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Etiopathogenesis, Prevention, Clinical Evaluation and Treatment of Scleroderma Skin Ulcers

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ABSTRACT

Introduction. Systemic Sclerosis (SSc) is a connective tissue disease, characterized by fibroblast dysfunction leading to increased deposition of collagen and variable degree of fibrosis of skin and internal organs, small vessels vasculopathy, immune system alterations. SSc skin ulcers (SSc-SU) occur in about 50% of patients, representing one of the most frequent and debilitating clinical manifestation, and with multifactorial pathogenesis. In the absence of a validated classification, we have adopted, since many years, a personal approach based on peculiar features characterizing the different sub-type of SSc-SU; namely, digital ulcers (DU) occurring on fingertips of hands and feet; SU on bony prominence, particularly at level of metacarpophalangeal and interphalangeal joints and elbow; SU occurring on calcinosis; SU of lower limbs. No validated guidelines for management of SSc-SU are still available.

Aim of the study. Aim of this Doctorate Thesis was to provide new data helpful to clarify some clinical and therapeutic points, not previously explored in literature.

General features of our SSc population, demographic, clinical and serological correlations of SSc patients with SU, defined according to the classification previously described, are herein reported. Personal experience of the last three years on SSc-SU is summarized.

Patients and methods. Patients affected by SSc referred to our Rheumatology Unit were enrolled. Demographic, clinical, serological and instrumental data were recorded baseline and at follow-up. SU were classified as described. Treatment with

vasoactive drugs was tailored on single SSc patient with SU. As suggested by personal experience, local therapy of SU was invariably based on the wound bed preparation according to "T.I.M.E" principles (removal of necrotic tissue, resolution of infection and inflammation, correct moisture balance, progression of the edge).

Results. Two hundred and eigthy-two SSc patients (254 females, 28 males) were enrolled; 84.2% showed limited cutaneous subset, 15.8% diffuse cutaneous subset; anti-topoisomerase antibodies (Scl-70) were positive in 33.5%, anticentromere antibodies (ACA) in 44.8%, Antinucleolar antibodies (ANoA) in 15.7%. SU were recorded in 156 patients characterized by: lower mean age at Raynaud's and SSc onset and at the beginning of follow-up, diffuse cutaneous subset, sex male, calcinosis, teleangectasia, melanodermia and increased value of erythrocyte sedimentation rate (ESR) and reactive C protein (RCP). The following associations with subgroups were observed: DU of the hands: lower mean age at Raynaud's phenomenon and SSc onset, and at beginning of follow-up, male gender, diffuse cutaneous subset, melanodermia, teleangectasia, calcinosis; with inverse correlation with ACA. DU of the feet: male gender, calcinosis, teleangectasias and increased value of ESR and RCP. SU on bony prominence: lower mean age at SSc onset and at the beginning of follow-up, with longer disease duration, while non differences were observed about gender. Moreover this group of SU corretated with diffuse cutaneous form of the disease and positivity for anti-topoisomerase, calcinosis, melanodermia, teleangectasia increased value of ESR and RCP. SU secondary to calcinosis correlated with lower mean age at SSc onset,

presence of calcinosis; SU of the lower limbs*:* mean increased value of derived PAPs at echocardiography, ESR and RCP.

Starting from this SSc cohort, the following points were explored: **1**. the efficacy and the safety of platelet gel in 12 SSc-SU, observing a resolution of the lesion or a significant improvement in 83.3% of patients; **2.** the role of fecal pathogens in DU complicated by infection, showing that in 42 infected DU, the pathogens involved were *Escherichia coli* or *Enterococcus faecalis* in 26%, *Staphylococcus aureus* 50%, *Pseudomonas aeruginosa* 12%, other less frequent pathogens in the remaining 12%; **3.** the prevalence of ostomyelitis complicating SU in SSc patients, resulting in 7.7 % in the entire SSc patients' series examined. **4.** Definition of a systematic approach to analgesic treatment for procedural pain during surgical courretage during local medications, obtaining an optimal compliance and good response to local treatment. **5.** Validation study of the capillaroscopic skin ulcers risk index (CSURI) able to predict the appearance of SSc-DU within three months since evaluation. The score showed high specificity and sensitivity. **6.** Building of a risk chart considering the multifactorial pathogenesis of SSc-DU, able to identify SSc patients at high risk to develop DU in the next six months; According to multivariate analysis it was based on gender, previous history of ulcers, erythrocyte sedimentation rate, high value of CSURI. The chart showed a predictive positive value of 90.7%, a negative predictive value of 89.8%, and very high sensitivity and specificity. **7**. Preliminary, non published, data about possible role of nano-particles in SSc ethiology revealed increased value of silicon,

aluminium, chromium, titanium and zinc in SSc patients when compared with controls.

Conclusions: SU represent a very frequent and complex clinical manifestation in course of SSc. Because of the lack of data in literature we elaborated a clinical-prognostic classification also suggesting the importance of an olistic approach including both systemic and local treatment. Moreover, in the last years we have suggested a new approach to predict DU appearance, we have described some important features of infection in SSc-SU suggesting careful attention to this complication responsible for non-healing, also suggesting a systematic approach to management of analgesia during surgical debridement.

1. INTRODUCTION

1.1 Systemic Sclerosis (Scleroderma)

Systemic Sclerosis (SSc) is a connective tissue disease, characterized by fibroblast dysfunction leading to increased deposition of collagen and variable degree of fibrosis of skin and internal organs, small vessels vasculopathy with consequent diffuse typical microangiopathy, immune system alterations included production of autoantibodies[1,2].

1.1.1 Epidemiology

Epidemiological data from literature are scarce and often divergent. In 2002, Ferri C. et al. published a survival multicenter study, describing a series of 1,012 Italian SSc patients, which represents the largest observational study on SSc[1].

The actual incidence and prevalence of the disease are generally underestimated: in fact, they may reach 20/million/year and 1500/million of habitants, respectively. The clinical spectrum, including a wide range/grade of signs and symptoms, may explain, at least in part, this underestimation.

Generally, the exitus is due to heart, lung or renal (rare, nowadays) involvements[3].

SSc is 3-8 times more frequent in females, and presents an incidence peak in the 4°-6° decades of age[1,2].

In 2014 Ferri et al. published an Italian study on 821 SSc patients recruited between 2000 and 2011 about evolution, pathomorphosis and survival of the disease [4]. Comparing with older Italian SSc series,

these data showed a significantly increased prevalence of limited cutaneous SSc and serum anti-centromere antibodies with a significant reduction of lung, heart, and renal involvement, and skin ulcers. Cumulative 10th-year survival showed a clear-cut increase (80.7%) compared to previous series (69.2%). These data confirmed the results of other survival studies published during the last five decades, grouped according to the time periods of patients" recruitment at the referral centers. This favorable evolution of SSc pathomorphosis and prognosis during the last decades might be related to more diffuse physician/patient awareness of this harmful disease and availability of diagnostic tools, the consequent wider recruitment of patients in the early stages of the disease, as well as to the improved therapeutic strategies.

1.1.2 Etiopathogenesis

The etiopathogenesis of SSc is almost unknown, but surely multifactorial. As other autoimmune disorders, exogenous triggers (toxic – aromatic hydrocarbons, pentazocine, bleomycin, silicon – or infectious – parvovirus B19, cytomegalovirus) have been proposed[5]. Therefore, peculiar triggers would start an autoimmune process in genetically susceptible individuals. However, the impossibility to define genetic models of the disease implies that a number of probable different loci be involved. On the other hand, a few elements suggest that genetic factors should be included in the etiopathogenesis: the variations of prevalence and phenotype of SSc in different ethnic groups (see the high prevalence of SSc among Choctaw Indians because of the mutation of the gene FBN1)[6]; the existence of familiar

SSc forms, with a disease risk amount of 11-15 times higher than general population[7]; the positivity of autoantibodies in the relatives of SSc patients; the known HLA associations, such as the HLA-DR5 with the diffuse SSc subset, the HLA-DR1 with the limited one, the HLA-DR3/DRw52a with pulmonary fibrosis, the allele DQA1*0501 with a subset of diffuse cutaneous male SSc patients[8]; few animal models, such as tsk-1 mice or UCD L200 chickens; finally, the associations between SSc and several genetic polymorphisms, such as collagen (COL1A2 among Japanese patients), fibronectin, CXCR-2, TGF-beta, TNF-alpha, and others[8].

SSc pathogenesis may be simplified into three pivotal points: the activation of the immune system against self epitopes; the endothelial dysfunction; the activation of fibroblasts. Briefly, these points will be discussed.

Fibroblasts produce the components of the connective tissue, and different subtypes of fibroblasts perform a variety of functions in different locations. In fact, although morphologically similar, these different subpopulations are distinguishable by their gene expression profiles and functional activities[9]. After a tissue injury, quiescent fibroblasts are activated, producing granulation tissue and a provisional matrix, a healing process that is subsequently reversed to remodel the scar. In SSc, this scar tissue is not correctly terminated or remodelled, resulting in excessive fibrotic tissue formation[9]. Moreover, the phenotype of SSc fibroblasts is comparable with that of fibroblasts exposed to excessive signaling by TGF-beta, which suggests a role in pathogenesis[10].

Even if activated, these fibroblasts may not be terminally differentiated and in cultures they can exhibit an activated phenotype after several passages. SSc fibroblasts may derive from mesenchymal precursors recruited from the bone marrow or be quiescent fibroblasts cells activated by direct cell–cell contact, or by soluble mediators, such as CTGF, PDGF, endothelin-1, or by modulation of cell–matrix interactions[9]. Apart from connective tissue production, SSc fibroblasts may secrete cytokines, such as IL-6 and PDGF, which in turn stimulate MCP-1 (monocyte chemoattractant protein-1) secretion in an autocrine manner[11]. Actively synthesizing fibroblasts were frequently located near small blood vessels and surrounded by infiltrating mononuclear cells, suggesting an interaction between lymphocytes and fibroblasts.

Overall, the role of fibroblasts as effectors in the SSc pathogenesis is almost established.

In course of SSc, both humoral and cellular immunological alterations have been observed[11]. Activated lymphocytes and autoantibodies are detectable, as well as elevated levels of interleukins (IL-2, IL-4, IL-6, IL-10, IL-13) and chemokines (including MCP-1 and IL-8). Activated T cells have been demonstrated in both the circulation and the affected organs (namely, skin and lungs), with increase of CD4/CD8 ratio; moreover, there is evidence of clonal expansion in response to one or more specific antigens (probably exposed by fibroblasts). Also the IL-2 receptor level is increased and correlates with the extension of cutaneous fibrosis. In skin biopsies, infiltration of B lymphocytes has been evidenced; moreover, the depletion of these cells in tsk-1 mice, model of SSc, suppresses fibrosis. High circulating levels of specific

autoantibodies has been showed, in particular against nuclear antigens (topoisomerase, RNA polymerase), against endothelial cells (antibodies which induce apoptosis), against fibrillin-1 (able to activate fibroblasts to release TGF-beta), and anti-PDGF receptor (which may stimulate fibroblasts to the production of collagen type I and to the shift to an activated phenotype). AECA, antibodies against endothelium, may also participate in the pathogenesis of SSc by inducing the expression of adhesion molecules and enhancing the adherence of leukocytes; nonetheless, AECA-dependent ADCC was shown to induce apoptosis in human dermal microvascular endothelial cells, via Fas. On the whole, it is well accepted that the activation of the immune system produces a number of humoral mediators, which induce also the overproduction of connective tissue. In patients with SSc, endothelial function and vascular remodeling are dysregulated; in particular, vasculopathy may result from a disrupted or inappropriate repair process following the endothelial injury[12]. The latter expresses the up-regulation of vasoconstrictive, aggregant, and mitogenic factors compared to the vasodilatory, anti-thrombogenic, and anti-mitogenic ones. The unbalance of the vascular milieu produces structural changes of vessel walls, apart from the functional disorder; therefore, in situ thrombosis, intima thickening and media fibrocells proliferation may be observed. Obviously, fibrosis of the whole vessel wall produces an impairment of elasticity, while vascular narrowing plus the eventual thrombi lead to peripheral ischaemia, oxygen radicals formation, and reperfusion tissue damages. Consistent evidences suggest that vascular alterations are preceded by endothelial dysfunction, which is ubiquitous and early in the course of SSc[13].

Endothelial cells express adhesion molecules (ICAM-1 and VCAM-1), which promote perivascular lymphocyte and monocyte migrations, and platelet activation. Moreover, endothelium secretes several circulating mediators, such as endothelin-1, PDGF e TGF-beta1, which stimulate fibroblasts and fibrocells of the media; however, even though these angiogenic factors are increased, the response to tissue ischaemia remains inefficient[14]. Indeed, the up-regulation of VEGF (one of the strongest angiogenic factors) and its receptors could represent a tentative of compensation; alternatively, a chronic and uncontrolled over-expression might produce a paradox effect[14], as evidenced in other physiological situations. According to the hypothesis of a compensation, we may read the recent findings about endothelial progenitors cells (CD34+ CD133+ VEGF2R+)[15]; these cells are produced in the bone marrow from the hemangioblasts, precursors also of erythrocytes, entered into blood circulation, and addressed to ischaemic tissues in order to build new vessels. In SSc patients, the production and the function of these progenitor cells resulted disrupted.

In conclusion, a complex link between immune system cells, endothelial cells and fibroblasts may be found. It remains to be studied which is the *primum movens*.

1.1.3 Anatomopathology

Generally, typical SSc features are tissue fibrosis, lymphomonocyte infiltration, and vascular alterations.

Skin fibrosis is characterized by levelling of dermal papillae, loss of skin annexes, thickening of the dermis due to abnormal collagen and

interstitial matrix deposits. Fibrosis also involves internal organs where parenchyma is destroyed and replaced by fibronectin, proteoglycans, collagen fibers type I and III, less IV and VI[16].

The microvascular alterations appear early and it is characterized by intima thickness and fibrocells proliferation of the tunica media[17]. Capillaries' alterations may be well visualized by means of nailfold capillaroscopy; dilatations, typical megacapillaries (diameter usually > 100 micron) microhemorrages, or arborescent capillaries. In general, the microvascular architecture is deranged, with rarefaction or even desertification areas[18].

As autoimmune disorder, SSc presents a lymphomonocyte infiltration in involved tissues, involving mainly T cells, but also B cells and macrophage[19].

1.1.4 Classification and diagnosis

SSc is classified in two or three subsets on the basis of skin involvement: limited and diffuse (LeRoy classification)[20], or limited, intermediate, and diffuse (Italian classification)[21] (fig. 1).

- *Limited cutaneous SSc* = sclerosis of the fingers (sclerodactyly) with or without sclerosis of face, neck, and armpits.
- *Intermediate cutaneous SSc* = sclerosis of face, neck, limbs, but not trunk.
- *Diffuse cutaneous SSc* = sclerosis of the trunck and the limbs.

  "Sine scleroderma" SSc is a rare subset characterized by visceral involvement, exclusively.

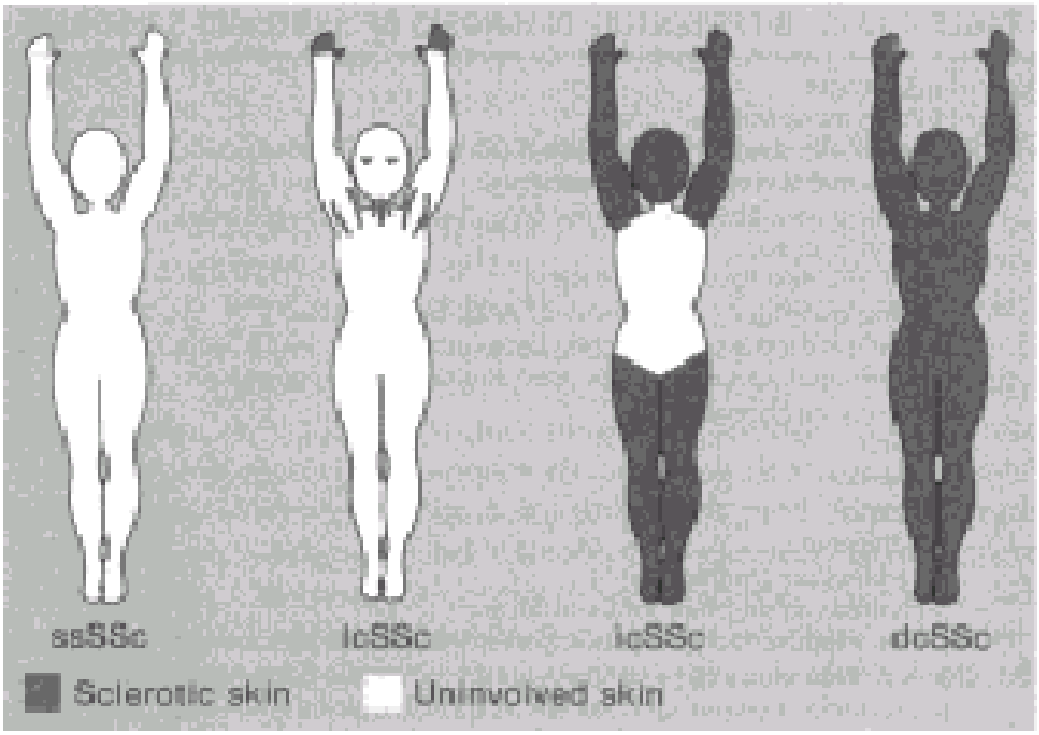


Fig. 1 – Systemic sclerosis classification according to skin involvement (black area): sine scleroderma SSc (ssSSc): absence of skin sclerosis but typical visceral involvement, capillaroscopic alterations, autoantibodies. Limited SSc sclerodactily with or without sclerotic lesions of the neck, of the face, of axillas. Intermediate SSc sclerosis of legs and arms, neck, face, without thorax involvement. Diffuse SSc skin sclerosis of legs, arms and thorax. From: Ferri C., Valentini G., Cozzi F. Systemic sclerosis: demographic, clinical and serologic features and survival in 1012 Italian Patients. Medicine 2002; 81:139-53.

A diagnostic test proving the absence or the presence of systemic sclerosis is not available; several sets of classification criteria have been developed with the aim to enroll patients really affected by SSc in research studies. In 1980 preliminary criteria for classification of SSc were developed by American College of Rheumatology using patients with longstanding SSc leading to miss correct classification of about 20% of limited cutaneous SSc[22] (tab 1).

Because of the low sensitivity of these criteria and the acquisition of new knowledge about SSc along the years, in 2013 a committee of

ACR and European League Against Rheumatism has proposed a new, more sensitive and specific, set of classification criteria[23].

The new criteria include 1 criterion that is alone sufficient for classification as SSc, namely skin thickening of the fingers extending proximal to the metacarpophalangeal joints, if this criterion is absent, the point system is applied and patients with a score of 9 are classified as having SSc (tab 2).

Tab 1 - Classification with the major criterion or at least 2 minor ones.

Classification Criteria – ACR, revised in 1987
Major criterion:
sclerosis of the skin proximal to fingers
Minor criteria:
Sclerodactyly
necrotic ulcers or substance loss at the fingers
XR-evident bibasilar pulmonary fibrosis

Tab 2 - The American College of Rheumatyology/European League Against Rheumatyism criteria for the classificatipon of systemic sclerosis

ITEM	SUB-ITEM	WEIGHT SCORE
Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints (sufficient criterion)	-	9
Skin thickening of the fingers (only count the higher score)	Puffy fingers Sclerodactily of the fingers (distal to metacarpophalangeal joints but proximal to the proximal interphalangeal joints)	2 4
Fingertips lesions (only count the higher score)	Digital tip ulcers Fingertips pitting scars	2 3
Teleangectasia	-	2
Abnormal nailfold capillaries	-	3
Pulmonary arterial hypertension and/or interstitial lung disease	Pulmonary arterial hypertension Interstitial lung disease	2 2
Raynaud's phenomenon	-	3
SSc-related autoantibodies (centromere, anti-topoisomerase I, anti-RNA polymerase III) (maximum score is 3)	Anticentromere Anti-topoisomerase I Anti-RNA polymerase III	3

These criteria are appliocable to any patient considered for inclusion in an SSc study. The criteria are not applicable to patients with skin thickening sparing the fingers or to patients who have a scleroderma-like disorder that better explains their manifestations (e.g. nephrogenic sclerosing fibrosis, generalized morphea, eosinophilic fasciitis, scleroderma diebeticorum, scleromyxedema, erythromyalgia, porphyria, lichen sclerosis, graft-versus-host-disease, diabetic cheiroarthropathy). The total score is determnined by adding the maximum weight (score) in each category. Patients with a total score of ≥9 are classifiied as having definite SSc

1.1.5 Clinical features [1,2,24 25]

SSc presents a wide spectrum of clinical features, with possible involvement of almost all organs and systems. The beginning may be shifty and underecognized; in other cases the disease may appear abruptly and in a striking way.

Raynaud's phenomenon is generally the first symptom and can sometimes precede the onset of SSc from few months to several years (fig 2).

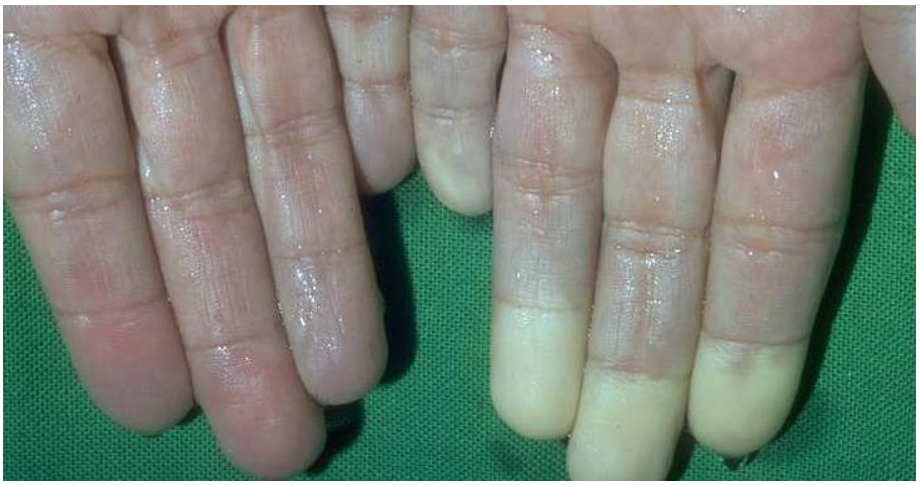


Fig 2 – Raynaud's phenomenon

Other possible manifestations at the beginning of scleroderma could be oligo-arthritis, myositis or visceral symptoms.

Skin involvement (hence, the name "scleroderma") is generally present in all patients, with variable extension; the first step is the "scleredema", that is an edematous thickening of the cutis. In a second time, skin becomes stiff, adherent to lower level, without wrinkles, so inelastic to limit articular excursions. Finally, skin becomes atrophic, thin, xerotic, and hyperpigmented.

Fingers involvement, particularly of flexor tendons, produces sclerodactyly (fig 3) and a deformation in a claw manner. Ischaemia leads to ulcerations of the tips, possibly to phalanx amputations and bone reabsorption. Face involvement is known as "sclerodermic facies" (fig 3), characterized by thin lips, reduced opening of oral cavity with teeth exposure, levelling of the wrinkles, nose sharpening, eyebrows alopecia, and teleangectasias.

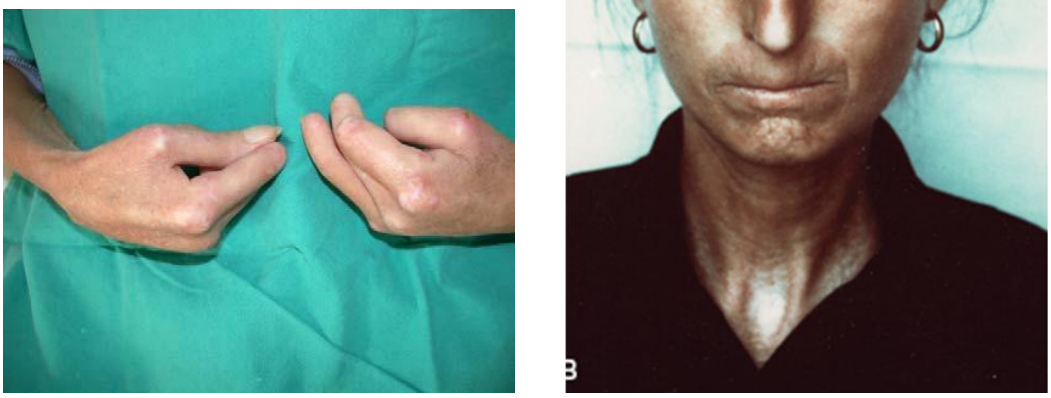


Fig 3 - Sclerodactyly and sclerodermic facies

Among the other typical skin lesions, there are *calcinosis*, subcutaneous deposits of calcium phosphate or carbonate, which may surface inducing an ulcer. Nonetheless, sclerodermic skin may be irregularly hypopigmented *(false vitiligo)* or hyperpigmented *(melanodermia)*, sometimes involving the whole body.

As regards the vascular involvement, the more common feature is the Raynaud's phenomenon (almost the totality of SSc patients), due to

acute vasospasm of microcirculation. This is well evidenced to the extremities, particularly the fingers, but also to feet, ears, and the tip of the nose. Furthermore, Raynaud's has been observed during angiographies of kidneys, coronaries, or lungs. Sclerodermic microangiopathy is responsible not only of Raynaud's, but also of several pathologic events, such as skin ulcers, pulmonary hypertension, and renal crisis.

SSc skin lesions represent the object of this thesis

Also the large and medium arteries are involved in SSc vasculopathy: namely, a media-intima thickness may be well recognized by means of carotid echography[26], and an impairment of vessel wall elasticity may be evidenced by pulse wave velocity measurement[27].

Visceral involvement is variable and not exactly predictable. Lungs, heart, digestive tract, and kidneys may be interested.

Pulmonary involvement is one of the most important features of SSc, and often the leading cause of exitus[28]. Typically, an interstitial lung disease is observed, usually symmetric, which preferentially occupies the sub-mantellar and the bottom areas. Two main histologic patterns may be observed: the "*non specific interstitial pneumonia*" and the "*usual interstitial pneumonia*", corresponding to the "ground-glass" (fig 4) and the "honeycombing" (fig 5) aspects at high-resolution computed tomography, respectively. Microscopically, an eosinophilic and/or neutrophilic alveolitis is present, plus a variable fibrosis of the parenchyma. Its destruction leads to the formation of pseudocysts and band scars.

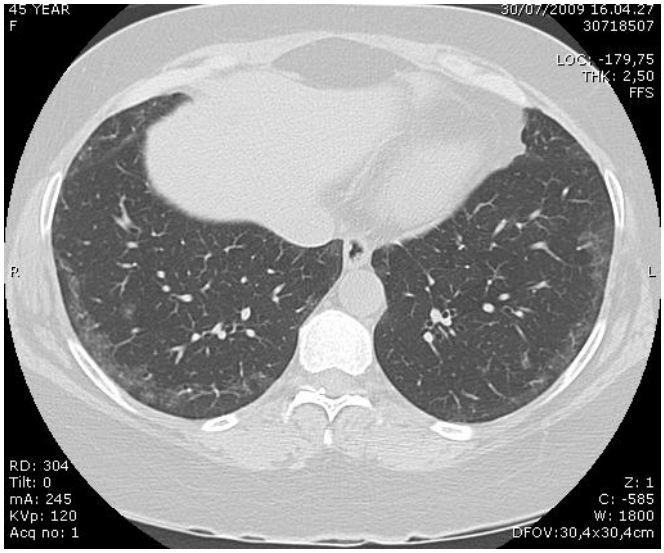


Fig 4 - Ground-glass at High Resolution Computed Tomography

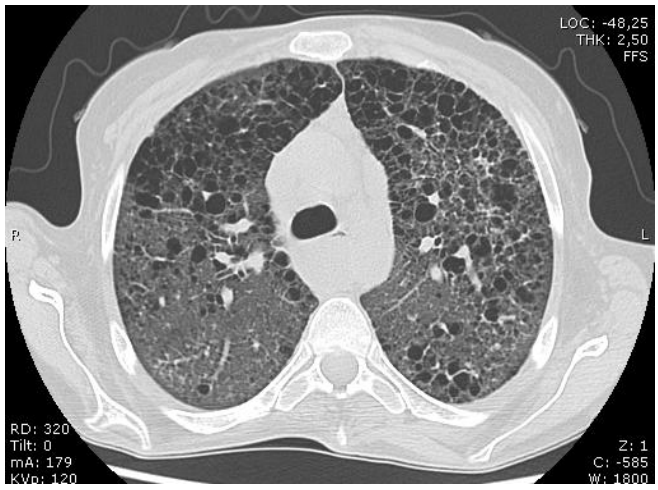


Fig 5 - Honeycombing at High Resolution Computed Tomography

Besides interstitial lung disease, pulmonary arterial hypertension could be associated; more rarely, the latter could be isolated. It is the pathologic result of vessel wall thickening, which consequently leads to lumen narrowing, along with the frequent formation of microthrombi. Later, glomerular-like coiled microvessels appear; those plexiform lesions are characteristic of a sever hypertension. Consequently, the air-blood gases exchanges are impaired, as evidenced by DLCO test; moreover, the raise of pre-capillary

resistances determines a chronic overload for the right ventricle, developing a *cor pulmonaris*.

SSc patients further present an increased incidence of lung carcinoma[29].

Heart involvement is clinically detectable in about 30-50% of cases[30]. Albeit myocardial function is not significantly reduced, a diastolic dysfunction is characteristic, due to reduction of elasticity of left ventricle walls. Furthermore, ECG anomalies are frequently observed, such as the disappearance of the circadian heart rate variation, several types of arrhythmias, causing sudden death in worse cases.

At anatomopathologic examination, sclerodermic microangiopathy causes parcellar band-shaped ischaemic lesions, in absence of coronary involvement or evident myocardial necrosis. On the whole, SSc patients could be considered individuals with high cardiovascular risk.

The most dangerous feature of renal involvement in course of SSc is the *sclerodermic renal crisis*[31]. It is a rapid and malignant deterioration of kidneys function associated to arterial hypertension, due to a reverberant activation of the renin-angiotensin aldosterone system; this vicious cycle can be broken using ACE inhibitors. Renal crisis was found more frequent in diffuse-SSc subset, in presence of anti-U3 RNP autoantibodies, and in patients dehydrated or treated with steroids.

Gastrointestinal dysmotility in SSc is prevalent in 90% of patients, increasing morbidity and in some cases mortality[32]. The resultant gastrointestinal complications are usually extensive, involving many regions of the gut from the oesophagus to the anus. Collagen

replacement of vascular and enteric smooth muscle results in hypomotility, lumen dilatation, tensile rigidity and eventual loss of organ functions. Clinically, oesophageal dysmotility and gastro-oesophageal reflux disease are the most common manifestations in SSc; other features are gastroparesis, dyspepsia, constipation, diarrhea due to microbial flora overgrowth. More rarely, in severe cases, intestinal malabsorption or subocclusions may be described.

Arthralgias are very common during the course of the disease, mainly in diffuse-SSc subset; furthermore, frank arthritis or overlapping with rheumatoid arthritis may be present. More frequent the inflammatory involvement of tendons, which produces a stenosant tendonitis, typically of flexors at the forearms. Also fibrosis of articular capsules needs to be mentioned, with consequent impairment of motion ranges.

Muscle involvement in course of SSc can be classified in three patterns: a torpid, underhand, chronic damage with a slight raise of creatin-kinases; muscle atrophy due to disuse; an overt myositis (overlap syndrome).

Frequently, in course of SSc, it is present a secondary Sjogren's syndrome, that is a mucosal xerosis due to glandular fibrosis/atrophy.

To our knowledge, the central nervous system is spared by SSc; otherwise, there is the possibility of an involvement of the peripheral one, namely a compressive neuropathy (typically at the wrists).

Finally, it is important to consider the psychological field; often, SSc patients present a depressive syndrome of variable entity, or (more characteristic) such a "dysmorphophobic disorder"[33].

1.2 Skin ulcers in systemic sclerosis

Skin ulcers (SU) occur in about 50% of SSc patients in the course of the disease representing one of the most frequent and debilitating disease's manifestation. A multi-factorial pathogenesis is certainly responsible for SU appearance with some differences according to the kind and the localization of ulcer. Because of the lack of a validated classification, we have adopted, since many years, a personal approach based on peculiar features characterizing the different sub-type of SU occurring in course of SSc. An ulcer is defined as "loss of substance", so digital pitting scars, typical of SSc, that is not an active lesion, should be excluded from definition of SSc-SU.

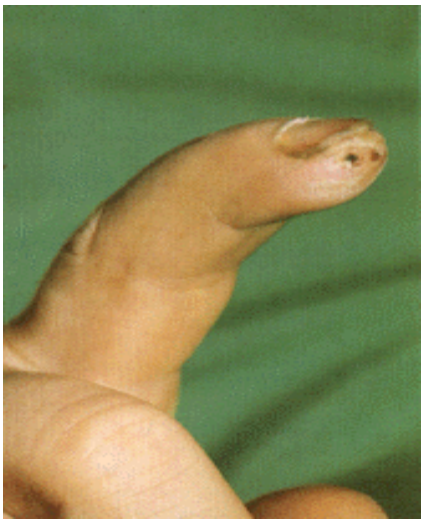


Fig 6 – digital pitting scar

Proposed classification allows to distinguish the following sub-group (tab 3):

Digital Ulcers (DU) (fig 7): occurring on fingertips are the most typical and frequent and represent the most visible manifestation of scleroderma vasculopathy. Interestingly, some bioptical studies have showed that hystopatological alterations detected in DU, intimal

proliferation with fibrosis, resulting in greater than 75% luminal narrowing, are the same of the ones found in life-threatning vascular manifestations, namely scleroderma renal crisis and pulmonary arterial hypertension[34]. DU are also due to progressive tissue ischemia with resultant injury induced by oxygen free radicals, persistent vasospasm secondary to Raynaud's phenomenon, endothelial cell injury, possibly mediated by anti-endothelial cell antibodies in some patients, resulting in an increased production of vasoconstrictors such as endothelin, and a decreased production of vasodilators such as prostacyclin and nitric oxide. Another factor that may play a role in the development of ischemic ulcers at fingertips is intraluminal thrombosis. Platelet activation has been shown to be a prominent feature of scleroderma vasculopathy, leading to clot formation as well as release of vasoconstrictors such as thromboxane[35].

SU on bony prominence (fig 8): particularly at level of metacarpophalangeal joint, interphalangeal joint and elbow are prominently caused by repetead microtraumatism at the site of chronic contractures and skin sclerosis with consequent avascularity and atrophy resulting in major vulnerability to injury and impaired healing[35]. These lesions are less affected by vasodilating therapies than DU located at distal digits. Some Authors report a similar prevalence for DU and extensor surface SU with analogue impact on hand functional impairment and disability[36].

SU occurring on calcinosis (fig 9): are mostly typical of patients with limited SSc are the for the mechanic stress induced by erosion of the skin from the underlying deposits of calcium hydrossiapatite at dermis and epidermic level. Pathogenetic mechanism is not completely

understood but presumably due to intracellular deposits of calcium secondary to the effect of traumatism and inflammation on cellular membrane. Calcinosis can erupt the skin inducing the formation of SU that are frequently complicated by infection.

SU of lower limbs[37-42] (fig 9): Differently from how believed in the past, an increased prevalence of peripheral vascular disease in SSc patients has been more recently observed. Macrocirculation involvement can induce the occurrence of both digital and no digital ulcers in course of SSc. These latter represent a significant cause of morbidity. Some Authors suggest that SSc should represent an independent risk factor for peripheral artery involvement of both upper and lower extremities [41]. Also prothrombotic risk should always be considered in case of SSc-SU of lower limbs. A recent study estimated a prevalence of 4% of lower extremities ulcers, bilateral in 70% of cases; 50% patients were positive for antiphospholipid antibodies and genetic prothrombotic screen was positive in 70% of cases[42].

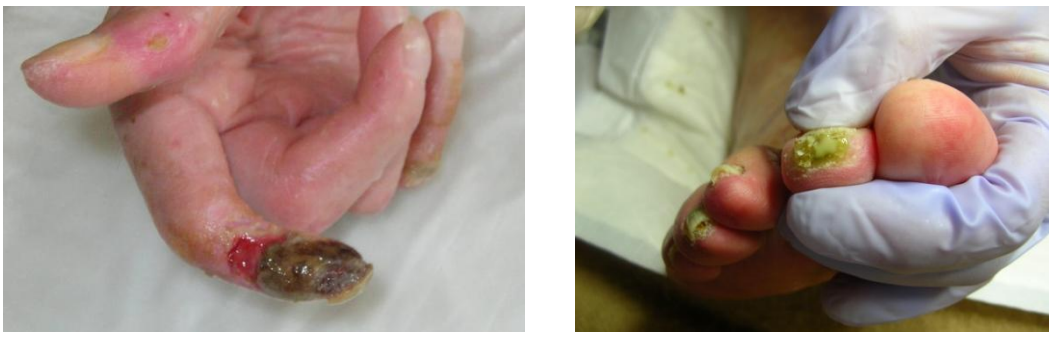


Fig. 7 - Necrotic DU of the hand and infected DU of a foot

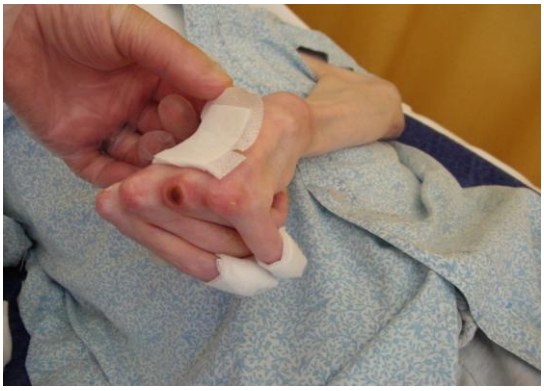
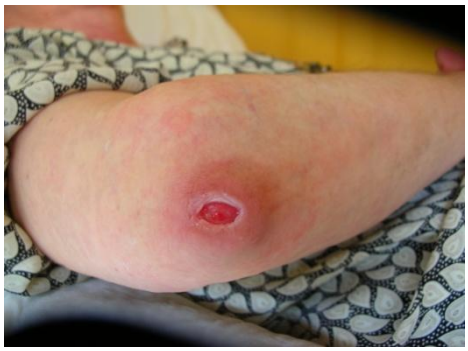


Fig. 8 - SSc-SU bony prominence



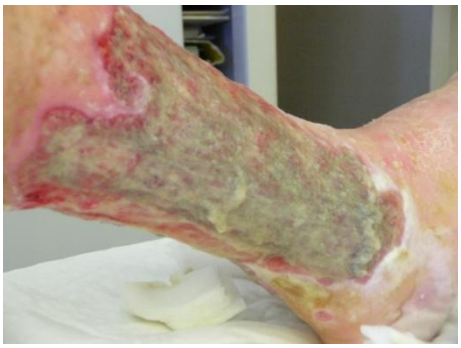


Fig. 9 - ulcer secondary to calcinosis and leg skin ulcer

Tab 3 – Classification of SSc-SU

TYPE OF SSC-SU	DIAGNOSIS	TREATMENT
DIGITAL ULCERS - hand - feet	- Clinical - Color doppler ultrasound	- WBP - Vasoactive systemic treatment - Revascularization
SU ON BONY PROMINENCE	- Clinical	- WBP
SU ON CALCINOSIS	- Clinical - X-rays	- WBP - Surgical removal - Lithotripsy
SU OF THE LOWER LIMBS	- Clinical - Color doppler ultrasound - Arteriography	- WBP - Revascularization

1.2.1 Non pharmacological measurement and physical

A correct education to SSc patients inducing life-style changes is mandatory. Clinicians should suggest to all SSc patients to wear a hat, mittens or gloves, scarf, coat with snug cuffs, and warm socks and shoes during cold weather, to use hand and foot warmers (the ones in small heat packs, or the others with battery-operated) in mittens, boots, socks, or pockets, warm up your car before driving in cold weather, wear gloves or mittens when taking food out of the refrigerator or freezer. It is also important to learn handling stress by mean of physical activity, yoga, tai chi, or meditation. Some drugs can trigger Raynaud's attacks: migraine headache medicines that contain ergotamine or diet aids, beta blockers, hormonal contaceptives. Smoking habit must be abolished while use of caffeine and alcohol should be limited.

Tailored rehabilitation and occupational therapy should also be included in correct management program of SSc patients. These physical therapy should be tailored on the bases of the different prevalent clinical manifestation or according to the involvement that mostly affects patients' quality of life.

A Canadian national survey aimed to evaluate the proportion to SSc patients with hand involvement referred to physical or occupational therapy showed that few SSc patients use these services, even thought they would need[43].

Many studies investigated the role of rehabilitation program in SSc. Maddali Bongi et al enrolled randomly 20 patients and interventional group was treated with hand and face specific and global rehabilitation[44]. Results showed an improvement of all parameters in

interventional group. In regards of hand function and consequently prevention of a percentage of SU on hands, another study evaluated the efficacy of a program based on combined connective tissue massage, Mc Mennel joint manipulation and home exercise program, and compared with an home exercise program only. The former approach allowed an improvement of hands functionality and patients' quality of life.

New data on larger population will clarify the best rehabilitative approach to SSc hands and impact on SU occurrence.

1.2.2 Systemic treatment of scleroderma skin ulcers.

Pharmacologic treatment for SSc-SU needs to be tailored on each patient, considering the disease features, the prognosis, the age and the individual aspects of the patients.

According to knowledge about SSc peripheral vasculopathy pathogenesis, we can assume that drugs to be used for the treatment should have some common actions: inhibiting vasoconstriction, increasing vasodilation, antithrombotic power and endothelium protection. In clinical practice it is suggested the use of combined therapy if a single drug is not efficacy enough, also if no ad hoc studies investigate efficacy of association therapies[45,46,47] (fig 10).

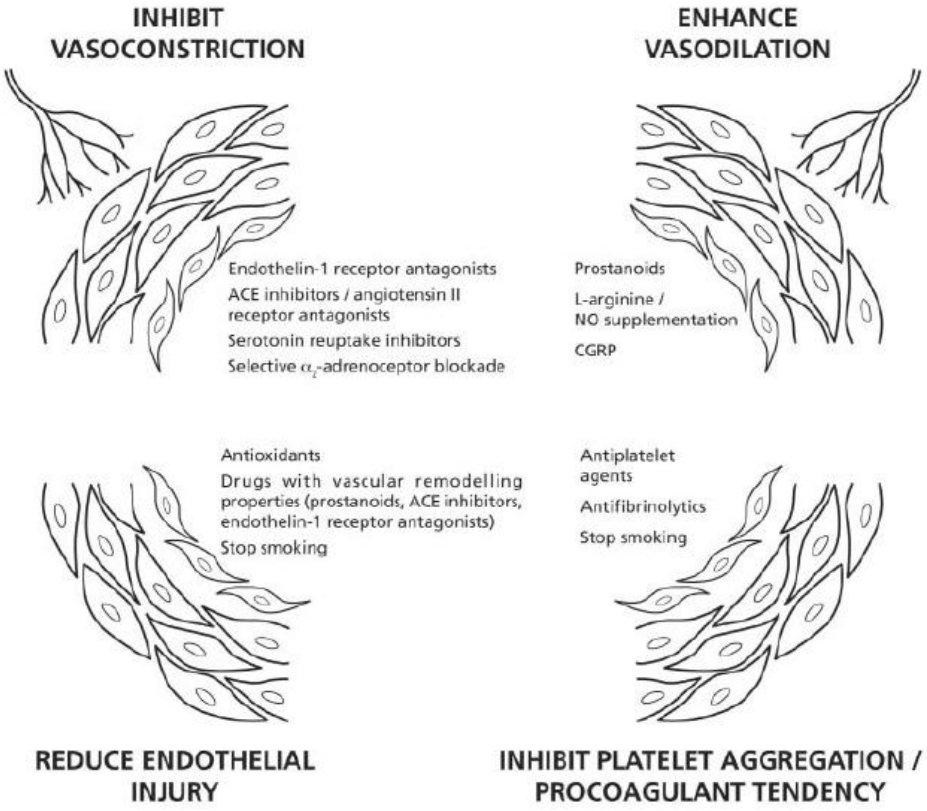


Fig 10 - Present and future approaches to the treatment of scleroderma microangiopathy. Image from Herrick et al, (REF 51).

Calcium channel blockers: Calcium channel blockers are vasodilators effective on smooth muscle cells of the vessels and activation of platelet inhibition. A meta-analysis on dihydropiridine-type calcium antagonists indicates that nifedipine and intravenous iloprost reduce the frequency and severity of SSc-related Raynaud's phenomenon (SSc-RP) attacks. Dihydropiridine-type calcium antagonists, usually oral nifedipine, should be considered for first-line therapy for SSc-RP[48]. Another meta-analysis, including eight randomised controlled trials (RCT; seven with nifedipine and one with nicardipine) with 109 SSc patients involved, indicates that dihydropiridine type calcium antagonists reduce the frequency and

severity of ischaemic attacks in SSc-RP. Two RCT comparing intravenous iloprost (0.5–2 ng/kg per minute for 3–5 days, every 6–8 weeks) with nifedipine (30–60 mg/day) indicate that iloprost is only slightly superior to nifedipine in improving symptoms of SSc-RP[49]. In view of costs and feasibility, the experts recommended that calcium antagonists are first-line therapy in the treatment of SSc-RP. Intravenous prostanoids are recommended when calcium antagonists have failed. As both types of drugs may induce side effects of vascular origin, the experts recommend particular attention if prostanoids are combined with calcium antagonists[47].

Intravenous prostanoids: *Iloprost* is a intravenous (i.v.) prostacyclin with different actions: vasodilation, inhibition of platelet aggregation, prevention of vascular remodeling[50, 51, 52, 53]. Two RCT[52,53] indicate that i.v. prostanoids (particularly i.v. iloprost) are efficacious in healing digital ulcers in patients with SSc. I.v. iloprost at the dosage of 0.5–2 ng/kg per minute for 3–5 consecutive days should be considered in the treatment of active DU in patients with SSc. In one small RCT it significantly reduced the number of DU in comparison with placebo, in another RCT including 73 SSc patients with active DU, iloprost improved DU healing. In addition, two RCT comparing i.v. iloprost with oral nifedipine suggest that both medications have a beneficial effect on DU healing, but the number of patients with DU in both trials was small.

Moreover, i.v. epoprostenol, administered continuously for severe SSc-related pulmonary arterial hypertension (SSc-PAH), revealed a tendency towards a reduction in the number of new DU (by 50%)[54.]

Endothelin receptor antagonist: *bosentan* is a dual endothelin receptor antagonist (ERA). It binds Eta and ETb receptors expressed on endothelial cells, fibroblasts, smooth cells muscle. Two high-quality RCT has confirmed efficacy of bosentan to prevent DU in diffuse SSc patients, in particular in those with multiple DU. Bosentan was evaluated in two placebo-controlled RCT (RAPIDS-1 and RAPIDS-2)[55,56] involving 210 SSc patients in total. Bosentan, at an oral dose of 62.5 mg twice a day for 4 weeks followed by 125 mg twice a day for another 12 weeks, significantly reduced the number of new DU by 48% compared with placebo (ES 0.4; 95% CI 0.0 to 0.8). The efficacy of bosentan in preventing new digital ulcer formation was corroborated by the results of the RAPIDS-2 study, which was performed in SSc patients with active DU (this is population is considered to be at high risk of peripheral digital necrosis) and new ulcers per patient over 12 and 24 weeks. The beneficial effect on new DU formation was accompanied by a significant improvement in overall hand function (specific health assessment questionnaire (HAQ) score ES 0.4; 95% CI 0.0 to 0.8) in RAPIDS-1, and a significant improvement in the Scleroderma-HAQ dressing domain ($p=0.03$) in RAPIDS-2. As discussed above, intravenous iloprost and epoprostenol were shown to improve the healing of active DU. There are two major concerns related to the use of bosentan and other ERA: potential liver injury and teratogenicity. Hormonal contraceptives may not be reliable if co-administered with bosentan, because bosentan may reduce their efficacy by interference with the cytochrome P450 system.

Anti-phosphodiesterase. *Sildenafil* is a phosphodiesterase type 5 inhibitor able to increase cyclic GMP in the smooth muscle cells. Sildenafil has been approved by the US Food and Drug Administration for the treatment of PAH patients in WHO functional classes II, III and IV, and it should be considered for the treatment of SSc-PAH patients in whom bosentan has been ineffective, or can not be used for safety reasons[57,58]. Numerous anecdotal reports in litterature suggest a possible role of sildenafil's efficacy in SSc severe non-healing DU, but its role should be still investigated[59, 60, 61,62].

1.2.3 Local treatment

Local treatment is a fundamental step in correct management of SSc-SU. Since many years, our approach is based on principles of wound bed preparation (WBP) of the European Wound Management Association and the World Union of Wound Healing Societies' consensus statements[63,64] The wound bed preparation was first described in 2000 by Sibbald et al[65] and Falanga[66] .

This approach to wound management stresses that successful diagnosis and treatment of patients with chronic wounds requires holistic care and a team approach. The whole patient, the underlying causes, and patient-centered concerns must be considered before looking at the wound itself is considered the best approach to chronic non-healing wound. In the last years we have tailored these knowledge to specific features of SSc-SU, suggesting that WBP represents the best method for local treatment of SSc related SU. Principles of WBP are summarized in the acronymous TIME, which usefully recall to the most important aspects of ulcer evaluation and

management: necrotic tissue, infection/inflammation, moisture balance, epithelization, in order to accelerate endogenous healing or to facilitate the effectiveness of therapeutic measures[67-74].

More in detail, *T is for* ***necrotic tissue.*** Its removal by mean of debridement allows elimination of phenotypically alterated cells and local bacterial load that represent a strong obstacle to healing process. These tissues can obscure the underlying wound bed for proper assessment and bacteria may also be responsable for scarce local resources such as oxygen, nutrition, that are crucial for wound healing. Therefore, debridement of the necrotic tissue is an important component of the management of SSc SU.

The *debridement* is the removal of unhealthy tissue from a wound to promote healing. Different kind of debridement can be performed: surgical, chemical and autolytic are considered the most selective, able to remove only necrotic tissue, while mechanical and biological debridement are considered less selective, allowing sometimes to vital tissue damage. Autolytic debridement is the most natural since it accelerates the physiologic process of self-cleaning of the wound through phagocitic cells and proteolitic enzymes. Our approach always includes also surgical debridement, performed with sharp or courette. It is very selective since the doctor has control over the tissue to be removed or left. This method is extremely effective allowing the fastest results also in removal of infected tissue. It requires appropriate cleaning and disinfection of surrounding skin; the main limit of surgical debridement is represented by procedural pain that can reduce the patients' compliance to local medication.

I is for ***infection and inflammation***, in fact chronic wounds always contain bacteria at levels ranging from contamination, through colonization, critical colonization to infection. Several systemic and local factors can increase the risk of infection, namely bacterial burden, characteristics of the wound and host resistance lowered by poor tissue perfusion, poor nutritional status, local edema, smoking and drug and alcohol abuse, comorbidities and medications.

Diagnosis is primarily a clinical skill, with the supplement of microbiological data. The signs of infection in chronic wounds include pain, erythema, edema, purulent discharge, and increased heat, delayed healing, increased exudate, bright red discoloration of granulation tissue, friable and exuberant granulation, new areas of slough or breakdown on the wound surface, undermining, foul odor, and new areas of wound breakdown, carefull evaluation of presence of biofilm is always necessary.

In our clinical practice, microbiological data about predominant flora are provided by bacterial swabs. These qualitative data are required in case of systemic antibiotic therapy. The wound bed must be cleaned with saline solution and superficially debrided so the cultures from the superficial wound compartment more closely resemble those in the deep wound compartment.

According to clinical and microbiological data, appropriate antiseptic local treatment or systemic antibiotic should be considered for management of infected ulcer. Clinical signs of osteomyelitis should always be carefully investigated and evaluated.

Data on pathogens mainly involved in SSc-SU infection and osteomyelitis are discussed later.

M is for ***moisture balance*** since a delicate control of the wound and surrounding area moisture balance has been proven to accelerate wound healing in terms of re-epithelization, promote granulation tissue formation and prevent maceration of the surrounding skin, without increasing the infection rate[69,70]. Moisture balance allows to stimulation of component with a positive effect on healing, such as growth factors, cytokines, keratinocytes, endothelial cells and fibroblasts. On the other hand excessive moisture containing matrix metaloproteinases, and serine proteinases that can break down or damage essential extracellular matrix material, leading also to maceration of wound edges and slow healing. In our clinical practice this delicate balance is favoured by the use of the wide array of "advanced" moisture retentive dressing (occlusive, semiocclusive, absorbitive, and hydrating dressing) according to the moisture status of the wound bed.

E is for ***epithelization***: Wound healing can be defined only in case of complete progression of the edges and wound contraction. In case of arrest of these processes, every step of the TIME should be re-considered, since necrotic tissue, biofilm, hypergranulation tissue, hyperkeratotic edges can obstacle keratinocytes proliferation and motility.

2. AIM OF THE STUDY

Aim of this Doctorate Thesis was to provide new data helpful to clarify some clinical and therapeutic points, not previously explored in literature.

General features of our SSc population, demographic, clinical and serological correlations of SSc patients with SU, defined according to the classification previously described, are herein reported. Personal experience of the last three years on SSc-SU is summarized.

3. PATIENTS

Studies performed from 2010 to 2014 on SSc-SU, and here reported, took in account patients from our SSc population, classified according to ACR/EULAR criteria for classification of SSc[23], referred to our Rheumatology Unit. All patients were evaluated baseline and during follow-up about visceral involvement; clinical, serological and instrumental data were regularly reported in clinical records, such as treatment. SU were classified as: DU on hands, DU on feet, SU on bony prominence, SU on calcinosis, SU of the lower limbs.

4. METHODS

All patients affected by SSc and complicated by SU were treated with systemic and local therapy. Systemic treatment was mainly based on acetylsalicylic acid, calcium channel blockers, iloprost, bosentan, besides steroids, immunosuppressant. Local treatment was regularly performed according to wound bed preparation as previously described. To obtain the best results from local treatment, we use *active dressing* when required; these medications actively interact with the wound tissues in developing of cellular matrix and promoting the entire healing process. Among the large number of available active medications, their use is defined in the course of wound management

according to careful clinical evaluation of the single lesion. The most used products were the alginate (non-woven absorbent fiber derived from different types of algae and seaweeds), hydrocolloid (wafer type of self-adhering dressing containing gel-forming agents in an adhesive compound laminated onto a flexible, water-resistant outer layer), hydrofiber (soft, sterile, non-woven pad or ribbon dressing composed of sodium carboxymethylcellulose), hydrogel (water based-gel wafer able to promote moist environment and removal of necrotic tissue through autolytic debridement), and polyurethane foam or film (open-cell hydrophilic polyurethane foam sheets, permeable to gas and water vapor, with an hydrophobic surface).

As previously described, SSc-SU are extremely painful. ***Analgesia*** is mandatory for both background pain and procedural pain during local debridement. The former is responsible of decreased patients' quality of life, the latter generally causes low compliance during local medications with consequent delay in healing process. Our patients with SSc-SU were treated, when required, with long-lasting analgesic treatment by means of opioids, according to evaluation of pain severity by mean of visual analogue scale (0-10)[75-77].

The approach to procedural pain, usually treated with topical application of lidocaine/prilocaine cream (EMLA), was revised and re-elaborated

Finally, SSc-SU refractory to conventional therapies were usefully treated by means of ***emathopoietic growth factors*** as recombinant human erythropoietin (RhuEPO) and granulocyte-colony stimulating factor (G-CSF).

Occasionally, for more severe and hard-to-heal skin lesions, skin grafting, skin bank bioproducts, especially cyropreserved skin and de-epidermized dermis (DED) and/or others skin substitutes were used[78-84].

5. RESULTS

5.1 SSc population features and clinical, serological, demographic correlations with SSc-SU

Two hundred and eigthy-two SSc patients were enrolled. 254 patients are females (90.1%) and 28 males (9.9%); age at the beginning of follow-up was 54.6±13.5 years with a follow-up of 5.8±4.6 years; SSc duration was 3.1±5.7, Raynaud's phenomenon duration was 44.6±16.2 years, age at Raynaud's onset was 44.6±16.2 years, age at SSc onset was 51.5±13.9 years. 84.2% SSc patients showed limited cutaneous subset, while 15.8% had diffuse cutaneous subset; anti-topoisomerase antibodies (Scl-70) were positive in 33.5% of SSc patients, anticentromere antibodies (ACA) in 44.8%, Antinucleolar antibodies (ANoA) in 15.7%; increased value of erythrocyte sedimentation rate was recorded in 29.9% of SSc patients, and 20.9% showed increased value of reactive C protein.

90.3% of patients had Raynaud's phenomenon, melanodermia was detected in 38.5%, telengectasias in 64.9%, calcinosis in 13%, sicca syndrome in 54.9%, lung fibrosis in 47.5%, smoking habit 32.4%. Altered derived pulmonary arterial pressure (PAPs) at echocardiography was detected in 14% of patients. SSc patients underwent therapy with calcium channel blockers in 86.4%, 75.1%

underwent therapy with acetylsalicylic acid, 74.6% underwent prostanoids, 25% underwent bosentan, 65.9% steroids, 32.8% immunosuppressant.

Occurrence of SU during follow-up was recorded in 156 patients (55.4%), 86.5% females, 13.5% males.

Patients with SU showed a lower mean age at Raynaud's and SSc onset and at the beginning of follow-up (p=0.035, 0.024 and 0.030, respectively). Diffuse cutaneous subset was more frequently detected in SSc patients with SU (p=0.003), sex male, calcinosis, teleangectasia, melanodermia were more frequent among patients with SU (p= 0.009, 0.001, 0.009, 0.001 and 0.001 respectively). Increased value of ESR and RCP was detected in SSc-SU patients (p=0.039 and 0.001), and PAPs > 40 mmHg was more frequently detected in SSc-SU patients (p=0.048). (tab 4)

Percentage of different sub-groups of SSc-SU were: DU of the hands 139/282 (49.4%), DU of the feet 29/282 (10.4%), SU on bony prominence 52/282 (18.4%), SU on calcinosis 13/282 (4.6%), SU of lower limbs 22/282 (7.8%).

Tab 4 - Demographic, serologic and clinical features of SSc population and comparison between patients with or without SU

	SSc tot	SU +	SU-	*P*
TOT	282	156 (55,4%)	126 (44.6%)	
Follow-up (yrs ± SD)	5.8±4.6	6.3±4.6	5.4±4.4	Ns
Raynaud's duration (yrs ± SD)	9.3±12.3	9.8±12.4	8.5±12.0	Ns
SSc duration (yrs ± SD)	3.1±5.7	3.3±5.6	2.7±6.1	Ns
Age at Raynaud onset (yrs ± SD)	44.6±16.2	43.1±16.6	47.4±15.7	0.035
Age et SSc onset (yrs ± SD)	51.5±13.9	50.0±14.5	54.0±12.8	0.024
Age at beginning of follow-up (yrs ± SD)	54.6±13.5	53.3±13.9	56.8±12.7	0.030
F/M (%)	90.1/9.9	86.5/13.5	95.5/4.5	0.009
L/D (%)	84.2/15.8	79.2/20.8	91.8/8.2	0.003
Scl70 %	33.5	34.7	31.5	Ns
ACA %	44.8	41.8	49.5	Ns
ANoA %	15.7	17.1	13.5	Ns
Calcinosis %	13	18.6	4.6	0.001
Telenagectasias %	64.9	71.2	55.6	0.009
Melanodermia %	38.5	46.3	26.9	0.001
Raynaud's phenomenon%	90.3	91	89.2	Ns
Sicca Syndrome %	45.9	42.2	51.4	Ns
Lung fibrosis %	47.5	52.2	40	0.056
PAP s>40 %	14	17.5	8.1	0.048
Smoking habit %	32.4	32.8	31.5	Ns
ESR %	29.9	35	20	0.039
RCP %	20.9	26.5	10	0.011
Therapies (%)				
Calcium channel blockers	86.4	88	83.3	Ns
Acetilslycilic acid	75.1	78.6	68.3	Ns
Prostanoids	74.6	86.3	51.7	0.001
Bosentan	25	32.5	10.2	0.001
Steroids	65.9	73.3	51.7	0.007
Immunosoppressant	32.8	35.9	26.7	Ns

SSc: systemic sclerosis, SU: skin ulcers, L: limited subset of the disease, D: diffuse subset of the disease, Scl70: anti-topoisomerase, ACA: anticentromere antibody, ANoA: antinuclear antibody, PAPs: pulmonary arterial pressure, ESR erythrocyte sedimentation rate, RCP: reactive C protein

DU of the hands

DU of the hands showed a lower mean age at Raynaud's phenomenon and SSc onset, and at beginning of follow-up (p=0.003, < 0.001, 0.001 respectively). No differences were observed about mean disease duration of both Raynaud's phenomen and SSc. DU occurrence correlated with male gender (p=0.003), diffuse cutaneous subset

(p=0.001), melanodermia (p<0.01), teleangectasias (p=0.039), calcinosis (p=0.001); increased value of ESR were more frequently observed in patients with DU of the hands (p=0.037), while an inverse correlation was observed with ACA (p=0.008). Prostanoids, bosentan and steroids were more frequently used in patients with DU of the hands (p <0.01; <0.01; 0.014). (tab 5)

Tab 5 - Comparison between patients with or without DU of the hands

	DU hand +	DU hand -	*P*
TOT	139 (49.4%)	143 (50.6%)	
Follow-up (yrs ± SD)	6.4V4.5	5.7±4.7	Ns
Raynaud's duration (yrs ± SD)	9.6±12.1	8.8±12.2	Ns
SSc duration (yrs ± SD)	3.7±6.1	2.6±5.6	Ns
Age at Raynaud onset (yrs ± SD)	41.5±15.6	47.5±15.7	0.003
Age et SSc onset (yrs ± SD)	47.6±14.2	54.4±12.9	<0.001
Age at beginning of follow-up (yrs ± SD)	51.3±13.3	57.1±12.9	<0.001
F/M (%)	84.4/15.6	95.4/4.6	0.003
L/D (%)	74.6/25.4	93.1/6.9	<0.001
Scl70 %	38.3	30.5	Ns
ACA %	34.4	51.1	0.008
ANoA %	18	13	Ns
Calcinosis %	19.8	6.3	0.001
Telenagectasias %	70.8	58.3	0.039
Melanodermia %	51.3	26.8	<0.001
Raynaud's phenomenon %	91.2	88.5	Ns
Sicca Syndrome %	40.8	48	Ns
Lung fibrosis %	50	40.2	Ns
PAP s>40 %	13.1	10.6	Ns
Smoking habit %	35.4	28.4	Ns
ESR %	48.3	28	0.037
RCP %	21.6	20	Ns
Therapies (%)			
Calcium channel blockers	86.6	84	Ns
Acetilslycilic acid	79.4	72	Ns
Prostanoids	90.7	57.3	<0.01
Bosentan	39.2	9.5	<0.01
Steroids	75	56	0.014
Immunosoppressant	38.1	28	Ns

SSc: systemic sclerosis, SU: skin ulcers, L: limited subset of the disease, D: diffuse subset of the disease, Scl70: anti-topoisomerase, ACA: anticentromere antibody, ANoA: antinuclear antibody, PAPs: pulmonary arterial pressure, ESR erythrocyte sedimentation rate, RCP: reactive C protein

DU of the feet.

SSc patients with DU of the feet showed a longer follow-up (p=0.003), correlated with male gender, calcinosis and teleangectasias (respectively, p=0.026, p=0.002, and p=0.001); increased value of ESR and RCP were more frequently detected in SSc patients with DU of the feet (p=0.015 and p<0.001). treatment with bosentan was significantly more frequent in patients with DU of the feet than patients without. (tab 6)

Tab 6 - Comparison between patients with or without DU of the feet

	DU feet +	DU feet -	*P*
TOT	29 (10.4%)	253 (89.6%)	
Follow-up (yrs ± SD)	8.6±4.8	5.8±4.5	0.003
Raynaud's duration (yrs ± SD)	11.6±11.3	8.9±12.2	Ns
SSc duration (yrs ± SD)	4.0±5.2	3.0±5.9	Ns
Age at Raynaud onset (yrs ± SD)	41.8±16.1	44.8±15.9	Ns
Age et SSc onset (yrs ± SD)	49.5±13.6	51.2±14.0	Ns
Age at beginning of follow-up (yrs ± SD)	53.8±12.2	54.3±13.5	Ns
F/M (%)	77.8/22.2	91.4/8.6	0.026
L/D (%)	80.8/19.2	84.3/15.7	Ns
Scl70 %	44.4	33.2	Ns
ACA %	44.4	42.7	Ns
ANoA %	22.2	14.7	Ns
Calcinosis %	34.6	10.3	0.002
Telenagectasias %	92.3	61.1	0.001
Melanodermia %	46.2	37.7	Ns
Raynaud's phenomenon %	88.9	89.9	Ns
Sicca Syndrome %	44.4	45.9	Ns
Lung fibrosis %	44	45.2	Ns
PAP s>40 %	24	10.2	Ns
Smoking habit %	50	29.9	Ns
ESR %	52.2	26.2	0.015
RCP %	56.5	15.4	<0.001
Therapies (%)			
Calcium channel blockers	91.3	84.6	Ns
Acetilslycilic acid	87	74.5	Ns
Prostanoids	91.3	73.8	Ns
Bosentan	52.2	22.3	0.005
Steroids	72.7	65.8	Ns
Immunosoppressant	43.5	32.2	Ns

SSc: systemic sclerosis, SU: skin ulcers, L: limited subset of the disease, D: diffuse subset of the disease, Scl70: anti-topoisomerase, ACA: anticentromere antibody, ANoA: antinuclear antibody, PAPs: pulmonary arterial pressure, ESR erythrocyte sedimentation rate, RCP: reactive C protein

SU on bony prominence

Patients with SU localized on bony prominence showed lower mean age at SSc onset and at the beginning of follow-up, with longer disease duration, while non differences were observed about gender. Diffuse cutaneous form of the disease and positivity for anti-topoisomerase were correlated with occurrence of SU on bony prominence, while an inverse correlation was observed with ACA positivity. Calcinosis, melanodermia and teleangectasia were more frequently detected (p=0.001; <0.001; 0.047), increased value of ESR and RCP were observed (p=0.003 and <0.001).

The use of prostanoids, bosentan and immunosuppressant was more frequent in SSc patients with SU on bony prominence. (tab 7)

Tab 7 - Comparison between patients with or without SU on bony prominence

	SU on bony prominence +	SU on bony prominence -	*P*
TOT	52 (18%)	231 (82%)	
Follow-up (yrs ± SD)	6.3± 4.5	6.1±4.6	Ns
Raynaud's duration (yrs ± SD)	8.54±7.8	9.3±12.5	Ns
SSc duration (yrs ± SD)	5.4 ±6.5	2.9±5.8	0.045
Age at Raynaud onset (yrs ± SD)	39.6±13.9	45.0±16.0	Ns
Age et SSc onset (yrs ± SD)	42.7±14.9	51.9±13.6	0.007
Age at beginning of follow-up (yrs ± SD)	48.3±12.8	54.8±13.3	0.023
F/M (%)	83.3/16.7	90.6/9.4	ns
L/D (%)	47.8/52.2	87.6/12.4	<0.001
Scl70 %	62.5	31.5	0.006
ACA %	20.8	45.1	0.029
ANoA %	20.8	14.9	Ns
Calcinosis %	37.5	10.5	0.001
Telenagectasias %	95.8	68	<0.001
Melanodermia %	58.3	36.5	0.047
Raynaud's phenomenon %	100	88.8	Ns
Sicca Syndrome %	30.4	46.2	Ns
Lung fibrosis %	56.5	43.9	Ns
PAP s>40 %	14.3	11.6	Ns
Smoking habit %	38.1	31.7	Ns
ESR %	60	25.7	0.003
RCP %	60	15.8	<0.001

Therapies (%)			
Calcium channel blockers	90	84.9	Ns
Acetilslycilic acid	80	65.7	Ns
Prostanoids	100	73	0.004
Bosentan	50	23.2	0.015
Steroids	78.9	65.1	Ns
Immunosoppressant	60	30.3	0.012

SSc: systemic sclerosis, SU: skin ulcers, L: limited subset of the disease, D: diffuse subset of the disease, Scl70: anti-topoisomerase, ACA: anticentromere antibody, ANoA: antinuclear antibody, PAPs: pulmonary arterial pressure, ESR erythrocyte sedimentation rate, RCP: reactive C protein

SU secondary to calcinosis

Patients with SU on calcinosis showed significant lower mean age at SSc onset and a longer SSc duration (*p* 0.029 and 0.037). As expected, a correlation with the presence of calcinosis was observed (p<0.001)

Tab 8 - Comparison between patients with or without calcinosis

	Calcinosis +	Calcinosis -	*P*
TOT	13 (4.6%)	269 (95.4%)	
Follow-up (yrs ± SD)	8.4±5.5	6±4.5	Ns
Raynaud's duration (yrs ± SD)	10.5±10.4	9.1±12.3	Ns
SSc duration (yrs ± SD)	6.6±7.8	3±5.7	0.037
Age at Raynaud onset (yrs ± SD)	38.6±16.4	44.8±15.8	Ns
Age et SSc onset (yrs ± SD)	42.5V13.8	51.5±13.8	0.029
Age at beginning of follow-up (yrs ± SD)	49.4±11.1	54.5±13.5	Ns
F/M (%)	83.3/16.7	90.3/9.7	Ns
L/D (%)	81.8/18.2	84.1/15.9	Ns
Scl70 %	33.3	34.3	Ns
ACA %	58.3	42.1	Ns
ANoA %	16.7	15.4	Ns
Calcinosis %	100	10.5	<0.001
Telenagectasias %	81.8	63.6	Ns
Melanodermia %	63.6	37.4	Ns
Raynaud's phenomenon %	100	89.3	Ns
Sicca Syndrome %	16.7	46	Ns
Lung fibrosis %	50	44.8	Ns
PAP s>40 %	11.1	11.9	Ns
Smoking habit %	45.5	31.6	Ns
ESR %	36.4	29.2	Ns
RCP %	36.4	19.9	Ns
Therapies (%)			
Calcium channel blockers	90.9	85.1	Ns
Acetilslycilic acid	72.7	76.4	Ns
Prostanoids	90.9	75.2	Ns
Bosentan	45.5	25	Ns
Steroids	63.6	66.9	Ns
Immunosoppressant	36.4	33.5	Ns

SSc: systemic sclerosis, SU: skin ulcers, L: limited subset of the disease, D: diffuse subset of the disease, Scl70: anti-topoisomerase, ACA: anticentromere antibody, ANoA: antinuclear antibody, PAPs: pulmonary arterial pressure, ESR erythrocyte sedimentation rate, RCP: reactive C protein

SU of the lower limbs

A mean increased value of derived PAPs at echocardiography, ESR and RCP was observed in SSc patients with SU of the legs (p respectively 0.058; 0.002; <0.001). No other statistically significant differences were observed.

Tab 9 - Comparison between patients with or without SU on lower limbs

	Lower limbs +	Lower limbs-	*P*
TOT	22 (7.8%)	260 (92.2%)	
Follow-up (yrs ± SD)	7.9±4.6	5.9±4.6	Ns
Raynaud's duration (yrs ± SD)	10.3±10.8	9.1±12.3	Ns
SSc duration (yrs ± SD)	2.3±4.0	3.2±6.0	Ns
Age at Raynaud onset (yrs ± SD)	44.7±14.3	44.4±16.1	Ns
Age et SSc onset (yrs ± SD)	52.8±13.8	50.9±14.0	Ns
Age at beginning of follow-up (yrs ± SD)	55.4±12.6	54.1±13.5	Ns
F/M (%)	90/10	89.9/10.1	Ns
L/D (%)	84.2/15.8	83.9/16.1	Ns
Scl70 %	40	34	Ns
ACA %	50	42	Ns
ANoA %	15	15.5	Ns
Calcinosis %	21.1	12.2	Ns
Telenagectasias %	78.9	63.4	Ns
Melanodermia %	47.4	38.1	Ns
Raynaud's phenomenonb %	85	90.2	Ns
Sicca Syndrome %	30	45.6	Ns
lung fibrosis %	40	45.8	Ns
PAP s>40 %	26.3	10.5	0.058
smoking habit %	31.3	32.7	Ns
ESR %	64.7	25.8	0.002
RCP %	64.7	16.1	<0.001
Therapies (%)			
Calcium channel blockers	94.1	84.5	Ns
Acetilslycilic acid	94.1	74.2	Ns
Prostanoids	88.2	74.8	Ns
Bosentan	41.2	24.7	Ns
Steroids	76.5	65.6 ns	Ns
Immunosoppressant	47.1	32.3	Ns

SSc: systemic sclerosis, SU: skin ulcers, L: limited subset of the disease, D: diffuse subset of the disease, Scl70: anti-topoisomerase, ACA: anticentromere antibody, ANoA: antinuclear antibody, PAPs: pulmonary arterial pressure, ESR erythrocyte sedimentation rate, RCP: reactive C protein

Comparison between DU (considering both hands and feet) and SU on bony prominence showed that diffuse disease subset, detected in both subgroups when compared with controls, was significantly more frequent in group of patients with SU on bony prominence (p=0.008), such as anti-Scl70 positivity (p=0.018)

Tab 10 - Comparison between DU (hands and/or feet) and SU on bony prominence

	DU hands and/or feet	SU on bony prominence	*P*
TOT	147	26 (9.3%)	
Follow-up (yrs ± SD)	7.4±4.4	6.3± 4.5	Ns
Raynaud's duration (yrs ± SD)	10.6±11.9	8.54±7.8	Ns
SSc duration (yrs ± SD)	3.9±6.0	5.4 ±6.5	Ns
Age at Raynaud onset (yrs ± SD)	41.9±15.9	39.6±13.9	Ns
Age et SSc onset (yrs ± SD)	48.4.±14.4	42.7±14.9	Ns
Age at beginning of follow-up (yrs ± SD)	52.0±13.5	48.3±12.8	Ns
F/M (%)	84./15.6	83.3	Ns
L/D (%)	75.9/24.1	47.8/52.2	0,008
Scl70 %	37	62.5	0,018
ACA %	36.3	20.8	Ns
ANoA %	17.8	20.8	Ns
Calcinosis %	20.3	37.5	Ns
Telenagectasias %	71.7	95.8	0,006
Melanodermia %	48.4	58.3	Ns
Raynaud's phenomenon %	91.9	100	Ns
Sicca Syndrome %	40.2	30.4	Ns
Lung fibrosis %	49.2	56.5	Ns
PAP s>40 %	14	14.3	Ns
Smoking habit %	35.6	38.1	Ns
ESR %	50.1	60	Ns
RCP %	24.3	60	0,002

SSc: systemic sclerosis, SU: skin ulcers, L: limited subset of the disease, D: diffuse subset of the disease, Scl70: anti-topoisomerase, ACA: anticentromere antibody, ANoA: antinuclear antibody, PAPs: pulmonary arterial pressure, ESR erythrocyte sedimentation rate, RCP: reactive C protein

5.2 Studies performed on this SSc population in the last four years

5.2.1 Platelet gel in the treatment of severe scleroderma skin ulcers[85]

(Published on Rheumatology International in February 2011)

In 2011 we enrolled 12 consecutive SSc patients during a period of 6 months (10 women and 2 men, mean age 49.5 ± 13.6 SD years, mean disease duration 7.4 ± 3.5 SD years), with one (or more) non-venous leg SU refractory to conventional treatments with the aim to evaluate the effect of platelet gel (PG) in the treatment of severe, non-healing SU of the lower limbs in SSc patients. PG was prepared at the Blood Transfusion Centre of our Hospital, according to internal standard protocols, under sterile conditions, as follows: 50 ml of 24 h fresh, ABO/Rh compatible, platelet concentrates from BuVy coat was transferred to a sterile 50-ml Falcon tube and centrifuged at 3,000 g for 15 min. The plasma supernatant (20 ml) was used to obtain thrombin: 4 ml calcium gluconate was added to plasma and further incubated at +37°C for 15–30 min, and 10 ml of supernatant, rich in thrombin precursors, was added to 30 ml platelet concentrates into Falcon tube and gently shaken until an elastic paste was formed. Exclusion criterion of the PG treatment was the presence of infections, assessed by clinical and microscopic evaluation of the swab. The wound was preliminary irrigated with lactated Ringer's solution; if needed, a surgical debridement was also carried out. Secondly, PG was applied on the wound bed and covered by an occlusive dressing; the latter was removed after 48 h. The treatment schedule consisted of PG applications twice weekly for 2 weeks, then once a week for the following weeks. In all cases, the ongoing treatments remained

unchanged during PG applications. The effect of PG applications was evaluated at baseline, after 12 and 24 weeks by the size and depth of ulcers, wound bed conditions (presence of exudate, necrosis, infection and/or granulation tissue) and by patient self-evaluation using pain VAS and Disability Index of Health Assessment Questionnaire (HAQ) At the end of the 24-week follow-up, a median of 10 PG applications (range 5–24) was performed. Since a wound infection was observed in the first 5 patients, successfully treated with oral antibiotic, we decided to cover the next PG applications using a silver-releasing dressing, which was able to revent infectious complications. A complete response was observed in 4/12 patients (33%), and a decrease in wound area (>50%) was achieved in 6/12 (50%), whereas there was no response in 2/12 patients (16%). The baseline median wound size signifcantly decreased, while patients' quality of life improved as showed by both VAS (from 87.08 ± 13.5 SD to 57.9 ± 12.6 SD; $p = 0.001$) and HAQ-DI (from 0.73 ±0.43 to 0.57 ± 0.22; p = ns)

5.2.2 Scleroderma Digital Ulcers Complicated By Infection With Fecal Pathogens [86]

(Published on Arthitis care and research in February 2012)

In 2012 we have reported the observation that fecal pathogens should be frequently involved in SSc-DU complicated by infection. We evaluated 82 SSc patients with complicating digital ulcers. In 42 (51.2%) of 82 subjects with clear signs of possible bacterial infection, microbiologic examinations were performed, all swabs were performed at the level of necrotic areas, and dried exudates, slough,

and/or dressing residue was removed with sterile saline solution and surgical debridement. Then a sterile swab with a cotton tip was used to better recover infectious agents from the site. No patients underwent immunosuppressant therapy. Moreover, no patients activated the home nursing service, providing the ulcer care by themselves or with the help of relatives. All 42 SSc patients showed at least 1 infected lesion. Interestingly, 11 of 42 patients evaluated showed skin lesions infected with typical pathogens from intestinal origin; in particular, 7 patients were positive for *Escherichia coli* and 4 for *Enterococcus faecalis*. More habitual infectious agents were detected in the remaining 31 of 42 patients; in particular, *Staphylococcus aureus* was detected in 21 (50%) of 42 and *Pseudomonas aeruginosa* was detected in 5 (12%) of 42, while other less frequent pathogens were found in the remaining 5 (12%) of 42 individuals. All of the observed infections were responsive to common antibiotic therapy. Ulcers infected with fecal pathogens were more sensitive to antibiotics than those infected with *P aeruginosa*. While a faster resolution of infection was generally observed with fecal pathogens, reinfection was more frequently observed in these patients. Statistical analysis between SSc patients with ulcers infected with intestinal bacteria versus the others evidenced a significantly increased percentage of diffuse cutaneous SSc among the first ones (6 of 11 versus 5 of 31 SSc patients; Fisher's $P = 0.011$).

Tab 11 – Data of 42 SSc patients with infected DU

	Value
Clinicoserologic data	
Male/female, no.	5/37
Age, mean ± SD years	56.3 ± 15.8
Disease duration, mean ± SD years	11.1 ± 7.9
Skin subsets, no.	
Limited cutaneous	31
Diffuse cutaneous	11
Serology, no.	
Anti–Scl-70	19
Anticentromere	15
Others	8
Smokers/nonsmokers, no.	4/38
Microbiologic data, no. (%)	
Infections	
Escherichia coli, *Enterococcus faecalis*	11 (26)
Pseudomonas aeruginosa	5 (12)
Staphylococcus aureus	21 (50)
Others*	5 (12)

* *Proteus mirabilis*, *Streptococcus agalactiae*, *Citrobacter koseri*, and *Stenotrophomonas maltophilia*.

5.2.3 Osteomyelitis complicating scleroderma digital ulcers [87]

(Published on Clinical Rheumatology in May 2013)

Since infection is one of the worst complication of chronic SU, we have also focused attention to prevalence of osteomyelitis (OM) in SSc-DU in order to investigate the possible correlations between OM and SSc clinico-serological features, including some possible predisposing factors.

The study evaluated a population of 248 SSc patients (21 men and 227 females, mean age 61±13.5SD years, disease duration 12.3±6.7 SD years). All patients with skin ulcers and clear signs of possible bacterial infection underwent microbiological examinations. OM was diagnosed on the basis of clinical, laboratory, and radiological findings; namely, the presence of at least four clinical signs/symptoms (local pain, swelling, erythema around the skin ulcer, warmth,

purulent discharge, fever), blood chemistry alterations (ESR, CRP, and/or increased white cell count), and typical radiological findings at plain X-ray examinations (soft-tissue swelling, periostal reaction or elevation, loss of cortex with bony erosion, focal loss of trabecular pattern, new bone formation, bone sequestrum involucrum, and/or cloacae). Besides clinical and laboratory alterations, the diagnosis of OM was done only in the presence of concordant radiological findings observed by two blinded radiologists. Clinically, OM was classified according to the time of diagnosis from the detection of ulcer infection in acute (within 2 weeks), subacute (within 1-2 months), and chronic (>2 months). Moreover, following the probable way of microorganism spread, OM was classified as hematogenous, secondary to a contiguous focus (surgery, trauma or wounds), or secondary to vascular insufficiency.

During the follow-up period, one or more episodes of isolated or multiple skin ulcers were observed in 119/248 (48%) SSc patients. In particular, digital ulcers were recorded in 110/119 (92%) SSc patients, while both digital and skin ulcers of lower limbs were observed in 41 subjects (17%). SSc patients with ulcers presented a significantly lower age (59±14.5SD vs. 64±12.2SD years; p=0.005) and a longer disease duration (11.9±6.5SD vs. 9.97±6.3SD years; p=0.0007) if compared with those without; moreover, the presence of anticentromere autoantibodies resulted significantly lower in subjects with skin lesions (40/119, 33.6% vs. 66/129, 51.2%; p=0.007). Clinical signs suggesting bacterial infection of digital ulcers were observed in 45/119 (37.8%) SSc patients, and invariably confirmed by laboratory microbiological examinations. The prevalence of OM in

the entire SSc patients' series was 7.7% (19/248); it was invariably found in the setting of patients with infected digital ulcers, showing a surprisingly high percentage of underlying bone involvement (19/45, 42%). In particular, all SSc patients with complicating OM presented typical clinical and laboratory features, while plain X-ray showed typical alterations, mainly bone erosions (Fig. 11). The OM was localized at the hands in 14 patients and feet in 5. The OM was classified as subacute in 15 patients and chronic in 4; moreover, in all cases a contiguous spread of bacteria from skin ulcers was considered highly probable in the absence of other detectable causes. Patients with infected digital ulcers complicated by OM presented significantly lower mean age ($p<0.016$), higher percentage of serum anti-Scl70 autoantibodies ($p<0.0128$) compared to those without OM. Moreover, patients with OM showed a lower percentage of serum anticentromere antibodies (21% vs 50%), while they were more often treated with low-dose steroids (42% vs 19%). The most frequently isolated pathogens from infected digital ulcers were Staphylococcus Aureus and Escherichia Coli. All patients with complicating OM started antibiotic therapy, soon after the isolation of the involved infectious agent and antibiotic sensitivity analysis; the treatment was carried out until clinical resolution of the wound infection (medially 12 weeks; range 8-16).

Finally, in 16/19 patients the local and systemic treatments lead to complete resolution of OM and digital ulcers, while 3 (16%) subjects needed surgical digit amputation

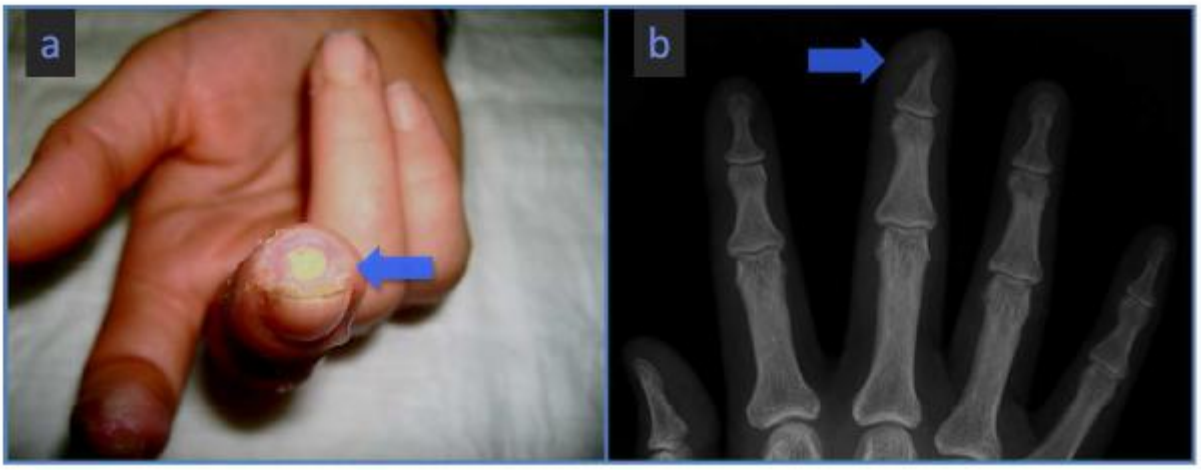


Fig 11 - Scleroderma digital ulcer complicated by osteomyelitis in a 51-year-old female.
(a): digital ulcer at the third fingertip of the right hand.
(b): standard x-ray examination shows marked bony erosion of the 3rd phalanx of the same finger.

5.2.4 Procedural pain during local treatments. [88]

(Accepted on Clinical Experimental Rheumatology in July 2014)

SSc patients with skin wounds almost invariably need analgesic treatment for long-lasting, chronic pain, usually defined as background pain, as well as procedural pain management caused by local wound treatment. In particular, extensive and in depth debridement of slough and necrotic tissue is an extremely painful procedure; as a consequence, the patients often ask clinicians to stop local treatment before the debridement is complete because they are unable to tolerate the resulting pain. Because of these considerations, we proposed a systematic approach to analgesic treatment of procedural pain during the local treatment of scleroderma DU.

In this study 51 DU in 32 consecutive SSc patients were evaluated (6 males and 26 females, mean age 53±14.6SD years, disease duration 10.6 ±5.2 SD years, 18 patients with limited and 14 with diffuse

cutaneous subset). In the absence of validated severity scale for DU, we graded the severity of the lesions according to modified Wagner's scale; namely, the scoring of severity included the following parameters: width and depth of DU, presence of necrosis, infection, and/or gangrene. On these basis, DU severity ranged from score 1 for small and superficial lesions to score 5 for large ulcers with extensive gangrene.

The pain severity was scored using a 10 cm visual analogue scale (VAS), graduated from 0 ("no pain') to 10 ("worst imaginable pain"). According to the previously suggested cut-off, persistent VAS scores ≤ 4 were regarded as the expression of tolerable discomfort, which may allow the prosecution of the local treatments, while scores > 4 were considered to indicate moderate-severe pain. Patients with moderate-severe pain were basically treated with analgesics for background pain due to skin lesions (oxycodone, from 5mg bid to 20mg bid).

The eutectic mixture of the two local anesthetic lidocaine and prilocaine was applied in all DU. (Fig. 12).

At the baseline evaluation, medially higher values of pain VAS were recorded in infected DU (p=.0001), as well as in the presence of more severe lesions (p=.0001). According to pain VAS levels, the DU-related pain was classified as 'mild' (VAS ≤ 4; 12 DU) or 'moderate-severe' (VAS >4; 39 DU). Before local management, all DU were invariably treated with the application of EMLA; a good compliance during the entire session of debridement and dressing was observed in patients with DU characterized by mild pain VAS at the baseline, as well as in few cases with pain VAS of 5-6. While, the majority of DU

with moderate-severe pain VAS (34/39) needed a combined therapy with EMLA and local morphine (8/34) or with EMLA, local and oral morphine (26/34) before the debridement. In only one case of particularly severe DU, with infection and gangrene, resistant to analgesic treatments, intravenous morphine was also administered during the debridement. Pain management during DU debridement required only EMLA application in 33% of cases, EMLA plus local morphine in 16%, and EMLA plus local and oral morphine in the remaining 51%. Combined treatment with EMLA, local and oral morphine was necessary in the presence of DU with higher pain VAS as evidenced by pain VAS variations during DU debridement'.

In all instances, the use of analgesics, in particular local and oral morphine, revealed manageable and well tolerated; in no cases sensitization phenomena were observed. Oxicodone administered for background pain due to skin lesions was generally well tolerated, with the exception of mild constipation. Moreover, all patients showed a good compliance for the entire treatment cycle, without relevant side effects to analgesic treatments.

During the study (12 months), the combination of systemic and local treatments leads to the improvement (reduction >50% of DU clinical features: size, depth, perilesional erythema, and signs of infection) or healing of 19 (37%) and 31 skin lesions (61%), respectively. Only one DU (2%) did not improve after 6 months of treatment, needing digit amputation for gangrene and osteomyelitis.

Generally, the response to treatments was correlated to the severity of the DU at baseline, as well as the duration of local treatment and the number of dressing/debridement sessions.

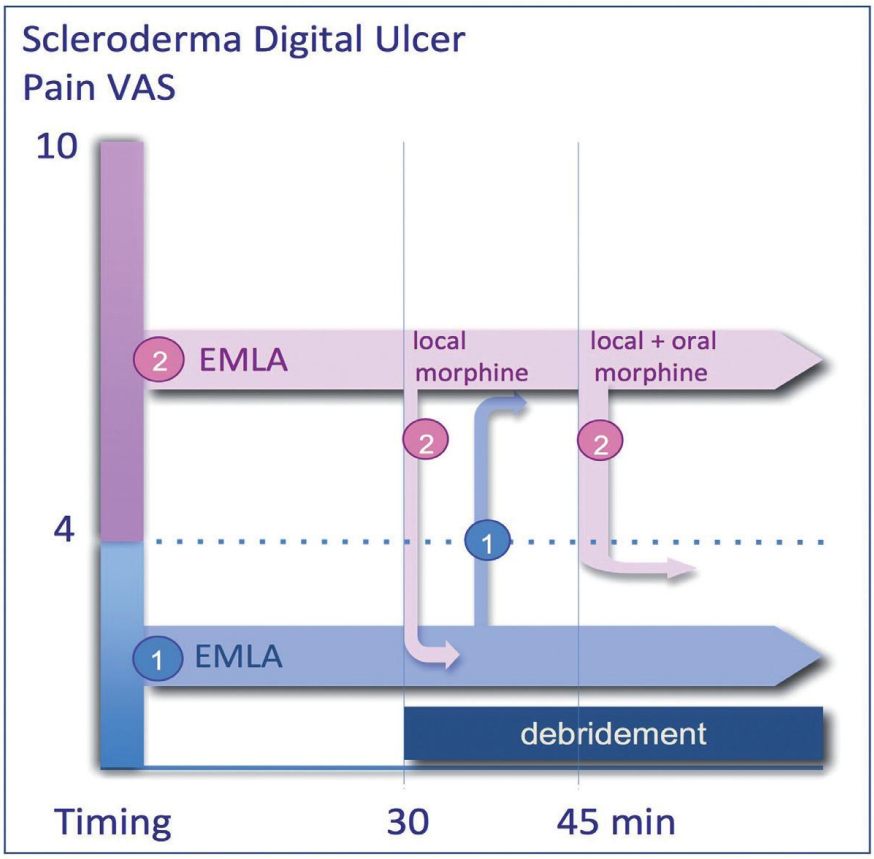


Fig. 12. The figure describes the analgesic treatment strategy during local debridement sessions of digital ulcers (DU). The pain severity was scored using a 10 cm visual analogue scale (VAS) and monitored following a standardised timing; namely at baseline, after 30, 45 and 60 minutes of first analgesic application. VAS scores >4 indicated moderate-severe pain, while scores ≤4 were classified as tolerable discomfort, allowing the prosecution of local treatments. In all cases, two local anesthetics (lidocaine and prilocaine EMLA 5% cream) were applied (see text for details). After 30 minutes a sharp debridement of DU was performed in those patients with stable, tolerable pain (VAS ≤4). When pain VAS remained persistently high (>4) or abnormally increased during debridement, the cleaning was suspended and local morphine was added; after 15 minutes the debridement was started over if pain VAS decreased <4. Finally, if even topical morphine was ineffective (VAS >4), oral morphine was administered to patient; after 15 minutes from the sublingual morphine administration, the cleaning of the DU was completed in patients with tolerable pain (VAS ≤4).

5.2.5 Predictive role of capillaroscopic skin ulcer risk index in systemic sclerosis: a multicentre validation study [89-90]

(Published in Annals of the Rheumatic diseases in 2011)

Background: In 2009, our group proposed a quantitative score, termed the 'capillaroscopic skin ulcer risk index' (CSURI), which was able to recognize those patients with a higher risk of developing digital ulcers within 3 months from the initial capillaroscopic evaluation with high sensitivity and specificity. The aim of the study was to validate the predictive value of CSURI within 3 months of the videocapillaroscopic evaluation in a larger population of scleroderma patients from a multicentre study. The secondary endpoint was to evaluate the predictive value of CSURI in different subgroups of patients, with respect to the clinical history of digital ulcers.

CSURI was analysed in 229 unselected SSc patients by nailfold videocapillaroscopy (NVC).

As described in our preliminary work, we have defined the exact method to consider all capillaroscopic parameters included in CSURI formula as follows:

1. Number of capillaries (N): all the capillaries detectable in the entire distal front line, including capillaries placed at different levels; namely, by counting all the capillaries that one can see from the top of the picture.

2. Megacapillary (M): a capillary with a homogeneously enlarged loop with a diameter of 50 μm or greater.

3. Maximum diameter of megacapillary (D): the largest distal row giant capillary diameter present in a capillaroscopic image, except at the level of a microaneurysm.

Among all images, we chose only those with the lowest N and, second, with the highest M; the presence of at least one giant capillary is required to perform the score. All patients were re-evaluated 3 months later with regard to the persistence and/or appearance of new digital ulcers.

57 of 229 patients presented with digital ulcers after 3 months. The receiver operating characteristic curve analysis showed an area under the curve of 0.884 (95% CI 0.835 to 0.922), with specificity and sensitivity of 81.4% (95% CI 74.8 to 86.89) and 92.98% (95% CI 83.0 to 98.0), respectively, at the cut-off value of 2.96. The reproducibility of CSURI was validated on a random sample of 81 patients, with a κ-statistic measure of interrater agreement of 0.8514.

5.2.6 Prediction risk chart for scleroderma digital ulcers[91]

(Published on Clinical Hemoreology and Microcirculation in January 2014)

Because of the deep impact of DU in patients affected by SSc especially in prognosis quod-valetudinem, and the difficulties in management of this clinical complication, we proposed a predictive risk chart for DU appearance within six months from baseline evaluation taking into account demographic, clinico-serological, and capillaroscopic parameters.

For this study, two hundred and nineteen unselected SSc patients, classified according to the preliminary American College of Rheumatology classification criteria, from 8 Italian Rheumatology Centers were consecutively enrolled during a 6-month period.

Demographic, clinical, serological and instrumental data, and Capillaroscopic skin ulcers risk index (CSURI) evaluation were recorded at baseline . This latter is a capillaroscopic quantitative score previously validated from our group able to detect SSc patients at high risk to develop DU within next three months since evaluation. Six months after the enrollment all patients were clinically re-evaluated for the development of DU;

During the 6-month follow-up period, a total number of 67 new DU were observed in our patients'series (67/219; 30.6%). Among analyzed parameters, cutaneous SSc subsets, disease duration, history of DU in the last year, smoke, DLCO, CSURI, ESR, and CRP showed a significantly positive association with DU.

There was not significant association between DU and treatment with vasodilators and/or immunosuppressors (data not shown). In the multivariate logistic regression analysis, CSURI≥2.96, DU history, the ESR≥20, and also the male gender were significant predictors of DU. A prognostic index was derived by the algorithm obtained from multivariate regression analysis. In addition, a prediction risk chart, including CSURI, ESR, gender and DU history, was built in order to facilitate the interpretation and application in clinical practice of this prognostic index. For each possible combination of included parameters, the chart shows the risk to develop DU during a 6 month period from CSURI evaluation. For an easier use of chart risk, males and females are graphically described separately. According to the risk level, four classes of risk were proposed, concerning the probability to develop DU within six months fromclinical and capillaroscopic evaluation: low (≤19.3%), medium (>19.3% and

≤58.6%), high (>58.6% and ≤89.2%), and very high risk (>89.2%) (Figure 13). The predictive positive value was 90.7%, while the negative predictive value was 89.8%, with a sensitivity and specificity of 90.74 (95%CI 79.7 - 96.9) and 90.51 (84.3–94.8), respectively. The predictive model fit measures showed an adequate fit. The Chi-square Pearson was 9.39 (*p*-value=0.59) and the Hosmer–Lemeshow test was 6.48 (p = 0.37). The area under the ROC curve was 40 96% (95%CI: 0.922–0.983). (fig 13-14)

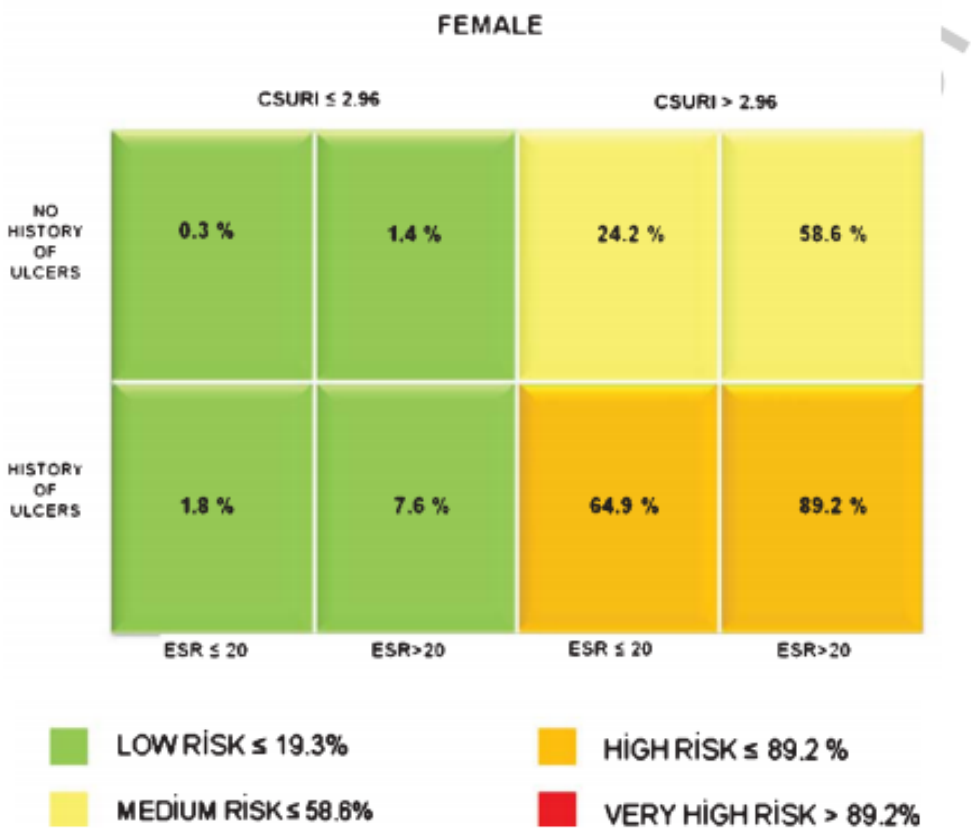


Fig. 13. Risk chart for DU appearance within six months from capillaroscopic evaluation in females

Fig. 14. Risk chart for DU appearance within six months from capillaroscopic evaluation in males

5.2.7 Preliminary data on the role of micro and nano-particles as possible etiologic or prognostic factors in SSc

Unpublished data

Background: as previously described, the ethiology of SSc is unknown; although we can hypotize that upon a genetic predisposition some toxic or infective stimuli can be involved, in a multifactorial process.

This study aimed to investigate the possible role of micro and nano particles as further ethiologic or prognostic factors in SSc, in

particular if an exposure to occupational or environmental to fine and ultrafine powder could induce occurrence or evolution of the disease.

30 SSc patients and 20 controls matched for age, gender and geographic area were enrolled. Exposure risk was evaluated by mean of a questionnaire including clinical and demographic features of each patient and information about occupation, environmental exposure, smoking and food habit, joint and dental protesis and drugs. Increased risk to develop SSc was correlated to occupational exposure, in fact SSc group worked for more than 5 years in ceramic, manufacturing metalworking and textile. Qualitative and quantitative analysis were performed to evaluate the presence of micro or nanoparticles. Qualitative analysis was performed by mean of electronic microscopy. A significant higher frequence of micro-nanoparticles was observed in the serum of SSc patients compared with controls, namely Si, Fe, Zn, Al, Ti and Mg. On the contrary, Cu, Mn, Ni, Cr, Ba, Zr e Sn were less frequently detected.

Interestingly, silicon was present in serum of all SSc patients evaluated, frequently associated to Fe and Al.

Quantitative analysis was performed by mean of inductive coupled plasma mass spectometry. Results showed a significative increase of Al, Cr, Si, Ti and Zn concentration, while a significant decrease of Fe concentration compared with controls was observed.

These preliminary data need to be confirmed on larger population and evaluation of correlation between micro-nanoparticles concentration and clinical manifestations of the disease with particular attention to SSc-SU is object of "work in progress"

(Legenda Si silicon, Fe iron, Zn zinc, Al aluminium, Ti titanium, Mg magnesium, Cu copper, Mn manganese, Ni nickel, Cr chromium, Ba barium, Zr zirconium, Sn tin)

6. DISCUSSION

SSc-SU, particularly SU occurring on hands and feet, represent a burden clinical manifestation of the disease with possible severe complications, such as gangrene, osteomyelitis, infection of soft tissue, requiring sometime hospitalization, and always frequent clinical evaluation and treatment. The impact on patients' quality of life is often very deep, especially when multiple lesions occur contemporary, for the consequent hands and feet dysfunction and severe pain.

A study evaluating the impact of DU on disability and health-related quality of life (HRQoL) in SSc showed a reduced wrist and hand mobility, increased global and hand disability and decreased mental component of HRQoL[92.] In another study evaluating the impact of SSc-DU on daily living and professional activity involving 189 patients, the results showed significant impact on activities of daily living and work disability with increased need for external home help[93]. These data were confirmed in a study on larger population (2327 SSc patients enrolled) from Digital Ulcers Outcome (DUO) registry aimed to assess functional limitations due to presence of DU. Results showed that increasing number of DU was associated to worst impairment in work and daily activities with need for more support from others[94].

Although there is agreement about the importance of SU in course of SSc, no validated guidelines are available about their management and correct classification. Moreover, possible risk factors for SU appearance were often explored but results of different studies are not conform[95-100]

Classification is mandatory for correct therapeutic approach, since, as previously described, different kind of SU can occur in course of SSc. Different diagnostic and therapeutic strategies are mandatory in case of DU of hands and feet, SU on bony prominence, SU on calcinosis or SU of the legs. This classification adopted by our group has been effectively used in the last years in our large SSc cohort.

Our results underline specific correlations of different kind of SU in SSc patients.

Patients younger at SSc onset seem to be more frequently affected by both DU of the hands and SU on bony prominence. A longer follow-up correlates with appearance of DU on the feet, probably because influenced also by macrocirculation abnormalities. Male gender, widely associated to more severe form of SSc, correlates only with occurrence of DU. Diffuse subset of the disease is more frequent in both patients with DU and SU on bony prominence when compared with controls, but significantly more frequent in patients with SU on bony prominence when compared with DU group. Positivity of Scl70 correlates with SU on BP, but not with DU. Increased value of ESR and RCP are more or less detected in SSc-SU patients except for SU on calcinosis.

In case of DU appearance, systemic treatment of SSc should be reconsidered and in presence of suggestive clinical signs, an

evaluation of possible role of macroangiopathy should be excluded. Especially in cases of DU occurring at the fingers of feet micro- and macrocirculation can be sometimes contemporary responsible of non-healing lesions and, in these cases, a more specific treatment is required.

In case of SU occurring on bony prominence, the use of specific orthosis in opposition with joint flexion induced to skin retraction can be considered, such as the local use of growth factors to promote edges proliferation.

SU on calcinosis are frequently recurrent, so in cases of voluminous subcutaneous calcinosis surgical removal or lithotripsy could be considered. Occurrence of SU of the legs impose an evaluation of macrocirculation, and in cases of diffuse subset of the disease, when the skin is particularly sclerotic, the recourse of skin grafting should be considered.

Once the diagnosis is performed, including the assessment of possible comorbidities with possible involvement of other specialists, namely vascular surger, infectivologist, plastic surger, physioterapist, etc, the local treatment is invariably based on wound bed preparation. In this regard it is also very important to score the severity of the skin lesions. In the absence of validated severity scale for DU, we use to grade the severity of the SSc-SU according to modified Wagner's scale, validated for diabetic ulcers[101]; namely, the scoring of severity included the following parameters: width and depth of DU, presence of necrosis, infection, and/or gangrene. On these basis, DU severity ranged from score 1 for small and superficial lesions to score 5 for

large ulcers with extensive gangrene. Validation of this severity scale is in course.

In 2010 Amanzi et Al published a very interesting paper elaborating data about 1614 digital lesions occurring in SSc patients. In this work lesions are classified considering also pitting scars as active ulcers, and DU defined as "*a loss of epithelialization and tissues involving, in different degrees, the epidermis, the dermis, the subcutaneous tissue and sometimes also involving the bone*". DU are subsequently classified according to the origin, localization and specific features of the wound. Authors conclude that pitting scars, DU in general, calcinosis and gangrene are the most frequent kind of skin lesions occurring in course of SSc and a more detailed classification is useful in clinical practice [102].

In 2011 Denton et Al presented data from DUO registry. This database enrolled 2439 patients with SSc ongoing DU. Anyway, a definition of DU is not reported in the work. Results showed that DU developed earlier in patients with dcSSc compared with lcSSc. Almost all patients (95.7%) tested positive for antinuclear antibodies, 45.2% for anti-scleroderma-70 and 43.6% for anticentromere antibodies (ACA). The first digital ulcer in the anti-Scl70 positive patient cohort occurred approximately 5 years earlier than the ACA positive patient group [103.]

These data, although very interesting and helpful for a better understanding of SSc-SU, particularly DU, confirm the need for a more detailed and evidence-based classification.

The works presented in this thesis have been elaborated with the aim to provide adjunctive data for clinicians involved in SSc to improve the complex management of SSc-SU.

The prevention should be a primary goal to achieve.

In the last years we have validated the capillaroscopic score with the higher predictive value for appearance of SSc-DU in a period of three months. CSURI is a prognostic index which is a combination of capillaroscopic alterations able to identify patients at significantly higher risk to develop DU; it was firstly proposed in 2009 and subsequently validated in a multicenter study on 229 scleroderma patients. CSURI showed a very high negative predictive value (97.2%), while in unselected patients the positive predictive value was relatively low (62.3%). So, a negative CSURI allows us to exclude new digital ulcers, while a positive value of CSURI doesn't justify us to start a preventive therapy, in particular for patients without history of DU, where the positive predictive value of CSURI is not satisfactory (about 45%). The combination of CSURI with other features of SSc patients could increase the power of videocapillaroscopy to predict DU development.

The building of the predictive risk chart aimed to take into account the multifactorial etiology of DU. Starting from the evaluation of clinical serological and instrumental parameters, we obtained a composite model based on few risk factors namely gender, DU history, CSURI, ESR. The prognostic index allows to calculate the risk to develop DU within six months from the evaluation, considering all the possible combination of the risk factors evidenced. Except for the gender, all parameters included in the proposed risk chart are modifiable over

time and easily achievable, so that the routinely use of this chart might be recommended in the follow-up of SSc patients and it could help the rheumatologist for the therapeutical strategy. Despite the possible value of this chart in the clinical practice, other important factors as weather, work activities and microtraumatism should be considered. These conditions are sometimes difficult to accurately quantify; however, the actual risk can be evaluated and discussed individually according to patient's lifestyle and clinical condition, suggesting suitable changes and, if necessary, preventive vasoactive therapies.

However many steps have been performed in the last years about preventive therapy for DU appearance, the management of active, recurrent and multiple skin lesions still remains a daily clinical challenge for rheumatologist involved in SSc management.

Information provided about infections and relative complications are very useful.

No data were available about the prevalent pathogens involved in SSc-SU infection, particularly DU. Our data show that P. aeruginosa, S. aureus, E. coli and E. faecalis are preminently responsible for infections. The prevalence of fecal pathogens suggest the possible contamination from patient himself during personal hygiene, or from relatives. Consequent consideration is that correct education about procedures for medications performed at home is mandatory. Physicians and nurses should effectively explain how to change bandages, how to perform personal hygiene and some domestic activities promoting the use of gloves. Expert patients program should also be encouraged and planned.

The importance to prevent infection is also underlined by results of our retrospective analysis about OM that is one of the most fearsome complication. Our data showed, for the first time, an higher prevalence of OM than expected: 7,7% of the whole SSc population, detecting it in 42% of patients with SSc-SU. It is well known that foot OM is a common complication of diabetic foot ulcers but data regarding the appearance and treatment of OM in SSc patients were still lacking.

Patients with digital ulcers complicated by OM showed a significantly higher percentage of serum anti-Scl70 autoantibodies and lower mean age compared to those without OM; this particular correlation suggests that OM might complicate patients with more severe SSc clinical variants, characterized by more pronounced immune-system depression and marked deterioration of the patient's general conditions, including nutritional status.

The correct approach to treatment of SSc-SU should always be based on combination of local and systemic treatment. No data are available about local therapy and our personal experience is mainly based on knowledge acquired in field of chronic non-healing wound. A non-healing wound is defined when normal healing process fail and there is no the expected improvement. This condition can occur for different reason and at each moment of the healing.

A SSc-SU quickly acquires typical feature of non-healing wound, for this reason the application of principles of wound bed preparation are mandatory for a correct evolution of the process.

Because of the lack of data about WBP in SSc, all new information provided are particularly helpful.

The proposal of a systematic strategy for a better control of procedural pain during surgical debridement performed in our dedicated ambulatory, can significantly increase patients' compliance allowing to faster healing of chronic lesions.

In the last years our group suggested the use of emathopoietic growth factors in SSc-SU, namely the use of granulocyte-colony stimulating factor in 26 SSc patients (5 microg/kg subcutaneously for 5 days) showed an improvement in 24/26 and complete healing in 22/26 [83]; the use of recombinant human erythropoietin (rHuEPO) was carried out in 14 patients with SSc with nonhealing, severe cutaneous ulcers (subcutaneously at a dosage of 150 IU/kg 3 times weekly for 2 weeks, twice weekly for the next 2 weeks, and then once weekly for 1 month). At follow-up 3-6 months from the beginning of the treatment, six patients showed complete resolution of the skin ulcers, while a significant reduction (> 60%) in lesional areas was obtained in the other eight patients [104]. About rHuEPO, we also reported the case of a patient with nonhealing cutaneous ulcers successfully treated with recombinant human erythropoietin (rHuEPO)[84]. The possible biological effects of this drug were also investigated. Before rHuEPO treatment, the bone-marrow sample contained reduced numbers of EPCs, which were functionally impaired. After a 6-month rHuEPO cycle, a marked increase in endothelial progenitor markers was seen, along with a significant reduction in their apoptotic rates.

Results of the work reported in the thesis about the use of platelet gel in SSc-SU complete the picture suggesting that in selected case in which healing is not obtained nevertheless correct use of standard

therapy, growth factors can represent an interesting therapeutic opportunity also in SSc-SU.

Finally, since ethiology of SSc is not definitely defined, and it is hypotized that different infectous or exogen triggers can stimulate the complex pathogenetic steps of the disease (REF), we consider very interesting the exploration of the possible role of micro- nanoparticles in SSc ethiology. Different possible ethiologic factors have been proposed and evaluated in the last years, if a more strong correlation with different clinical manifestation was evidenced, this could be helpful in prevention strategy.

SU occurring in course of SSc still represent a major clinical problem. Multidisciplary approach is sometimes required for a better result, but rheumatologists should always play a central role not only for systemic but also for local treatment, since they are generally strictly associated and need to be frequently re-evaluated according to carefull evaluation of both skin lesion and systemic aspect of the disease. Evidence-based guidelines about classification, scoring and management are desiderable to improve and guarantee a standard approach in clinical practice.

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