The overall microscopic picture is that of a thrombotic microangiopathic process [26, 27]. Similar to idiopathic malignant hypertension, and in contrast to hemolytic uremic syndrome and thrombotic thrombocytopenic purpura, primary small vessel changes usually predominate over glomerular alterations in SRC. Small vessel thrombi outnumbered glomerular thrombi in SRC [11/17 (65%) versus 3/17 (18%), $P = .01$] [13], while the opposite was found in hemolytic uremic syndrome; thrombotic microangiopathy changes were more commonly detected in the glomeruli compared to small vessels [11/12 (92%) versus 4/12 (33%), $P = .009$] [28].

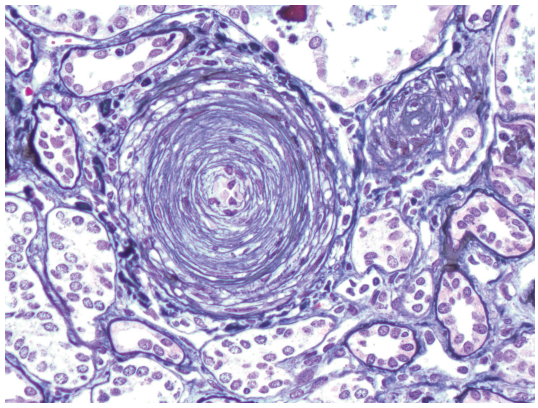


Figure 2: Prominent arterial onion skin lesion in a patient with scleroderma renal crisis. Such lesions often cause severe vascular narrowing leaving only a pinpoint open lumen (Methenamine silver stain; original magnification x400).

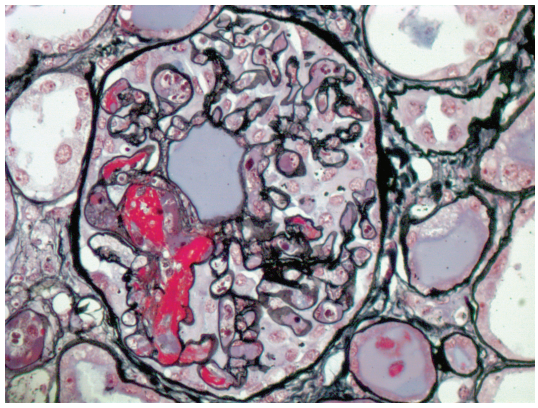


Figure 3: Glomerular capillary thrombosis in a patient with scleroderma renal crisis. This finding is rather infrequent in scleroderma renal crisis and is more commonly observed in hemolytic uremic anemia and thrombotic thrombocytopenic purpura (Methenamine silver stain; original magnification x600).

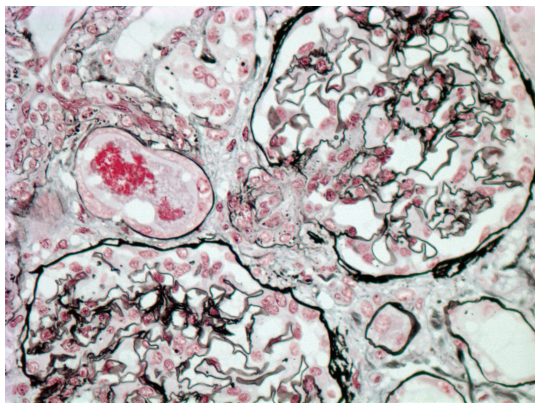


Figure 4: Prominent juxtaglomerular apparatus containing sparse silver positive renin granules in a patient with scleroderma renal crisis (Methenamine silver stain; original magnification x400).

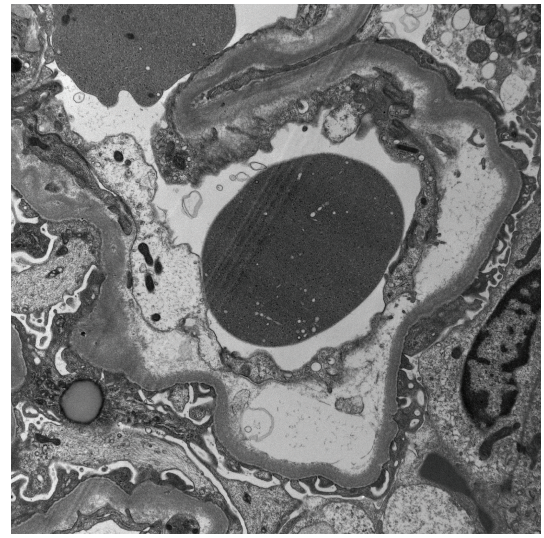


Figure 5: Electron microscopy from a patient with scleroderma renal crisis reveals detachment of the endothelium and prominent electron lucent fluffy material (Electron microscopy; original magnification x5600).

Histologic manifestations may vary during the course of the disease. Early vascular changes can manifest as intimal accumulation of myxoid material, thrombosis (Figure 1), and/or fibrinoid necrosis. Onion-skin lesions develop later (Figure 2), while fibrointimal sclerosis with or without adventitial fibrosis may be the only manifestation of chronic ongoing damage or organization resulting from previous episodes of acute injury. Acute glomerular changes can occur primarily or often develop secondary to the vascular injury and reduction in renal perfusion. Primary glomerular changes appear to be related to glomerular endothelial injury. These can manifest as endothelial swelling and glomerular capillary thrombosis (Figure 3). The latter is relatively infrequent [13]. Chronic glomerular changes, which include basement membrane double contours (tram tracking) and glomerulosclerosis, may develop later. Secondary glomerular changes may result in ischemic glomerular collapse. JGA hyperplasia, a histologic sequel of increased renin production can be observed microscopically (Figure 4). Prominent JGA hyperplasia was found to be present in 2/17 (12%) of our SRC cases [13]. Tubulointerstitial changes, which are also secondary to vascular pathology, are frequently manifested as ischemic acute tubular injury/necrosis or, if more chronic, as tubular atrophy and interstitial fibrosis. A lymphohistiocytic interstitial inflammatory infiltrate can occasionally be observed.

Finally, even though SRC represents an acute form of renal involvement, vascular pathology may be observed in SSc patients in the absence of SRC. Trostle et al. [14], in a case control autopsy study, compared the intimal surface areas of renal arteries in SSc autopsy cases (SRC, dcSSc without SRC, and lcSSc without SRC) to age- and sex-matched autopsy controls. Using morphometric techniques, these investigators confirmed that SRC patients had a significant

increase in renal arterial intimal thickening. Interestingly, they also found that, in the absence of SRC, a significant increase in arterial fibrointimal thickness was observed in dcSSc patients, and to a lesser extent in lcSSc patients, compared to controls. Such vascular changes may be due to the presence of mild ongoing renal vascular injury below the threshold which triggers SRC.

5. Ancillary Studies

Immunofluorescence and electron microscopy are routinely used ancillary studies for evaluating native renal biopsies. Immunofluorescence studies are mainly utilized to characterize the presence, nature, pattern of staining, and anatomic distribution of immune deposits. Electron microscopy is used for ultrastructural assessment of renal biopsies. It is extremely helpful in localizing and characterizing immune complex and protein deposits with organized substructures (amyloidosis, fibrillary etc.) and to assess the glomerular endothelium, basement membrane, and podocytes.

Routine ancillary studies are of limited value in confirming the diagnosis of SRC. There are only a few reports which have characterized the immunofluorescence findings in SRC. Immunoglobulin deposits in the glomeruli and/or blood vessels were identified in most but not all of these studies. Among the immunoglobulin deposits, IgM, which might be considered the result of a nonspecific entrapment, was the most frequently detected [29–31]. This was often accompanied by complement deposits.

In SRC, electron microscopic evaluation frequently fails to detect discrete electron dense deposits. Hyaline material often accumulates in the subendothelium of the glomeruli and/or blood vessels in SRC [29–32]. Of note, hyaline deposits can sometimes be difficult to distinguish from definite immune complex deposits. Evidence of endothelial injury such as endothelial swelling and prominent accumulation of glomerular subendothelial electron lucent material have been described in SRC (Figure 5) but also in malignant hypertension [33]. Myointimal cells were detected in the extended fibrointima in SRC patients [34].

C4d is a complement split product which is generated following complement activation via classical or mannose-bound lectin pathways. C4d can be detected using immunoperoxidase on formalin fixed tissue or immunofluorescence on frozen tissue. The former is technically easier to perform while the latter is considered slightly more sensitive and specific. We identified finely granular C4d staining in the peritubular capillary of a subset of SRC patients who had associated poor renal outcome [13]. In allograft kidneys, the detection of peritubular capillary C4d staining is usually associated with antibody-mediated rejection and poor allograft outcome [35–37]. Confocal immunofluorescence can potentially play an important role in localizing C4d as a target. Although a larger multicenter study using immunofluorescence technique is needed to validate our preliminary findings and to further characterize the cause of such deposits, evidence supporting the role of antibody-mediated injury in SSc/SRC patients is accumulating. First, disease-specific serum autoantibodies have been found to be associated with certain clinical manifestations [22]. Second, antiendothelial antibodies and increased expression of endothelin-1/endothelin-B have been detected in a subset of SRC patients [12]; it is noteworthy that overexpression of endothelin-1 gene has been recently discovered in allograft kidneys with antibody-mediated rejection [38]. Lastly, antiglobulin antibodies have been found in the eluate of several SRC kidney samples [30].

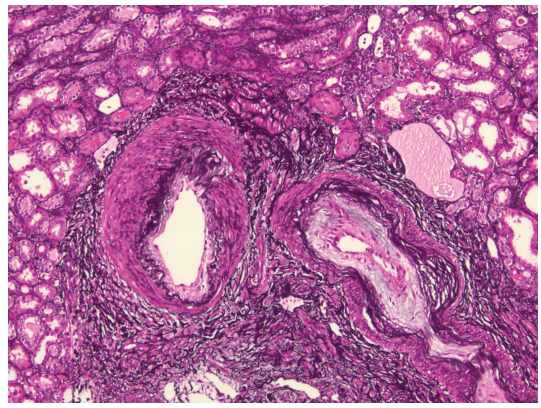
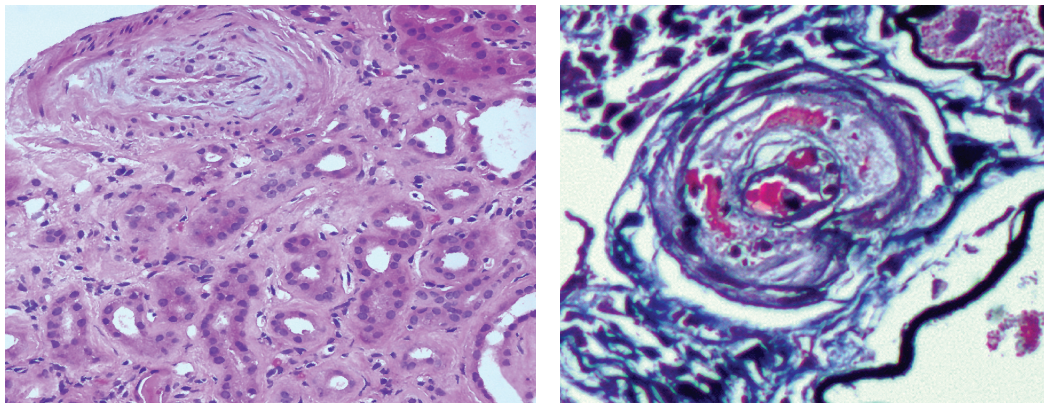


Figure 6: Prominent arterial adventitial fibrosis in a patient with scleroderma renal crisis. Note that the arteries also have mild intimal accumulation of myxoid material. (Methenamine silver stain; original magnification x100).

6. Differential Diagnosis

Clinically, SRC should be suspected when acute renal failure (ARF) develops in SSc patients. Nevertheless, ARF occurring in SSc patients is not always due to SRC. Renal artery stenosis, hypovolemia, crescentic glomerulonephritis (GN), and other renal diseases may also occur in SSc patients [27]. These disorders may result in a similar clinical picture. A thrombotic microangiopathy-like clinical picture can even be encountered in patients with renal arterial stenosis [27]. Distinguish SRC from crescentic GN is critical since immunosuppressive therapy is used to treat the latter. As the name implies, the presence of crescents is the hallmark of crescentic GN. In typical SRC cases, crescents are extremely rare and, when detected, are very small [39]. In SSc patients, most of the encountered crescentic GN are ANCA-associated. These are pauci-immune on immunofluorescence studies, associated with anti-myeloperoxidase antibodies, and usually triggered by penicillamine [40, 41]. Less often, one may encounter immune complex GN [39] or antiglomerular basement membrane GN [42]. Immunofluorescence studies reveal granular glomerular basement membrane and/or mesangial immune complex deposits in the former and linear glomerular basement membrane IgG staining in the latter.

Histologically, thrombotic microangiopathic changes can be observed in several disorders. Although it is often impossible to ascertain the specific cause of thrombotic microangiopathy based on histologic evaluation alone, it



(a) (b)

Figure 7: Two renal allograft biopsies with histologic features suspicious for recurrence of scleroderma. Note the prominent myxoid changes in the artery in biopsy (a) as well as the severe intimal thickening of blood vessels, which is accompanied by thrombosis and schistocytes within the arteriole wall in biopsy (b). The differential diagnosis includes acute antibody-mediated rejection and acute calcineurin inhibitor toxicity. Clinical correlation with the presence of C4d stain, detection of circulating donor-specific antibodies, and calcineurin inhibitor levels are usually warranted to achieve a correct diagnosis [(a) H&E; original magnification x200 and (b) Methenamine silver stain; original magnification x400].

is important to note that extraglomerular small vessel vascular lesions often predominate in SRC while primary glomerular capillary microangiopathic changes (glomerular capillary thrombosis) are a relatively infrequent histologic finding in SRC [13]. The presence of JGA hyperplasia has been described in SRC patients [43, 44]. Similarly, vascular adventitial fibrosis (Figure 6) [45] has been regarded by some investigators to be characteristic for SSc [46] and SRC [47]. Clinicopathological correlation, however, is often required to achieve the correct diagnosis. In advanced stages of SRC, the histologic findings are usually nonspecific and reflect advanced chronic renal damage. At such late stages, it is often difficult to distinguish chronic vascular changes associated with organized SRC from preexisting chronic accelerated essential hypertension. The presence of peri-adventitial fibrosis, if prominent, might be helpful as a point in favor of SRC.

7. Prognosis

Several retrospective studies have investigated the role of renal biopsy in predicting prognosis in SRC. Penn et al. showed that the presence of acute vascular changes (myxoid intimal thickening and thrombosis) were associated with poor prognosis [18]. We showed that the severity and extent of acute vascular injury, including fibrinoid changes and/or thrombosis, was most predictive of poor outcome [13]. We also showed that severe glomerular ischemic collapse, and to a lesser extent acute tubular necrosis, may also be associated with poor prognosis [13]. Both of the latter are secondary changes reflecting the severity of vascular lesions. In contrast to acute changes, we observed that chronic renal changes did not significantly correlate with poor outcome [13]. A recently published abstract suggested that chronic pathological changes might be associated with a favorable prognosis [48]. The latter observation is difficult to explain since chronic changes are typically irreversible and are expected, if any, to have an adverse impact on renal survival, as was described in other kidney diseases such as lupus nephritis [49].

8. Treatment/Outcome

Blood pressure should be vigorously and aggressively controlled in patients with established SRC. The mortality associated with SRC has significantly decreased due to early diagnosis and aggressive angiotensin-converting enzyme (ACE) inhibitor therapy [50, 51]. Still, a subset of SRC patients may be refractory to ACE-inhibitor and other hypertensive therapy. These patients often remain on dialysis or die [17, 51, 52]. Kidney transplantation should be considered if the condition does not reverse despite aggressive treatment (usually within two years) [53].

9. Posttransplant Outcome

Although renal transplantation offers superior survival in SRC patients, graft survival is frequently reduced in SSc-induced renal failure compared to the general renal transplant population [54, 55]. We retrospectively studied the posttransplantation course of 10 SRC patients [56]. One, three, and five year graft survivals in this SRC cohort of patients were 70%, 70%, and 25%, respectively, compared with approximately 90%, 79%, and 75% graft survival in miscellaneous patients who received kidney transplants from deceased donors at the same institution [57]. Recurrence of scleroderma (Figure 7) may play a role in this poor post-renal transplant outcome [58, 59]. Two of our 10 patients had histologic features suspicious for SRC recurrence, manifested by both exacerbated development of arterial fibrointimal

thickening with thrombotic microangiopathy-like changes [56]. Pham et al. [59] found that recurrent SRC occurs early in the course of transplantation (within 2 years post-transplantation). However, Cheung et al. [58] challenged the conventional experience by reporting a recurrence of SRC which occurred seven years post-transplantation. This SRC occurred following switching therapy from an ACE inhibitor to an angiotensin II receptor blocker.

Establishing a histologic diagnosis of recurrent scleroderma/SRC in an allograft is more challenging than diagnosing SRC in a native kidney biopsy. In addition to recurrent SRC, the pathologic differential diagnosis in allograft biopsies with a thrombotic microangiopathy-like picture also includes antibody-mediated rejection, calcineurin inhibitor toxicity, infection, and other less common allograft-related abnormalities [60]. More chronic changes can also be difficult to distinguish from de novo transplant glomerulopathy.

In summary, SRC is a severe complication of systemic sclerosis. Although not always clinically warranted, renal biopsy can play an important role in establishing the diagnosis and in excluding other pathologic conditions such as vasculitis and connective tissue disease related and nonrelated syndromes. Furthermore, renal biopsies can help to predict renal prognosis and may contribute to our better understanding of the mechanisms and pathologic manifestations of SRC, ultimately leading to optimization of treatment strategies.

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Research Article

An International, Web-Based, Prospective Cohort Study to Determine Whether the Use of ACE Inhibitors prior to the Onset of Scleroderma Renal Crisis Is Associated with Worse Outcomes—Methodology and Preliminary Results

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Background. To describe the methodology of a study designed to determine whether systemic sclerosis (SSc) patients with incident scleroderma renal crisis (SRC) on angiotensin converting enzyme (ACE) inhibitors prior to the onset of SRC have worse outcomes. *Methods*. Prospective, international cohort study of SRC subjects identified through an ongoing web-based survey. Every second Friday afternoon, an e-mail was sent to 589 participating physicians to identify new cases of SRC. Death or dialysis at one year after the onset of SRC will be compared in patients exposed or not to ACE inhibitors prior to the onset of SRC. *Results*. Fifteen months after the start of the survey, we had identified 76 incident cases of SRC. Of these, 66 (87%) had a hypertensive SRC and 10 (13%) a normotensive SRC. Twenty-two percent (22%) of the patients were on an ACE inhibitor immediately prior to the onset of the SRC. To date, we have collected one-year follow-up data on approximately 1/3 of the cohort. Of these, over 50% have died or remain on dialysis at one year. *Conclusion*. An international, web-based cohort study design is a feasible method of recruiting a substantial number of patients to study an infrequent vascular manifestation of SSc.

1. Introduction

Scleroderma renal crisis is an infrequent but life-threatening complication of systemic sclerosis (SSc) [1]. It was previously associated with significant morbidity, including chronic renal failure and dialysis, and high mortality. However, since the advent of angiotensin converting enzyme (ACE) inhibitors, the outcome of SRC has improved dramatically [2]. There is also a perception among experts that the incidence of SRC has fallen over the past years. This is thought to be due in part to the more liberal use of ACE inhibitors to treat Raynaud's phenomenon and hypertension in SSc [3].

Given the benefits of ACE inhibitors in SRC and the perceived decrease in incidence in SRC, some experts have advocated the use of prophylactic ACE inhibitors even in the absence of Raynaud's or hypertension [3]. However, others have argued that there is no clear rationale for this since it has been demonstrated that most SSc patients do not have hyper-reninemia prior to the onset of SRC [4]. In addition, recent retrospective data in patients with SRC suggested that ACE inhibitors prior to the onset of SRC may have worse outcomes than those not taking these drugs [5–7]. This has been hypothesized to be due to the fact that those on ACE inhibitors may have normotensive SRC and diagnosis may thus be delayed in these patients.

Given the belief that the incidence of SRC seems to have fallen over the past years due to the increasing use of ACE inhibitors, some experts have proposed undertaking a large, simple randomized trial to confirm this finding [8]. However, concerns based on the preliminary data that suggests that patients taking ACE inhibitors who develop

Hypertensive SRC:
Systolic blood pressure >140 mmHg
Diastolic blood pressure >90 mmHg
Rise in systolic blood pressure >30 mmHg compared to baseline
Rise in diastolic blood pressure >20 mmHg compared to baseline
AND
One of the following features:
(a) Increase in serum creatinine >50% over baseline OR serum creatinine >120% of upper limit of normal for local laboratory,
(b) Proteinuria: >2+ by dipstick and confirmed by protein:creatinine ratio >upper limits of normal (ULN),
(c) Hematuria: >2+ by dipstick or >10 RBCs/HPF (without menstruation),
(d) Thrombocytopenia: ≤100,000 plts/mm^3,
(e) Hemolysis: by blood smear or increased reticulocyte count,
(f) Hypertensive encephalopathy.
Normotensive SRC:
Increase in serum creatinine >50% over baseline OR serum creatinine >120% of upper limit of normal for local laboratory
AND
One of the following features:
(a) Proteinuria: >2+ by dipstick and confirmed by protein:creatinine ratio > upper limits of normal (ULN),
(b) Hematuria: >2+ by dipstick or >10 RBCs/HPF (without menstruation),
(c) Thrombocytopenia: ≤100,000 plts/mm^3,
(d) Hemolysis: by blood smear or increased reticulocyte count,
(e) Hypertensive encephalopathy.

Box 1: Proposed characteristics of SRC.

SRC may have worse outcomes remain. Thus, prior to undertaking a large, randomized trial, we believed that there was a real need to obtain additional data to assess whether in fact the use of ACE inhibitors prior to the onset of SRC was associated with worse outcomes.

We therefore undertook a study to determine whether SSc patients with incident SRC on ACE inhibitors immediately prior to the onset of SRC have worse outcomes, defined as dialysis dependence or death after one year than those not on these drugs prior to the onset of SRC. The purpose of this paper is to describe the methodology and preliminary data of this study. In particular, we wish to highlight the advantages and disadvantages associated with using survey methodology via the internet to study uncommon vascular manifestations of SSc.

2. Methods

2.1. Design. Prospective, international cohort study of subjects identified through an ongoing web-based survey.

2.2. Study Subjects. In September 2008, we compiled an e-mail list of physicians with an interest in SSc from the Canadian Scleroderma Research Group, the Scleroderma Clinical Trials Consortium, the EULAR Scleroderma Trials and Research (EUSTAR) group, and other international collaborators, in particular from Colombia, Mexico, and Australia. They were contacted and invited to participate in the web-based survey. Thereafter, 589 participating physicians were sent an e-mail every second Friday afternoon asking them simply: "Have you diagnosed a case of SRC in the past two weeks". They were asked to check a yes/no box. If the answer was no, and in most cases it was, then that was all that was required of them for that period. If the answer was yes, they were then asked to answer a simple, short survey about their case requiring about 5 minutes to complete. The survey was developed and conducted using SurveyMonkey, a simple, inexpensive, web-based survey tool. We initially intended to collect cases over a 52-week period, but since we were still identifying new cases at the end of that period, we chose to continue the survey beyond that point.

2.3. Definition of SRC. For the purposes of the study, a patient was diagnosed with SRC if he/she was *diagnosed with SRC by the recruiting physician*. We nevertheless collected data on the signs and symptoms that the physicians relied on to make their diagnosis (Box 1).

2.4. Covariates. The survey allowed us to collect data on the following variables:

(1) patient demographics (age, sex, race/ethnicity),
(2) disease characteristics (limited versus diffuse, disease duration, autoantibodies),
(3) blood pressure and renal function prior to the onset of the SRC,
(4) current use of ACE inhibitor or ARB immediately prior to SRC onset, and if so, reasons for such use (Raynaud's, hypertension, prophylaxis because of concurrent corticosteroid use, simple prophylaxis); name of drug, current dose,
(5) concomitant medications, including glucocorticoids, cyclosporine and nonsteroidal anti-inflammatories,
(6) signs and symptoms used to diagnose SRC.

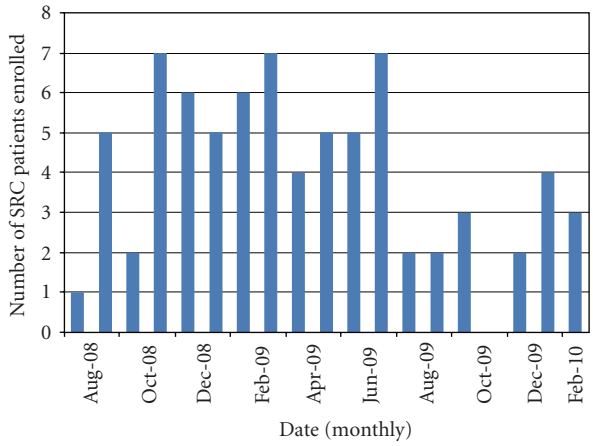

Figure 1: Rate of recruitment of study subjects.

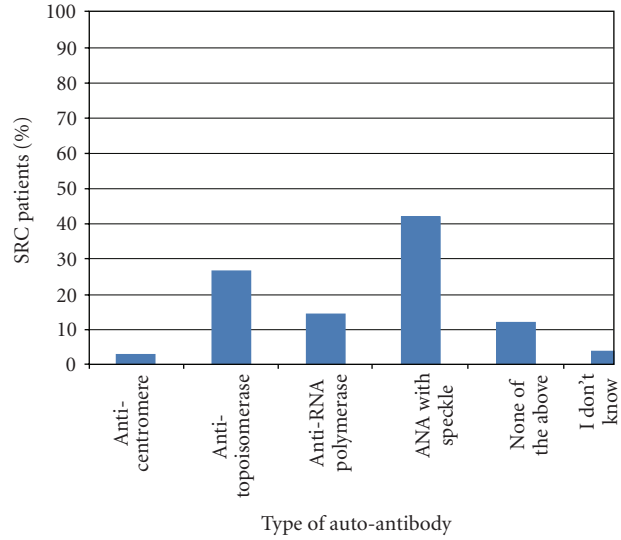

Figure 2: Autoantibodies.

2.5. Outcomes. The primary outcomes of interest were defined as death or dialysis one year after the onset of SRC. Secondary outcomes included renal function after one year. One year after a patient was identified, a simple follow-up case report form is sent to the recruiting physician. At study completion, the rates of dialysis or death after one year in SSc patients with SRC exposed to ACE inhibitors at the time when they developed SRC will be compared to the rates in those not on ACE inhibitors at the time they developed their SRC.

2.6. Sample Size Considerations. The main objective of this study was to determine whether there was harm associated with using ACE inhibitors prior to the onset of SRC. In computing an estimated sample size, we made the following assumptions: (1) estimated prevalence of ACE inhibitor exposure prior to the onset of SRC approximately 25% (ratio of exposed to non-exposed 1 : 3); (2) prevalence of death or dialysis after one year in the nonexposed of approximately 50% [2]; (3) risk of death or dialysis associated with exposure approximately twofold [5], and (4) loss to follow-up of about 10%. We thus calculated that a total sample of approximately 60 subjects would be needed to have 80% power to detect the estimated increased risk in poor outcomes in those exposed compared to those unexposed to ACE inhibitors.

2.7. Ethical Considerations. Central research ethics approval was obtained for this study from the ethics review board of the Jewish General Hospital, Montreal, Canada. Some recruiting physicians also sought local ethics approval prior to enrolling patients into the survey.

3. Results

As of February 2010, fifteen months after the start of the survey, we had identified 76 incident cases of SRC (Figure 1). Mean age of the cohort was 53 (±12 years), 68% were women, 72% were White, 68% had diffuse SSc, and median disease duration since the onset of the first non-Raynaud's symptom was 1.5 years (Table 1). Approximately half of the cases were from Canada and the United States, and the other half from elsewhere around the world. Fifteen percent (15%) of patients were positive for an RNA polymerase autoantibody (although not all centers tested for this antibody) and 42% for a speckled antinuclear antibody (Figure 2).

Of the 76 patients, 66 (87%) had a hypertensive SRC and 10 (13%) a normotensive SRC according to the recruiting physician. Twenty-two percent (22%) of the patients were on an ACE inhibitor and 5% on an ARB immediately prior to the onset of the SRC (Table 2). Of these, 16 of the 66 (24%) with hypertensive crisis and 5 of the 10 (50%) with normotensive crisis were on an ACE inhibitor or an ARB prior to the onset of SRC. Over 50% of the patients were also on glucocorticoids immediately prior to the onset of SRC, at a mean dose of 17 mg/d of prednisone (or its equivalents).

Of the 66 patients classified as hypertensive, 64 satisfied the proposed criteria for hypertensive SRC mentioned in Box 1. Of the 10 patients classified as normotensive, 4 satisfied the proposed criteria for normotensive SRC mentioned in Box 1.

To date, we have collected one-year follow-up data on approximately 1/3 of the cohort. Of these, over 50% have died or remained on dialysis after one year. Collection of one-year follow-up data on the remainder of the patients is ongoing.

4. Discussion

Whether ACE inhibitors are associated with a worse prognosis for patients with SRC is an important clinical question, in particular given the widespread availability of these drugs and their perceived benefits in reducing the incidence of SRC. However, given the rarity of SRC, designing a prospective study to address this question is not without considerable logistical problems. Using an international, web-based cohort study design, we identified 76 incident SRC cases over

Table 1: Baseline characteristics of cohort ($N = 76$).

Mean (SD) age, years (SD)	53.3 (12.4)
Women, *N* (%)	52 (68.4)
Ethnic groups, *N* (%)	
White	55 (72.4)
Black	12 (15.8)
Asian	4 (5.3)
Hispanic	3 (4.0)
Native American	2 (2.6)
Disease subsets, *N* (%)	
Diffuse disease	52 (68.4)
Limited disease	19 (25.0)
Sine scleroderma	5 (6.6)
Disease duration (since first non-Raynaud's symptom)	
Median disease duration, years (IQR)	1.47 (0.87, 4.21)
Number (%) with disease duration < 1 year	24 (32)
Countries of origin of study subjects, *N*	
Australia	2
Belgium	1
Brazil	1
Canada	13
Denmark	2
Dominican Republic	1
France	2
Germany	2
Ghana	1
Greece	1
Haiti	2
Hungary	3
Israel	1
Italy	2
Korea	1
Norway	3
Pakistan	3
Poland	1
Spain	2
Switzerland	2
The Netherlands	1
Turkey	3
USA	26

Table 2: Characteristics of study patients ($N = 76$).

	N (%)
Hypertensive SRC	66 (86.8)
Normotensive SRC	10 (13.2)
ACE inhibitor immediately prior to SRC onset	17 (22.4)
ARB immediately prior to SRC onset	4 (5.3)
Glucocorticoids immediately prior to SRC onset	39 (51.3)
Mean prednisone dose in prednisone equivalents	16.7 mg/day
Nonsteroidal anti-inflammatory drugs immediately prior to SRC onset	9 (11.8)
Cyclosporine immediately prior to SRC onset	1 (1.3)

SRC: scleroderma renal crisis, ACE: angiotensin converting enzyme, and ARB: angiotensin receptor blocker.

approximately 15 months. We thus believe that this is a feasible method of recruiting a substantial number of patients to study this infrequent vascular manifestation of SSc.

We had made several assumptions to compute our desired sample size, including that approximately 25% of subjects would be exposed to ACE inhibitors and that the rate of death and/or dialysis after one year would approach 50%. The numbers presented in this preliminary analysis support these assumptions. Thus, after approximately one more year of follow-up, we should have sufficient power to address the important clinical question of the prognosis of patients who develop SRC while on ACE inhibitors compared to those not on these medications.

In order to maximize enrollment for this study, we compiled an extensive list of 589 physicians from around the world with an apparent interest in SSc (i.e., identified from well-established SSc research groups and through international SSc networks), we designed a simple survey requiring less than 5 minutes to complete and we sent out the survey every 2 weeks so as to increase the possibility that the recruiting physician would have easy access to the clinical data. We were especially careful in including incident cases. Indeed, many physicians contacted us to enquire whether they could enroll patients who had had their SRC in the past and were being seen in follow-up. Unfortunately, those patients were not eligible because, having survived their SRC sufficiently long, these prevalent cases were in fact "survivors" and including them could have biased our results.

We encountered several problems in the course of the study. The most important one was that there was no gold standard to define SRC. Given that the recruiting physicians were identified through SSc research groups and had an apparent interest in SSc, we chose to rely on their "expert" opinion. Moreover, we also collected data on the signs and symptoms that they relied on to make their diagnosis. In this preliminary analysis, most patients satisfied the proposed criteria for hypertensive SRC (Box 1). However, additional work will be needed to validate a more sensitive definition of normotensive SRC.

Another issue that arose was that of ethics approval. The study had been approved by the principal investigators' ethics committee, the data collected for the purposes of this study were obtained through chart review by a treating physician, no direct patient contact was required, and patients were identified using depersonalized study codes. Nevertheless, some recruiting physicians preferred to obtain ethics approval from their local ethics committees. Unfortunately, this imposed a certain workload on them and we did not have funds to support them in this regard. Although this

Table 3: Signs and symptoms of SRC.

Patients with hypertensive SRC ($N = 66$)	N (%)
Systolic blood pressure >140 mmHg	64 (97)
Diastolic blood pressure > 90 mmHg	54 (82)
Rise in systolic blood pressure >30 mmHg compared to baseline	46 (70)
Rise in diastolic blood pressure >20 mmHg compared to baseline	37 (56)
Increase in serum creatinine >50 % above baseline OR serum creatinine > 120% of upper limit of normal for local laboratory	60 (91)
Proteinuria: >2+ by dipstick and confirmed by protein:creatinine ratio > upper limits of normal	25 (38)
Hematuria: >2+ by dipstick or >10 RBCs/HPF (without menstruation)	18 (27)
Thrombocytopenia: < 100,000 platelets/mm^3	20 (30)
Hemolysis: by blood smear or increased reticulocyte count	27 (41)
Hypertensive encephalopathy	9 (14)
Patients with normotensive SRC ($N = 10$)	N (%)
Systolic blood pressure >140 mmHg	2 (20)
Diastolic blood pressure >90 mmHg	2 (20)
Rise in systolic blood pressure >30 mmHg compared to baseline	3 (30)
Rise in diastolic blood pressure >20 mmHg compared to baseline	2 (20)
Increase in serum creatinine >50% above baseline OR serum creatinine > 120% of upper limit of normal for local laboratory	9 (90)
Proteinuria: >2+ by dipstick and confirmed by protein:creatinine ratio > upper limits of normal	5 (50)
Hematuria: >2+ by dipstick or >10 RBCs/HPF (without menstruation)	4 (40)
Thrombocytopenia: <100,000 platelets/mm^3	2 (20)
Hemolysis: by blood smear or increased reticulocyte count	3 (30)
Hypertensive encephalopathy	1 (10)

may have delayed initial recruitment in those centers, many participants nonetheless pursued ethics approval presumably based on enthusiasm for the project.

Thirdly, although the tool that we used to create the survey was very user-friendly, inexpensive, and allowed us to integrate important data quality checks, it allows only basic data analysis. More detailed analysis will require time-consuming data manipulation to transfer the data into more sophisticated programs. Alternative data acquisition formats are available and could be considered for future studies involving more complicated analyses.

Finally, we have had to invest a lot of effort in obtaining one-year follow-up data. The follow-up case report form is somewhat longer and requires approximately twenty minutes to complete. A central research assistant has had to work diligently to encourage recruiting physicians to complete these forms. Contacting them personally by telephone has resulted in improved follow-up data collection. We did not have funds to pay for local research assistants to fill the follow-up forms. Whether this could also have contributed to more efficient collection of follow-up data thus remains unknown.

Our study will be unable to answer another very important question; that is, whether ACE inhibitors are associated with a reduction in the incidence of SRC. That study would require following patients with mostly early SSc, some exposed and others unexposed to ACE inhibitors, until the occurrence of SRC. Since SRC is infrequent, the sample size for such a cohort study exceeds 1000. Nevertheless, that study using our current design could be feasible. Recruitment would most likely have to occur over several years and strategies to maintain interest in recruitment would have to be developed. Careful collection of follow up data would also be necessary. On the other hand, the costs of maintaining an ongoing web-based survey are really quite minimal.

This study has some limitations. First, our response rate remains largely uncertain. When we sent the biweekly e-mails, we asked the participants to answer whether or not they had seen a case of SRC in the past two weeks, and if so, go on to fill out the survey. Unfortunately, many participants did not respond to the biweekly e-mails. Thus, it is difficult to know whether they indeed had not seen a case or whether they were not participating (during that particular time period). It is possible that some cases were seen but not entered into the survey, and it is conceivable that their disease characteristics may have been different from those of the cases included in the survey (e.g., some may have had worse and others milder disease). Thus, the response rate and the effect of a nonresponse bias in this study are uncertain. Second, patients who did not have access to a participating physician or those with subclinical disease (e.g., normotensive SRC) whose SRC may have been overlooked by a physician were not captured in this survey. Thus, our results are generalizable to patients diagnosed with SRC and entered into this survey by a participating physician. On the other hand, every two weeks we contacted well over 550 participants identified as members of well-established scleroderma research groups or colleagues of such groups

from around the world. SRC is a serious complication of SSc and we thus believe that many if not most SRC cases were, at some point, brought to the attention of one of these perceived SSc experts.

In conclusion, using an international, web-based prospective cohort design, we identified 76 incident of SRC cases over approximately 15 months. Twenty-two percent (22%) of them were on an ACE inhibitor immediately prior to the onset of their SRC. Follow-up data collection to determine rates of death and/or dialysis after one year according to exposure to ACE inhibitor prior to SRC onset is ongoing. The methodology used for this study is innovative and emphasizes that interinstitutional and international collaboration can contribute significantly to the study of infrequent vascular manifestations of SSc. The ultimate success of this study will depend largely on the goodwill of the recruiting physicians who will have to invest additional time and effort in collecting and providing us with the most complete follow-up data possible. Their dedication will hopefully allow us to answer one of the most pressing ongoing questions related to SRC in the near future.

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Review Article

Vascular Complications of Systemic Sclerosis during Pregnancy

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Systemic sclerosis (SSc) is a chronic autoimmune disorder characterized by progressive fibrosis of the skin and visceral tissues as well as a noninflammatory vasculopathy. Vascular disease in systemic sclerosis is a major cause of morbidity and mortality among nonpregnant patients with SSc and is even a bigger concern in the pregnant SSc patient, as the underlying vasculopathy may prevent the required hemodynamic changes necessary to support a growing pregnancy. Vascular manifestations including scleroderma renal crisis and pulmonary arterial hypertension should be considered relative contraindications against pregnancy due to the high associations of both maternal and fetal morbidity and mortality. In contrast, Raynaud's phenomenon may actually improve somewhat during pregnancy. Women with SSc who are considering a pregnancy or discover they are pregnant require evaluation for the presence and extent of underlying vasculopathy. In the absence of significant visceral vasculopathy, most women with SSc can expect to have reasonable pregnancy outcomes.

1. Introduction

Systemic sclerosis (SSc) is a chronic autoimmune disorder characterized by progressive fibrosis of the skin and visceral tissues as well as a non-inflammatory vasculopathy manifesting as Raynaud's phenomenon, digital ulcerations, pulmonary arterial hypertension, and scleroderma renal crisis. Vascular disease in systemic sclerosis is a major cause of morbidity and mortality among non-pregnant patients with SSc. There are two main subsets of SSc: diffuse cutaneous disease is characterized by cutaneous fibrosis proximal to the forearms and is associated with higher incidence of scleroderma renal crisis and pulmonary fibrosis; limited cutaneous disease has more limited cutaneous involvement to the distal extremities and face and is associated with higher prevalence of digital ulcers and pulmonary arterial hypertension.

Akin to many autoimmune diseases, SSc has a strong female predominance, with an approximate female- to male-ratio of 5 : 1 [1, 2]. SSc is a relatively rare disease, with a prevalence in North America estimated to be approximately 49,000–55,000, between 25 and 44 per 100,000 persons [1, 2]. Symptom onset of SSc usually begins in the 5th decade, approximately 10 years prior to the mean age of menopause. In more recent decades, many women have postponed childbearing into their 30 s and 40 s for career and other personal reasons. For this reason, the number of women who develop SSc and have not yet completed their families and may be considering pregnancy is likely to increase. When counseling women with SSc who are considering pregnancy or who discover a pregnancy, it is critical to understand the potential vascular complications that may lead to adverse pregnancy outcomes.

2. Normal Hemodynamic and Vascular Changes of Pregnancy

In order to accommodate the enlarging uterus and growing fetus, a pregnant woman's body must make numerous physiologic hemodynamic changes. Many of these changes involve changes in blood volume, vascular resistance, cardiac output, and oxygen consumption. Most physiologic changes begin early in gestation and peak by the late second trimester of pregnancy. Although not definitive, it has been suggested that an initial fall in systemic vascular resistance leads to the other well-established vascular and hemodynamic changes

of pregnancies [3]. Activation of the renin-angiotensin-aldosterone system results in a 7-8 liter increase in total body fluid during gestation (distributed between maternal intra- and extracellular spaces, fetus, and amniotic fluid) [4]. Cardiac output increases by 50% over the course of pregnancy, with the majority of increase occurring within the first 8 weeks, followed by a gradual increase until plateau mid-third trimester [5]. This is mediated by an increase in both stroke volume early in pregnancy and heart rate throughout pregnancy. During pregnancy, cardiac output is preferentially distributed to the uterus, kidneys, skin, and breast tissue and away from skeletal muscle [6]. The pulmonary system additionally undergoes significant physiologic changes during pregnancy. Pulmonary vascular resistance decreases. Basal oxygen consumption increases by 50 mL/min by term, and alveolar ventilation increases, lowering PCO2 by about 8 torr.

The exact mechanisms underlying the hemodynamic changes during pregnancy remain to be completely understood. An increase in prostacyclin in both the fetoplacental tissues and maternal system plays a clear role in systemic vasodilation; however, additional mediators are required to explain the degree of reduction in vascular resistance [3]. Nitric oxide (NO) is likely candidate to play a significant role in the hemodynamic accommodations during pregnancy. There appears to be a permanent basal activity of NO in utero- and fetoplacental vasculature in pregnant women; the trophoblast may be a significant source of NO production [3]. NO donors administered to women between 8 and 10 weeks gestation resulted in a further fall of peripheral resistance [7]. Other studies have demonstrated a deficiency of NO in pregnancy complications associated with defective placental perfusion: preeclampsia and intrauterine growth restriction [3]. In addition to the important roles of prostacyclin and NO in the maintenance of low peripheral vascular resistance, the maternal vasculature appears to be resistant to the effects of angiotensin II.

Physiologic hemodynamic changes are an integral part of human pregnancy. In some cases, changes in vascular blood flow and decreased vascular resistance may lead to improvement in manifestations of systemic sclerosis. In other cases, an intrinsic inability to adapt to the necessary hemodynamic changes in pregnancy, possibly due to underlying vasculopathy of systemic sclerosis, may contribute to adverse pregnancy outcomes including hypertensive disorders of pregnancy and intrauterine growth restriction.

3. Maternal and Fetal Outcomes of the SSc Pregnancy

Given the prominent vasculopathy associated with SSc, there is concern for an increased risk of adverse pregnancy outcomes associated with vascular compromise or inability to make appropriate hemodynamic adaptations to support a pregnancy. Indeed, early reports suggested very high rates of maternal death during pregnancy [8–11]. It is likely for this reason that women with SSc in past decades had been strongly advised against pregnancy and often counseled to terminate pregnancies that have occurred [12]. More recently, a series of retrospective and prospective studies have provided more detailed analysis of pregnancy outcomes and have demonstrated that, for most women with SSc, pregnancy outcomes are reasonably good [13–18]. Reported rates of early pregnancy loss of 14%–15% [13, 14] are somewhat increased from the estimated 10% in the general population. Late pregnancy losses were few, and generally occurred in women with severe diffuse SSc [14, 16]. Preterm delivery rates have ranged from 8%to40% [13, 14, 16]. The majority of preterm deliveries were on or after gestational age 34 [14]. Small for gestational age infants (<10th% tile for gestational age) [19], ranged from 0% to 50% [2, 14, 16]. Cases of preeclampsia were isolated [14, 16]. It must be remembered, however, that these studies reflect the experience of approximately 200 patients followed at tertiary-care centers over a period of 10 years. A population-based study of pregnancy outcomes among women with SSc in the United States that utilized administrative hospital discharge databases identified 504 SSc women who delivered between 2004 and 2006 [18]. This study found a 22.9% rate of hypertensive disorders including preeclampsia, a four-fold increased odds compared to the general population (85% CI, 2.4–6.6). Similarly, a nearly four-fold increased rate of intrauterine growth restriction was found. Overall, the majority of patients with SSc appear to have reasonable obstetric outcomes although women with rapidly progressive diffuse disease may be at higher risk for complications.

4. Scleroderma Renal Crisis

Scleroderma renal crisis, characterized by malignant hypertension, proteinuria, acute renal failure, microangiopathic changes, and the pathognomonic "onion skin" appearance of renal arteries on pathology, is one of the most severe complications of SSc [20]. Affecting 5%–10% of SSc patients, renal crisis characteristically occurs in patients with rapidly progressing diffuse skin disease of relatively recent onset. Prior to the widespread use of angiotensin-converting enzyme (ACE) inhibitors, renal crisis was one of the leading causes of mortality. Many of the perinatal deaths reported among SSc patients involved scleroderma renal crisis [7–10]. Even in more recent series, episodes of renal crisis during pregnancy were reported. Steen et al. reported two cases of scleroderma renal crisis in a retrospective study of 86 pregnancies occurring after the diagnosis of SSc [12]. Both cases occurred abruptly in the third trimester of pregnancy and resulted in preterm delivery. One woman developed end- stage renal disease, and the other died from status epilepticus. In a prospective study of 91 pregnancies, Steen reported two additional cases of renal crisis [13]. Both women required hemodialysis after delivery. All of these cases occurred in women with early, rapidly progressive diffuse disease. It remains unclear if rates of renal crisis are increased in pregnant women compared to nonpregnant women with severe diffuse disease. The severity of scleroderma renal crisis during pregnancy with very high risks of maternal and fetal morbidity and mortality and the benefits of treatment with ACE inhibitors to both mother and fetus if renal

crisis is suspected are highly likely to outweigh the risks of fetotoxicity associated with use [14, 21, 22].

Unfortunately, scleroderma renal crisis can be difficult to distinguish from preeclampsia (abrupt onset hypertension and proteinuria) in the pregnant SSc patient; both conditions carry a high risk of severe maternal and fetal complications if not treated aggressively. In situations where the woman has a history of renal crisis or is at high risk for developing renal crisis (early rapidly progressive renal disease), an immediate trial of ACE inhibitors may be indicated. In cases of profound maternal or fetal distress, emergent delivery may be the most appropriate option followed by initiation of ACE inhibitor therapy. In this situation, the definitive therapy for preeclampsia has been completed (delivery), and ACE inhibitors can be instituted without concern of risk of fetotoxicity of antenatal ACE inhibitor exposure [21]. Renal biopsy may be indicated in cases where the distinction between renal crisis and preeclampsia is necessary for management [23].

5. Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) has been increasingly recognized as a major cause of morbidity and mortality in the SSc population. Estimates of PAH in the SSc population range from 7.85% to 26.7% [24, 25]. In contrast to scleroderma renal crisis, PAH can occur with both limited and diffuse cutaneous disease and may be more prevalent in patients with limited cutaneous disease. PAH in SSc is a result of long standing vasculopathy involving the pulmonary arterial vasculature. Pulmonary arterioles exhibit the same "onion skin" appearance as seen in renal crisis. Symptom onset is often insidious, delaying diagnosis and the introduction of appropriate therapy. Experts recommend routine screening for PAH to ensure early diagnosis and therapy in hopes of preventing remodeling and permanent occlusion of the vessel lumen.

It is clear that women with PAH are at extremely high risk for severe hemodynamic complications during pregnancy as there is significantly less reserve in the pulmonary arterioles to reduce vascular resistance to accommodate the increased blood volume and cardiac output that occurs during pregnancy. Reports estimate a 36%–50% maternal death rate in women with PAH, with the most vulnerable period occurring with delivery and the first two weeks postpartum [26, 27]. A more recent study suggested a slightly lower maternal death rate of 17%–33%, but this is arguably still unacceptably high [28]. Death is usually due to acute cardiovascular collapse. Rates of preterm delivery with resultant neonatal morbidity and mortality are similarly high in the PAH population. For all of these reasons, women with known PAH should be strongly discouraged from becoming pregnant; additionally, women with SSc (diffuse or limited cutaneous disease) should undergo careful screening for subclinical PAH when considering a future pregnancy. Noninvasive evaluation for PAH includes an echocardiogram looking for a pulmonary arterial pressure of >30 mm Hg at rest or an isolated reduced diffusion capacity (DLCO) in the absence of restrictive lung disease on standard pulmonary function testing [24, 25]. Furthermore, all complaints of dyspnea in pregnant SSc patients should prompt an immediate evaluation for development or worsening of PAH. Assessing diffusion capacity using carbon monoxide is considered safe during pregnancy [29].

If a woman with PAH discovers a pregnancy and wishes to continue with the pregnancy or if PAH is diagnosed during an established pregnancy, careful hemodynamic monitoring and comanagement with pulmonologists experienced with PAH is essential. Case reports have described successful use of epoprostenol and sildenafil during pregnancy [24, 26–28, 30–32]. Anticoagulation with low-molecular-weight heparin is recommended to reduce risk of thromboembolism [26], and some have suggested use of supplemental oxygen to maintain a PO2 greater than 70 mm Hg [26]. Inhaled NO has been used in extreme circumstances during labor and delivery [26, 27].

Delivery is a period of extremely high risk in the woman with PAH. Acute hemodynamic changes including increase in cardiac output of 25%–50% during the second stage of labor through delivery with the return of blood volume from the uterus to the main circulation [26, 27]. Increased maternal mortality has been described with general ansthesia [28]. The preferred mode of delivery remains controversial: vaginal delivery is associated with less shifts in blood volume but has a prolonged second stage of labor and issues regarding increased pressure with contractions. Cesarean delivery reduces the second stage of labor and may be necessary in cases of extreme maternal or fetal distress but increases risks of infection and thrombosis [28].

6. Raynauds Phenomenon and Digital Ulceration

Of all vascular complications of SSc, Raynauds phenomenon and digital ulcers are most likely to improve during pregnancy. Raynauds phenomenon is characterized by vascular hyperreactivity and vasospasm. However, when digital vasculopathy becomes more fixed, as is often the case in SSc, chronic poor perfusion leads to digital ulceration. Raynauds phenomenon tends to improve during pregnancy, only to worsen postpartum [15]. Less is known about the development of or healing of digital ulcerations. Improvements may be because the increased blood volume and reduced systemic vascular resistance may improve peripheral circulation. Return to pre-pregnancy hemodynamics leads to return of baseline peripheral circulatory complications. A study of pregnancy outcomes among women with primary Raynauds phenomenon (without SSc or other connective tissue diseases) found a slightly increased risk of preterm delivery and smaller weights among full-term infants [33] but concluded that these outcomes did not have any adverse clinical significance for mother or infant. The study did not evaluate the course of Raynaud's phenomenon during the pregnancy or postpartum period.

7. Decidual/Placental Vasculopathy

Given the diffuse vasculopathy present in patients with SSc, there are concerns that the same pathophysiologic changes may occur in the placental vasculature. The higher rates of prematurity and small for gestation age babies seen in SSc pregnancies may be a direct result of placental vascular insufficiency. Most studies did not examine placental tissue, thus not allowing for direct correlation between placental vascular abnormalities and adverse pregnancy outcomes. Histopathologic examination of placentas from a limited number of SSc pregnancies has been reported; all of which reported normal placenta weight for gestational age [34–36]. In one study of three placentas from SSc patients (gestational age at delivery between 34 and 38 weeks) found evidence of decidual vasculopathy with stomal fibrosis and infarcts in chronic villi despite an absence of reported adverse pregnancy outcomes [33]. In another case of a pregnancy SSc patient with intrauterine growth restriction was found to have increased resistance in the umbilical artery by dopper examination at 31-week gestational age. Examination of the placenta after delivery at 37 weeks found numerous placental infarcts, placental mesenchymal dysplasia, decreased vascularity, and stromal fibrosis all consistent with decidual vasculopathy [35]. In the largest study to date, 13 placentas from SSc patients were examined and correlated with perinatal outcomes [36]. Five of 13 placentas demonstrated marked decidual vasculopathy, four of which were associated with intrauterine fetal demise between weeks 16 and 30. Chorioamnionitis and accelerated placental maturation complicated the majority of other placentas. These findings are similar to what is seen in pregnancies complicated by pregnancy-induced hypertension. Indeed, in a nationwide study of pregnancy outcomes of SSc patients, 23% carried a diagnosis of pregnancy-induce hypertension [18]. Thus, placental abnormalities may be present in SSc pregnancies, even in the absence of clinical perinatal complications, and these abnormalities may be more severe correlating with perinatal growth restriction and death.

8. Conclusion

Healthy pregnancies require extensive pulmonary, cardiovascular, and vascular changes to support the fetus during development. An inability to accommodate such changes due to preexisting pulmonary, cardiac, or vascular disease may lead to pregnancy complications and an inability to support the continuance of the pregnancy. Vasculopathy is a prominent feature of SSc in general and may play an important role in adverse pregnancy outcomes in women with preexisting disease. Women with SSc who are contemplating pregnancy or who discover an inadvertent pregnancy should undergo a thorough evaluation for systemic and organ-specific vasculopathy. Those with a history of scleroderma renal crisis or pulmonary arterial hypertension (or patients at high risk for developing these complications) are at the highest risk for maternal and fetal death and other highly morbid complications of pregnancy. Those with or at high risk for renal or pulmonary arterial vasculopathy should be strongly advised against becoming pregnant and to consider termination if a pregnancy occurs. If a woman wishes to continue with a pregnancy after appropriate counseling of risks, aggressive monitoring and co-management with experts in renal or pulmonary arterial disease is mandatory. Medications such as ACE inhibitors and prostaglandins, which can carry risks for congenital malformations or fetal toxicity, need to be considered as the benefits to both mother and fetus may outweigh known risks of antenatal exposure. Labor and delivery is a very vulnerable period in these cases, and patients may require extended observation in the hospital following delivery to watch for acute cardiovascular collapse in cases of PAH.

However, if after thorough evaluation, a woman with SSc does not appear to have vasculopathy of the internal organs, pregnancy may be considered. Raynaud's phenomenon should not be considered a contraindication to pregnancy in the absence of renal disease or PAH. With careful monitoring by high-risk obstetrics, most women without advanced vasculopathy can expect to have reasonable perinatal outcomes although preterm delivery is common. Advances in neonatology have helped improve the short- and long-term outcomes of premature infants.

Whenever possible, placentas and umbilical cords should be subject to histopathologic examination for evidence of decidual vasculopathy and stromal fibrosis. This will hopefully lead to better understanding of the role of placental vasculopathy in adverse pregnancy outcomes and may additionally provide histopathologic clues to systemic or organ- specific vasculopathy in SSc patients.

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Review Article

Vascular Alterations and Sexual Function in Systemic Sclerosis

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Sexual dysfunction is common in systemic sclerosis (SSc). Male erectile dysfunction (MED) has been reported in around 80% of subjects and more than half of female patients fulfill criteria for diagnosis as female sexual arousal Disorder (FSAD). While some evidence supports a role for cavernosal fibrosis, abundant data suggest that MED is yet another clinical feature of SSc related to vasculopathy. The contribution of vasculopathy to the more complex issues of female sexual dysfunction is less clear. Inhibitors of Type V phosphodiesterase are effective in men with MED secondary to SSc. Limited study in women suggests inconsistent effects on behavior (frequency) but not on measures related to perfusion. Sexual activity is an important component of quality of life and an important domain for the caregiver to address; it is not clear that it warrants primary consideration as a consistent measure of scleroderma-related vasculopathy.

1. Introduction

Any comprehensive hypothesis concerning the pathogenesis of systemic sclerosis (SSc, scleroderma) must account for the varying contributions of vascular damage, extravascular tissue fibrosis, and inflammation [1]. While certain clinical features of SSc are overt expressions of vascular injury (renal crisis, pulmonary arterial hypertension, and Raynaud phenomenon), our understanding of other organ involvements is less well understood. The clinical and physiologic expressions of SSc on sexual function are one such example.

Complications from scleroderma do have a negative impact on sexual function and in turn on the overall quality of life. In spite of the 80% female predominance of SSc [1], most of the studies on the effect of SSc on sexual function have involved men [2–9]. Male erectile dysfunction (MED) has been noted in as many as 81% of afflicted persons [9]. MED in SSc can be reasonably attributed to scleroderma vasculopathy although cavernosal fibrosis has been implicated as well [10]. Little is known of the impact of scleroderma on female sexual functioning and on quality of female sex life. The general difficulties in researching female sexual function might explain some of the lack of interest in this area although there has been a marked change in the past year with the publication of several studies [11–13]. Daily and long-acting PDE-5 inhibitors have been proven to be safe and effective for MED in males with SSc [10]. To our knowledge, no studies have been published on successful pharmacological interventions in female SSc patients with sexual impairment.

2. Male Erectile Dysfunction

MED is defined as the consistent inability to attain and maintain an erection sufficient to permit satisfactory sexual performance [14]. The first report of an association between erectile dysfunction and SSc was as recently as 1981 [15]. Prevalence of MED in SSc has been found to range from 12% to 81% [9, 16]. The causes of ED associated with SSc are unclear although a number of factors have been suggested including vascular, fibrotic, and neuropathic/dysautonomic factors [10, 15, 17]. While some studies reported correlations between ED in SSc and testosterone and prolactin levels [15, 17] others have not [18–20]. Walker et al. [10] conclude that there is no support for a hormonal basis for ED in SSc

as no consistent abnormalities in serum testosterone, follicle-stimulating hormone, luteinizing hormone, prolactin, estradiol, or thyroid hormones have been found.

Several studies have ruled out a neurological basis for ED in SSc [17, 21]. Recent studies have found links between ED in SSc and penile blood pressure [22] and penile temperature [23], leading to a link between ED in SSc and vasculopathy. A strong connection between endothelial function and ED has been reported in the general population [24], supported by the strong linkage of MED with coronary artery disease. In this construct, endothelial injury and dysfunction result in diminished nitric oxide (NO) production. During normal penile erection, nitric oxide is released at the nerve endings of the penis. Endothelial cells are also a source of nitric oxide. Nitric oxide diffuses into the vascular smooth muscle cells in the penile corpus cavernosum stimulating guanylyl cyclase and production of cyclic guanosine monophosphate (cGMP). This in turn activates cGMP-dependent protein kinase (PKG), phosphorylation of several proteins, lowering of intracellular cell calcium, or sensitivity to calcium leading to muscle relaxation. This relaxation leads to an increase of blood in the corpus cavernosum leading to penile erection [25]. An insufficient release of nitric oxide from nerve endings or endothelium can lead to an attenuated production of cGMP.

Penile vascular damage in SSc patients was assessed using Duplex ultrasonography [2, 23] showing that penile thermal abnormalities are present in almost all SSc patients. Penile fibrosis has also been shown to be present in almost all SSc patients. Nehra et al. [19] report severe veno-occlusive dysfunction based on histological analysis confirming severe corporeal fibrosis from a biopsy evaluation of a single SSc ED patient. They postulate that fibrosis could lead to veno-occlusion leading to a reduction of the trabecular fibroelastic compliance and trabecular smooth muscle tone which depend on neurogenic-and endothelial-dependent vasomotility [23]. Penile fibrosis has also been shown to occur in almost all SSc patients with the presence of thickening of the tunica albuginea and hyperechoic spots inside the corpera carnevosa [22]. Merla et al. conclude that their results showing fibrosis and thermoregulatory dysfunction in SSc patients with ED support the hypothesis that structural modifications induced by SSc lead to a reduced capability of heat exchange and vascular damage [23].

Phosphodiesterase type-5 (PDE5) is an enzyme which degrades cGMP. PDE-5 inhibitors enhance erectile function by blocking degradation of cGMP leading to an increase in intracellular cGMP in the corpus cavernosum and penile vasculature. The result is an increase in the relaxation of the smooth muscle leading to an increased blood flow and penile erection [25]. There are three PDE-5 inhibitor compounds licensed for treatment of MED: sildenafil, vardenafil, and tadalafil. These three compounds are similar in pharmacologic and clinical characteristics. Studies in the general population have shown these to be safe and generally effective depending on the etiology and severity of the erectile dysfunction [26]. MED in SSc has been less well-studied. Ostojic and Damjanov [21] described a small case series with unsatisfactory response to on demand sildenafil (25–50 mg). An open label randomized cross-over study by Aversa et al. [27] compared 20 mg tadalafil on demand versus 20 mg in a fixed alternate day schedule. On the fixed alternate day schedule, a significant improvement was reported for the flow-mediated dilatation, peak flow velocities of cavernous arteries, reduced plasma levels of ET-1 and vascular cell adhesion molecules (markers of endothelial function), and morning erections. No such results were found for the on-demand regimen suggesting that a constant plasma level of PDE-5I might be important for the treatment of MED in SSc [10]. Proietti et al. [2] report the results of a study with a daily 10 mg dose of tadalafil for 12 weeks in 14 SSc patients with varying degrees of ED. Improvements were found in erectile function, vascular measures of cavernous arteries, morning erections, and plasma ET-1 levels (reduced levels).

3. Female Sexual Dysfunction

There is an inequality in the number of studies focusing on male versus female sexual dysfunction (FSD) in the general population [28] and the same holds true in SSc although the disease primarily affects women. The complexity and multifactorial nature of female sexual response and female sexual dysfunction adds to the difficulty in research in this area. FSD can have psychological and social components in addition to medical and physiological components. FSD is defined as persistent or recurring decrease in sexual desire, persistent or recurring decrease in sexual arousal, dyspareunia, and a difficulty in or inability to achieve orgasm [29]. It is reported to affect 20%–50% of all women and can severely impact quality of life and interpersonal relationships [28, 29].

Changes associated with SSc can have a negative impact on female sexuality and sexual functioning. Symptoms such as skin tightening around the vaginal introitus, joint contractures, muscle weakness, changes in skin around the breasts and breast muscle, and joint pain have been found to be associated with lower levels of sexual functioning, desire, arousal, lubrication, and satisfaction [4, 6, 30]. Changes in the vaginal mucosal may lead to difficulties with lubrication. Many SSc patients experience significant limitations on exercise capacity with dyspnea, decreased stamina, and coughing that may interfere with certain sexual behaviors [4, 30]. Medications used to treat SSc-related symptom are also known to impact sexual desire and sexual functioning [30].

The number of women with SSc reporting sexual dysfunction is higher than those reported in the general population and also higher than those reported in studies on other chronic conditions [11–13]. Bhadauria et al. [30] reported that more than 50% of women with SSc had significantly fewer orgasms and that they reported a decrease in the intensity of their orgasms compared to fewer than 20% of RA patients. In a study by Sampaio-Barros et al. [31], 37% of sexually active SSc patients mentioned dyspareunia. Some female SSc patients are sexually inactive because of complications related to their disease. One study reported that 17% of subjects were sexually inactive because of issues caused by their disease [11].

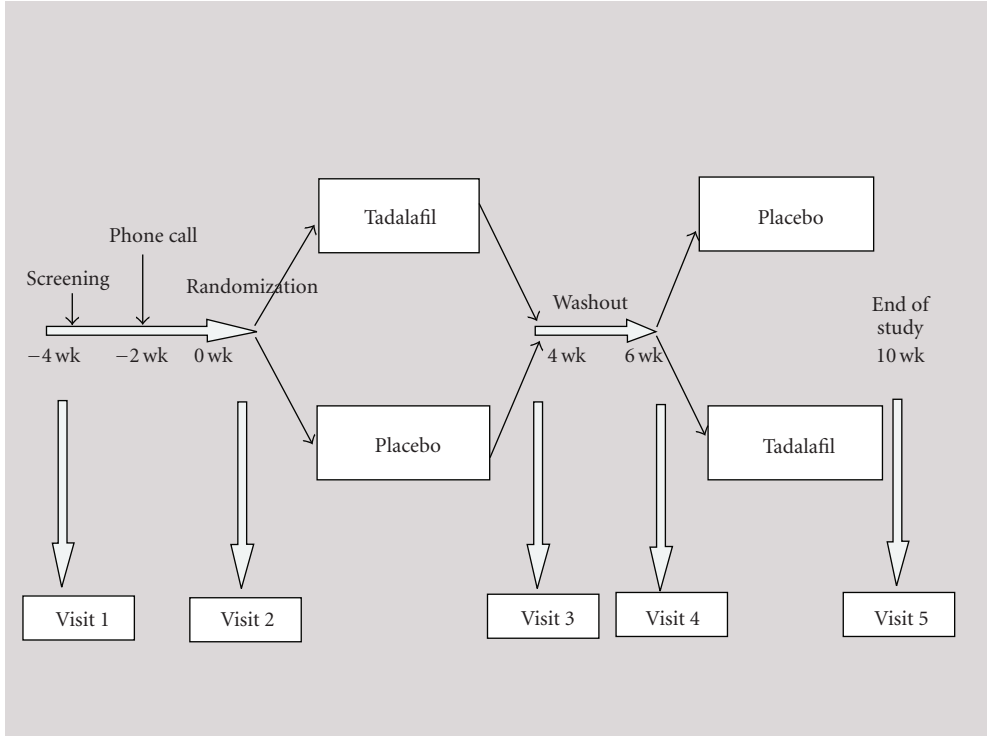

Figure 1: Study design.

While there are many studies on the use of PDE-5 inhibitors in the treatment of ED there are few studies on their use in the treatment of female sexual arousal problems and their effectiveness is in question [32]. Our understanding of the effects of these drugs on female sexual psychophysiology is limited. Animal models of female sexual response show a similar physiological effect of PDE-5 on vaginal and clitoral tissues compared to males with NO synthase (NOS) being active in the vaginal epithelium and PDE5 enzyme found in vaginal smooth muscle tissue and clitoral shaft. PDE5 inhibition with sidenafil in female rabbits have been shown to lead to increased smooth muscle relaxation. Chivers and Rosen [32] conclude that there is clear evidence for the role of NO neurotransmitter mechanisms in mediating blood flow and smooth muscle relaxation in the clitoral shaft, vaginal endothelium, and uterine tissues. However, PDE5 inhibitors are not found to be as efficacious in women with sexual dysfunction as in men with ED [32, 33]. Studies focusing on the physiological effects of PDE5 on genital vasocongestion consistently report effects on genital sexual response while those using self-reported measures provide mixed results. This is likely due to problems related to the classification and measurement of FSD in women and difficulties related to finding meaningful endpoints in clinical trials due to the complexity of female sexual response compared to men [32]. Chivers and Rosen [32] attribute this lack of efficacy of PDE-5 inhibitors in women to gender differences in the concordance between physiological and psychological components of sexual response.

While there are few studies on the effectiveness of PDE-5 inhibitors for FSD in the general population, no studies were found in SSc patients. The paper by Chivers and Rosen [32] regarding the effectiveness of PDE-5 inhibitors for FSD did not include any studies on the longer acting drug, tadalafil. As part of a study on the safety, tolerability and effectiveness of tadalafil for Raynaud phenomenon (RP) [34], female subjects were asked to attempt sex at least once weekly to evaluate potential effects on quality of female sexual functioning. This prospective randomized, double-blinded, placebo-controlled, and cross-over study compared oral tadalifil at a fixed 20 mg dose daily for a period of 4 weeks versus placebo in female SSc patients. Tadalafil was found to be well tolerated but no statistically significant differences were found in Raynaud Condition Score, frequency or duration of RP episodes between treatment periods. The study design can be found in Figure 1. There were a total of 5 visits: a screening visit (Visit 1), three intermediate visits (Visits 2–4), and an exit Visit. Data obtained specifically regarding sexual function included the female sexual function index (FSFI) and a sexual activity log including the frequency of sexual activity. This was obtained at Visit 2 (baseline) and Visits 3 and 5 (after treatment or no treatment period). The FSFI quantitatively assesses six domains of sexual functioning: desire, subjective arousal, lubrication, orgasm, pain, and satisfaction [35].

A total of 39 patients met all inclusion criteria, agreed to attempt sexual activity at least once weekly and successfully completed all 5 visits. When comparing change from baseline

between drug and placebo on the six domains of the FSFI no statistically significant differences were found although patients reported greater change when on drug versus when on placebo. A significant difference was found between drug and placebo in the number of sexual activities with SSc patients reporting more frequent sexual activities when receiving drug than when on placebo (mean number sexual activity drug = 7.24 with sd = 4.53 and a mean of 6.12 and sd of 3.3 on placebo). This difference was statistically significant (P = .021; 2-tailed) as was the change from baseline (1.49 and sd = 2.55 on drug and x = .36 and sd = 2.16; P = .024 2 tailed). The only other statistically significant difference in this study was found in a change from baseline in the sexual desire domain of the FSFI for drug although this was not statistically different from the change in the placebo group.

Some of the more recent studies on female sexual functioning in SSc confirm the complexity of studying female sexual function as well as the psychological components of it. Depressive symptoms were found to be associated with impaired sexual functioning and sexual distress in SSc [11, 13]. In our own studies of quality of female sexual function, we found stronger correlations with the mental component of the SF-36 than with the physical component score [11]. One lesson is that health care professionals should not dismiss consideration of sexual care and advice in any patient with systemic sclerosis.

4. Conclusions

Sexual dysfunction is a common problem in both men and women with systemic sclerosis. In males, the dominant issue is erectile dysfunction which in turn seems tightly linked to vascular dysfunction. Sexual dysfunction in the female patient is no less prevalent but is considerably more complex. Aspects of female sexual response thought to be vascularly influenced—clitoral engorgement, lubrication, orgasm—are but some of the components of quality of sexual life. To our knowledge, only one study with PDE5-I has been performed in women with confusing and small effects whereas PDE5-I are a mainstay agent in MED. The clinician should be reminded that women with scleroderma remain sexually active overall (60%) in spite of a host of physical and psychological difficulties associated with their disease. Complete care should consider agents for vaginal lubrication, advice about positioning and attention to sexual adverse events of therapies.

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Review Article

Penile involvement in Systemic Sclerosis: New Diagnostic and Therapeutic Aspects

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Systemic Sclerosis (SSc) is a connective tissue disorder featuring vascular alterations and an immunological activation leading to a progressive and widespread fibrosis of several organs such as the skin, lung, gastrointestinal tract, heart, and kidney. Men with SSc are at increased risk of developing erectile dysfunction (ED) because of the evolution of early microvascular tissutal damage into corporeal fibrosis. The entity of penile vascular damage in SSc patients has been demonstrated by using Duplex ultrasonography and functional infra-red imaging and it is now clear that this is a true clinical entity invariably occurring irrespective of age and disease duration and constituting the "*sclerodermic penis*". Once-daily phosphodiesterase type-5 (PDE5) inhibitors improve both sexual function and vascular measures of cavernous arteries by improving surrogate markers of endothelial dysfunction, that is, plasma endothelin-1 and adrenomedullin levels, which may play a potential role in preventing progression of penile fibrosis and ED. Also, the beneficial effect of long-term PDE5i add-on therapy to SSc therapy in the treatment of Raynaud's phenomenon is described.

1. Introduction

Systemic Sclerosis (SSc) is a connective tissue disorder featured by vascular alterations and immunological activation leading to progressive and widespread fibrosis of several organs such as skin, lung, gastrointestinal tract, heart, and kidney [1, 2]. The typical hallmark of SSc is a microvascular involvement, while macrovascular involvement is not well documented in these patients, but the majority of authors agree that its prevalence is similar to general population [3]. Vascular involvement in SSc has been believed to be limited to digital arteries [4]. It is extremely rare that SSc patients without vascular risk factors have macrovascular lesions above the elbow or knee. However, a relatively high incidence of vascular involvement between the digits and the elbow or knee has been described [5]. Early disease is mediated through microvascular dysfunction secondary to a number of factors including endothelial damage, overexpression of specific adhesion molecules, and perivascular inflammatory cell infiltration [6]. These changes make endothelium unable to carry out its functions in the regulation of vascular tone, coagulation, adhesions and migration of blood cells, transportation of nutrients, achieved through production of a complex array of molecules including vasodilators (e.g., nitric oxide: NO), vasoconstrictors (e.g., endothelin-1: ET-1), and cell adhesion molecules (e.g., selectins and integrins) [7]. The endothelial dysfunction can explain some major clinical symptoms of SSc such as Raynaud's phenomenon (RP), fingertip ulcers and gangrene, pulmonary arterial hypertension and erectile dysfunction (ED) (Figure 1). Irrespective of the classification of the disease, SSc is typically associated with RP that is characterized by microvascular damage, high plasma adrenomedullin and ET-1 levels, reduced production of NO [8–11].

Similarly, at the penile level, there occurs a consistent vascular damage that almost invariably determines,

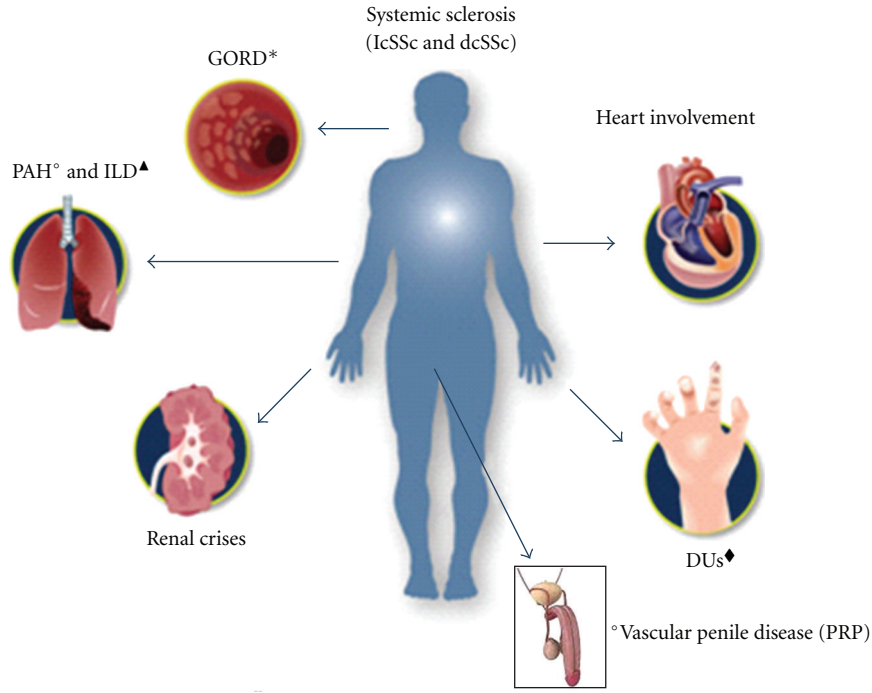

Figure 1

as a consequence of both endothelium damage [12–16] and increased fibrogenesis [17], the so-called "*sclerodermic penis*". In fact, the prevalence of ED in men with SSc has been reported as high as 80% [18] and it can be considered an end-organ disease involving both macro and microvascular damage.

2. Pathophysiology of Sclerodermic Erectile Dysfunction

Hormonal derangement is a common finding in SSc patients even if a hormonal basis for impotence, that is, abnormalities in serum testosterone, follicle-stimulating hormone, luteinizing hormone, prolactin, and oestradiol, has never been demonstrated. Neurological causes could also be excluded. In contrast, penile blood pressure, but not ankle blood pressure indices, were found to be diminished [19]. Duplex sonography measurements in male SSc patients show impaired peak systolic velocities (PSVs) in penile arteries and also the presence of veno-occlusive dysfunction. The latter is often associated with the identification of diffuse hyperechogenic "spots" inside the corpora cavernosa, along with a thickening of the tunica albuginea and is consistent with the presence of a high degree of corporeal fibrosis [20]. In a recent angiographic study it has been demonstrated that the prevalence of coronary artery disease in SSc patients is not different from a control group [21]. In accordance, our studies found that the intima-media-thickness (IMT) of the common carotid artery of patients with ED in the context of SSc is normal, thus confirming that advanced atherosclerosis occurs late in the course of disease. By contrast, endothelial dysfunction is present early, as we were able to demonstrate with impaired thermal recovery of the penis after cold exposure [22]. Taken together, these data indicate an altered arterial blood flow in the absence of general atherosclerosis as it is common in end-organ disease. Indeed, an increased collagen synthesis by smooth muscle cells and the accumulation of extracellular matrix had been already demonstrated in patients with SSc [23], and it is known that under hypoxic conditions of various origin, transforming growth factor beta (TGFβ1), platelet-derived growth factor (PDGF) and its receptors are overexpressed in the corpora cavernosa [24]. TGFβ1 and PDGF have both been identified as important regulators of the collagen and extracellular matrix synthesis by smooth muscle cells and also act as smooth muscle mitogens. Under hypoxic conditions, human penile smooth muscle cells also release ET-1 and induce ET-B receptor expression, processes that in turn are strongly increased by TGFβ1 and ET-1 itself [25]. These results suggest that the molecular mechanisms by which penile hypoxia of any cause induce penile fibrosis are similar to those implicated in the fibrotic transformation of tissues in SSc patients [26]. The hypoxic and the SSc-specific processes may thus contribute to, and even perpetuate, each other in the manifestation of ED.

Contradictory attitudes exist about the mechanisms of the vasospastic arteriolar paroxysms—from hyperactivity of the sympathetic nervous system and "local defect" with receptor and nerve endings' dysfunctions, endothelial dysregulation and blood cells' activation to statements of primary central nervous mechanism. The venoarteriolar reflex is a local mechanism protecting the capillary bed against high hydrostatic pressure and therefore the tissue against edema. The underlying mechanism of the venoarteriolar reflex is still debated, although it is commonly believed to depend on an intact innervations of the arterioles by sympathetic vasoconstrictor fibers [27]. The modulating role of endothelium with its influence on the contractile behaviour of precapillary resistance vessels is also assumed so we can hypothesize that an autonomic nervous system dysregulation can play an important role in determining microvascular damage in sclerodermic patients. Evidence-based studies aimed to investigate neurological involvement in SSc patients are lacking.

3. Diagnostic Approach

The diagnosis of scleroderma is not always easy. If scleroderma is suspected, tests should be ordered to confirm the diagnosis, as well as to determine the severity of the disease. Major body changes determined by SSc are often responsible for increased morbidity and mortality, and may contribute to the occurrence of psychological disturbances such as anxiety and depression, whereas the incidence of psychotic symptoms is very low in SSc patients [28]. It is known that SSc patients are more vulnerable to depression [29], and psychological interventions including counseling with special psychotherapists and even antidepressant medication may prevent the development of depression in such patients. Further studies are needed to confirm our findings in a prospective manner if possible, and also to determine whether depression is an important prognostic indicator for quality of life in SSc. and an important breakthrough can be made in understanding the underlying mechanisms of psychiatric manifestations, in order to improve therapeutic management and quality of life. Furthermore, an accurate psychological assessment in SSc men with ED is recommended.

ED should always be investigated with appropriate questionnaires and dynamic penile Duplex ultrasound. In fact, penile vascular damage occurs in almost all SSc patients, regardless of clinical symptoms and we have suggested that investigation of these patients with Duplex ultrasound is mandatory for documenting the degree of vascular involvement since the self-administered International Index of Erectile Function questionnaire does not often match with vascular findings [20]. In addition, we have demonstrated that penile thermal proprieties of SSc patients differ from healthy controls, by using functional infrared imaging [22]. Data collected in this late pilot study demonstrate that SSc patients' penile temperature appears to be lower than that of the healthy controls. In particular, it seems that major differences are found at the level of the corpora, while minor but still significant differences are pointed out for the temperature of the glans penis. Since cutaneous temperature depends on cutaneous blood flow and thermal exchanges with deeper tissues (by convection through the arterovenous network and by conduction from vessel walls to tissues), the results seem to suggest the existence of functional alterations of both tissue properties and blood flow. Penile temperature response to thermal stress seems to confirm such a hypothesis since SSc patients counteract to and recover from cooling differently from controls. Therefore, assessing whether thermal properties and temperature control processes of the penis in SSc patients are altered could provide clues on potential ED, the progression of the illness and the effectiveness of possible treatments [22].

After careful review of clinical and instrumental data already published in our previous papers, we suggest that there is a relationship between ED and SSc vascular damage evaluated by capillaroscopic pattern and vascular domain of Medsger Disease Severity Scale (DSS) [30]. In our opinion ED is present at onset in all patients with SSc, but early stage of ED it can be attributed to a reduced penile arterial inflow that is similar to RP of the hands and that configures the "*sclerodermic penis*". With the progression of micromacrovascular damage in the natural course of the disease, a concomitant penile fibrosis and venocclusive dysfunction occur leading to difficult-to-treat ED.

For these reasons, a multidisciplinary approach to SSc patients would involve the rheumatologist, the uro/andrologist and the psychiatrist to comprehensively evaluate all diagnostic aspects.

4. Treatment

The treatment of ED in SSc suggest to modify or revert risk factors for ED, including lifestyle, psychological or drug-related factors, but such treatments are often unsatisfactory and limited by frequent side effects in the long-term. Three different phosphodiesterase type-5 (PDE5) inhibitors are currently available: sildenafil, vardenafil and tadalafil. Several randomized trials have demonstrated the efficacy of this class of medications, but there are no compelling data to support the superiority of one PDE5 inhibitor over another in non-SSc patients. The three PDE5 inhibitors share many pharmacological and clinical characteristics. Initial studies involving animal models, data from open-label, uncontrolled trials involving patients with pulmonary arterial hypertension, and a small randomized, controlled studies involving patients with idiopathic pulmonary arterial hypertension suggest that PDE5 inhibitors are beneficial in the treatment of pulmonary arterial hypertension [31–33]. Recently some authors have shown that sildenafil may be complimentary in the treatment of RP refractory to conventional drugs [34, 35]. Several studies have corroborated the efficacy of PDE5 inhibitors in the treatment of ED related to several internistic disease such as atherosclerosis, mellitus diabetes, arterial hypertension [36]. This improvement is confirmed also by sexual questionnaires and suggests PDE5 inhibitors as an adjunctive therapy that might possibly reverse the process

of endothelial damage leading to vascular disease in SSc. In a recent study, we demonstrated that once-daily tadalafil is able to decrease the mean number of Raynaud's attacks by reducing surrogate markers of vascular damage such as adrenomedullin and ET-1 plasma levels. The results of that study lead us to postulate the beneficial effect of adding daily long-term PDE5 inhibitors to the medical treatment of SSc [37]. The use of endothelin-receptor antagonists (ERAs) in SSc is actually deserved to patients with severe pulmonary arterial hypertension. The possibility of an add-on effect of ERAs to PDE5i is to be considered in future studies. At the moment, no proof-of-fact that the use of ERAs alone may improve ED is present.

In the era of PDE5 inhibitors, we believe that the implantation of a penile prosthesis may be considered only in patients who fail pharmacotherapy or who prefer a permanent solution of their problem. The choice of prosthesis is depends from patients' preference and his manual dexterity. This solution may have an high satisfaction rate as in the non-SSc population but must take into account that it is not reversible. Finally, mechanical failures and infection has the same occurrence (1%–3%) than the general population [38].

5. Conclusion

Long-standing SSc is almost always associated with the presence of some degree of ED, because of micro and macrovascular disseminated damage through cavernosal penile arteries and capillaries and subsequent cavernosal fibrosis. As a consequence, ED should be systematically questioned and counselled in any patient presenting with SSc by a specialized team. Although robust data on treatment options for ED in the SSc population are not yet available, empirical treatment should be started with a daily or alternate day regimen of a long-acting PDE5i after addressing modifiable risk factors for ED. Second-line treatment decisions require the cooperation between expert specialists in order to maximize medical therapy benefits for underlying conditions.

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Review Article

Microscopic Polyangiitis in Systemic Sclerosis

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AAV in SSc is described from the point of view of MPA. Some of reported SSc cases with AAV are thought to exhibit the characteristic clinical manifestations of MPA, although ANCA positivity in SSc is uncommon. MPA is clinically characterized by a multisystemic disease such as RPGN, pulmonary hemorrhage, mononeuritis, and skin involvement, as well as other manifestations in conjunction with high levels of inflammatory activity such as high ESR or CRP. It is also characterized by a high frequency of MPO-ANCA, showing predominant pANCA by IIF. When rapid renal failure or RPGN with active urine sediments, pulmonary hemorrhage and/or systemic inflammatory manifestations are observed in patients with SSc having positive ANCA, the possibility of MPA should always be considered. If SSc patients with MPA have life-threatening visceral involvement such as the above clinical manifestations, the patients should be treated with induction therapy using cyclophosphamide, methotrexate, corticosteroids, or plasmapheresis, etc. according to the severity of the disease soon after the diagnosis of MPA. It is important not to overlook characteristic clinical manifestations of AAV during the course of the disease in SSc in order to diagnose MPA early.

1. Introduction

Systemic sclerosis (SSc), which mostly affects middle-aged women, is a chronic disorder of connective tissue characterized by inflammation and fibrosis and by degenerative changes in the blood vessels, skin, synovium, skeletal muscle, and certain internal organs, notably the gastrointestinal tract, lung, heart, and kidney [1]. The life-threatening visceral involvements in SSc include scleroderma renal crisis, pulmonary hypertension, and lung fibrosis.

On the other hand, microscopic polyangiitis (MPA), which mostly affects elderly people, is a systemic disease characterized by vasculitis involving small blood vessels, particularly the glomerular and pulmonary capillaries, and serologically by antineutrophil cytoplasmic antibody (ANCA) positivity [2–4]. MPA is also well known to present as one form of primary ANCA-associated vasculitis (AAV) as well as Wegener's granulomatosis (WG) [2]. Clinical manifestations in this disease include rapidly progressive glomerulonephritis (RPGN) and pulmonary hemorrhage, which are life-threatening visceral involvements.

Cases of SSc patients with AAV have been reported, although ANCA positivity in SSc is uncommon. Some of these cases are thought to exhibit the characteristic clinical manifestations of MPA. In this paper, AAV in SSc will be described from the point of view of MPA.

2. ANCA and MPA

ANCA is well known to be associated with small-sized vasculitic disorders such as MPA, WG, and allergic granulomatous angiitis (AGA) in primary systemic vasculitides. These diseases are also known as primary ANCA-associated vasculitides. There are two major distinct subtypes of ANCA. One subtype is an antibody against myeloperoxidase (MPO), which stains in a perinuclear pattern (pANCA) by indirect immunofluorescence (IIF) using a neutrophil substrate, and the other subtype is an antibody against proteinase 3 (PR3), which stains in a diffuse granular cytoplasmic pattern (cANCA) by IIF. Enzyme-linked immunosorbent assay (ELISA) is used for target-specific ANCA determination [5]. MPO-ANCA can be found in MPA and AGA more frequently than in WG. On the other hand, PR3-ANCA is more specific in WG than the other primary systemic vasculitides. The association of MPA with MPO-ANCA is reported to be in

Table 1: Comparison of clinical manifestations in MPA between French and Japanese studies.

	French study [4]	Japanese study [19]
Cases	85	63
Age, mean (range) years	56.8 (16–86)	59.0 (13–91)
Sex ratio, Male : Female	47 versus 38	19 versus 44
Fever	55.30%	71.00%
Weight loss	72.90%	42.90%
Skin involvement	62.40%	24.20%
Arthralgias	50.60%	62.90%
Myalgias	48.20%	50.80%
Renal involvement	78.80%	87.30%
Renal insufficiency	(47/67) 70.1%	49.20%
Rapidly progressive GN	—	66.70%
Proteinuria	(54/67) 80.6%	93.10%
Hematuria	(45/67) 67.2%	76.70%
Lung involvement	24.70%	63.50%
Alveolar hemorrhage	11.80%	22.20%
Pneumonitis	10.60%	33.30%
Pleiritis	5.90%	19.00%
Mononeuritis multiplex	57.60%	30.00%
Central nervous system involvement	11.80%	6.30%
Gastrointestinal tract involvement	30.60%	6.30%
Hypertension	34.10%	41.30%
Myocardial infarction	2.40%	1.60%
Pericariditis	10.60%	3.20%
Cardiac failure	17.60%	3.20%

the range of 40–80%, and MPA with MPO-ANCA is noted to be frequently associated with necrotizing glomerulonephritis and/or pulmonary capillaritis, namely, a pulmonary-renal syndrome similar to that observed in Goodpasture syndrome or WG [3, 4]. Furthermore, renal-limited vasculitis, which is characterized by pauci-immune focal necrotizing crescentic glomerulonephritis (pFNCGN), is classified as MPA using European Medicines Agency (EMEA) algorithm [6]. Interestingly, MPO-ANCA-associated vasculitides are more common in Asian countries than in the USA or Europe, where 80% of AAV is PR-3 ANCA [6, 7] and ANCA-positive, while anti-MPO-negative patients with MPA most often have antibody specificity for PR3 [3].

In Japan, MPO-ANCA has been reported to account for 90% of cases of AAV [8, 9]. Therefore, renal-limited AAV in Japan is almost exclusively MPA, whereas in the UK only 41% of cases of renal limited AAV have MPA, although the overall incidences of renal vasculitis are similar in the UK and Japan [6].

On the other hand, ANCA has also been described in patients with rheumatic autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, polymyositis/dermatomyositis, and antiphospholipid syndrome. The fluorescence patterns encountered in these diseases are mostly pANCA. A multitude of target antigens, including not only MPO but also lactoferrin, elastase, lysozyme, cathepsin G, and bactericidal/permeability-increasing protein (BPI) have been described in rheumatic autoimmune diseases [3, 10]. However, ANCA in SSc is uncommon, showing a positive rate ranging from 0 to 18% according to investigators [3, 11–17], in spite of the development of many autoantibodies, such as antitopoisomerase-I (Scl-70) antibodies, anticentromere antibodies, and rheumatoid factors, in SSc. ANCA in SSc usually shows pANCA and/or anti-MPO-ANCA, although BPI and cathepsin G were reported to be the major antigenic targets of ANCA seen in patients with SSc [18].

3. Characteristics of MPA

MPA is clinically characterized by a multisystemic disease such as RPGN, alveolar hemorrhage, mononeuritis, and skin involvement, as well as other manifestations. It is also characterized by a high frequency of MPO-ANCA, showing predominant pANCA by IIF as mentioned above. Pathologically, inflammation and necrosis in various sites of vessels including arteries, arterioles, capillaries, and venules are observed, and pFNCGN characteristically occurs in the context of MPA as well as WG. The severity of the injury requires immediate treatment with immunosuppressive drugs including cyclophosphamide and corticosteroids.

There are also some differences in the clinical manifestations of MPA, in addition to the clinical phenotype of renal AVV, between Europe and Japan. Table 1 shows

Table 2: Proposed diagnostic criteria for microscopic polyangiitis (MPA). (Research Committee on Intractable Vasculitis and Research Committee on Epidemiology of Intractable Diseases, The Ministry of Health and Welfare of Japan, 1998).

(1) Clinical manifestations
(i) rapidly progressive glomerulonephritis
(ii) pulmonary hemorrhage or interstitial pneumonia
(iii) organ involvement besides kidney and lung: purpura, subcutaneous bleeding, gastrointestinal bleeding, mononeuritis multiplex, and so forth
(2)Histological findings
necrotizing vasculitis in capillaries, venules, or arterioles, with perivascular inflammatory infiltrate
(3) Laboratory findings
(i) positive MPO-ANCA
(ii) elevated level of CRP
(iii) proteinuria and/or hematuria, or elevated levels of BUN and/or creatinine
(iv) chest X-ray findings: infiltration (pulmonary hemorrhage), and/or interstitial pneumonitis
(4) Diagnosis
(i) Definite
(a) At least two clinical manifestations with the histological findings
(b) At least two clinical manifestations including items (i) or (ii), and positive MPO-ANCA
(ii) Probable
(a) At least three clinical manifestations
(b) One clinical manifestation and positive MPO-ANCA
(5) Exclusion diseases
(i) polyarteritis nodosa
(ii) Wegener's granulomatosis
(iii) allergic granulomatous angiits (Churg-Strauss syndrome)
(iv) Goodpasture syndrome

a comparison of clinical findings of MPA in the French Vasculitis Study Group reported by Guillevin et al. [4] and the Japanese Nationwide Epidemiological Survey [19] reported by Hashimoto et al. The observed differences might be expected because the same diagnostic criteria were not used, although the inclusion criteria for the diagnosis of MPA were almost the same. Table 2 shows the diagnostic criteria of MPA used in the Japanese Nationwide Epidemiological Survey. Interestingly, the female : male sex ratio was 1.23 in the French study, which contrasts with 0.43 in the Japanese study, although the mean age at diagnosis was almost the same. The incidence of clinical manifestations including fever and weight loss in the French study was greater than that in the Japanese study, but the incidences of arthralgias and myalgias were almost the same. Concerning visceral involvement, the incidence of renal involvement and lung involvement, such as alveolar hemorrhage, pneumonitis, and pleuritis, in the Japanese study was greater than that in the French study; in contrast, the incidence of mononeuritis multiplex, central nervous system involvement, and gastrointestinal tract involvement in the French study was greater than that in the Japanese study. The frequencies of hypertension were 34.1% in the French study and 41.3% in the Japanese study.

In the French study, ANCA was present in 74.5% of patients, of whom 86.8% had pANCA and the remainder had cANCA. In the Japanese study, pANCA was present in 90.4% of patients and cANCA was present in 9.6%. Among pathological findings in the Japanese study, necrotizing vasculitis was present in 32 out of 55 patients and crescentic glomerulonephritis was present in 41 out of 47 patients (not indicated in the French study). In the Japanese study, most of the patients were treated with corticosteroids (93.4%) and immunosuppressant drugs (71.0%). The mortality rate was 12.9% and the most frequent cause of death was infection (36.5%) followed by alveolar hemorrhage (17.3) and renal failure (13.5%). Guillevin et al. [4] noted that in the French study, deaths were less frequent when patients had been treated with corticosteroids and immunosuppressive drugs, but relapse of MPA was common.

4. AAV in SSc

SSc is divided into two major clinical subsets, namely diffuse cutaneous and limited cutaneous disease, which are distinguished from one another primarily by the degree and extent of skin involvement [20]. Overlap syndrome, which has either diffuse or limited cutaneous disease and typical features of one or more of other connective tissue diseases, is also present [1]. Since the identification of antitopoisomerase-I (anti-Scl-70 antibody) and anticentromere antibodies, it has been well known that antitopoisomerase-1 antibodies are associated with diffuse cutaneous disease as well as evident renal involvement and pulmonary interstitial fibrosis, and anticentromere antibodies are associated with limited cutaneous disease as

well as pulmonary hypertension. Anti-U1RNP and anti-Ku antibodies are observed in patients with overlap syndrome of SSc and myositis. Although the number of cases with overlap syndrome of SSc and MPA among the cases of SSc with AAV that have been reported is unknown, several cases of overlap syndrome were suggested to be present by Rho et al. [21] who analyzed and reviewed the clinical characteristics of SSc with AAV in 50 cases reported in 31 articles. In this study, MPO-ANCA was the predominant type (72%), but PR-3 ANCA was also found in some cases (24%). The authors noted that 33 out of 50 cases had definite features of AAV with pathological or clinical evidence including crescentic glomerulonephritis, RPGN, pulmonary hemorrhage, skin and/or nerve vasculitis, and necrotizing vasculitis of muscle. High levels of inflammatory activity, such as high ESR or CRP, or abnormal urinary sediments, were also indicated. Cases with MPO-ANCA had a higher prevalence of renal impairment and pulmonary hemorrhage than those with PR-3 ANCA. Having antitopoisomerase-1 antibodies made the development of AAV in SSc three times more likely than that in patients who had neither antibody. The mortality rate was 39.4% with virtually all of the deaths occurring within 1 year. The major causes of deaths included infection or septic shock, pulmonary hemorrhage, intracerebral hemorrhage due to coagulopathy, and acute cardiac failure. These findings strongly suggest the coexistence of MPA and strongly contrast with the survival rates and causes of deaths that are generally recorded for patients with SSc.

Although the association of normotensive rapid renal failure and MPO-ANCA that suggested the existence of a renal specific subset apart from scleroderma renal crisis was noted [13], nearly one-third of the MPA patients with renal involvement were hypertensive [4, 19], and hypertension was not an essential concomitant of renal failure due to SSc even though renal arterial stenosis was observed pathologically in SSc [22]. Blood pressure measurement cannot indicate whether or not scleroderma renal crisis or RPGN associated with AAV exists. However, the association of normotensive renal failure with microangiopathic hemolytic anemia in SSc was indicated [23]. When rapid renal failure or RPGN with active urine sediments or systemic inflammatory manifestations is observed in patients with SSc, the possibility of MPA should always be considered. In these cases, a renal biopsy should be considered to evaluate histological findings because the use of basic laboratory indicators, such as hematuria, proteinuria, or serum creatinine level, is considerably limited in facilitating the prediction of the site affected by vasculitis [2, 4, 24]. The characteristic renal histopathological findings of MPA are pauci-immune necrotizing and/or crescentic focal necrotizing glomerulonephritis as well as small vessel arteritis, which is identical to the lesions seen in WG or renal-limited vasculitis, which is a subtype of MPA [6, 9]. However, this finding distinguishes the disease from polyarteritis nodosa and SSc with fulminating course of malignant hypertension in which mucoid thickening of proximal interlobular arteries and fibrinoid necrosis in distal interlobular arterioles are specific for SSc [25].

Table 3: Differences in vascular clinical findings between SSc and MPA.

	SSc	MPA
Raynaud's phenomenon	+	−
digital ischemia	+	−
skin ulcers	+	+
skin nodules	−	+
Purpura	−	+
nail bed change/telangiectasia	+	−
Myositis	+	+
interstitial pneumonia	+	+
honeycomb lung	+	−
alveolar hemorrhage	−	+
pulmonary hypertension	+	−
RPGN	−	+
scleroderma renal crisis	+	−
mononeuritis multiplex	−	+
CNS vasculitis	−	+
Histological findings;		
fibrinoid necrosis	arteries, arterioles	capillaries, arterioles
intimal hyperplasia	+	−
capillaritis	−	+
pFNCGN	−	+

SSc: scleroderma
MPA: microscopic polyangiitis
RPGN: rapidly progressive glomerulonephritis
CNS: central nervous system
pFNCGN: pauci-immune focal necrotizing crescentic glomerulonephritis.

Mortality is greatest in the setting of pulmonary-renal syndrome in MPA. Pulmonary lesion is a small-vessel vasculitis, with fibrinoid necrosis of capillaries, leading to alveolar septal disruption, blood-filled alveoli, and the clinical sequelae of dyspnea, cough, and/or hemoptysis. The relative risk of death in MPA has been calculated to be 8 times higher in patients with pulmonary hemorrhage [26]. On the other hand, pulmonary hypertension, which is mostly observed in limited cutaneous scleroderma, is rare in MPA, although a few cases with WG and MPA accompanied with pulmonary hypertension have been reported [27]. In SSc, interstitial fibrosis was found to be the most common pulmonary lesion, showing bilateral fibrosis of lower lung or honeycomb lung in chest X-ray. Its presence correlated well with clinical measurements of restrictive lung disease and decreased diffusing capacity. Arteriolar thickening, described as medial hypertrophy or concentric intimal proliferation, was the most specific lesion in lungs, being noted in 29% of SSc patients in autopsy cases [22]. The differences in vascular clinical findings between SSc and MPA are shown in Table 3.

No treatment for SSc is proven to be effective in preventing progression of disease, reversing fibrosis, or improving long-term outcome, although a number of novel agents including anti-interleukin-6, transforming growth factor-β-directed therapies, and other novel biological agents are being developed [28]. However, if SSc patients with MPA have life-threatening visceral involvement such as rapid renal failure or RPGN and pulmonary hemorrhage, the patients should be treated with induction therapy using cyclophosphamide, methotrexate, corticosteroids, or plasmapheresis, and so forth, according to disease severity soon after the diagnosis of MPA, although attention should be paid to reducing treatment toxicity [29].

5. Significance of MPO-ANCA in Overlap Syndrome of MPA and SSc

The etiology of MPA is unknown, but is generally considered to be the result of an interaction between triggering agents and disease susceptibility genes. In primary AAV, MPO-ANCA as well as PR3-ANCA has been established as a marker for diagnosis and has been implicated in the pathogenesis of vasculitis [2, 30]. In most patients with MPA and/or SSc with AAV, high titers of MPO-ANCA are associated with disease activity, rises in MPO-ANCA titers precede relapses, and even a case of MPO-ANCA seroconversion associated with fulminant vasculitis in antitopoisomerase-1 antibody positive SSc has been reported [31], but MPO-ANCA titers do not necessarily correlate with disease activity or vasculitis syndrome. There are also the cases without clinical manifestations related to AAV in spite of MPO-ANCA positivity. The same can be seen for other autoantibodies, such as anti-U1-RNP antibodies, antiphospholipid antibodies, and rheumatoid factor, in rheumatic diseases. Although the reason for cases without clinical manifestations showing MPO-ANCA positivity is not known, the following reasons may be considered: (1) it is a predictive marker for the development of AAV in the future, (2) it is an epiphenomenon, and (3) false positivity. The pathogenic potential of MPO-ANCA to small-vessel vasculitis may be due to not only quantities but also qualities of MPO-ANCA such as immunoglobulin phenotypes, affinity and/or avidity of MPO-ANCA, and specific risk epitope for MPO-ANCA.

Concerning the source of antigens, attention has been focused on neutrophil extracellular traps (NETs)[32], which are chromatin fibers and are released by ANCA-stimulated neutrophils and contain the targeted autoantigens of PR3 and MPO [33] as well as topoisomerase-1. This may be circumstantial evidence that antitopoisomerase-1 antibodies occur more frequently in SSc with AAV than are usually found in SSc as described in the paper of Rho et al. [21].

Some investigators pointed out that ANCA positivity in SSc is a red flag and draws attention [17, 21]. This is true, and it is important not to overlook characteristic clinical manifestations of AAV during the course of the disease in SSc.

Abbreviations

AAV:	ANCA associated vasculitis
AGA:	Allergic granulomatosis angiitis
ANCA:	Anti-neutrophil cytoplasmic antibody
BPI:	Bactericidal/permeability-increasing protein
cANCA:	Cytoplasmic ANCA
ELISA:	Enzyme-linked immunosorbent assay
EMEA:	European Medicines Agency
IIF:	Indirect immunofluorescence
MPA:	Microscopic polyangiitis
MPO:	Myeloperoxidase
NETs:	Neutrophil extracellular traps
pANCA:	Perinuclear ANCA
pFNCGN:	Pauci-immune focal necrotizing crescentic glomerulonephritis
RPGN:	Rapidly progressive glomerulonephritis
SSc:	Systemic sclerosis
WG:	Wegener's granulomatosis.

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Review Article

Vasculitis in Systemic Sclerosis

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Systemic sclerosis (SSc) is a multiorgan connective tissue disease characterized by autoantibody production and fibroproliferative stenosis of the microvasculature. The vascoluopathy associated with SSc is considered to be noninflammatory, yet frank vasculitis can complicate SSc, posing diagnostic and therapeutic challenges. Here, we have reviewed the literature for reports of small-, medium-, and large-vessel vasculitis occurring in SSc. Amongst 88 reported cases of vasculitis in SSc, patients with ANCA-associated vasculitis appear to present a unique subclass in that they combined typical features of SSc with the renal manifestation of ANCA-associated glomerulonephritis. Other vasculitic syndromes, including large-vessel vasculitis, Behcet's disease, cryoglobulinemia, and polyarteritis nodosa, are rarely encountered in SSc patients. ANCA-associated vasculitis needs to be considered as a differential diagnosis in SSc patients presenting with renal insufficiency, as renal manifestations may result from distinct disease processes and require appropriate diagnostic testing and treatment.

1. Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by fibrosis and vasculopathy involving multiple organ systems. Classification into diffuse or limited cutaneous forms depends on the extent of skin thickening, with the former affecting areas proximal to the elbows or knees, and the latter limited to the face and distal extremities [1]. Many clinical complications of SSc are due to dysfunction of vascular beds throughout the body. Involvement of the microvasculature leads to cutaneous and mucosal telangiectasias, digital ulcers, and tissue ischemia. If medium-sized blood vessels are involved, manifestations include gangrene, digital loss, renal crisis, and pulmonary arterial hypertension [2]. While occlusive vasculopathy is a well-recognized feature of SSc, less is known about the occurrence and the consequences of frank vascular inflammation. Albeit rare, typical vasculitis with inflammatory infiltrates damaging blood vessels has been reported in patients with SSc. Distinguishing between noninflammatory vasculopathy and vasculitis can pose a significant diagnostic challenge in the absence of histologic examination. Here, we review reported cases of large-, medium-, and small-vessel vasculitis in association with SSc.

2. Vasculopathy versus Vasculitis

The distinction between SSc vasculopathy and vasculitis can be difficult to make based on clinical presentation alone, but knowledge of the underlying pathogenesis and histopathology can be very helpful. In the current pathogenic model of SSc, a vascular injury of unknown cause leads to endothelial apoptosis and initiates the process of SSc vasculopathy. Autoantibodies, reperfusion injury, infection, and defects in vascular repair have all been implicated as possible instigators [3]. Increased levels of endothelial cells in the circulation have been cited as evidence that the intactness of the vascular lining is jeopardized [3, 4]. Subsequent endothelial dysfunction results in the imbalance of vasoactive factors: decreased levels of vasodilators such as endothelial nitric oxide synthase and prostacyclin synthase, as well as increased levels of the vasoconstrictor endothelin-1 and vascular endothelial growth factor [5, 6]. Continuous endothelial dysfunction likely contributes to activation of adventitial fibroblasts with resultant intimal proliferation, eventual luminal narrowing, and tissue hypoxia [4, 7]. Histopathology of SSc vasculopathy reflects the underlying pathogenesis, with myofibroblast proliferation and matrix

deposit in the subendothelial layer leading to obliterative thickening of vessel walls. Inflammatory infiltrates are absent, and the internal elastic lamina remains intact [8].

In contrast to vasculopathy, concurrent vasculitis in SSc shows histopathologic evidence of inflammation, with presence of mononuclear infiltrates and destruction of the vascular wall. Notably, both vasculopathic and vasculitic changes were seen in five of nine (55%) digital amputation specimens from SSc patients, emphasizing that small vessel vasculitis and stenosing vasculopathy may coexist [8]. Further support has come from autopsy studies of SSc patients, where 24% of 58 cases showed noninflammatory intimal proliferation in two or more organs, but 9% had features of inflammatory polyarteritis [9]. Thus, vasculitis is known to occur even in the setting of a disease predisposing towards vasculopathy, and histology is required to distinguish the two pathogenic processes.

3. Large-Vessel Vasculitis

3.1. Giant Cell Arteritis. Giant cell arteritis is a common vasculitis of the elderly involving large- and medium-sized arteries, typically the temporal, ophthalmic, vertebral, and axillary arteries as well as the aorta. The American College of Rheumatology (ACR) criteria include at least three of the following: (1) onset at age >50, (2) new headache, (3) claudication of the jaw or tongue, (4) temporal artery tenderness to palpation or decreased pulsation, (5) ESR >50 mm/h, and (6) temporal artery biopsy showing vasculitis with mononuclear inflammatory infiltrate or granulomatous inflammation with presence of giant cells [10]. Typical histomorphologic findings include disruption of the internal elastic lamina, thinning of the media, and occlusion of the lumen by hyperplastic intima. Pathogenic studies have established that giant cell arteritis is a T-cell driven disease with participation of Th1 and Th17 lineages [11, 12]. Steroids remain the mainstay of therapy, with many cases resolving after one to two years.

While giant cell arteritis is relatively common among the vasculitides, it has only been reported in three cases of concurrent SSc, all of which were women in their sixth decade with limited skin involvement [13–15] (Table 1). Two of the three had the classic presentation of headache, jaw claudication, and elevated sedimentation rate (ESR), with evidence of vessel wall inflammation and giant cells on temporal artery biopsy. The case reported by Sari-Kouzel was unusual in that the presenting symptom was pain and discoloration of the right foot in the setting of normal ESR. The lower extremity ischemia eventually progressed to gangrene necessitating a below-the-knee amputation. While SSc vasculopathy may have contributed to ischemic tissue damage, the histology from the amputation specimen yielded evidence for vasculitis with the presence of inflammatory infiltrates, giant cells in the vessel wall, and vascular lumen obliteration. All patients were started on corticosteroids (prednisolone or prednisone 30 to 80 mg daily) with slow taper over 5–6 months and resolution of symptoms.

3.2. Takayasu Arteritis. Takayasu arteritis is a relatively rare large vessel vasculitis (incidence 0.4–2/million/year) affecting mostly young women of Asian origin although the incidence among the middleaged with atherosclerosis has been rising [20, 21]. The aorta and its main branches are typically involved. ACR diagnostic criteria include at least three of the following: (1) onset before age 40, (2) claudication of an extremity, (3) decreased brachial artery pulse, (4) >10 mmHg in systolic blood pressure between the arms, (5) bruit over the subclavian arteries or aorta, and (6) stenosis/occlusion of the aorta, its major branches, or large arteries in proximal upper or lower extremities [22]. Similar to giant cell arteritis, the histopathology in Takayasu arteritis shows mononuclear infiltrates in the vessel wall, intimal thickening, destruction of elastic laminas, giant cell formation, and expansion of the adventitial layer. Elastic lamina destruction can lead to aneurysm formation while transmural inflammation drives intimal proliferation, adventitial scarring, and vascular lumen narrowing [23]. Treatment with steroids leads to remission in 40% of patients while 40% may relapse or require addition of a second drug such as methotrexate or azathioprine [24, 25].

Four cases of Takayasu arteritis in the setting of SSc have been reported. As the overwhelming majority of patients with Takaysu arteritis are female, all four of these cases were women, with ages ranging from 29 to 68 [16–19]. Three of the patients had diffuse skin involvement. The presenting symptoms included arm claudication and lightheadedness, and physical examination revealed pulselessness in upper extremities, blood pressure discrepancies >10 mmHg as measured in both arms, and in one case bruits involving the neck and the back. Computed tomographic angiography in all cases showed stenosis of various aortic branches, including the brachiocephalic trunk, common carotid, subclavian, celiac, and renal arteries. Thoracic outlet syndrome was concurrently diagnosed in one case of arteritis, with imaging demonstrating compression of the brachial artery by the scalenus muscle. Two of the four patients were older than 40 years of age at the time of Takayasu arteritis diagnosis, raising the question whether they indeed had typical large vessel vasculitis or whether a component of vessel-obstructive and progressive atherosclerosis was part of the disease process. The reports did not provide information about therapeutic management.

4. Medium- and Small-Vessel Vasculitis

4.1. Polyarteritis Nodosa. Polyarteritis nodosa (PAN) is a necrotizing vasculitis affecting medium-sized vessels, with a constellation of clinical findings that reflect multiorgan involvement. It can be associated with hepatitis B viral infection. PAN can be distinguished from the small-vessel vasculitides such as microscopic polyangiitis by the absence of antineutrophil cytoplasmic antibodies. The ACR diagnostic criteria for PAN include at least three of the following: (1) weight loss >4 kg, (2) livedo reticularis, (3) testicular pain or tenderness, (4) myalgias, weakness, or leg tenderness, (5) mono- or polyneuropathy, (6) hypertension, (7) elevated blood creatinine or urea, (8) serum hepatitis B antigen or

Table 1: Seven cases of large-vessel vasculitis associated with SSc.

Case	Vasculitis	Age/Sex	Age at SSc Dx	SSc type	Age at Vasculitis Dx	Presentation	Diagnosis	Outcome
Perez-Jimenez et al. [13]	GCA	68/F	68	L	70	Headache, jaw claudication; ESR 53	Temporal artery biopsy: inflammatory infiltrate, giant cells	Prednisone 60 mg daily x 5 weeks then tapered. Symptom-free at 5 years
Hupp [14]	GCA	70/F	?	L	70	Headache, jaw claudication; ESR 53	Temporal artery biopsy: mononuclear infiltrate, giant cells, destruction of internal elastic lamina	Prednisone 80 mg daily, tapered to 30 mg daily over 5 months. Symptom-free and ESR 3 at 5 months.
Sari-Kouzel et al. [15]	GCA	64/F	49	L	64	Gangrene of right 2nd and 3rd toes; ESR 14	Right knee amputation biopsy: mononuclear infiltrate, giant cells, vascular lumen occlusion	Prednisolone 30 mg daily, tapered to 10 mg daily over 6 months. Symptom-free at 6 months.
Passiu et al. [16]	TA	29/F	29	D	21	?	?	?
Yago et al. [17]	TA	68/F	66	D	66	Vertigo, bruits in neck, abdomen, and back; asymmetric blood pressure in arms	Stenosis of right brachiocephalic trunk, celiac and left renal arteries	?
Kocabay et al. [18]	TA	48/F	48	D	47	Pulseless in both arms with intermittent claudication	Obliteration of bilateral subclavian arteries distal to vertebral artery bifurcation	?
Kim et al. [19]	TA	37/F	33	L	37	Weak left radial pulse	Stenosis of right common carotid artery, right external carotid artery, thoracic and abdominal aorta, left brachial artery compression by scalenus muscle on abduction	?

GCA = giant cell arteritis; TA = Takayasu's arteritis; SSc = systemic sclerosis; L = limited; D = diffuse; ESR = erythrocyte sedimentation rate.

antibody, (9) aneurysms or occlusions of visceral arteries, or (10) granulocytes on biopsy of small- or medium-sized arteries [26]. A recent retrospective study of 348 patients with PAN found general symptoms in 93.1%, neurologic involvement in 79%, and skin involvement in about 50% [27]. Five-year relapse-free survival was 59.4% for nonhepatitis-B-associated PAN and 67% for HBV-associated PAN.

Predictors of mortality included age greater than 65 years, new onset hypertension, and gastrointestinal manifestations requiring surgery. Treatment relies on glucocorticoids in mild disease and a combination of glucocorticoids and cyclophosphamide in moderate to severe disease.

Only one case of PAN has been described in a 28-year-old woman with diffuse SSc, characterized by Raynaud's phenomenon and skin sclerosis over the hands, arms, and chest [28] (Table 2). The patient developed brownish tender nodules on her legs over three months. Biopsy of these lesions revealed necrotizing arteritis in the deep dermis. Despite the histologic appearance of the nodules, the patient technically did not meet ACR criteria for PAN as she had normal blood pressure and renal function, negative hepatitis B serologies, and no other symptoms such as weakness or neuropathy. Symptoms responded to weekly methotrexate at 20 mg and remainedstable at five months.

4.2. Primary Angiitis of the Central Nervous System. Primary angiitis of the central nervous system (PACNS) is a rare poorly characterized entity affecting small- and medium-sized vessels of the central nervous system (CNS) but not organs or vessels outside the CNS. In general, PACNS is distinguished from secondary CNS vasculitis with the exclusion of infections, malignancy, systemic vasculitis or connective tissue disease, or drug-induced vasculitis. Clinical presentations of PACNS include confusion, new onset headache, seizures, stroke or cerebral hemorrhage, and myelopathy [35]. The duration from symptom onset to diagnosis can range from 3 days to 3 years [36]. Multiple laboratory data abnormalities can occur but none is specific for the diagnosis, with ESR described to be normal in a number of cases. Characteristic changes on cerebral angiography include multifocal segmental stenosis, dilatation, or occlusion of small- and medium-sized leptomeningial and intracranial vessels as well as formation of collateral vessels. Further supportive evidence can be obtained from leptomeningeal or parenchymal biopsies, which are specific but not sensitive for vasculitis given the focal segmental nature of the disease; therefore, a negative biopsy does not rule out the diagnosis. Histology can show either granulomas in small vessel walls, lymphocytic infiltrates, or necrotizing vasculitis [36]. The rarity and heterogeneity complicate classification, diagnosis, and management. Calabrese and Mallek have proposed the following diagnostic criteria for PACNS: (1) recent onset of headache, confusion, or multifocal neurologic deficits, (2) cerebral angiographic changes suggestive of vasculitis, (3) exclusion of systemic disease or infection, and (4) leptomeningeal or parenchymal biopsy to confirm vasculitis and to exclude infection, malignancy, and noninflammatory vascular occlusive disease [37]. Once the diagnosis is made, aggressive treatment with high-dose steroids and cyclophosphamide has been suggested as the management of choice.

One case of PACNS has been described in SSc [29] (Table 2). A 45-year-old woman with limited SSc diagnosed at age 21 presented with new onset headache for 3 days and confusion, later developing hypertension to the 230s/190s and generalized seizure. Computed tomography of the head and spinal fluid was unremarkable. Cerebral angiogram showed an occluded medium-sized branch of the middle cerebral artery as well as narrowing of several distal medium-sized arteries in the anterior and middle cerebral artery distribution. The patient was empirically started on methylprednisolone 100 mg IV every 4 hours for suspicion of PACNS, and her mental status was normalized within 14 hours. A repeat cerebral angiogram of the posterior circulation showed multiple 1.5 cm segments of smooth narrowing in medium-sized arteries. The steroid dosage could not be tapered below prednisone 50 mg daily, as each time the patient developed right facial and arm paresthesias and expressive aphasia. Despite a negative leptomeningeal biopsy, cyclophosphamide was started at 100 mg daily and gradually increased to 200 mg daily with complete resolution of symptoms.

4.3. Mixed Cryoglobulinemia and Cryofibrinogenemia. Mixed cryoglobulinemia is the presence of polyclonal immunoglobulins that precipitate in the serum with cold exposure, often secondary to a connective tissue disease such as systemic lupus erythematosus or Sjogren's syndrome. The presence of cryoglobulins (CGs) may be asymptomatic or may lead to manifestations of the cryoglobulinemic syndrome, including purpura, arthralgia, myalgia, glomerulonephritis, and peripheral neuropathy [38]. The diagnosis of the latter entails a combination of clinical presentation, laboratory testing showing the presence of circulating cryoglobulins, and histopathologic appearance such as leukocytoclastic vasculitis (most common). Similarly, cryofibrinogenemia is the presence of cold-induced precipitants in the plasma but not in the serum. Connective tissue diseases, malignancy, and infection have been known to be associated with this condition, which can be asymptomatic or can manifest as painful ulcers, purpura, or perniosis, reflecting possible underlying cold-induced thromboses, increased blood viscosity, or vascular reactivity. The diagnosis of clinically significant cryofibrinogenemia requires not only circulating cryofibrinogen (CF) but also clinical features and histopathologic evidence of small-vessel thrombosis and perivascular infiltrate [39]. For both mixed cryoglobulinemia and cryofibrinogenemia, treating the underlying disease (whether infection, connective tissue disease, or malignancy) can sometimes improve symptoms. Plasmapheresis and immunosuppression with glucocorticoids and/or cytotoxic therapy have also been used in severe disease although with unclear efficacy.

Connective tissue diseases have been associated with the presence of both CG and CF, perhaps more so than CF alone [40]. In the few studies and reports involving SSc, these cold-induced precipitants do not appear to trigger symptoms. In one study, one out of 19 patients with both CG and CF carried the diagnosis of SSc [40]. In another study, 10 out of 20 SSc patients had the presence of polyclonal IgG and IgM cryoglobulins in the serum, but none exhibited clinical signs of cryoglobulinemic syndrome [41]. In one report of long-standing SSc with the presence of cryoglobulins (both IgG and IgM), the patient presented with paresthesias, transient aphasia, vision changes, and delirium [30] (Table 2). Cerebral angiogram was normal, and electroencephalogram revealed generalized slowing of action potentials, and computed tomography of the extremities revealed calcinosis. While peripheral neuropathy can be a manifestation of mixed cryoglobulinemia, central nervous system involvement would be highly unusual; therefore it, is unclear whether the presence of cryoglobulins in this case is an incidental finding. Another man with long-standing SSc presented with sudden onset gangrene in the fingers and toes after cold exposure and was found to have very elevated cryofibrinogen [31]. He did not respond to prostaglandin E1 or subcutaneous heparin and died shortly after presentation. No biopsy was done to ascertain the etiology for the gangrene, therefore, either underlying SSc vasculopathy or thrombosis from the cryofibrinogenemia could have been possible causes.

4.4. Behcet's Disease. Behcet's disease is characterized by recurrent oral aphthous ulcers and other systemic manifestations believed to be due to systemic vasculitis, including genital aphthous ulcers, ocular disease, skin involvement,

Table 2: Seven cases of medium- and-small vessel vasculitis associated with SSc.

Case	Vasculitis	Age/Sex	Ag at SSc Dx	SSc type	Age at Vasculitis Dx	Presentation	Diagnosis	Outcome
Pathak and Gobor [29]	CNS	45/F	21	L	45	Headache, confusion, seizure, aphasia; right face/arm/leg numbness	Angiography with abrupt cutoff in MCA branch, narrowing in ACA and MCA branches; leptomeningeal biopsy negative	Improved with methylprednisolone then cyclophosphamide
Kang et al. [28]	PAN	28/F	28	D	28	Brownish tender nodules on legs	Skin biopsy: necrotizing arteritis in deep dermis	Stable on weekly methotrexate 20 mg
Jiménez López et al. [30]	Mixed CG	50/F	50	L	50	Paresthesias, vision changes, aphasia, delirium; rash on lips, palms, soles	Cryoglobulin > 0.1mg/100mL Electroencephalogram: generalized slowing Cerebral angiogram: normal	?
Barrett et al. [31]	CF	49/M	38	L	49	Sudden onset gangrene of fingers and toes; confusion	Cryofibrinogen 435 mg/L (<40)	Died despite Subcutaneous heparin and prostaglandin E1
Choy et al. [32]	BD	54/F	54	D	16	Oral/genital ulcers, superficial throm-bophlebitis, rash, esophageal dysmotility, no tear production, left knee arthritis	Grade II esophagitis ILD with restrictive PFTs Rash with pathergy	Unresponsive to topical steroids; later azathioprine 100 mg daily, eye drops, D-penicillamine, colchicine; arthritis continued
Yokota et al. [33]	BD	62/M	57	L	60	Oral/genital ulcers, erythema nodosa, esophageal and gastric ulcers, chronic hepatitis C, pancytopenia	?	Esophageal ulcers resistant to prednisolone 30 mg daily; died from pneumonia
Sugisaki et al. [34]	RPC	35/M	31	L	35	Left auricular ulcer and swelling; polyarthralgia	MRI: partial defect in nasal septum	Improved with prednisolone 15 mg daily and not with antibiotics

CNS = central nervous system vasculitis; PAN = polyarteritis nodosa; Mixed CG = mixed cryoglobulinemia; CF = cryofibrinogenemia; BD = Behcet's disease; RPC = relapsing polychondritis; SSc = systemic sclerosis; L = limited; D = diffuse.

gastrointestinal ulcers, neurologic disease, and arthritis. It is more common along the ancient Silk Road, with 0.11% prevalence in Turkey and 2.6% per 100,000 in Southern China [42]. Diagnosis is made based on clinical features including presence of recurrent oral aphthae plus two of the following without other systemic disease: (1) recurrent genital aphthae, (2) eye lesions including uveitis or retinal vasculitis, (3) skin lesions including erythema nodosum, pseudovasculitis, papulopustular lesions, or acneiform nodules, or (4) positive pathergy test [43]. Treatment for mucocutaneous and joint disease includes colchicine (mixed results), glucocorticoids, and other immunosuppressives such as azathioprine. More serious disease with internal organ involvement has been treated with cyclophosphamide and high-dose steroids.

Two cases of Behcet's disease with concurrent SSc have been reported [32, 33] (Table 2). Recurrent oral and genital ulcers were the predominant symptoms in both cases, and neither had eye involvement. In the case of the 54-year-old woman with diffuse SSc, the diagnosis of Behcet's disease was made based on recurrent oral and genital ulcers and presence of pathergy; skin involvement was nonspecific [32]. Her more bothersome symptoms were related to her SSc including restrictive lung disease, esophageal dysmotility, and polyarthritis, as well as secondary Sjogren's syndrome. The recurrent ulcers initially responded well to azathioprine 100 mg daily; later she was switched to colchicine with unknown effect. In the case of the 62-year-old man with limited SSc based on biopsy-proven sclerodactyly, the presence of recurrent oral and genital ulcers as well as erythema

nodosa led to the diagnosis of Behcet's disease [33]. He had concurrent chronic hepatitis C complicated by pancytopenia. He later developed esophageal and gastric ulcers that were felt to be consistent with enteric Behcet's. These ulcers were resistant to prednisolone 30 mg daily.

4.5. Relapsing Polychondritis. Relapsing polychondritis (RPC) is an inflammatory disease of unknown etiology involving cartilagenous tissues in multiple organs, typically the ears, nose, eyes, respiratory tract, and joints. Vascular and neurologic complications have also been reported. Association with systemic vasculitis, connective tissue disease, or myelodysplastic syndrome occurs in up to one-third of the cases. The original diagnostic criteria by McAdam required three of six clinical manifestations: (1) bilateral auricular chondritis, (2) nonerosive seronegative polyarthritis, (3) nasal chondritis, (4) respiratory tract chondritis, (5) ocular inflammation, or (6) cochlear and/or vestibular dysfunction [44]. The criteria were later modified to include the presence of three or more of the above, one clinical manifestation with corroborating histology, or chondritis at more than two sites responsive to steroids or Dapsone [45]. Given the rarity and relapsing nature of the disease, treatment has been empiric, with Dapsone and glucocorticoids favored for mild nonvisceral disease, and high-dose steroids and possible adjunctive immunosuppressants for organ-threatening disease.

Only one case of RPC has been reported in association with SSc [34] (Table 2). A 35-year-old man with limited SSc presented with a known nasal septal perforation presented with left auricular swelling and polyarthralgia. The left auricle later was ulcerated with drainage of pus and was felt to be super infected with *Pseudomonas aeruginosa*. However, the ulcer did not improve with antibiotics but rather with prednisolone 15 mg daily.

5. ANCA-Associated Vasculitis (AAV)

The spectrum of necrotizing small-vessel vasculitis known as ANCA-associated vasculitis (AAV) includes Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and Churg-Strauss syndrome (CSS). While formally classified as small-vessel vasculitis, AAV can involve medium-sized vessels. Cytoplasmic antineutrophil cytoplasmic antibodies (c-ANCAs) directed against proteinase 3 (PR3) are more commonly found in WGs whereas perinuclear ANCAs (p-ANCA) targeting myeloperoxidase (MPO) are more frequently seen in MPA and CSS. Variable organ involvement makes diagnosis a challenge, with alveolar hemorrhage and crescentic glomerulonephritis frequently occurring in WG and MPA, while polyneuropathy can be seen in ANCA-associated CSS [46]. Disease stage can range from localized without end-organ damage to severe generalized with organ failure. Treatment of generalized disease involves induction with cyclophosphamide (whether oral or intravenous) followed by maintenance with less toxic drugs such as azathioprine, methotrexate, or myclophenolate mofetil. Standard therapy leads to remission in 90% to 94% of AAV patients within six months although relapses frequently occur (18 to 40% in WG at 24 months) [47].

Of all the small vessel vasculitides, AAV is the most frequently reported in association with SSc, raising the question whether an overlap syndrome exists that combines features from both diseases. A study by Rho et al. found 31 reports containing 63 cases of AAV in SSc up to 1994 [56]. Fifty of the 63 cases provided sufficient clinical and laboratory information and were included in the analysis. Eighty-four percent were women with a mean age of 57.1 years. Cases of limited and diffuse SSc were equally represented. Autoantibody profiling showed 72% with positive ANA (titers unknown), 70% with anti-Scl-70 antibody, and 72% with positive anti-MPO antibody. The most common end-organ involvement included kidneys (82%) and lungs (70% had pulmonary fibrosis). Outcomes were not described in 17/50 (34%), but analysis of the remaining cases yielded a 7-year survival rate of 67.9%, with a high mortality rate within the first year. Furthermore, anti-Scl-70 antibody was found to be a significant predictor of developing AAV in SSc (OR 3.1, 95% confidence interval 1.11-8.55, P value .031). There was no difference in AAV occurrence between SSc subtypes (P value .998), the presence of MPO versus. PR3 ANCA (P value .196), or prior use of D-penicillamine (P value .143).

In our review of the literature, we found eleven additional cases of AAV in SSc that were not cited as part of the Rho study [48–55] (Tables 3 and 4). Seven (64%) were female with a mean age of 53 ± 14 years (range from 19 to 71 years). SSc disease duration was known for 9 of the 11 cases, with a mean of 4.49 ± 3.37 years (range from 0.42 to 10 years). The time from SSc diagnosis to AAV onset was known for 10 cases and reached a mean of 3.85 ± 2.67 years (range from 0.42 to 8 yrs). Seven patients (64%) had diffuse skin involvement, and four (36%) were previously treated with D-penicillamine. Renal involvement in the form of crescentic glomerulonephritis on renal biopsy was seen in 9 cases (82%) while pulmonary fibrosis was seen in 3 cases (27%). One case was complicated by multiple cerebral hemorrhages [55]. Eight cases (88%) had positive ANA of at least 1 : 320, 5 cases (45%) with positive anti-Scl-70 antibody, and only 1 case (9%) with Anticentromere antibody. Almost all cases (91%) had positive anti-MPO antibody while none had anti-PR3 antibody.

These findings highlight the importance of considering crescentic glomerulonephritis related to AAV as a potential cause of renal insufficiency in SSc patients. Classically, scleroderma renal crisis occurs in up to 20% of patients with diffuse SSc, and renal involvement manifesting as hypertension, proteinuria, or azotemia can be found in 45–60% [57]. However, causes other than scleroderma renal crisis should be considered as a differential diagnosis, especially in settings of normotension or ANCA positivity.

6. Conclusion

While lumen-occlusive vasculopathy is a prominent feature of SSc, frank vasculitis may also occur. Coexistent SSc and vasculitis have been reported for vessels of all sizes, either before or after SSc diagnosis, and in either SSc subtype (limited or diffuse). We found in the literature 88 cases

Table 3: Eleven new cases of ANCA-associated vasculitis in SSc.

Case	Age/Sex	SSc (yrs)	SSc to AAV (yrs)	SSc type	Use of D-penicillamine	Renal	Pulm fibrosis	ANA	Scl-70	MPO
Marlier et al. [48]	62/M	5	5	L	Yes	CGN	No	?	?	+
Martínez Ara et al. [49]	63/F	2	2	?	No	CGN	No	1 : 640	+	+
Herrera-Esparza et al. [50]	60/M	2	3	L	No	CGN	No	1 : 2560	?	+
Hidalgo-Tenorio et al. [51]	48/F	1	1.5	D	No	None	Yes	1 : 320	Neg	+
	71/F	?	?	L	No	None	No	1 : 320	Neg	+
Kamen et al. [52]	45/M	0.42	0.42	D	No	CGN	No	1 : 2560	+	+
	19/M	5	5	D	Yes	CGN	No	1 : 2560	+	+
	60/F	8	8	D	No	CGN	No	?	+	+
Arnaud et al. [53]	46/F	10	5.75	D	Yes	CGN	Yes	>1 : 1000	+	+
Ramaswami et al. [54]	52/F	?	0.83	D	No	CGN	No	1 : 640	?	?
Veetil and Schimmer [55]	62/F	7	7	D	Yes	CGN	No	?	?	+

Note: All but Ramaswami had positive MPO, and none had PR3. SSc = Systemic sclerosis; L = Limited; D = Diffuse; CGN = crescentic glomerulonephritis; ? = unknown.

Table 4: Characteristics of eleven cases of AAV in SSc.

Characteristic	Number (%)
Sex (M/F)	4/7 (36%/64%)
Age (mean ± SD)	53 ± 14 (range from 19 to 71)
SSc duration (mean ± SD in yrs); 9 pts	4.49 ± 3.37 (range from 0.42 to 10 yrs)
SSc Dx to AAV dx (mean ± SD in yrs); 10 pts	3.85 ± 2.67 (range from 0.42 to 8 yrs)
SSc type (Diffuse/Limited)	7/3 (64%/27%)
D-penicillamine use	4 (36%)
Renal involvement	9 (82%) all with crescentic GN on Bx
Pulmonary involvement	2 (18%)
ANA + (> 1 : 320)	8 (88%)
Scl-70 +	5 (45%)
Anticentromere +	1 (9%)
MPO +	10 (91%)
PR3 +	0

of vasculitis in SSc, with the vast majority being ANCA-associated vasculitides (74 cases: 63 cited by previous studies, 11 new). Rare cases of Takayasu's arteritis (4 cases), giant cell arteritis (3 cases), and Behcet's disease (2 cases) have been reported in patients affected by SSc. Only single-case reports have focused on other vasculitic syndromes, including mixed cryoglobulinemia/cryofibrinogenemia, polyarteritis nodosa, primary angiitis of the central nervous system, and relapsing polychondritis, suggesting that the association between SSc and these disorders may be a chance event. Although most patients exhibited classic symptoms and signs for the respective vasculitides, confirmation of diagnoses and distinction from SSc rested on histology. Prompt treatment with immunosuppression usually resulted in stabilization of symptoms. In SSc patients with renal insufficiency and ANCA positivity, crescentic glomerulonephritis related to AAV should be considered as a differential diagnosis.

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