

ASSESSMENT OF DISEASE ACTIVITY AND  
EVALUATION OF CLINICAL PARAMETERS AND  
BIOMARKERS IN SYSTEMIC SCLEROSIS

Ph. D. Thesis



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## **ABBREVIATIONS**

ACA – anticentromere antibody

anti-topo I - anti-DNA-topoisomerase-I antibody

APRIL - A Proliferation-inducing ligand

AT<sub>1</sub>R - angiotensin II type 1 receptor

BAFF - B-cell activation factor

BMI - body mass index

CAD- coronary artery disease

CATPCA- categorical principal component analysis

CFR - coronary flow reserve

CK - creatine kinase

CRP - C-reactive protein

CTGF - connective tissue growth factor

CTX-1 - crosslinked collagen I carboxiterminal telopeptide

DASH - Disabilities of Arm, Shoulder and Hand questionnaire

dcSSc - diffuse cutaneous systemic sclerosis

DLCO - carbon monoxide diffusion capacity

DRG - Disease Related Groups

DSS - Disease Severity Scale

ELISA – Enzyme-linked immunoassay

EScSG activity index - European Scleroderma Study Group activity index

ESR- erythrocyte sedimentation rate

ET – endothelin

ET<sub>A</sub>R - endothelin-1 type A receptor

EUSTAR - European League Against Rheumatism Scleroderma Trials And Research

FFR – fractional flow reserve

FVC - forced vital capacity

FVC/DLCO - ratio of forced vital capacity and carbon monoxide diffusion capacity

GP - general practitioner

HAI - hand anatomic index

HAQ-DI - Health Assessment Questionnaire Disability Index

HRCT - high resolution computed tomography

ICAM-1 - inter-cellular adhesion molecule 1

KL-6 - Kerbs von Lungren 6 antigen

lcSSc – limited cutaneous systemic sclerosis

LDH - lactate dehydrogenase  
LVEF - left ventricular ejection fraction  
MOA - maximal oral aperture  
mPAP – mean pulmonary pressure  
MRSS - modified Rodnan skin score  
MVD – microvascular disease  
PAH - pulmonary arterial hypertension verified by right heart catheterisation  
PDGF - platelet-derived growth factor  
PIIINP - procollagen type III N terminal propeptide  
PINP - procollagen type I N terminal propeptide  
PRP - primary Raynaud's phenomenon  
PsA – psoriatic arthritis  
RA - rheumatoid arthritis  
RHC- right heart catheterisation  
RIA – Radioimmunoassay  
RP – Raynaud's phenomenon  
sCD40L - soluble CD40 ligand  
S-HAQ – Scleroderma Health Assessment Questionnaire Disability Index  
sE-selectin - soluble E-selectin  
SP-D - surfactant protein D  
sPSGL-1 - soluble P-selectin glycoprotein ligand-1  
SSc – systemic sclerosis  
TGF- $\beta$  - transforming growth factor- $\beta$   
VAS- visual analogue scale  
VCAM-1 - vascular cell adhesion molecule-1  
VEGF - vascular endothelial growth factor  
vWF - von Willebrand factor

## INTRODUCTION

Systemic sclerosis (systemic scleroderma, SSc) is a connective tissue disease with complex pathomechanism. Compared to the other rheumatic diseases, the prognosis of SSc is unfavorable. The clinical manifestations are very heterogeneous because of the simultaneous presence of vascular involvement, fibrosis of different internal organs and skin and activation of the immune system. The severity and course of disease can differ substantially among patients.

There is an unmet need to measure properly the disease activity in SSc because the currently available instruments have their limitations. The Department of Rheumatology and Immunology, University of Pécs, Hungary has a long tradition in the treatment and follow-up of patients with scleroderma, treating approximately 320 patients with SSc. Taking into consideration the relatively high number of scleroderma patients attending our Department, longitudinal studies regarding disease activity, severity and survival are worthwhile to be carried out. Measures of disease activity in SSc are needed for multiple uses. In observational studies, they could be used to describe and compare study populations and identify potentially reversible aspects of disease. As research into new treatments for SSc is rapidly advancing, they could be used to determine eligibility and as a measure of outcome in upcoming clinical trials. Finally, in daily clinical practice it is necessary to assess disease activity in order to decide whether the actual treatment is effective or one should switch to a more aggressive therapy. Also the assessments of biomarkers must occur in large cohorts of patients with available fine clinical phenotyping and ideally with longitudinal data focusing on selected outcomes.

Although in recent years, a significant progress has been achieved in the development and validation of instruments used in the assessment of the disease, at this moment there is no unanimously accepted set of criteria in the assessment of disease activity. Because of the complexity and heterogeneity of the disease a reliable composite index should be developed, which reflects the variety of organ involvements, and preferably also contains information obtained from the patients.

The aim of our research team was to study the activity of systemic sclerosis in a prospective follow-up study, and to identify possible new markers, which could reflect disease activity. In collaboration with other institutes we also carried out further investigations on the socio-economic impact of disease activity and severity in systemic sclerosis, and characterisation of cardiopulmonary vasculopathy in systemic sclerosis, a leading cause of morbidity and mortality.

## **BACKGROUND**

### *ETIOLOGY OF SYSTEMIC SCLEROSIS*

With respect to the etiology of systemic sclerosis, the definite initiating mechanisms are not fully understood and remain controversial. Infection-caused stimuli, e.g., cytomegalovirus [1], or Epstein–Barr virus [2], as well as other environmental factors [3] such as exposure to silica [4] or to organic solvents [5, 6], but also genotypic modalities comprising certain susceptibility constellations [3, 5, 7-9] have been discussed to contribute to the development of SSc.

### *PATHOGENESIS OF SYSTEMIC SCLEROSIS*

The major characteristic pathogenic features of SSc are dysfunction of the vasculature, activation of fibroblasts and other resident cells as well as dysregulation of both the innate and adaptive immune system (Figure 1).

#### **Vascular disarrangement**

Vascular alterations as potentially very early events in SSc are believed to be a major pathogenic hallmark of the disease, albeit it still has to be clarified if it is the dominant primary one. Endothelial cell injury in microvessels and in small and medium arteries might be triggered by vasculotropic viruses, inflammatory cytokines, granzymes, endothelial cell-specific autoantibodies or elevated levels of reactive oxygen species due to oxidative stress [10]. Vascular injury leads to structural changes, appearance of megacapillaries, afterwards loss of capillaries (well demonstrated with nailfold capillaroscopy [11]), remodelling of the vessel wall with intimal and median layers hyperplasia and adventitial fibrosis resulting in progressive luminal narrowing and eventually occlusion. This proliferative intimal vasculopathy is mediated by molecules that regulate mainly cell apoptosis, proliferation and vasoconstriction including an increase production of endothelin (ET), a reduction of prostacyclin release and a reduced production of nitric oxide synthase. Moreover, there is an overexpression of adhesion molecules (E-selectin, P-selectin, VCAM-1, ICAM-1) [12]. The loss of capillaries in SSc is not compensated because of defective angiogenesis and vasculogenesis [13, 14].

The earliest manifestation of the vascular involvement is the Raynaud's phenomenon (RP). RP is essentially due to an excessive vasospasm of digital arteries, precapillary arterioles and cutaneous arteriovenous shunts, usually in response to cold exposure or other stimuli resulting in impaired oxygenation of the distal extremities. As long as primary Raynaud's phenomenon (PRP) consists of reversible vasospasm without vascular injury and further complications, secondary RP has a more severe course. It is initiated primary by endothelial cell injury and in

SSc it is frequently associated with development of digital ulcers and necrosis. The dysfunction of the autonomic nervous system leading to abnormal control of the vascular tone has also been described in SSc [15]. Digital ulcers are a frequent complication in SSc patients, which are persistent, difficult to heal and extremely painful, can cause tissue loss, autoamputation and impaired hand function, and sometimes may lead eventually to osteomyelitis, gangrene and septicaemia due to infection [16,17]. Obliterative vasculopathy appears after longer disease duration which consists of thrombotic events in small arteries, leading to complete occlusion and finally resulting in ischemic insult with hypoxia, further entailing necrosis and loss of tissue [18].

Although scleroderma primarily affects the microvessels, macrovascular obliterative disease has also been described in SSc. There have been reports of very severe clinical cases of obliterative peripheral arterial disease despite of the lack of traditional risk factors of atherosclerosis. About 15–20% of scleroderma patients exert multiple vascular abnormalities including the combination of cardiovascular diseases, stroke and peripheral arterial disease [19-21].

### **Role of autoimmunity and inflammation**

Another prominent feature of SSc is given by the implication of the immune system [22]. Altered function of both humoral and cellular immune mediators represents an indispensable linkage between vascular impairment and tissue fibrosis, as both B and T cells exert manifest effects on either. Despite a lot of interesting data (anti-DNA-topoisomerase I/anti-topo I/, anticentromere antibodies /ACA/, anti-platelet derived growth factor receptor /anti-PDGF receptor/ antibodies, and TH2 cytokines in skin biopsies, etc.) the causative role of B cells derived products is not well defined. Memory B cells were demonstrated to be chronically activated in patients with SSc [23]. As B cell depletion in a mouse model for SSc not only revealed reduction of skin fibrosis but also diminished autoantibody production and suppression of hypergammaglobulinemia [24], the typical appearance of autoantibodies was also shown to play active functional roles in SSc. Their generation might be explained by the increased occurrence or abnormal distribution of potential autoantigenic peptides, by molecular mimicry and endogenous B cell alterations leading to chronic hyperreactivity [25]. Putative pathogenic autoantibodies characteristic for SSc include such targeting endothelial cells as well as those against PDGF receptors and fibroblasts. Endothelial cell-specific antibodies were estimated to occur in approximately one third of SSc patients and are not unlikely to contribute to the apoptosis and activation of endothelial cells [26, 27]. Recently antibodies against angiotensin II type 1 receptor (AT<sub>1</sub>R) and endothelin-1 type A receptor



(ET<sub>A</sub>R) were identified with high prevalence (approx. 20%) in patients with SSc [28]. Anti-AT<sub>1</sub>R and anti-ET<sub>A</sub>R autoantibodies have been demonstrated to exert biological effects on endothelial cells and may contribute to the pathogenesis of SSc and could also represent a link between autoimmunity, endothelial injury and fibrosis [28].

Furthermore, activated B cells secrete IL-6 and thus additionally stimulate fibroblasts, which might also contribute to the pathogenesis of SSc by directly promoting fibrogenesis [24, 29].

The inadequate activation of T cells further contributes to microvascular dysfunction, autoimmunity, and tissue fibrosis. Activated endothelial cells express adhesion molecules, which, in part, promote the formation of perivascular inflammatory infiltrates by enabling the transmigration of inflammatory cells through the endothelium. Correspondingly, enhanced transendothelial migration of CD4<sup>+</sup> T cells could be observed in patients with SSc [30]. An imbalance in TH1 and TH2 cytokines is also seen in SSc patients with the TH2 products preponderating.

In summary, the inflammatory and autoimmune aspects of SSc enabling perivascular inflammation, release of fibrosis promoting cytokines and the production of stimulatory autoantibodies are essential drivers of the disease, leading to progression of vasculopathy as well as tissue remodeling by means of increased fibrogenesis in patients with SSc.

### **Fibrosis in systemic sclerosis**

Fibrosis might be considered as the most prominent hallmark of SSc. Newly formed broad and hyalinized collagen bundles occur within sclerotic lesions. Its occurrence is not restricted to the skin, but also very prominent in the lung and heart, the gastrointestinal tract, in tendons and ligaments as well as in endocrine glands and within the perivascular space, where it also accounts for vascular pathology and contributes to the vicious SSc circle. Fibroblasts are the key players in promoting extracellular matrix remodeling. In SSc, the fibrotic processes are typically characterized by prolonged and exaggerated activation of fibroblasts and maintenance of fibroblast-mediated effects. Transdifferentiation of fibroblasts and pericytes into contractile myofibroblasts may also occur [31, 32]. Myofibroblasts are specialized fibroblasts that share similarities with smooth muscle cell differentiation, since they possess prominent  $\alpha$ -SMA<sup>+</sup> stress fibers within their cytoplasm. Myofibroblasts also synthesize collagens and other ECM components. In contrast to physiological wound healing, where myofibroblasts only occur transiently within the granulation tissue, there is persistence in SSc fibrosis leading to contracture of the extracellular matrix with chronic scarring [33]. Myofibroblasts are also a major source of transforming growth factor- $\beta$  (TGF- $\beta$ ) which plays

a pivotal role in fibrosis [32], as it is both a direct mediator of fibrosis and can also induce itself the transdifferentiation of fibroblasts to myofibroblasts.

The bone marrow also releases fibroblast progenitor cells. Additionally, the transition of differentiated non-fibroblastic cell lineages (such as epithelial or endothelial cells or adipocytes) into fibroblasts and myofibroblasts in the context of deregulated fibrogenesis in SSc is an area of substantial research interest. Factors promoting the transition of various precursor and mature cell types toward an activated fibroblastic phenotype include TGF- $\beta$ , Wnt and hypoxia, whereas peroxisome proliferator-activated receptor gamma appears to play an important inhibitory role in these pathways and promotes the maintenance of cellular quiescence [34].

From a molecular point of view, several profibrotic mediators such as cytokines, chemokines and growth factors contribute to the pathogenesis of SSc by increasing the fibrogenic cellular response, which is achieved by inducing the expression of genes encoding for extracellular matrix constituents. TGF- $\beta$ , for instance, is considered to be the master regulator not only of physiological fibrogenesis during wound healing and tissue repair, but also during pathological scarring as seen in SSc [31, 35]. It can be secreted by various cell types including fibroblasts, T cells, monocytes and macrophages, as well as platelets, and many cell types also possess surface receptors specific for TGF- $\beta$  [31]. The SMAD integrating pathway is thought to be the most essential mechanism transmitting TGF- $\beta$ -mediated signals, however additional studies underline also the potential relevance of non-SMAD pathways [36]. Expressed at only very low levels in physiologically intact tissues, connective tissue growth factor (CTGF) was also found to play a profibrotic role in SSc, since its levels were detected to be markedly elevated in lesional tissues from affected patients [37]. PDGF is another growth factor additionally managing to modify the interaction of TGF- $\beta$ , ET-1, and CTGF. PDGF is produced by fibroblasts, endothelial cells, macrophages, and platelets upon activation [38]. As a strong mitogen and chemoattractant for fibroblasts, PDGF can activate the fibroblast response. The cytokines IL-4 and IL-13 also exhibit important roles in the pathogenesis of profibrotic and TH2 cell-mediated disorders [39]. By indirect effects of chemokines such as MCP-1, for example, TGF- $\beta$  and IL-4 can also be induced to promote further collagen production in vitro, and MCP-1 also directly stimulates collagen production itself [40].

#### **A. Investigation of disease activity in systemic sclerosis**

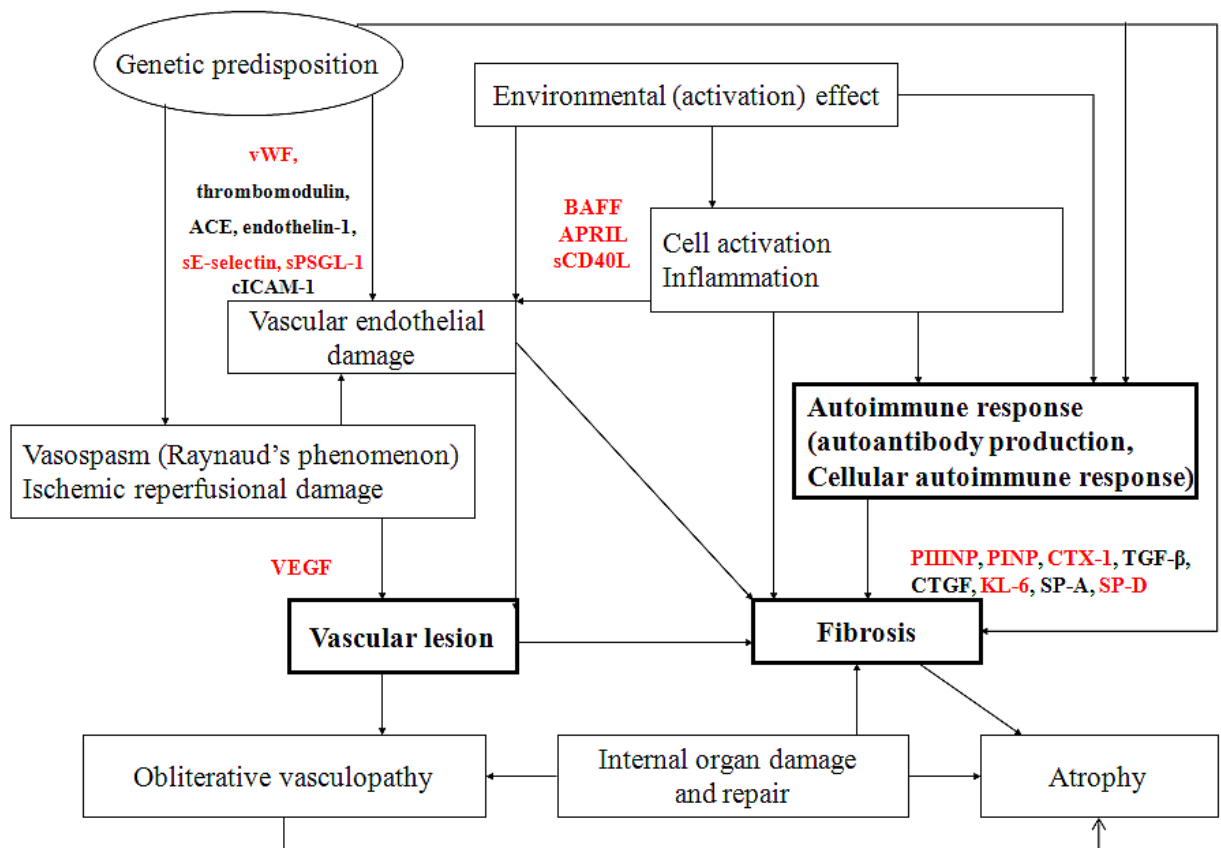
Systemic sclerosis is characterized by tightening and thickening of the skin and the presence of specific internal organ involvements. Particularly early in the disease, more extensive skin

involvement coincides with more severe internal organ manifestation(s), poor prognosis [41-43], and increased disability [44-46]. The evaluation of disease activity, especially in early cases with SSc is a crucial point, as in this phase of the disease more aggressive therapy is needed.

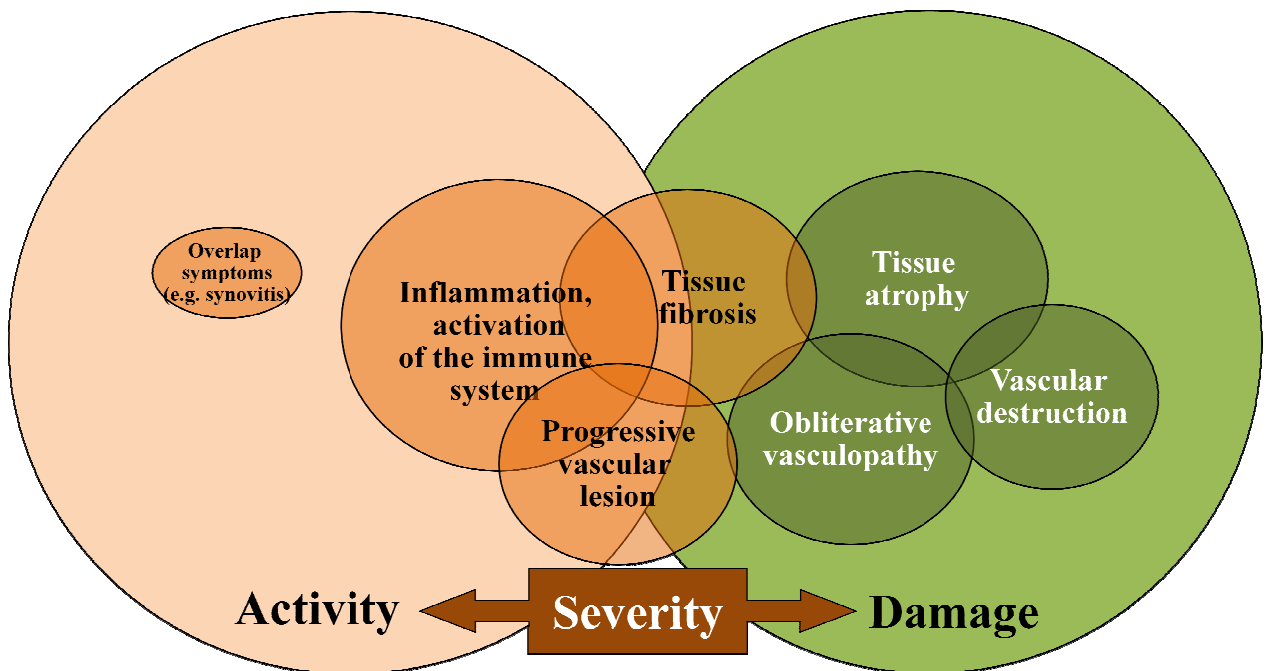
The key points in the disease process are the endothelial cell injury, inflammation/immune activation and collagen deposition by activated fibroblasts (Figure 1). Therefore, theoretically a disease activity measurement should reflect all these important factors, and also their impact on the different organ systems.

Disease activity usually measures the aspect of disease that varies over time and is potentially reversible spontaneously or under drug treatment [47-50]. On contrary, damage measures irreversible tissue injury that results from disease. Disease severity lacks an accepted definition. Some equate it to a measure of prognosis [48]. Most frequently it is considered as representing the total effect of disease on organ function at a given point in time, including both reversible (activity) and irreversible components (damage) phase response [49, 51] (Figure 2).

**Figure 1.** Schematic model of pathogenesis in systemic sclerosis



**Figure 2.** The main pathological processes of disease activity, damage and severity in systemic sclerosis



Measures of disease activity in SSc are needed for multiple uses. In observational studies, they could be used to describe and compare study populations and identify potentially reversible aspects of disease [48, 51]. Given that research into new treatments for SSc is rapidly advancing, they could be used to determine eligibility and as a measure of outcome in upcoming clinical trials. Finally, measuring disease activity in clinical practice helps to decide, whether the actual therapy gives satisfactory results or a more aggressive therapy is needed.

Measurement of disease activity in SSc is particularly difficult, because many patients, especially those with limited skin involvement, have an indolent course without clear signs of inflammation [47, 50]. Although our previous studies indicated that the inflammation (increased acute phase reaction) plays a significant role in SSc [52] the extent of inflammatory signs are less pronounced compared to the other connective tissue diseases, and even may be missing. The importance of the inflammatory signs and the activation of immune system is still a question of debate in SSc [53-55]. Furthermore, clinical features contributing to vascular damage and tissue fibrosis are also difficult to evaluate [47, 52]. When these become measurable, the process has often progressed to permanent damage (Figure 2).

For the investigation of disease activity in SSc, two major tools are used: **clinical investigation** including the assessment of skin thickening and changes in the internal organ involvements [47, 56, 57], and **laboratory parameters** indicating the inflammatory activity, ongoing fibrosis, and vascular changes during the disease course [58].

Derived from the original Rodnan skin score [59] several modified and simplified skin score measuring methods have been developed [60, 61]. The modified Rodnan skin score (MRSS) uses clinical palpation to estimate skin thickness. In present the MRSS is considered the most appropriate and reproducible technique for measuring skin involvement in SSc [41, 42, 60, 62, 63]. The method is easily used and fully validated [62, 63].

Although MRSS is an excellent, simple, and reproducible method, the intra- and interobserver variability still remain relatively high, and the application of this particular method requires a careful teaching process, and probably even a repeated teaching procedure [64, 65]. There is a need for an alternative, simple, independent method for measuring skin involvement, because the administration of at least two independent instruments substantially increases the reliability and accuracy of the particular evaluation of skin thickening. Other non-invasive methods for measuring skin thickness/hardness include ultrasound, durometer, elastometer and plicometer. Some of them may not be feasible enough for everyday clinical practice (e.g. ultrasound examination), others are not validated. The durometer is a digital, hand-held, spring-loaded device predominantly measuring skin hardness by applying an indentation load

on surfaces. Durometry has been recently demonstrated to be a valid, objective and reliable technique in skin hardness measurement, which correlates well with skin score, disability of patients, and also with the extent of skin fibrosis scored on skin biopsy [66]. Nevertheless, it captures similar, but not exactly the same domains of skin disease as these other measurements [67, 68].

We assumed that patients could provide valid and reliable data regarding their skin involvement, which could be a new skin thickness evaluation tool in addition to the MRSS. Therefore we have developed and partially validated a patient self assessment questionnaire for the measurement of skin thickening [69].

This independent, simple, quick and feasible parallel method for measuring skin thickness increases the reliability of clinical judgment in the evaluation of skin thickening in patients with SSc.

In 2001, the European Scleroderma Study Group (EScSG) developed preliminary disease activity indices to be used in patients with SSc [70, 71]. Investigators of 19 European Centers including our center completed an extensive 88 item, standardized chart for each of 290 consecutive patients with either diffuse (dcSSc) or limited SSc (lcSSc). Three experts blindly evaluated each chart and, by consensus, assigned a disease activity score to each chart on a scale of 0 to 10. This constituted the “gold standard”. By using the statistical methods of univariate analysis and multiple linear regression analysis, three separate weighted 10-point indices of disease activity were constructed, one for the group as a whole and one for each of the subsets with either diffuse or limited disease. During construct validity assessment of the 3 preliminary indices only the index for the whole scleroderma group and not the indices for disease subsets were judged to be valid [72]. At last, only the Disease Activity Index for all patients with SSc (EScSG activity index) was adopted by EScSG and the Scleroderma Clinical Trials Consortium. It consists of 10 variables with weights ranging from 0.5 to 2.0 and resulting in a total score ranging from 0 to 10. This index appears simple and easy to use as it evaluates both clinical items (MRSS, DLCO, presence of sclerodema, arthritis and digital ulcers) and some basic laboratory investigations (erythrocyte sedimentation rate, hypocomplementaemia) for the calculation of disease activity. Therefore it is now increasingly being used in research settings [73-75]. However, these criteria await for a full validation, as the construct validity has been confirmed only on a small cohort of 30 SSc patients. Also the responsiveness of the index has not been proved yet.

Furthermore, there is a chance, that by the introduction of some additional clinical-laboratory parameters a new disease activity instrument may be also constructed or at least the present one could be improved. Additional clinical parameters that could indicate the activation of the

disease might be the **appearance or worsening of ulcers; worsening in the musculoskeletal, gastrointestinal or renal symptoms** of the patients; and **signs of interstitial or vascular pulmonary involvement**.

Regarding laboratory parameters, many biomarker candidates have been identified in the past two decades; however, fully validated measures are still lacking with regard to supporting the early diagnosis and reflecting the disease activity, severity, prognosis, and response to therapy. This is in part because it is difficult to objectively measure many aspects of this particular multifactorial disease that may even change slowly with time or may smolder. Attention in biomarker development should focus on their discriminatory value for specific clinical features and outcomes within scleroderma, rather than searching for those markers that are uniformly and strikingly different compared with control populations.

An ideal biomarker should be highly sensitive and specific, reflecting the current status of disease; should be related to the disease activity and/or severity in accordance with the clinical evolution; should anticipate clinical changes before they occur; and should add independent information about the risk or prognosis that is reproducible and feasible [76].

### **Potential biomarkers for disease activity assessment in systemic sclerosis**

#### ***Markers of endothelial cell activation.***

**von Willebrand factor antigen (vWF<sub>Ag</sub>)** is a glycoprotein that plays a pivotal role in the interaction among endothelial cells and platelets. It is released mainly from injured or activated endothelial cells and has been reported to be a marker of vascular disease. The vWF level was reported as increased in SSc [77-80], the mean levels being higher in the dcSSc subset [81]. The elevated level seemed to correlate with the extent of internal organ involvement [82, 83]. In an earlier study an inverse correlation has been found between the glomerular filtration rate and vWF and a negative relation between pulmonary pressure and vWF in a small number of patients with isolated pulmonary hypertension. [84].

**E-selectin** (or Endothelial Leukocyte Adhesion Molecule-1 (ELAM-1)) belongs to the selectin family of adhesion molecules. Together with L-selectin and P-selectin, E-selectin mediates the initial interactions of leukocytes and platelets with endothelial cells. Selectins guide non-activated polymorphonuclear cells to the areas of inflammation in creating first, loose contacts with the endothelial layer. E-selectin is particularly interesting because it is found only on the activated endothelium in contrast to other adhesion molecules which have a wide tissue distribution [85]. The level of the soluble form of E-selectin (sE-selectin) was found to be elevated in SSc [86]. Increased tissue expression of E-selectin has been used to demonstrate

endothelial cell activation on the skin of patients with systemic sclerosis [87]. In another study higher levels of sE-selectin were found in SSc patients with pulmonary fibrosis [88].

**P-selectin glycoprotein ligand-1** (PSGL-1) is a high affinity ligand for P-selectin and to a lesser extent for E-selectin, which can be found on most leukocytes. Its soluble form acts as an antagonist for selectins. In SSc the elevated serum levels of sPSGL-1 were found to be associated with a lower frequency and severity of lung fibrosis [89], thus it could be a protective factor against the development of pulmonary fibrosis.

### ***Proangiogenic factors***

Inflammatory cell migration is also increased due to the stimulation of new blood vessel formation by endothelial growth factors.

**Vascular endothelial growth factor** (VEGF) as a fundamental modulator of angiogenesis is involved in the pathogenesis of SSc and other fibrovascular disorders. VEGF has been shown to stimulate von Willebrand factor release from endothelial cells and induce expression of tissue factor activity in endothelial cells as well as in monocytes. In vivo, VEGF can induce angiogenesis as well as increase microvascular permeability.

In one study significantly higher levels of VEGF were detected in patients with anti-topo I autoantibodies and in patients with diffuse SSc. Elevated serum levels of VEGF were a feature of the earliest disease stages. Patients without fingertip ulcers were found to have higher levels of VEGF than patients with fingertip ulcers [14]. In another study serum levels of VEGF in 48 patients with SSc were significantly higher than in 30 controls. Serum VEGF levels correlated well with the extent of skin sclerosis, as determined by modified Rodnan skin score. In particular, serum VEGF levels were inversely correlated with the capillary density of nailfold [90]. Its permanent up-regulation was observed in SSc and this could be essential in a paradox manner in the decreased angiogenetic activity that is characteristic to this disease [91].

### ***Markers of fibrosis.***

There are numerous well-characterized markers of increased fibrotic process, of which levels indicate the extent of fibrotic alteration of the connective tissue. The normal dermis contains several mature fibrillar collagen types, but type I collagen is the most abundant protein, constituting about 80-85% of skin collagens. Type III collagen is responsible for about 10-15% and the minor collagens for about 5%. In sclerodermatous skin, an increase in type I collagen production has been described from fibroblast cultures. The evaluation of collagen formation markers has revealed that serum procollagen I concentrations may increase in SSc,



but that this increase is not clearly correlated with fibrotic processes. Conflicting results have been reported with respect to collagen degradation in SSc. Both unchanged and decreased amounts of mRNA for interstitial collagenase and activities of the enzyme have been found in fibroblast cultures obtained from patients with this disease.

**Procollagen Type I N-Terminal Propeptide (PINP)** results from the cleavage of the two large extension domains from the longer precursor procollagen molecules during type I collagen synthesis, thus its concentration in the circulation reflects the synthesis rate of type I collagen. In a multicenter, randomized, placebo-controlled phase I/II trial with a recombinant human antibody that neutralizes transforming growth factor, the changes in the PINP level correlated with changes in the MRSS [92].

The recently described cross-linked **collagen I carboxiterminal telopeptide** (serum crosslaps, CTX-1) is an 8 amino acid telopeptide produced during resorption of the  $\alpha 1$  type I collagen chain. The concentration of CTX-1 was found to be higher in the serum of dcSSc patients than in the serum of those suffering from lcSSc. CTX-1 correlated with the extent of skin involvement (MRSS), the acute-phase proteins and indicators of decreased pulmonary function (DLCO<75%) [93].

In a previous study we have found, that the concentration of **procollagen type III N terminal propeptid** (PIIINP) in the serum was in direct proportion with the extension of skin involvement. The level of PIIINP also indicated the prognosis of the disease, beneath a higher concentration in the serum, the patients' life expectancy was lower. The highest values of PIIINP have been measured in the case of patients with rapidly progressive course of disease with deadly outcome. Serum PIIINP levels were significantly higher in SSc patients with restrictive pulmonary function (FVC < 80%) than in patients with normal pulmonary function. The PIIINP was found to be in correlation with the MRSS and negatively correlated with DLCO [52].

### ***Inflammation, markers of cellular/humoral activation.***

The acute-phase reactants in a remarkable part of the systemic sclerosis patients do not indicate the inflammatory phase of the disease; however the permanently elevated **erythrocyte sedimentation rate** (ESR) and elevated **C-reactive protein** (CRP) levels are secure signs of increased activity and unfavorable prognosis. Several studies including ours [52] showed that the value of ESR was significantly higher in patients with dcSSc than those with lcSSc, in elder male scleroderma patients who had a worse prognosis, in patients with a severe course of disease and fatal outcome, and in cases with severe interstitial alveolitis and/or lung fibrosis. The permanently elevated CRP was a bad prognostic factor in our

experience, too [52]. In those cases, when the CRP is elevated, its monitoring could help to follow the efficacy of the treatment.

#### ***Markers of pulmonary fibrosis/involvement.***

The **KL-6** (Kerbs von Lungren 6 antigen) is a mucin-like protein, which is produced by type II alveolar epithelial cells. Circulating KL-6 has been shown to be a sensitive marker of the disease activity of interstitial lung diseases. Circulating KL-6 concentration strongly correlated with the severity of interstitial lung disease (ILD), and also with disease activity in one study [94-97]. Beneath increasing levels of KL-6 in the serum a proportionally worsening ILD was evidenced by high resolution computed tomography (HRCT), in parallel with a decreasing vital capacity (VC) [94].

The type II alveolar epithelial cells also produce surfactant proteins, from which the concentration in the serum of **surfactant protein -A and -D** (SP-A, SP-D) are raised in scleroderma associated with interstitial lung involvement. The levels of SP-A and SP-D correlated with the activity of disease [95, 98]. In one study the level in the serum of SP-D proved to be more sensitive in the appreciation of lung involvement, than the KL-6 level, however the KL-6 was more specific, than the SP-D level [99].

#### ***T-cell activation marker.***

The **Soluble CD40 Ligand** (sCD40L) is released from activated CD4+ T cells, and is biologically active. The CD40/CD40L interactions activate B cells, upregulate endothelial adhesion molecules, and induce fibrosis. A recent study showed that patients with dcSSc exhibited relatively persistent elevations of sCD40L concentrations, whereas temporary elevations were observed in lcSSc patients during follow-up [100]. Another study reported the association of plasma sCD40L concentrations with the presence of digital ulcers in SSc patients. Concentrations of plasma sCD40L were significantly higher also in patients with PAH [101].

#### ***B-cell activation markers.***

The **B-cell activation factor** (BAFF), a marker associated with altered B-cell function, has multiple roles in B-cell development and homeostasis ranging from maturation of naïve B-cells to the maintenance of long lived plasma cells. A recent study demonstrated elevated BAFF level, and correlation of BAFF level with skin fibrosis in patients with SSc. Moreover, increasing levels of BAFF were associated with the new onset or worsening of organ involvement, while decreasing BAFF levels were accompanied by regression of skin sclerosis. BAFF mRNA

expression was up-regulated in the affected skin of patients with early diffuse cutaneous SSc [102, 103]. Concerning serum BAFF levels in localized scleroderma subgroups, patients with generalized morphea, the severest form of localized scleroderma, had higher serum BAFF levels than linear scleroderma or morphea patients. The BAFF levels of generalized morphea were comparable with those of SSc or SLE. By contrast, serum BAFF levels were not significantly elevated in patients with other autoimmune diseases of skin, such as pemphigus or pemphigoid [104]. In the tight-skin (TSK/+) mouse, a genetic model for systemic sclerosis, BAFF antagonist inhibited the development of skin fibrosis, hypergammaglobulinemia, and the autoantibody production [105].

**A Proliferation-Inducing Ligand** (APRIL) is also a tumor necrosis factor (TNF) superfamily member with close homology to BAFF, which stimulates B cells *in vitro* and *in vivo* and plays role in T cell survival and T-independent type II antigen responses also. In a recent study serum APRIL levels tended to be higher in patients with dcSSc compared with lcSSc, and SSc patients with elevated APRIL levels had significantly higher incidence of pulmonary fibrosis and decreased vital capacity (VC). Serum APRIL levels did not correlate with the extent of skin fibrosis and BAFF levels [103].

With regard to the **anti-DNA topoisomerase I** (anti-topo I) autoantibody, higher titres of it were found in patients with very active disease (based on clinical evaluation) compared with those with inactive disease, and a recent study also found that anti-topo I levels correlated with disease activity, MRSS, forced vital capacity (FVC) and DLCO [57, 106].

**B. Investigations of the socio-economic impact of disease activity and severity in systemic sclerosis, and characterisation of cardiopulmonary vasculopathy in systemic sclerosis, a leading cause of morbidity and mortality, by invasive techniques**

Due to the complex pathomechanism of the disease the diagnosis, screening and treating of systemic sclerosis represents one of the greatest challenges in the management of autoimmune rheumatic diseases. The interaction between the immune system, vasculature and components of connective tissue contribute towards the development of severe organ-based complications, including digital ulceration (DU; often secondary to RP), renal disease, cardiac or gastrointestinal (GI) manifestations of SSc and pulmonary disease. Organ-based complications produce the high case-specific mortality rate observed among patients with SSc [31, 107]. Despite the use of angiotensin-converting enzyme inhibitors to prevent scleroderma renal crisis (SRC), it occurs in ~6% of all patients with SSc, and in 10–15% of those with diffuse SSc. The outcome remains inadequate for many patients. Up to half of them will

require dialysis and the early mortality rate approaches 10% [107]. Cardiac manifestations of SSc include pericardial effusion, myocardial inflammation, conduction abnormalities and, eventually, cardiac failure. Such complications are usually underdiagnosed. Complications involving the gastrointestinal tract are common among patients with SSc. Reflux and difficulty with swallowing are two of the main features of GI involvement, though other problems may include malabsorption, weight loss, bleeding, diarrhoea/constipation, colonic perforation, intestinal pseudo-obstruction, faecal incontinence, early satiety and bloating. Pulmonary complications are the leading cause of death in SSc and most often comprise fibrosis or interstitial lung disease and pulmonary vascular disease leading to pulmonary arterial hypertension (PAH) [108]. The clinical significance of lung fibrosis in SSc is best defined by high-resolution CT, and can be monitored optimally using pulmonary function testing [109]. Currently, identification of PAH often occurs late in the course of SSc, with up to 81% of the patients categorized as New York Heart Association (NYHA) Class III or IV at the time of PAH diagnosis [110]. PAH and lung disease without PAH have a dramatic effect on survival in patients with SSc.

Patients with SSc are at risk for substantial morbidity and disability, so although this disease is relatively rare, it has a potentially important economic impact in terms of health care costs and lost productivity. However, published estimates of the economic impact of SSc are almost nonexistent.

#### ***Socio-economic impact of disease activity and severity in systemic sclerosis***

Taking into account the chronically debilitating character and increased mortality of the disease, SSc has a significant burden on society [111-114]. There is very little information on the economic impact of SSc. The annual average SSc related total costs were 14 959 US\$/patient in the United States in 1994 [115]. Another study analysing 3621 hospital discharges data revealed an average of US\$15 000 per hospitalization (median = US\$8441), amounting to US\$280 million in community hospital charges in the U.S. in 1995 [112]. In Canada, SSc related costs were mean 18 453 Canadian dollars/patient/year in 2007 and in Italy, applying an estimation for indirect costs, the total cost was €11 074/patient in 2001 [116]. Although the average per patient costs of SSc is comparable to or even can exceed the costs of rheumatoid arthritis (RA) (in 2006 RA related mean costs were 21 069, 10 459 and 16 441 euros/patient/year in the US, Canada and Italy, respectively) much less is known on its economic burden in most of the countries [117].

The European League Against Rheumatism Scleroderma Trials And Research group (EUSTAR) has been successful in establishing an international collaboration of data collection on demographics, clinical and laboratory parameters (the so called minimal

essential dataset, MEDS), involving over 7000 SSc patients from 150 centres in 4 continents [118]. As an example for regional differences, analysis of MEDS revealed that eastern centres care for more severe SSc cases in Europe. Large differences in patient referral and recruitment (diverse medical specialities take care of the patients, they attract different patient populations, there are differences in willing to participate in EUSTAR documentation) account for the large local variability of SSc presentations [119]. Hence country- or region specific health economic data have to be investigated.

### *Evaluation of different types of cardiopulmonary vasculopathy in SSc*

In present, pulmonary disease produces the highest mortality in terms of SSc-related problems [108], but cardiovascular deaths are consistently reported as being responsible for 20-30% of all premature deaths [120, 121]. Cardiac involvement in systemic sclerosis includes coronary artery disease (CAD), pulmonary arterial hypertension (PAH) related right ventricular changes and microvascular disease (MVD) [122-127]. The clinical presentation of these conditions is often atypical, making their distinction difficult. While some of this cardiac pathology is undoubtedly due to the involvement of the myocardium in the SSc process it can be atherosclerotic in origin and is often asymptomatic until death [128]. Although a high rate of CV related mortality is also common in the general population, it may occur in SSc patients more than a decade earlier [129].

Right heart catheterisation is the gold standard method to confirm the presence of PAH. The coexistence of coronary artery disease and microvascular abnormalities in symptomatic patients has not been previously investigated.

## AIMS OF THE STUDY

### A. Investigation of disease activity in systemic sclerosis

1. Up to now there were basically only two methods of assessing disease activity of systemic sclerosis: the assessment of the clinician, based on the global assessment of the clinical picture and available laboratory and investigation parameters; and the use of the European Scleroderma Study Group Activity Index, developed and partially validated in a European multicenter study in 2003. One of our main aims was to further study the validity of this composite activity index, in a large cohort of systemic sclerosis patients enrolled prospectively, and to repeat the same clinical and laboratory investigations one year later to test the reproducibility of the results.
2. We also aimed to analyze whether additional clinical or laboratory parameters would correlate with the EScSG activity index, and thus could be also used in the assessment of disease activity. We also examined some potential biomarkers reflecting the main pathways of pathogenesis in this disease /**endothelial cell activation** markers (sE-selectin, vWF, sPSGL-1), **proangiogenetic** factors (VEGF), markers of **collagen metabolism** (PINP, PIIINP, CTX-1), markers of **B-cell activation** (BAFF, APRIL), markers of **T-cell activation** (sCD40L), markers of **type II alveolar epithelial cell injury** (KL-6, SP-D) and the titer of anti-DNA-topoisomerase I antibody/ that maybe could be even built into the EScSG activity index, in order to enhance the sensitivity of this index to better reflect the activation of SSc.
3. There is only one fully validated and feasible method of analyzing skin thickness in systemic sclerosis, the modified Rodnan skin score, which is used widely in daily practice and also in clinical trials to assess disease activity and also as a primary endpoint for testing treatment effect. As two independent methods increase the reliability of the assessment of a certain phenomenon, we have developed and partially validated patient self assessment questionnaire of skin thickening in systemic sclerosis, based on the OMERACT ('Outcome Measures in Rheumatology') filter, which gives reliable information about the skin status that is comparable to the modified Rodnan skin score.
4. In our study we have analyzed the relationship of the newly developed patient reported skin thickness score to the EScSG activity index.

5. We have set ourselves the aim to try to modify the EScSG activity index based on our previous results, to develop a new index that better reflects disease activity.

**B. Investigations of the socio-economic impact of disease activity and severity in systemic sclerosis, and characterisation of cardiopulmonary vasculopathy in systemic sclerosis, a leading cause of morbidity and mortality, by invasive techniques**

1. As data about the scleroderma-related burdens of disease is almost nonexistent, we have proposed to estimate it in the Hungarian population (evaluation of costs related to activity and severity of SSc and examination of main cost-drivers). We also compared the costs-of-illness in SSc, psoriatic arthritis (PsA) and rheumatoid arthritis (RA) based on data collected from the Hungarian population.
2. In the present, the leading causes of death after pulmonary fibrosis in systemic sclerosis are the different types of cardiopulmonary vasculopathy (such as pulmonary arterial hypertension, coronary disease and microvascular involvement), therefore in our study we have aimed to evaluate their presence with invasive techniques (left- and right heart catheterisation).

## PATIENTS AND METHODS

### A. Investigation of disease activity in systemic sclerosis

#### a. Study groups

In our prospective study 131 consecutive, unselected patients with SSc were included and re-investigated  $12\pm 1.3$  month later. Diagnosis of scleroderma and classification into dcSSc or lcSSc subgroups was performed at the entry based on the criteria proposed by LeRoy *et al* [130]. Disease duration was defined as the period of time in years from the date of onset of first non-Raynaud's phenomenon symptom until the patient's first and second investigation during the study.

Demographic, clinical, and laboratory items, were recorded by our standard protocol [52, 131]. Patients underwent echocardiography, spirometry, DLCO measurement and high-resolution computed tomography if necessary. Pulmonary arterial hypertension was defined by right heart catheterisation [132, 133]. Esophageal involvement was established with barium swallow, or esophago-gastroscopy. Scleroderma renal crisis was recorded as kidney involvement. Musculoskeletal involvement (flexion contractures on hands, arthralgia, arthritis, symmetric muscle weakness /bilateral quadriceps muscle strength  $\leq 3$  on a 1-5 scale), and presence of myositis was also evaluated.

MRSS was performed according to the standard method [134]. Three investigators, unaware of the clinical presentation of the particular patients performed a parallel investigation of each case. Previously the examiners underwent an MRSS assessment validation process [64]. The presence and severity of skin ulcers on the whole body surface was encoded on a 0-3 scale (0- no ulcer, 3- extended ulcer or presence of gangrene) and summarized in the 'ulcer score' variable. The Hungarian validated version of the Scleroderma Health Assessment Questionnaire (S-HAQ) was used [135], and the HAQ Disability Index (HAQ-DI) was calculated, corrected with aids/devices. Disease Severity Scale (DSS) [51] and EScSG activity index [72, 136] were also evaluated. For the hand function assessment, the hand anatomic index (HAI) was used (measure of open hand span minus closed hand span divided by the maximal lateral height of the hand) [137]. The presence of joint contractures was blindly evaluated by experienced physical therapists. The 'number of contractures' variable was counted as the sum of contractures of the following joints bilateral (by summarizing the presence of contractures in all joint axes): shoulder, elbow, wrist, the metacarpophalangeal (MCP) joint of the 2<sup>nd</sup> and 3<sup>rd</sup> finger, the proximal interphalangeal (PIP) joint of the 2<sup>nd</sup> and 3<sup>rd</sup> finger, hip, knee, and ankle (range 0-30). Joint contracture was defined as a decrease  $>25\%$  of the normal range of motion in at least one joint axis.



Laboratory tests included blood cell counts, ESR, C-reactive protein, hemoglobin, hematocrit, serum creatine kinase (CK), lactate dehydrogenase (LDH), total protein, serum albumin, complement (C3, C4).

**b. Establishment and partial validation of a patient skin self assessment questionnaire in systemic sclerosis**

Patients also filled out our newly developed and validated skin self assessment questionnaire [69]. Originally, questions were posed in English and the questionnaire was then formally validated into Hungarian by the “forward-backward” translation method [138]. Questions covered skin thickening, tethering/thinning, changes in the extent/quality of skin involvement, and regional skin symptoms including some questions related to the recent changes in skin involvement. Regarding the skin thickness, tethering and thinness domains, each domain was constructed of 3 items. The overall evaluation of the particular skin involvement was performed by the patient on a 0 to 3 Likert type scale (overall thickness/tethering/thinness score). For the calculation of the 17-area thickness/tethering/thinness score the patients were asked to provide ratings from 0-3 regarding the skin involvement on their face, anterior chest, abdomen, upper arms, forearms, hands, fingers, thighs, lower legs, and feet. We doubled the scores of upper arms, forearms, hands, fingers, thighs, lower legs, feet and added to the scores of the other 3 regions. The maximum score was therefore 51. Furthermore, a visual analogue scale was used regarding the change in the given skin involvement during last month (VAS  $\Delta$ thickness/tethering/thinness score). We applied the OMERACT filter during the validation procedure for truth, discrimination and feasibility [139, 140].

**c. Enzyme-linked immunoassays (ELISA) and Radioimmunoassays (RIA)**

The serum concentrations of VEGF and BAFF (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany), SP-D (AntibodyShop, Gentofte, Denmark), CTX-1 (Nordic Bioscience Diagnostics A/S, Herlev, Denmark), anti-DNA-topoisomerase I antibody titer (Hycor Biomedical GmbH, Kassel, Germany), APRIL and sCD40L (Bender MedSystems GmbH, Vienna, Austria) were measured using commercial ELISA kits, the evaluation of KL-6 using an ELISA kit (Sanko-Junyaku, Tokyo, Japan) was performed as described [141]. Levels of sE-selectin, sPSGL-1 (Bender MedSystems GmbH, Vienna, Austria), vWF (United States Biological, Swampscott, Massachusetts), were determined from plasma samples, using commercial ELISA kits according to the manufacturer’s instructions.

Serum PIIINP and PINP concentrations were determined by radioimmunoassay using RIA kits from Orion Diagnostica, Espoo, Finland.

As controls for these particular tests sera of 51 patients with primary Raynaud's phenomenon and 30 healthy volunteers were used.

Informed consent was obtained from all patients and control subjects participating in the study. The project was approved by the local Research Ethics Committee (Approval No. 2720/2006).

#### **d. Statistical analysis**

Regarding the newly developed **skin self assessment questionnaire**, the criterion validity evaluation included the calculation of Spearman's correlation coefficients to estimate the association of the *skin thickness domain* with the gold standard MRSS. Other instruments and measurements, e.g. the S-HAQ, HAI, Disabilities of Arm, Shoulder and Hand questionnaire (DASH), and maximal oral aperture (MOA) were also used for comparison with the skin self assessment questionnaire. Discriminating ability of the questionnaire was examined by comparing scores of different groups using the Mann-Whitney U test. A principal component analysis was performed to evaluate the construct validity. Our variables had a non-normal distribution, therefore in the multivariate analysis we used non-parametric statistical methods. Namely, our variables were encoded into their ranks and the correlation matrix of the ranks (i.e. matrix of Spearman correlation coefficients) was entered into the principal component analysis. The number of factors was determined on visual inspection of the scree plot, the set of factors with an eigenvalue strikingly separated from the set of those with a value around 1 were selected. The retained factors were then rotated using a varimax procedure [142].

To assess reliability we analyzed the internal consistency of the different types of measures and the reproducibility based on the test-retest method. The self assessment questionnaire was given to 43 consecutive new patients at the time of their appearance for clinical investigation, and the same questionnaire was sent to these particular patients one and two weeks later. Test-retest reliability was assessed by calculating the intraclass correlation coefficient (ICC) [143]. To examine the sensitivity to change of the self assessed score using the changes of MRSS, as a gold standard indicator of change in skin thickening status, three subgroups were formed: worsening, no change and improved status. A change of 3 points was considered to represent the minimal important change in MRSS [144]. Changes in self assessed scores between the baseline- and 12 month reinvestigation were tested with paired *t*-tests in the three groups of change in MRSS.

During the investigation of **possible markers of disease activity**, the normality of distribution for the laboratory and clinical parameters was investigated by Kolmogorov-Smirnov test. As the majority of these parameters had non-normal distribution, median values

were determined and nonparametric tests (Mann-Whitney U test and Wilcoxon test, respectively) were used to compare patient and control subgroups, or baseline and one-year reinvestigation data.

As the clinical-laboratory data contained dichotomous, fractional and continuous variables we used categorical principal component analysis (CATPCA) to evaluate the relationship of these particular items to the EScSG activity index. CATPCA analysis determines the directions and magnitudes of correlations between numerous pairs of differently scaled variables in a simple (i.e., two-dimensional) space. In CATPCA, the relationships between the variables (represented by their correlations with the principal components) are graphically displayed by depicting the variables as vectors. The position of the particular vectors with respect to the axes of the graph indicates the component loadings. The angles between the vectors represent the correlations between the variables. Variables forming an angle less than  $45^{\circ}$  with the vector of the reference variable (namely the EScSG activity index) are considered as related to it [145]. In case of two principal components the visual inspection and interpretation of the graphical representation of the relationships is feasible. Parallel vectors indicate a direct correlation, projections with opposite slopes indicate inverse correlation, whereas perpendiculars indicate no correlation.

For the study of association between the clinical parameters and the EScSG activity index, the number of dimensions was determined depending on the percentage of variances contained by the axes of dimensions. When no remarkable increase in the total variances was detected by the introduction of an additional dimension, the optimal number of dimensions was reached.

We also attempted to generate a new index that reflected disease activity better, than the original EScSG index, therefore we introduced variables considered to be relevant in the assessment of disease activity one by one into the modified index. The relationship of the generated index to the basic clinical parameters was tested each time with CATPCA. The final version was reached, when the new index was associated with both dimensions (i.e. was placed at equal distance from both dimension axes).

The SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) program was used for all analyses.

**B. Investigations of the socio-economic impact of disease activity and severity in systemic sclerosis, and characterisation of cardiopulmonary vasculopathy in systemic sclerosis, a leading cause of morbidity and mortality, by invasive techniques**

***Socio-economic impact of disease activity and severity in systemic sclerosis***

**a. Patient groups**

A cross-sectional survey was performed involving 80 consecutive patients, aged 18 or over attending our Department. Diagnosis of scleroderma and classification into dcSSc or lcSSc subgroups was performed at the entry based on the criteria proposed by LeRoy *et al* [130]. Patients signed informed consent form regarding participation in the study. Authorization by the local Research Ethics Committee was also obtained (No. 2896/2007).

Demographic and clinical data were recorded by our standard protocol [52, 131], EScSG activity index and Disease Severity Scale were used to assess disease activity and severity [49, 72]. Patients completed the validated Hungarian version of Scleroderma Health Assessment Questionnaire [135]. The aggregated S-HAQ score was calculated as the mean of the 13 items (8 domains of the HAQ-DI, 5 VAS), the score range is 0-3, higher S-HAQ value refers to more pronounced disability.

General health status was measured by the validated Hungarian version of the EQ-5D self-completing questionnaire. As there are no available Hungarian time trade off scores offering country-specific EQ-5D index, weights of the United Kingdom's version were used in our study [146].

**b. Health care utilisation survey**

Based on patients' documentation and interviews, we completed a set of questions in reference to SSc related health care resource utilisations. Previous (past year) and current drug treatment, use of aids and assistive devices were recorded, diagnostic procedures, general practitioner (GP) and specialist visits, hospitalizations, surgical interventions, transportation, resorts to non-reimbursed services, home-remodelling (due to health status) were surveyed for the past 12 months. Resort to help from others for self care and everyday activities was asked for the past month. Present employment status and sick-leave taken in the past 12 months were also recorded.

**c. Cost calculation**

Social perspective was used for cost calculation, **direct medical**, **direct non-medical costs** and **indirect costs** (i.e. costs related to loss of productivity) were considered over one year

period. Hungarian official price, tariffs and reimbursement lists of 2006 were used for cost calculation. Direct medical costs were calculated by multiplying the number of utilisations by the unit prices. Diagnostic procedures (X-rays, gastroscopy, spirometry, CT, ultrasound) were evaluated on outpatient prices. Prices of the most commonly used aids and devices were considered for cost calculation. Physicians' and specialist services' costs were calculated on the basis of mean cost/visit obtained from the official statistical database in 2006. Cost calculation of hospitalisations and surgical procedures were based on Disease Related Groups (DRG) reimbursement list.

Distance between patients' residence and rheumatology department was considered to calculate transportation costs. Non-reimbursed medical services and disease related home remodelling costs were based upon patients' answers. Informal care (help from others for everyday activities) was calculated using the average hourly net wage in Hungary (2.7 euros/hour) multiplied by the number of hours.

The costs of sick leave, part time job and full disability due to SSc were calculated using human capital approach method [147]. The average gross income including net wage, personal income tax, pension contribution, health insurance contributions, employers' contribution (913 euros/month in 2006) was multiplied with the time off work. Conversion (250 Hungarian Forints = 1 Euro, at the time of investigation) was applied.

#### **d. Comparison with rheumatoid arthritis (RA) and psoriatic arthritis (PsA)**

A cross-sectional survey involving 255 RA patients was performed by the collaborative research group in Hungary in 2004, taking aim at assessing the RA related costs. Costs of PsA were surveyed in the same way in 2007 in a sample of 185 patients [148]. For the comparison, unit cost-based domains of the RA study were recalculated with prices of 2007 and patient reported amounts were inflated with the cumulative consumer price index 2004–2007, 116%. Total costs, rates of direct and indirect costs were compared by age-groups.

#### **e. Statistical analysis**

Data were analysed using the Statistical Package of Social Sciences, version 14.0 (SPSS Inc., Chicago, IL, USA). Due to skewed distribution of costs, Spearman's rank correlation was used to analyse the relation with variables and Kolmogorov-Smirnov test was applied to compare costs of lcSSc and dcSSc.

## *Evaluation of different types of cardiopulmonary vasculopathy in SSc*

### **a. Patients and methods**

A total of 120 consecutive SSc cases attending our Department were enrolled in the study. SSc was diagnosed by standard criteria [130], and the patients were asked to provide informed consent before the entry. Those with an ejection fraction <30% on echocardiography, or with known severe valvular disease were excluded. Patients with severe lung fibrosis (forced vital capacity <50% on a pulmonary function test) were also excluded from further studies (pulmonary fibrosis group).

Each patient underwent a baseline physical examination. In addition, electrocardiogram, echocardiography and a 6-min walk test were also performed. Lung involvement was investigated by using chest x ray, pulmonary function tests and high-resolution CT in cases where interstitial lung disease was suspected. Clinical and laboratory parameters were recorded.

Cardiac catheterisation was initiated by the cardiologist working in the Heart Institution, University of Pécs, in the presence of abnormalities suggestive of PAH (“suspected PAH” group) or suggestive of CAD (“suspected CAD” group).

Criteria for the “suspected PAH” included signs of right ventricular involvement on echocardiography; tricuspid insufficiency diagnosed by flow velocity over 3 m/s, or consistent with 2.5–3 m/s in the presence of unexplained dyspnoea; signs of right ventricular hypertrophy/dilatation, or right ventricular D sign; and effort related dyspnoea with disproportional decrease of CO diffusion capacity (DLCO) compared to the forced vital capacity (FVC/DLCO >1.8).

Patients were included in the “suspected CAD” group if they reported recent deterioration in physical activity, evolving exertional dyspnoea or evolving chest pain, if they fulfilled the criteria of the New York Heart Association functional class III–IV, or if their 6-min walking distance was <380 m, but did not fulfilled the criteria for PAH [149].

Right heart catheterisation (RHC) and coronary angiography, supplemented with thermodilution coronary flow reserve (CFR) assessment, were performed at the same time. The trial protocol was approved by the Medical Research Council Scientific and Ethical Committee.

### **b. Invasive cardiac investigations**

Right heart catheterisation and coronary angiography were performed at the Heart Institute, University of Pécs. At RHC, if the resting mean pulmonary pressure (mPAP) was lower than

30 mm Hg, a 3-min bench-fly physical stress test was performed with 1 kg dumbbells. mPAP was measured at rest and at peak exercise. mPAP values of >25 mm Hg at rest or >30 mm Hg upon exertion were considered abnormal. On coronary angiography, the extent of the CAD, fractional flow reserve (FFR) and CFR was recorded. Microvascular disease was defined as reduced flow reserve (CFR<2) in the absence of significant stenosis (FFR>0.75).

**c. Statistical analysis**

For the in-group comparisons, unpaired t tests,  $\chi^2$  tests, or Fisher exact tests were used. Correlation analysis was performed with the Pearson rho test.

## RESULTS

### A. Investigation of disease activity in systemic sclerosis

#### **Clinical characteristics of the SSc patients**

The clinical parameters of the 131 prospectively enrolled SSc patients are depicted in Table 1. The female/male ratio was 9.9:1 (17:1 in lcSSc group and 4.8:1 in dcSSc group, respectively), mean (SD) age at the entry into the study was 55.9 (11.6) years /57.4 (10.3) years in lcSSc group and 52.6 (13.8) years in dcSSc group, respectively/. The mean (SD) disease duration was 8.1 (7.2) years /8.6 (7.5) yrs in lcSSc group and 7.0 (6.3) years in dcSSc group/. 123 patients appeared for the one-year reinvestigation, 5 patients died during these 12 months of causes related to SSc, 3 were lost to follow-up.



**Table 1.** Clinical parameters of the 131 SSc patients enrolled into the follow-up study

	<b>Total (baseline)</b>	<b>Total (1st year)</b>	<b>lcSSc (baseline)</b>	<b>lcSSc (1st year)</b>	<b>dcSSc (baseline)</b>	<b>dcSSc (1st year)</b>
No of patients	131	123	90		41	
Female:Male ratio	9.9:1	9.2:1	17:1		4.8:1	
Age /Mean (SD)/	55.9 (11.7)	57.2 (11.4)	57.4 (10.3)		52.6 (13.8)	
Disease duration /Mean y's (SD)/	8.1 (7.2)	9.1 (7.3)	8.6 (7.5)		7.0 (6.3)	
<b>Clinical data</b>						
Raynaud's phenomenon	131 (100%)	123 (100%)	90 (100%)	87 (100%)	41 (100%)	36 (100%)
MRSS /Mean (SD)/	3.6 (3.9)	2.5 (3.2)	2.3 (2.3)	1.7 (1.7)	6.4 (5.0)	4.4 (4.8)
Lung involvement						
Bibasilar fibrosis <sup>1</sup>	46 (35.1%)	38 (30.9%)	32 (35.6%)	26 (29.9%)	14 (34.1%)	12 (33.3%)
Diffuse fibrosis <sup>1</sup>	23 (17.6%)	33 (26.8%)	11 (12.2%)	20 (23.0%)	12 (29.3%)	11 (30.6%)
Honeycombing <sup>1</sup>	17 (13.0%)	17 (13.8%)	10 (11.1%)	7 (8.0%)	7 (17.1%)	10 (27.8%)
FVC /Mean (SD)/	96.5 (19.7)	96.6 (19.8)	100.0 (17.9)	99.6 (18.5)	88.7 (21.4)	89.3 (21.1)
DLCO /Mean (SD)/	64.5 (17.5)	62.7 (18.1)	67.6 (16.2)	65.2 (16.7)	57.8 (18.5)	56.5 (20.2)
PAH <sup>2</sup>	10 (7.6%)	11 (8.9%)	8 (8.9%)	9 (10.3%)	2 (4.8%)	2 (5.6%)
Cardiac involvement						
LVEF<50%	4 (3.1%)	3 (2.4%)	3 (3.3%)	3 (3.4%)	1 (2.4%)	0 (0.0%)
Diastolic dysfunction	68 (51.9%)	68 (55.3%)	49 (54.4%)	50 (57.5%)	19 (46.3%)	18 (50%)
Esophageal involvement <sup>3</sup>	76 (58%)	78 (63.4%)	45 (50%)	50 (57.5%)	31 (75.6%)	28 (77.8%)
GI symptoms <sup>4</sup>	96 (73.2%)	94 (76.4%)	64 (71.1%)	64 (73.6%)	32 (78%)	30 (83.3%)
Muskuloskeletal involvement <sup>5</sup>	117 (89.3%)	86 (69.9%)	78 (86.7%)	59 (67.8%)	39 (95.1%)	27 (75%)
Renal involvement	2 (1.5%)	1 (0.8%)	1 (1.1%)	1 (1.1%)	1 (2.4%)	0 (0.0%)

<sup>1</sup>findings on HRCT; <sup>2</sup>Pulmonary artery hypertension; <sup>3</sup>signs of esophageal involvement on barium swallow or esophago-gastroscopy <sup>4</sup>gastrointestinal symptoms defined by heartburn, dysphagia, bloating, diarrhoea and/or fecal incontinence during last month; <sup>5</sup>defined by muscular weakness (strength of quadriceps  $\leq 3$  on a 1-5 scale), myositis, peripheral arthralgia or arthritis, or hand contracture (presence of contracture on physical examination in at least one of the PIP joints, MCP joints or wrists). For details, see Methods.

## Establishment and partial validation of a patient skin self assessment questionnaire in SSc

Correlation among the investigated items of skin evaluation

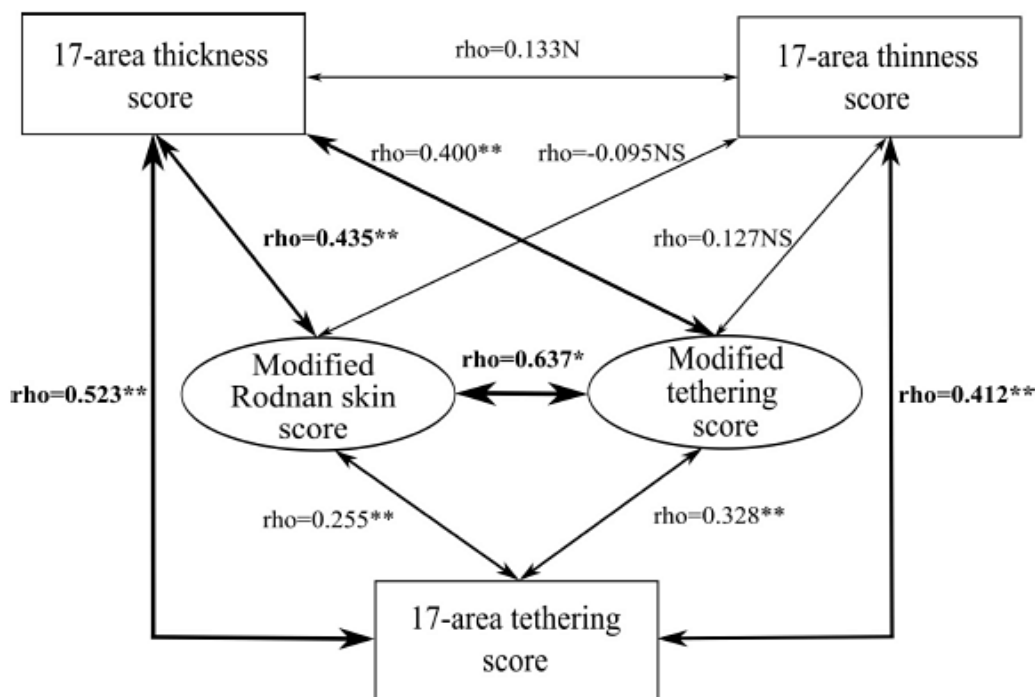
The *skin thickness related items* of the patient self assessment questionnaire correlated with both the MRSS and the modified tethering score. The highest correlation coefficient was seen between the MRSS and patient declared 17-area thickness score (Table 2). The items of *skin thinness domain* correlated neither to the MRSS, nor to the expert measured modified tethering score. The modified tethering score correlated with the MRSS to an extent similar to the patient reported tethering and thickness scores' correlation with each other (Figure 3). Thus we left the *skin tethering domain* out of the further validation process, as it was not independent from skin thickening.

**Table 2.** Correlation between the items of skin thickness domain and other instruments evaluating, at least partially, skin involvement

Instrument	Spearman's correlation coefficients							
	MRSS <sup>4</sup>		Modified tethering score		EScSG Activity Index		S-HAQ	
	Baseline	One year	Baseline	One year	Baseline	One year	Baseline	One year
Overall thickness score <sup>1</sup>	<b>0.332**</b>	<b>0.279**</b>	<b>0.290**</b>	<b>0.385**</b>	<b>0.291**</b>	0.108	<b>0.233**</b>	0.134
17-area thickness score <sup>2</sup>	<b>0.435**</b>	<b>0.331**</b>	<b>0.400**</b>	<b>0.408**</b>	<b>0.275**</b>	0.087	<b>0.241**</b>	<b>0.215*</b>
VAS thickness score <sup>3</sup>	<b>0.239**</b>	<b>0.274**</b>	<b>0.173*</b>	<b>0.185*</b>	<b>0.256**</b>	<b>0.192*</b>	0.163	<b>0.209*</b>
MRSS	–	–	<b>0.637**</b>	<b>0.509**</b>	<b>0.195*</b>	0.130	0.072	0.030
Modified tethering score	<b>0.637**</b>	<b>0.509**</b>	–	–	0.072	0.035	0.131	0.045
EScSG Activity Index <sup>4</sup>	<b>0.195*</b>	0.161	0.072	-0.031	–	–	<b>0.383**</b>	<b>0.329**</b>
S-HAQ <sup>5</sup>	0.072	0.030	0.131	0.045	<b>0.383**</b>	<b>0.305**</b>	–	–

\*. correlation significant at the 0.05 level (2-tailed); \*\*. correlation significant at the 0.01 level (2-tailed). <sup>1</sup>A score from 0 to 3 was provided by the patient characterizing his/her skin thickness in general. <sup>2</sup>17 body areas were scored by the patients (scores from 0 to 3) and summed afterwards. <sup>3</sup>Patients indicated the change in overall skin thickness during the previous month on a 100 VAS scale. <sup>4</sup>European Scleroderma Study Group activity index. <sup>5</sup>Scleroderma Health Assessment Questionnaire. For details, see Methods.

**Figure 3.** Relationship between patient self assessment questionnaire domains and clinical examination parameters



Correlation is significant at the \*0.05 level (2-tailed) and at the \*\*0.01 level (2-tailed). Overall skin thickness, tethering and thinness scores, and VAS thickness, tethering and thinness scores showed correlation coefficients of the same order of magnitude (data not shown).

### Validation of the skin thickness domain of the patient self assessment questionnaire

#### Truth

*Face and content validity.* The skin self assessment questionnaire was constructed by a recognized specialist in SSc, one experienced rheumatologist, and one physiotherapist working with scleroderma patients. For feedback on content and wording, 4 focus groups including a total number of 20 SSc patients were conducted. Patients were first asked to complete the questionnaire (the interviewer timed this process). The general impressions of the interviewees on the questionnaire were collected, and the complete questionnaire was reviewed question by question from the point of view of adequate wording, relevancy and comprehensiveness. There were no major problems with comprehension, the patients found the questionnaire 100% understandable, relevant to their disease, and feasible.

*Criterion validity.* We used the MRSS as the gold standard, and evaluated the correlation of thickness-related scores to this measurement. The baseline MRSS correlated significantly with the 17-area thickness score ( $\rho = 0.435$ ,  $p < 0.001$ ), overall thickness score ( $\rho = 0.332$ ,  $p < 0.001$ ), and VAS  $\Delta$ thickness score ( $\rho = 0.239$ ,  $p < 0.01$ ). One year follow-up data showed similar results (Table 2).

The 17-area thickness score showed a weak correlation to S-HAQ both at baseline- and re-investigation (Table 2). Although the European Scleroderma Study Group activity index showed weak correlation to the items of thickness domain at baseline evaluation, this could not be reproduced when the one year follow-up data were investigated (Table 2).

*Construct validity.* A principal component analysis was performed with Spearman's correlation coefficient matrix of the following variables: skin thickness, tethering and thinness scores, skin evaluation methods carried out by medical personnel (MRSS and modified tethering score), the EScSG activity index, indicators of chronicity and disability (disease duration, S-HAQ, DASH, MOA), indicators of damage (DLCO, LVEF, HAI of the dominant side, BMI) and the age at the entry into the study.

By principal component analysis the distribution of variables into five factors showed the most favourable results. The total variance was 61.6% and all factors exhibited an eigenvalue over 1. The patient assessed skin thickness domain values and the MRSS were sorted into the strongest factor 1 containing 25.3% of the total variances. Otherwise, two clearly separate factor groups could be distinguished: one comprising factor 1, which contained mostly the skin thickness and tethering related items, and the second one comprising all the remaining factors (factor 2-5), predominated by the S-HAQ, an indicator of disability and supported by disease chronicity- and damage-related parameters (e.g. HAI, MOA, disease duration, DASH, DLCO, LVEF).

The patient evaluated 17-area thinness score, and major indicators of disability/chronicity (DASH, S-HAQ, disease duration) were sorted into factor 2, while factor 3 and 4 contained mainly the further damage-related parameters caused by SSc (BMI, HAI, LVEF, DLCO). The MRSS was sorted here with a strong negative loading. The markers of heart and pulmonary involvement (LVEF, DLCO) were sorted into factor 4 together with the European Scleroderma Study Group activity index with high negative loading. The age of patients was placed with the highest loading into factor 5 among markers of disability (DASH, S-HAQ).

In summary, factor 1 predominantly contained skin thickness-related items, and apparently skin thickening and thinning were sorted into separate factors (Table 3).

**Table 3.** Principal component analysis performed to explore the underlying structure of the skin self assessment questionnaire domains, scored by the 131 SSc patients at baseline investigation

	Loading of Component*				
	1	2	3	4	5
MRSS (E)	<b>0,795</b>	<b>-0,299</b>	<b>-0,215</b>		
17-area thickness score <sup>1</sup> (P)	<b>0,776</b>	<b>0,235</b>			
Modified tethering score <sup>2</sup> (E)	<b>0,698</b>		<b>-0,466</b>		-0,189
17-area tethering score <sup>1</sup> (P)	<b>0,610</b>	<b>0,582</b>			
17-area thinness score <sup>1</sup> (P)		<b>0,706</b>			
S-HAQ <sup>3</sup>	0,134	<b>0,611</b>	<b>-0,396</b>	<b>-0,314</b>	<b>0,397</b>
DASH <sup>4</sup>		<b>0,543</b>	<b>-0,440</b>	<b>-0,320</b>	<b>0,459</b>
Disease duration	-0,179	<b>0,514</b>	-0,124	0,170	
HAI <sup>5</sup>	<b>-0,229</b>	-0,138	<b>0,723</b>		
MOA <sup>6</sup>		<b>-0,290</b>	<b>0,628</b>		
EScSG Activity Index	<b>0,287</b>	<b>0,241</b>		<b>-0,740</b>	
LVEF <sup>7</sup>	0,117			<b>0,682</b>	0,131
DLCO <sup>8</sup>		<b>0,269</b>	<b>0,496</b>	<b>0,537</b>	
BMI <sup>9</sup>		-0,160	<b>0,326</b>		<b>0,714</b>
Age at entry into the study		0,115	<b>-0,217</b>	0,107	<b>0,662</b>

*E-expert measurement; P-response of patient in the questionnaire*

<sup>1</sup>17 body areas were scored by the patients (scores from 0 to 3) and summed afterwards. <sup>2</sup>Tethering scores (from 0 to 3) of 17 regions were investigated. <sup>3</sup>Scleroderma-HAQ Disability Index was calculated from the answers of Scleroderma-HAQ Questionnaire <sup>4</sup>Disabilities of Arm, Shoulder and Hand score was calculated from the DASH Questionnaire <sup>5</sup>Hand Anatomic Index of the dominant side was determined. <sup>6</sup>Maximal oral aperture measured as the interlabial distance at maximal mouth opening (mm). <sup>7</sup>Left ventricular ejection fraction was determined on echocardiography. <sup>8</sup>Carbon monoxide diffusion capacity was determined on spirometry. <sup>9</sup>Body Mass Index. Bold numbers represent large values of loading (>0.2), reasonable for interpretation. Empty cells have low (<0.1) loadings without practical meaning. \*Factor loading represents correlation between the observable and the latent variable (i.e. factor).

A principal component analysis of patients with disease duration <3 years (n=29) was also performed. The changes of the skin related parameters, disability, internal organ involvements and disease activity were included in this particular subgroups analysis. The change in MRSS and change in 17-area thickness score were sorted into common factors (data not shown).

## **Discrimination**

*Classification.* There was a significant difference in the 17-area thickness scores between the two scleroderma subsets during the baseline evaluation: 7.5 (0; 16.3) in the lcSSc group and 11 (5.5; 20) in the dcSSc group (median (quartiles),  $p < 0.05$ ). The same difference could not be demonstrated in the one year follow-up data, the median (quartiles) being 4 (0; 12) in the lcSSc group and 7 (3; 14.5) in the dcSSc group.

*Responsiveness.* To examine sensitivity to change, we created 3 groups of patients based on the changes of MRSS of an increase or decrease of at least 3 points during the one year follow-up [31]. No significant differences in the 17-area thickness scores were observed among these groups by testing them with paired *t*-tests.

No correlation was found between change in MRSS and change in the items of *skin thickness domain* at the one year follow-up either. However, in the subgroup of patients with disease duration  $< 3$  years at entry into the study ( $n=29$ ), the change in MRSS showed a borderline correlation with the change in overall thickness score ( $\rho=0.442$ ,  $p < 0.05$ ).

At present, for the investigation of responsiveness of the skin self assessment questionnaire, we are conducting a multicenter study which involves the Departments of Rheumatology from Debrecen (Hungary), Belgrade (Serbia) and Bucharest (Romania).

*Reliability.* Reliability was investigated by analysing the reproducibility based on the test-retest method. Test-retest reliability was measured by intraclass correlation coefficient and good reliability was detected for all of the self reported scores (0.50 to 0.61).

## **Feasibility**

The patients participating in the focus group sessions stated unanimously that the skin self assessment questionnaire was feasible and easy to administer, with a filling out time of approximately 8-10 minutes.

## **Biomarker results in the SSc patients**

As we compared the median values of biomarker levels of the SSc patient group to healthy controls and patients with primary Raynaud's phenomenon, PINP, CTX-1, SP-D and KL-6 were found to be significantly higher in the SSc group both at baseline and one-year reinvestigation compared to both control groups ( $p < 0.01$ ). The sCD40L titer in the SSc group was elevated only compared to the PRP patients. On the contrary, the levels of sPSGL-1 were significantly lower in the scleroderma patients in comparison with the two control groups. Interestingly, the sE-selectin level was higher in the PRP group compared to the SSc patients and healthy controls (Table 4.).

**Table 4.** Median (percentiles) values of the investigated laboratory parameters in SSc patients, patients with primary Raynaud phenomenon and healthy controls

	<b>SSc baseline (n=131)</b>	<b>SSc 1 year (n=123)</b>	<b>Healthy controls (n=30)</b>	<b>Primary Raynaud's phenomenon (n=51)</b>
	Median (Percentiles)	Median (Percentiles)	Median (Percentiles)	Median (Percentiles)
PIIINP <sup>§§</sup> (µg/l)	3.8 (3.1;4.5)	<b>4.3*</b> <sup>††</sup> (3.8;5.2)	4.0 (3.7;4.4)	3.6 (3.4;4.2)
PINP (µg/l)	<b>45.0**</b> <sup>††</sup> (34;65)	<b>44.5*</b> <sup>††</sup> (30;60.1)	33.5 (28.3;44.5)	33.0 (25.7;37.8)
CTX-1 (ng/ml)	<b>0.4**</b> <sup>††</sup> (0.2;0.6)	<b>0.3**</b> <sup>††</sup> (0.2;0.6)	0.2 (0.2;0.3)	0.2 (0.1;0.3)
SPD (ng/ml)	<b>1997.4**</b> <sup>††</sup> (1367.1;3736.8)	<b>1961.4**</b> <sup>††</sup> (1218.8;3709.2)	1238.9 (648.6;1445.5)	1199.6 (734;1727.3)
vWF <sup>§§</sup> (µg/ml)	<b>30.0</b> <sup>††</sup> (21.9;38.6)	33.4 (25.8;41.3)	28.6 (22.6;40)	37.8 (28.1;48.8)
sPSGL-1 <sup>§§</sup> (U/ml)	<b>241.8**</b> <sup>††</sup> (170.6;298.4)	<b>262.3**</b> <sup>††</sup> (214;314.3)	324.2 (273.6;361.8)	322.9 (288.5;388.5)
VEGF (pg/ml)	122.1 (80.2;192.5)	129.2 (88.7;201.8)	93.8 (60;164.2)	111.7 (78.4;227.9)
sE-selectin <sup>§</sup> (ng/ml)	<b>34.4</b> <sup>††</sup> (25.5;46)	<b>34.4</b> <sup>††</sup> (24.7;47.8)	32.1 (21.3;41.3)	89.2 (34.7;226.7)
KL-6 (U/ml)	<b>802.2**</b> <sup>††</sup> (534.3;1246.7)	<b>935.3**</b> <sup>††</sup> (583.8;1323.3)	516.3 (316.5;644.4)	625.7 (387.7;744.8)
BAFF <sup>§§</sup> (pg/ml)	413 (338.2;561.3)	<b>556.9**</b> <sup>††</sup> (439;690.4)	383.7 (327.5;435.4)	440.4 (385.1;564.7)
APRIL <sup>§§</sup> (U/ml)	<b>9.8**</b> <sup>††</sup> (7.2;11.5)	3.2 (1.5;6.9)	2.9 (2.2;4.8)	4 (2.1;7.7)
sCD40L <sup>§§</sup> (U/ml)	<b>2.0*</b> <sup>††</sup> (1.4;2.6)	<b>1.7</b> <sup>††</sup> (1.3;2.2)	1.4 (1;2.3)	1.1 (0.6;1.7)

\*  $p < 0.05$ ; \*\*  $p < 0.01$  between the SSc and healthy control groups; <sup>†</sup>  $p < 0.05$ , <sup>††</sup>  $p < 0.01$  between the SSc patients and those with primary Raynaud phenomenon; <sup>§</sup>  $p < 0.05$ , <sup>§§</sup>  $p < 0.01$  between the baseline and one-year follow-up levels of laboratory parameters in SSc patients. Bold characters represent significant differences between the subgroups.

Comparing the lcSSc and dcSSc subsets, the CTX-1, SP-D and KL-6 differed significantly also between the two SSc subsets at baseline investigation, with higher median values in dcSSc patients. At the one-year reinvestigation the SP-D and KL-6 values also differed

significantly between the lcSSc and dcSSc patients, however the difference between the CTX-1 values in the two SSc subsets disappeared (Table 5.).

**Table 5.** Median (percentiles) values of the laboratory parameters in the SSc subsets during the baseline and one-year reinvestigation

	<b>lcSSc baseline (n=90)</b>	<b>dcSSc baseline (n=41)</b>	<b>lcSSc 1 year (n=87)</b>	<b>dcSSc 1 year (n=36)</b>
	Median (Percentiles)	Median (Percentiles)	Median (Percentiles)	Median (Percentiles)
PIIINP(µg/l)	3.8 (3.2;4.5)	3.7 (2.9;5)	4.3 (3.8;5.1)	4.2 (3.7;6.5)
PINP(µg/l)	45.2 (36.2;67.3)	41 (31.1;64.5)	45.5 (31.4;59.3)	42.1 (28.3;63)
CTX-1 <sup>†</sup> (ng/ml)	<b>0.4</b> (0.2;0.5)	<b>0.5</b> (0.3;0.7)	0.3 (0.2;0.6)	0.3 (0.2;0.5)
SPD <sup>††,‡‡</sup> (ng/ml)	<b>1829.6</b> (1198.6;3175.6)	<b>2899</b> (1762.8;5123.1)	<b>1737.2</b> (1089.4;3277.7)	<b>2799.7</b> (1815.4;5763.4)
vWF (µg/ml)	29.1 (22.6;36.3)	32.7 (19.3;43.1)	33.2 (25.5;39.8)	35 (27.6;50.1)
sPSGL-1(U/ml)	239.0 (172.9;297.7)	250.7 (164.8;303.4)	271.4 (213.4;317)	248.3 (216;294.3)
VEGF (pg/ml)	118.1 (80;190.6)	144.0 (81.7;225.7)	124.7 (87;197.8)	142.6 (93.1;231.7)
sE-selectin(ng/ml)	33.2 (24.9;45.8)	37.0 (26.1;51)	36.2 (24;50.4)	33.9 (25.6;44.7)
KL-6 <sup>††,‡‡</sup> (U/ml)	<b>760.2</b> (464.7;1076)	<b>1045.5</b> (676.4;1884.4)	<b>844.2</b> (533;1190.2)	<b>1176.3</b> (690.3;2345.7)
BAFF (pg/ml)	413.2 (344.9;510.8)	413.0 (311.4;691.4)	569.3 (465.9;711.2)	545.7 (424;683.2)
APRIL <sup>‡</sup> (U/ml)	9.6 (7;11.6)	9.8 (8.6;11.1)	<b>3.8</b> (2.1;10)	<b>2.5</b> (0.8;5.9)
sCD40L (U/ml)	2 (1.5;2.5)	1.9 (1.3;2.8)	1.8 (1.3;2.4)	1.7 (1.2;2)

<sup>†</sup>  $p < 0.05$ , <sup>††</sup>  $p < 0.01$  between the lcSSc and dcSSc subgroups at baseline evaluation; <sup>‡</sup>  $p < 0.05$ , <sup>‡‡</sup>  $p < 0.01$  between the lcSSc and dcSSc subgroups at one-year reinvestigation. Bold characters represent significant differences between the subgroups.

When the changes in the median biomarker levels at one-year follow-up were studied, the PIIINP and BAFF levels increased significantly in the SSc group. Their levels at the time of re-investigation were significantly higher in comparison with the two control groups (difference not seen at baseline investigation). The sPSGL-1 levels also increased significantly at one year follow up. On the contrary, the APRIL and sCD40L levels decreased significantly during the follow-up period (Table 4.).

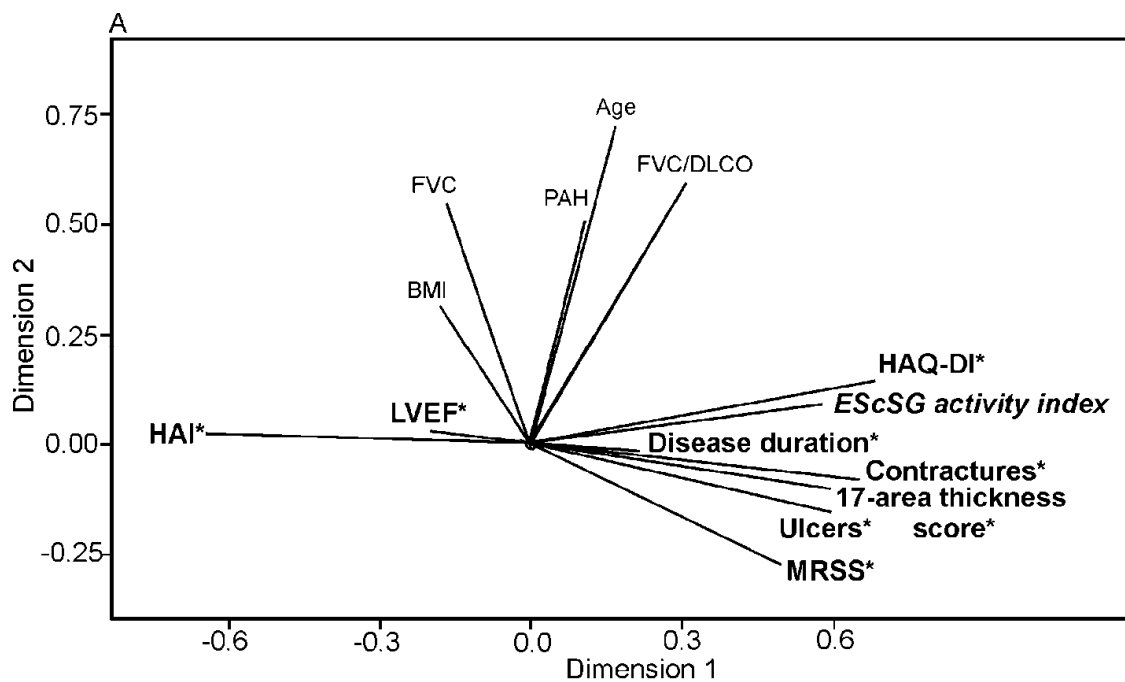


### **Relationship of the EScSG activity index to the clinical parameters**

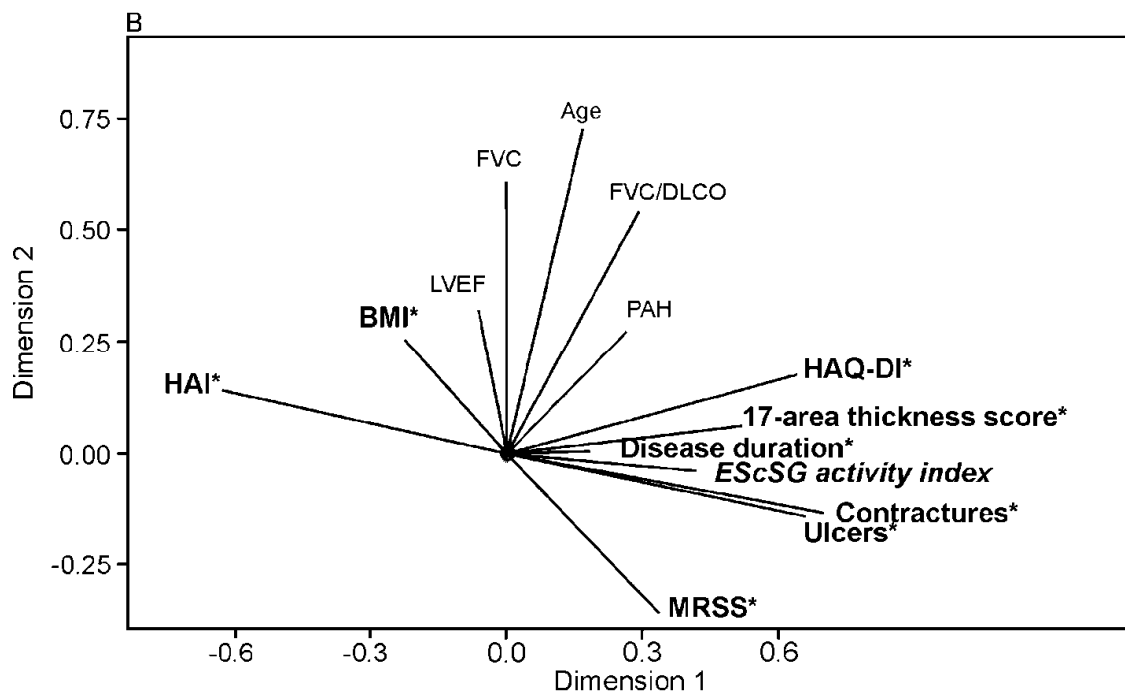
We evaluated the relationship between EScSG activity index and certain clinical parameters including MRSS, 17-area thickness score, disease duration, HAQ-DI, FVC, FVC/DLCO, LVEF, presence of PAH, HAI of the dominant side, BMI, ulcer score and number of joint contractures, and the age at the entry into the study. The disease activity related clinical parameters were distributed into two dimensions by CATPCA, explaining 32.9% of the total variance. The introduction of a third dimension has led to an increase of approximately 10% in the total variance, however only the age of patients was placed into this additional dimension. Therefore we decided to continue the examination of data with the two dimensional model.

The EScSG activity index loaded in dimension 1 and showed association with the HAQ-DI, ulcer score, MRSS, 17-area thickness score, and number of contractures and showed inverse correlation with the HAI (decrease in HAI meaning the worsening of hand function). The parameters of pulmonary involvement (FVC, FVC/DLCO) loaded in the second dimension, and the one year follow-up data showed the same settings (Figure 4. A. and B.).

**Figure 4. A.** Categorical principal component analysis with the clinical data and the EScSG activity index of 131 consecutive systemic sclerosis patients.



**Figure 4. B.** Categorical principal component analysis using the one year follow-up clinical data and the EScSG activity index of 123 consecutive systemic sclerosis patients

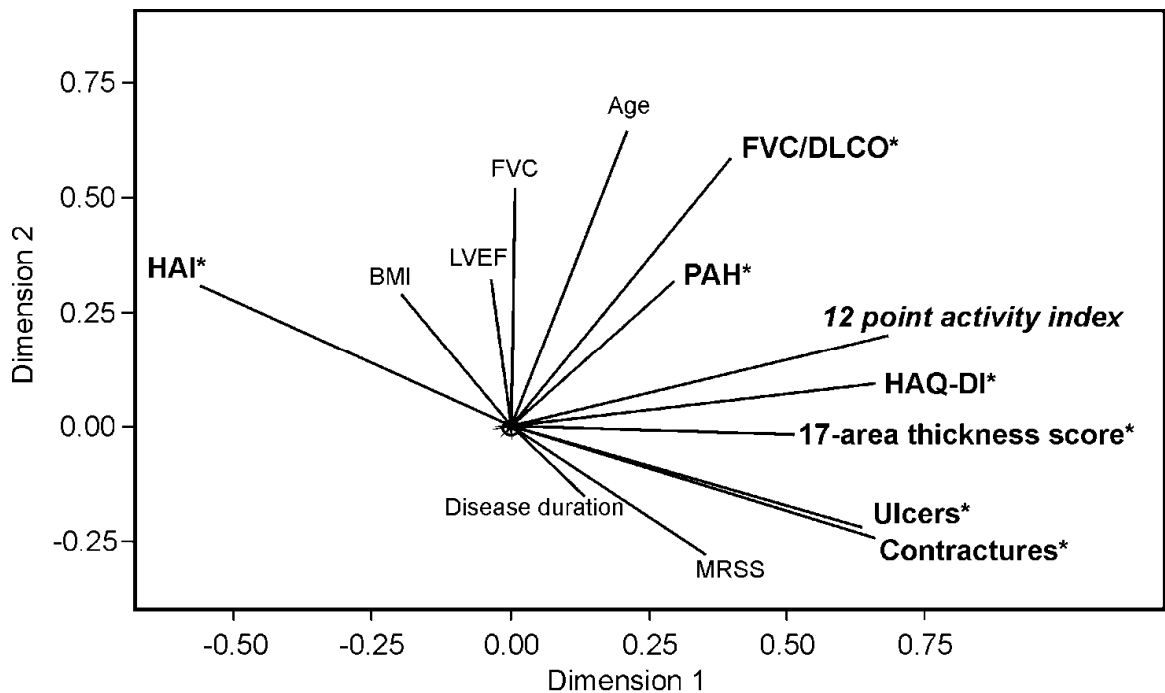


*Abbreviations: EScSG activity index: European Scleroderma Study Group activity index, HAQ-DI: Health Assessment Questionnaire Disability Index, Contractures: number of contractures, Ulcers: ulcer score, MRSS: modified Rodnan skin score, HAI: hand anatomic index, LVEF: left ventricular ejection fraction, BMI: body mass index, FVC: forced vital capacity, FVC/DLCO: ratio of forced vital capacity and carbon monoxide diffusion capacity, PAH: pulmonary arterial hypertension verified by right heart catheterisation. Bold characters together with the "\*" mark indicate those parameters which were found to be correlated with the EScSG activity index by CATPCA. For details, see Methods.*

## Development of a new 12 point activity index

As the lung-related parameters were independent from the EScSG activity index (Figure 4), we generated a new index that reflects lung-related disease activity somewhat better. The selection of variables was based on clinical judgment, and not by a statistical iterative method. We considered that two independent parallel methods for the assessment of a particular organ involvement may lead to a more appropriate result, therefore we introduced the patient reported skin thickness, skin ulcer score, HAQ-DI, change in DLCO and FVC/DLCO ratio. We decided to use the ‘minimal clinically relevant treatment effect’ values as threshold limits ( $\Delta$ MRSS: 3-7.5 point based on the baseline MRSS values,  $\Delta$ DLCO: 9-10%,  $\Delta$ HAQ-DI: 0.2-0.25) defined on a Delphi exercise [144]. In general these values are higher compared to the ‘minimally important difference’ values ( $\Delta$ MRSS: 3.2-5.3, and  $\Delta$ HAQ-DI: 0.1-0.14) [150]. We tested the model by CATPCA each time after each new introduced item. The final version was reached, when the newly generated activity index was at approximately equal distance from both dimensions (Figure 5).

**Figure 5.** Categorical principal component analysis with the one year follow-up clinical data and the 12 point activity index of 123 consecutive systemic sclerosis patients



Abbreviations used: see Figure 4.

Although the total variance of the newly generated 12 point index was not significantly higher than that of the EScSG activity index (32% vs. 30%), it belonged equally to both dimensions by CATPCA (Figure 5). The newly constructed activity index contained the following new variables: the change in DLCO $\geq$ 9% [144] over one year with 0.5 point value and FVC/DLCO $>$ 1.8 [151] with 1 point value were introduced as new objective parameters of heart/lung involvement. The patient reported worsening in ‘heart/lung component’ counted for only 1 point in the new index, and the patient reported change in skin thickness was also scored at half of the original weight (0.5 point). As an objectively measurable parameter of change in skin status, we introduced the change in MRSS $\geq$ 3-7.5 points at one year follow-up (based on the minimal clinically relevant treatment effect defined by a Delphi exercise, depending on the baseline MRSS score) [144]. Additionally we introduced the ‘change in ulcer score’ variable which scored the appearance of new ulcers on patients who had none at baseline investigation and the increase in the ulcer score at one year follow-up. This variable counted as an additional 0.5 points. Another half point was added to the index, if the HAQ-DI $\geq$ 1 (which means moderate or severe disability that may be caused by extensive skin involvement, characteristic to early diffuse scleroderma) and the change in HAQ-DI $\geq$ 0.2 point at one year assessment was also scored with 0.5 points. Thus the total score of the newly generated activity index was 12.0 points (Table 6).

**Table 6.** The differences between the structure of the EScSG activity index and the newly generated 12 point activity index in systemic sclerosis

EScSG activity index			12 point activity index		
Domain	Item	point	Domain	Item	Point
Skin	MRSS>14	1.0	Skin	MRSS>14	0.5
				17-area patient score>14 <sup>e</sup>	0.5
	Scleredema	0.5		Scleredema	0.5
	$\Delta$ Skin <sup>a</sup> (Patient)	2.0		$\Delta$ Skin <sup>a</sup> (Patient)	1.0
			$\Delta$ MRSS $\geq$ 3-7.5 <sup>f</sup>	1.0	
Vascular	Digital ulcers	0.5	Vascular	Digital ulcers	0.5
	$\Delta$ Vascular <sup>b</sup> (Patient)	0.5		$\Delta$ Vascular <sup>b</sup> (Patient)	0.5
				$\Delta$ Ulcer score <sup>g</sup>	0.5
Joints	Arthritis	0.5	Joints	Arthritis	0.5
				HAQ-DI $\geq$ 1	0.5
				$\Delta$ HAQ-DI $\geq$ 0.2 <sup>h</sup>	0.5
Lung/heart	DLCO<80%	0.5	Lung/heart	DLCO<80%	0.5
	$\Delta$ Lung/heart <sup>c</sup> (Patient)	2.0		$\Delta$ Lung/heart <sup>c</sup> (Patient)	1.0
				$\Delta$ DLCO $\geq$ 9% <sup>i</sup>	0.5
				FVC/DLCO>1.8	1.0
Laboratory	ESR>30mm/h	1.5	Laboratory	ESR>30mm/h	1.5
	Hypocomplementaemia <sup>d</sup>	1.0		Hypocomplementaemia	1.0
<b>Total score</b>		<b>10.0</b>			<b>12.0</b>

(Patient) – reported by the patients.

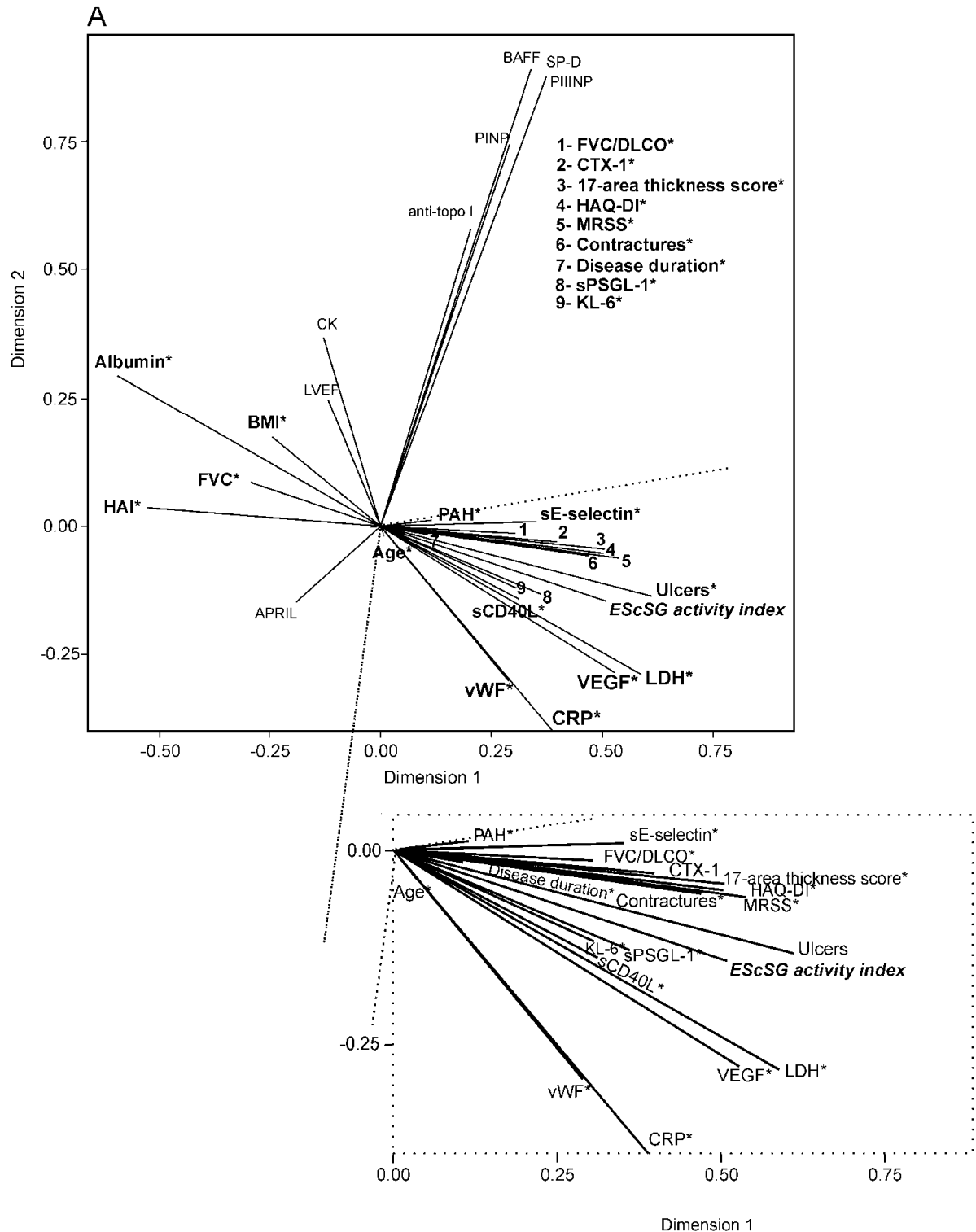
<sup>a</sup>change in skin symptoms during last month, <sup>b</sup>change in vascular symptoms during last month, <sup>c</sup>change in cardiopulmonary symptoms, <sup>d</sup>C3, C4 or total complement decreased, <sup>e</sup>change in patient reported 17-area thickness score over one year, <sup>f</sup>change in modified Rodnan skin score over one year, <sup>g</sup>appearance of ulcers and/or increase in severity of ulcers over one-year reinvestigation, <sup>h</sup>change in Health Assessment Questionnaire Disability Index over one year, <sup>i</sup>change in carbon monoxide diffusion capacity during one year. For details, see Methods.

The relationship of the 12 point activity index to the clinical parameters of one-year reinvestigation was also examined by CATPCA. The disease activity related clinical parameters were distributed into two dimensions, identical to those seen in the previous CATPCAs (Figure 5). However, the 12 point activity index was equally associated with both dimensions, as it appeared at almost equal distance between the axes of the two dimensions indicating that pulmonary involvement and vascular and fibrotic processes (predominantly characterized in dimension 1) were equally represented in this new index. The total variance was 32% in the CATPCA.

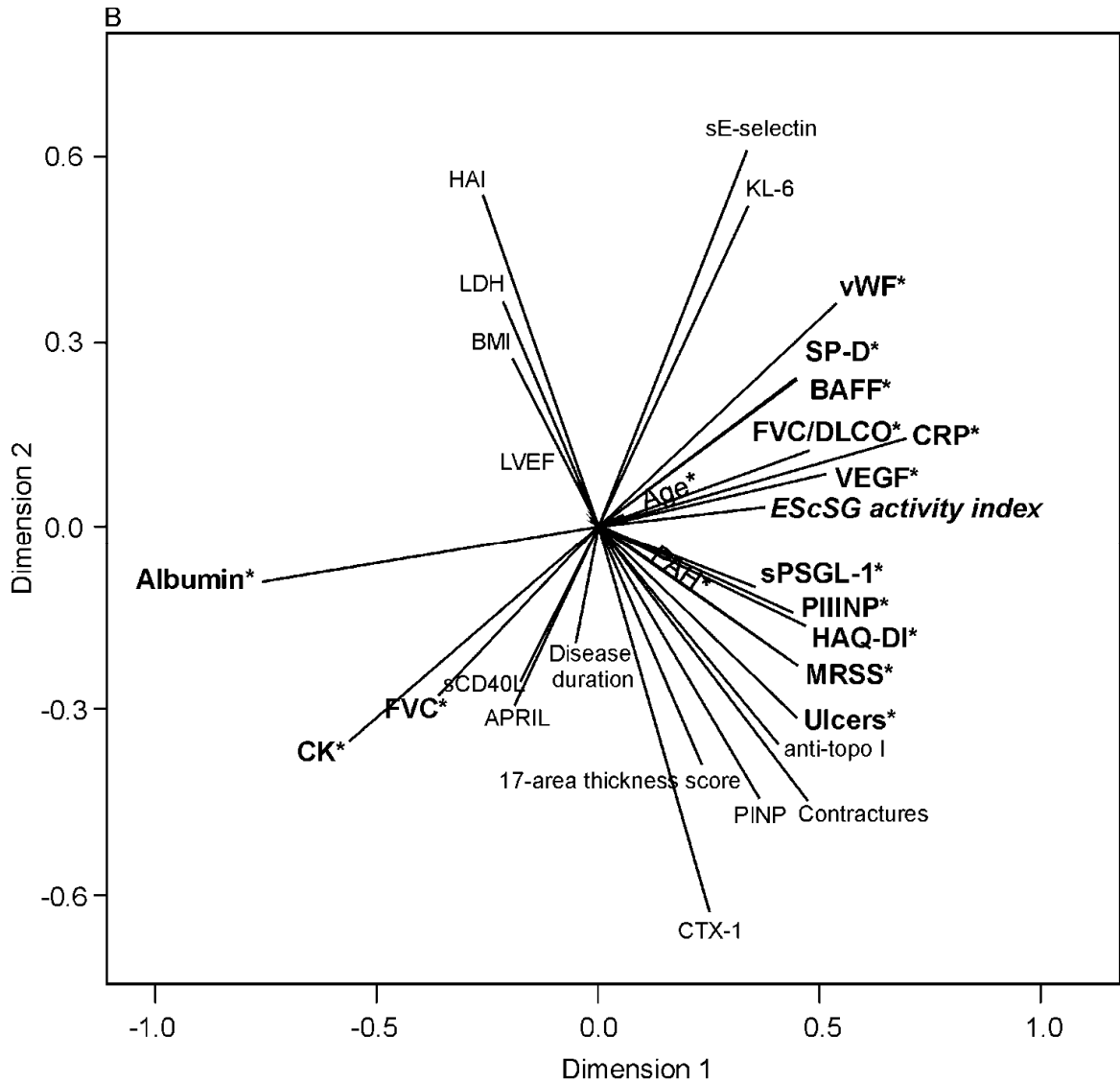
### **Relationship of the investigated biomarkers to the EScSG and 12 point activity index**

Another aim of the study was to investigate the possible correlation of laboratory parameters and biomarkers with the EScSG activity index. A series of laboratory parameters were introduced in the CATPCA analysis, and those markers were considered to be related to the activity index, which were associated with it both at baseline and at one-year reinvestigation (Figure 6).

**Figure 6. A.** Relationships between the EScSG activity index, clinical data and various laboratory parameters of 131 consecutive SSc patients by categorical principal component analysis (baseline investigation).



**Figure 6. B.** Relationships between the EScSG activity index, clinical data and various laboratory parameters of 123 consecutive SSc patients by categorical principal component analysis (1 year follow-up data)



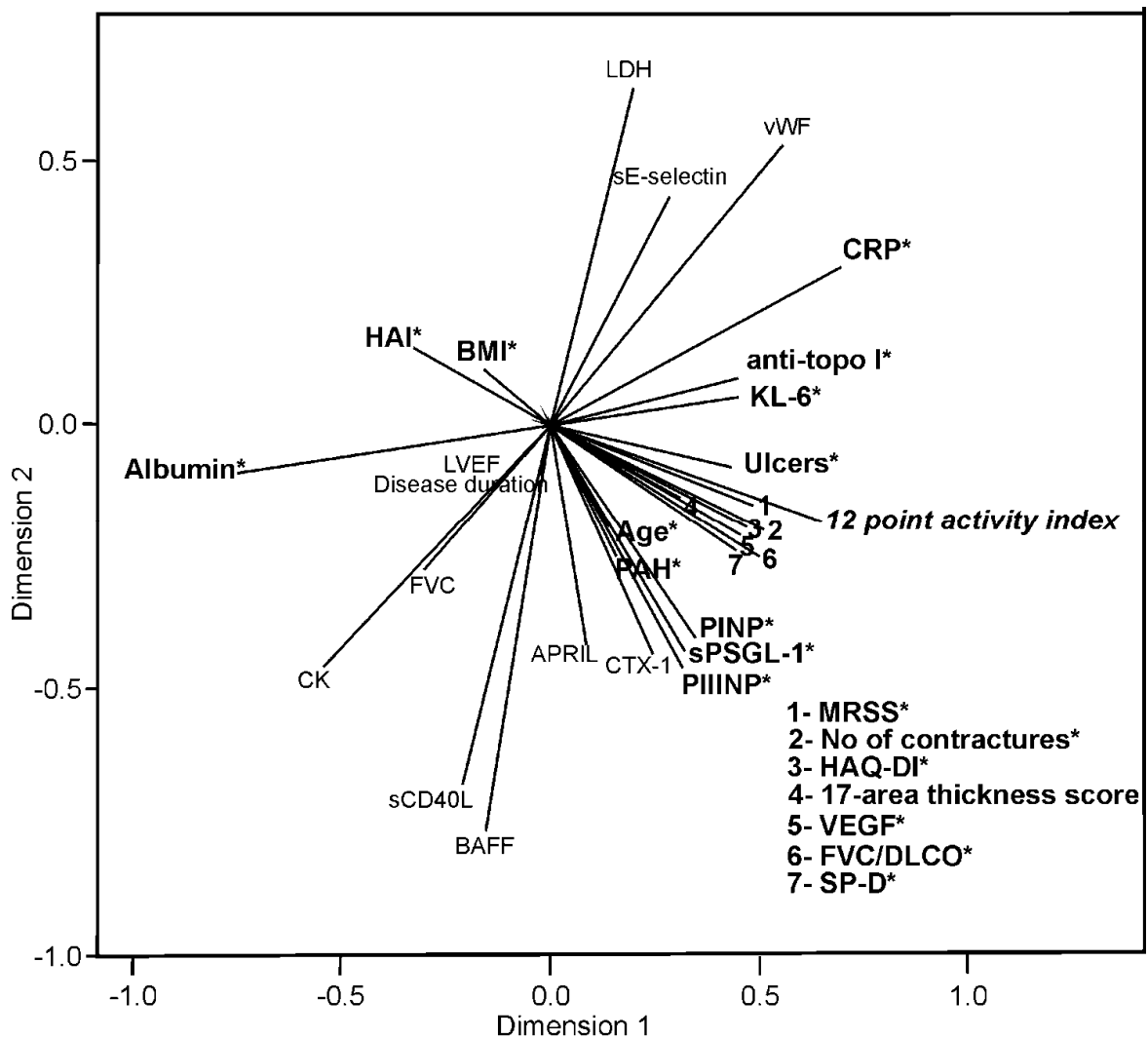
Abbreviations used: EScSG: European Scleroderma Study Group activity index, HAQ-DI: Health Assessment Questionnaire Disability Index, Contractures: number of contractures, Ulcers: ulcer score, MRSS: modified Rodnan skin score, HAI: hand anatomic index, LVEF: left ventricular ejection fraction, BMI: body mass index, FVC: forced vital capacity, FVC/DLCO: ratio of forced vital capacity and carbon monoxide diffusion capacity, PAH: pulmonary arterial hypertension verified by right heart catheterisation, sE-selectin: soluble E-selectin, CTX-1: crosslinked collagen I carboxiterminal telopeptide, KL-6: Kerbs von Lungren 6 antigen, sCD40L: soluble CD40 ligand, sPSGL-1: soluble P-selectin glycoprotein ligand-1, LDH: lactate dehydrogenase, VEGF: vascular endothelial growth factor, vWF: von Willebrand factor, CRP: C-reactive protein, APRIL: A Proliferation-inducing ligand, anti-topo I: anti-DNA-topoisomerase-I antibody, PINP: procollagen type I N terminal propeptide, PIIINP: procollagen type III N terminal propeptide, BAFF: B-cell activation factor, SP-D: surfactant protein D. Bold characters together with the '\*' mark indicate those parameters which were found to be correlated with the EScSG activity index by CATPCA. For details, see Methods.



**Serum albumin, VEGF, vWF, sPSGL-1 and CRP** were found to be in consistent correlation with the EScSG activity index at both investigations.

We also examined the relationship between these laboratory parameters and 12 point activity index. The **VEGF, albumin, sPSGL-1 and CRP** were related to the EScSG activity index and also our 12 point activity index. Furthermore, our modified index was associated to the **anti-topoisomerase I titer, KL-6, SP-D, PINP and PIIINP** (Figure 7). The total variance of this model was similar to the original (28% vs. 26.1% in the original EScSG model).

**Figure 7.** Relationships between the 12 point activity index, clinical data and various laboratory parameters of 123 consecutive SSc patients by categorical principal component analysis (1 year follow-up data)



Abbreviations used: *see Figure 6.*

As the 12 point activity index proposed by our research team cannot be used at first visit of the patient at the physician's office, in a further step we studied the simplified activity index, omitting the parameters of change ( $\Delta$ MRSS,  $\Delta$ Ulcer score,  $\Delta$ HAQ-DI,  $\Delta$ DLCO), without modifying the weight of the remaining variables. Thus, in comparison with the EScSG activity index, this 8.5 point activity index contained the 17-area thickness score reported by the patient, the moderate to severe disability reflected by the HAQ-DI and pulmonary vascular involvement characterized by the FVC/DLCO ratio. This particular index showed a good correlation with the EScSG activity index both at baseline and one-year reinvestigation (Spearman's  $\rho=0.911$ ,  $p<0.001$ , respectively  $\rho=0.831$ ,  $p<0.001$ ), and furthermore the total variance of the 8.5 point activity index was also similar to the EScSG activity index (34.6% vs. 32.9% at baseline investigation, and 32.2% vs. 30% at 1 year follow-up, respectively) (data not shown). The distribution of the variables was very similar to that seen with the EScSG activity index. The 8.5 point activity index also showed the same correlations with the biomarkers, as seen with the EScSG activity index at baseline, and with the 12 point activity index at the one-year reinvestigation (data not shown).

**B. Investigations of the socio-economic impact of disease activity and severity in systemic sclerosis, and characterisation of cardiopulmonary vasculopathy in systemic sclerosis, a leading cause of morbidity and mortality, by invasive techniques**

***Socio-economic impact of disease activity and severity in systemic sclerosis***

Among the 80 patients there were 8 (10%) men and 72 (90%) women, with mean age (SD) of 57.4 (9.6) and disease duration (SD) of 6.2 (6.6) years /lcSSc: mean 6.2 (7.2) years, dcSSc: mean 6.2 (4.8) years/, 5 (6.3%) patients had disease duration <2 years. Limited SSc was present in 60 (75%) and diffuse SSc in 20 (25%) patients. The distance between patients' residence and the care centre was mean 143.2 (SD 99.6) km. The main characteristics of the patients are presented in Table 7.

**Table 7.** Characteristics of patients with SSc (n=80)

Variable	Value, mean (SD)		
	lcSSc (n=60)	dcSSc (n=20)	Total (n=80)
<b>Clinical characteristics</b>			
Disease duration (years)	6.2 (7.2)	6.2 (4.8)	6.2 (6.6)
BMI (kg/m <sup>2</sup> )	26.4 (5.2)	25 (4.4)	26.1 (5)
C-reactive protein (mg/l)	6.3 (11.8)	8 (9.1)	6.7 (11.1)
Erythrocyte sedimentation rate (mm/h)	25.4 (15.4)	30.4 (19.6)	26.7 (16.9)
Modified Rodnan skin score (0-51)	2.3 (2.0)	3.3 (3.4)	2.5 (2.4)
EScSG Activity Index <sup>a</sup> (0-10)	2.03 (1.42)	1.78 (1.43)	1.96 (1.42)
Disease Severity Scale:			
- general health (0-4)	0.38 (0.69)	0.75 (1.02)	0.48 (0.8)
- peripheral vascular (0-4)	1.23 (0.56)	1.25 (0.55)	1.24 (0.56)
- skin (0-4)	0.68 (0.47)	0.75 (0.44)	0.7 (0.46)
- joint/tendon (0-4)	0.45 (0.89)	0.6 (1.27)	0.49 (0.99)
- muscle (0-4)	0.5 (0.68)	0.45 (0.51)	0.49 (0.64)
- gastrointestinal tract (0-4)	0.28 (0.72)	0.6 (0.75)	0.36 (0.73)
- lung (0-4)	1.47 (0.79)	2.0 (0.92)	1.6 (0.85)
- heart (0-4)	0.25 (0.63)	0.6 (1.1)	0.34 (0.78)
- kidney (0-4)	0.03 (0.18)	0.05 (0.22)	0.04 (0.19)
HAQ-DI <sup>b</sup> (0-3)	0.8 (0.67)	0.93 (0.70)	0.83 (0.68)
S-HAQ <sup>c</sup> (0-3)	0.75 (0.56)	0.83 (0.51)	0.77 (0.55)
Raynaud phenomenon VAS <sup>d</sup> (0-3)	1.1 (0.74)	0.94 (0.66)	1.06 (0.72)
Digital ulcer VAS (0-3)	0.48 (0.8)	0.42 (0.45)	0.46 (0.73)
Digestive VAS (0-3)	0.35 (0.61)	0.43 (0.63)	0.37 (0.62)
Pulmonary VAS (0-3)	0.51 (0.73)	0.76 (0.6)	0.58 (0.7)
Overall disease severity VAS (0-3)	0.87 (0.74)	0.81 (0.61)	0.86 (0.7)
EQ-5D score (-0.594-1)	0.58 (0.27)	0.58 (0.32)	0.58 (0.29)
EQ-5D Visual Analogue Scale (0-100)	57.32 (19.3)	53.05 (16.98)	56.25 (18.73)
<b>Presence of organ involvement*</b>	<b>Number of patients (%)</b>		
-Cutaneous manifestations	43 (72)	17 (85)	60 (75)
-Gastrointestinal	21 (35)	9 (45)	30 (38)
-Heart, lung, kidney	41 (68)	15 (75)	56 (70)
-Musculoskeletal	41 (68)	12 (60)	53 (66)

**Table 7.** Characteristics of patients with SSc (n=80) (continuation)

	lcSSc (n=60)	dcSSc (n=20)	Total (n=80)
<b>Current drug treatment</b>	<b>Number of receiving patients (%)</b>		
Cyclophosphamide	5 (8.3)	9 (45)	14 (17.5)
Azathioprine	5 (8.3)	3 (15)	8 (10)
Other cytostatic drugs	4 (6.7)	0 (0)	4 (5)
Corticosteroids	13 (21.7)	12 (60)	25 (31)
Proton pump inhibitors	27 (45)	5 (25)	32 (40)
H2-receptor inhibitor	19 (31.7)	7 (35)	26 (33)
Other gastrointestinal drugs	4 (6.7)	5 (25)	9 (11)
Ca antagonists	29 (48.3)	10 (50)	39 (49)
ACE – inhibitors <sup>e</sup>	28 (46.7)	7 (35)	35 (44)
Other (bosentan, pentosan polysulfate sodium, PGE1 <sup>f</sup> )	7 (11.7)	1 (5)	8 (10)

*\*according to the evaluation of the rheumatologist*

*Abbreviations used: <sup>a</sup>European Scleroderma Study Group activity index, <sup>b</sup>Health Assessment Questionnaire disability index, <sup>c</sup>Scleroderma Health Assessment Questionnaire, <sup>d</sup>Visual analogue scale, <sup>e</sup>Angiotensin-converting enzyme inhibitors, <sup>f</sup>Prostaglandin E1*

Regarding organ involvement during the course of the disease, gastrointestinal, heart/lung/kidney and musculoskeletal symptoms occurred in 65 (81%), 77 (96%), 76 (95%) patients respectively, and 11 (13.4%) had neoplasm (uterus, kidney, breast, malignant melanoma, fibroadenoma, basocellular carcinoma, adenocarcinoma) in their case history. Osteoporosis was present in 30 (37.5%), diabetes mellitus in 8 (10%) patients, thyroid disorder and ophthalmological disorders (glaucoma, cataracta, conjunctivitis) were present equally in 5 (6.3%).

### **Health care utilisation**

Use of diagnostic procedures, rates of outpatient and inpatient care and other services are presented in Table 8.

**Table 8.** Utilisation of main diagnostic procedures and other health care services due to SSc in the past 12 months

	Patients		Annual utilisation frequency of services: number of cases per year	
	Number	Rate (%)	Mean	SD
<b>Diagnostics</b>				
Hand x-ray	60	75	1.0	0.8
Chest x-ray	74	92.5	1.5	1
Barium swallow	47	58.8	0.6	0.6
HRCT	18	22.5	0.3	0.6
Echocardiography	72	90	1.6	1
Doppler echocardiography	8	10	0.1	0.4
Spirometry	80	100	2	1.1
Gastroscopy	21	26.3	0.4	1
Abdominal ultrasound	48	60	0.9	0.9
<b>Consultations</b>				
GP visit	71	88.8	10.3	6
Specialist visit	70	87.5	7.1	7.6
Admission to hospital	78	97.5	4.9	3.1
Surgical procedures	4	5.0	1.5	0.6
Diet consultation	22	27.5	0.6	1.7
Physical therapy	49	61.3	3.6	10.5
Electrotherapy	15	18.3	1.4	4.8
Dermatologic ulcer care	11	13.8	2.6	20.3
Home care	2	2.5	0.0	0.16
<b>Transportation</b>				
Ambulance	14	17.5	1.4	4.1
Reimbursed travel vouchers	50	62.5	3.8	5.9
<b>Aids and devices</b>				
Orthopaedic shoes, cane, elbow-crutches, frame, other	25	31.2	n.a.	n.a.
<b>Not reimbursed services*</b>				
Physiotherapy, private specialist visits	18	22.5	-	-
Home remodelling	11	13.7	-	-
Informal care (hours/week)	31	38.7	7.23	21.5

\* = Yearly costs of non-reimbursed services and home remodelling was asked in the questionnaire but not the number of cases.

n.a. = not applicable

The most commonly visited out-patient cares were (number of patients, %): rheumatology 58 (72.5%), cardiology 34 (42.5%), ophthalmology 33 (41.3%), pulmonology 18 (22.5%), dermatology 17 (21.3%), orthopaedics 11 (13.6%), although other specialist visits occurred as well (gynecology 10, surgery 9, internal medicine 8, gastroenterology 5, angiology 4, rehabilitation 3, nephrology 2, other 11 patients). Mean annual number of visits among receiving patients was: rheumatology 4 (SD 3.8), cardiology 1.7 (SD 0.8), ophthalmology 2.1 (SD 2.1), pulmonology 1.4 (SD 0.86), dermatology 1.8 (SD 0.9), orthopaedics 1.5 (SD 1.2).

Nearly all patients, 78 (97.5%) were admitted to rheumatology department at least once in the past 12 months but other hospitalisations also occurred, i.e. ophthalmology 11 (13.8%) patients (3 of them had cataract surgeries). Admittance to other departments (internal medicine, cardiology, pulmonology, dermatology, nephrology, surgery) occurred for 19 (23.8%) patients. Two patients (2.5%) were admitted to rehabilitation department and 3 (3.8%) received inpatient balneology care. Four patients (5%) underwent surgical procedures in the past 12 months (5 cases of cataract surgery, 1 water-melon stomach argon plasma coagulation) during in-patient care. Several aids and devices were used by the patients at the time of the survey: orthopaedic shoes 18 (22.5%), cane 6 (7.5%), fixed elbow-crutches 2 (2.5%), frame 1 (1.25%), other (e.g. grips, sock) 7 (8.75%) patients.

Ambulance transportation to attend health care was used by 8 (13.3%) lcSSc patients living mean 91.3 (SD 55.1) km from the care centre, average usage among them was 5.3 (SD 5.3) cases/year. In the dcSSc group these variables were 6 patients, 151.4 (SD 63.1) km and 11.3 (SD 7.9) cases/year.

### **Employment status**

Only 7 (8.75%) patients were in a full time job at the time of the survey, 2 (2.5%) were employed in a part time job, 1 (1.25%) was on permanent sick-leave, 39 (48.8%) were receiving disability allowance and 32 (40.0%) patients were retired.

### **Determinants of costs**

Mean yearly costs of SSc are presented in Table 9.

**Table 9.** Average annual costs of patients with SSc, in 2006 converted into euros

	lcSSc (n=60)	dcSSc (n=20)	All patients (n=80) (proportion of total costs)
<b>DIRECT MEDICAL COSTS</b>			
X-ray (hand, chest, barium swallow)	16.5	19.6	17.3
Diagnostic procedures (chest CT, echocardiography, doppler echocardiography, spirometry, gastroscopy, abdominal ultrasound)	25.9	45.9	30.9
Drugs	393.6	397.5	394.6
GP visits	35.2	36.5	35.5
Specialist visits	47.2	35.4	44.3
Other therapies (diet consultation, physiatry, dermatologic ulcer care, professional home care)	16	5.9	13.5
Hospitalisation	2 514.4	3 329.6	2 718.2
Surgical procedures	40.1	27.2	36.9
Aids and devices	10	5.9	8.9
<b>Total direct medical costs</b>	<b>3098.9</b>	<b>3903.5</b>	<b>3300 (34.3%)</b>
<b>DIRECT NON-MEDICAL COSTS</b>			
Home remodelling	172.7	220	184.5
Transportation (ambulance, travel voucher)	155.7	1 343.2	452.6
Transportation (not reimbursed)	33.5	83.4	46
Informal care	196.8	393.2	245.9
<b>Total direct non-medical costs</b>	<b>558.7</b>	<b>2039.8</b>	<b>929 (9.7%)</b>
<b>INDIRECT COSTS (productivity loss)</b>			
Disability pensioners	5025	6142	5305
Sick leave	110.8	8	85.1
<b>Total indirect costs (productivity loss)</b>	<b>5135.8</b>	<b>6150</b>	<b>5390 (56%)</b>
<b>Total costs</b>	<b>8793.7</b>	<b>12093.4</b>	<b>9619 (100%)</b>

Correlation between costs and disease duration, disease activity and health status is presented in Table 10.

**Table 10.** Correlation between clinical characteristics and costs, presented by rho values

Variable	Direct costs	Indirect costs	Total costs
SSc duration, years	-0.09	0.08	0.05
Modified Rodnan skin score	0.06	-0.12	-0.07
EScSG Activity Index	<b>0.23</b>	<b>0.23</b>	<b>0.28</b>
Disease Severity Scale	<b>0.29</b>	-0.01	0.14
- general health	0.06	0.08	0.14
- peripheral vascular	0.20	<b>0.23</b>	<b>0.26</b>
- skin	-0.06	-0.09	-0.10
- joint/tendon	0.11	0.06	0.11
- muscle	0.21	0.08	0.18
- gastrointestinal tract	<b>0.24</b>	-0.04	0.10
- lung	0.19	0.11	0.01
- heart	0.08	0.01	0.06
- kidney	0.05	-0.19	-0.12
HAQ-DI (0-3)	<b>0.33</b>	-0.07	0.08
S-HAQ (0-3)	<b>0.36</b>	-0.04	0.11
Raynaud's phenomenon VAS (0-3)	0.12	0.00	0.08
Digital ulcer VAS (0-3)	-0.05	0.01	0.00
Digestive VAS (0-3)	-0.14	-0.04	-0.03
Pulmonary VAS (0-3)	0.02	0.10	0.14
Overall disease severity VAS (0-3)	-0.05	0.14	0.09
EQ-5D score (-0.594-1)*	0.17	-0.16	-0.04
EQ-5D Visual Analogue Scale (0-100)*	<b>-0.30</b>	0.21	<b>-0.35</b>

\*Higher value refers to better health status. Bold characters represent significant correlations.

Average direct, indirect and total costs were higher in patients with dcSSc than with lcSSc, the differences were 2286, 1014 and 3300 euros/patient/year, respectively. The difference between direct costs of dcSSc and lcSSc groups was significant (P=0.005). Disease activity had significant impact on both direct and indirect costs whilst disease severity, disability (measured by DSS, S-HAQ and HAQ-DI) and patients' perception on health status (VAS) correlated significantly only with direct costs.



### **Comparison with costs of RA and PsA**

In our previous collaborative RA survey (n=255, women 86%, mean age 55 years, disease duration 9.1 years, DAS28 5.1, HAQ-DI 1.38, EQ-5D score 0.46) the average annual costs per patient was 6868 (SD 6196) euros on 2007 prices, which is lower than the present costs of SSc. Rate of women and mean age were alike to the SSc sample but disease duration of the RA patients was higher (plus 3.4 years). Regarding health care utilisation, a lower hospital admission rate was observed in the RA sample (62.6% vs. 97.5%) but 88% were taking disease modifying drugs whilst only 32.5% of the SSc patients were on cytostatic or immunomodulating therapy. (None of the RA patients were on biological therapy as the reimbursement started only in 2006 in Hungary. Leflunomide, a relatively costly disease modifying drug was taken by 21%.) Less RA than SSc patients were on disability pension (35.3% vs. 48.8%).

Costs of psoriatic arthritis (PsA) according to a cross sectional survey in Hungary (n=183, women 57%, age 50.1 years, disease duration 9.2 years, DAS28 4.1, HAQ-DI 1.0, EQ-5D score 0.47) involving 6% of patients on biological therapy was also much lower (5574 euros/patient/year, in 2007) than the costs of SSc. Both hospital admission (42.1%) and work disability pension (24.6%) rates in PsA were lower than in SSc or RA.

Among total costs, the proportion of productivity loss related costs (indirect costs) were the highest in all three diseases (SSc 56%, RA 67.4%, PsA 52.1%), the rate of direct medical costs was the highest in SSc (SSc 34.3%, RA 18.4%, PsA 33.6%).

### ***Evaluation of different types of cardiopulmonary vasculopathy in SSc***

Of the 120 cases, 2 patients were excluded due to severe pulmonary fibrosis. Characteristics of the patients are depicted in Table 11. Concerning the cardiovascular risk profile, hypertension and diabetes were more frequent in the “suspected PAH” group, whereas the distribution of hypercholesterinaemia, male gender and obesity were similar among groups. No significant difference was detected in any other laboratory results.

**Table 11.** Patient characteristics of the 120 systemic sclerosis patients included in the study

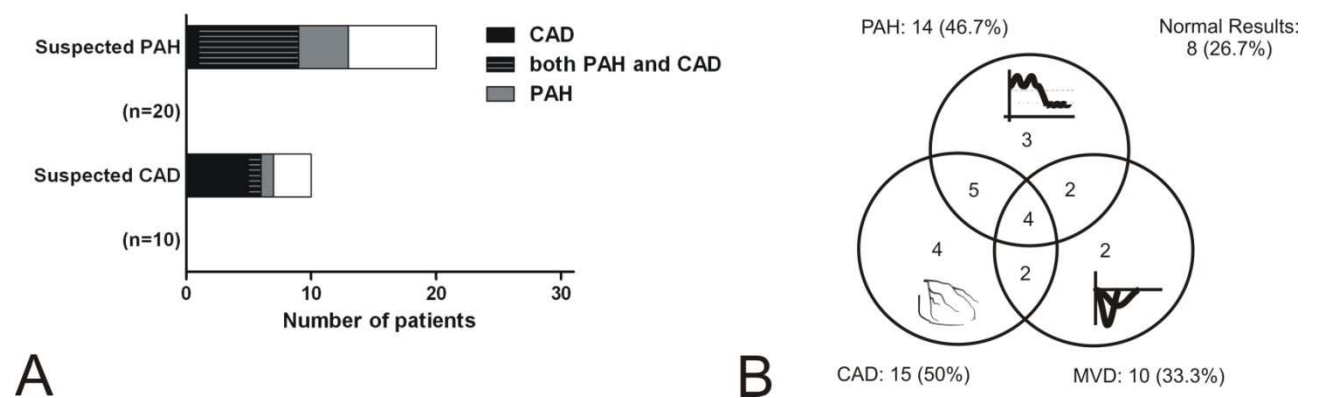
	<b>All patients (n=120)</b>	<b>Patients with suspected PAH (n=20)</b>	<b>Patients with suspected CAD (n=10)</b>	<b>Non catheterized patients (n=90)</b>
Age (years)	55.5±12.8	62.7±9.6*	53.3±12.0	54.2±13.0
Gender (male:female)	14:106	3:17	1:9	10:80
Follow-up (months)	53.3±45.1	41.0±39.6	67.1±40.9	54.4±46.5
Disease duration (years)	10.9±8.5	13.3±9.1	11.1±5.2	10.3±8.6
lcSSc:dcSSc	81:39	16:4	6:4	59:31
EScSG Activity Index	2.6±1.9	2.5±1.8	3.8±2.8	2.5±1.8
HAQ-DI	1.0±0.7	1.0±0.7	1.1±0.6	1.0±0.8
Hypertension	52 (43.3%)	13 (65.0%)*	5 (50.0%)	34 (37.8%)*
Diabetes mellitus	3 (2.5%)	2 (10.0%)*	0 (0.0%)	1 (1.1%)
Hypercholesterinaemia	8 (6.7%)	1 (5.0%)	0 (0.0%)	7 (7.8%)
BMI (kg/m <sup>2</sup> )	25.2±4.8	25.7±5.3	26.3±4.2	24.9±4.7
<b><u>Presentation:</u></b>				
Dyspnea, breathlessness	71 (59.2%)	19 (95.0%)**	7 (70.0%)	45 (50.0%)**
NYHA class	1.7±0.7	2.2±0.4*	2.0±0.5	1.6±0.7**
Chest pain	38 (31.7%)	8 (40.0%)	5 (50.0%)	25 (27.8%)
Anasarca and/ or nycturia	50 (41.7%)	11 (55.0%)	6 (60.0%)	33 (36.7%)
Dizziness	32 (26.7%)	7 (35.0%)	3 (33.3%)	22 (24.4%)
Faint	11 (9.2%)	2 (10.0%)	1 (10.0%)	8 (8.8%)
Palpitation	59 (49.2%)	8 (40.0%)	7 (70.0%)	44 (48.9%)
6-minutes walk test	359.2±74.4	323.0±53.9*	349.2±68.0	368.3±76.9*
<b><u>Clinical manifestations, case history</u></b>				
Raynaud's phenomenon	117 (97.5%)	19 (95.0%)	10 (100.0%)	88 (97.8%)
Subcutaneous calcinosis	18 (15.0%)	4 (20.0%)	1 (10.0%)	13 (14.4%)
Myositis	6 (5.0%)	1 (5.0%)	1 (11.1%)	4 (4.4%)
Modified Rodnan skin score	4.1±5.1	4.8±6.2	3.4±4.4	4.0±4.9
Abnormal Shirmer test	39 (32.5%)	8 (47.1%)	3 (33.3%)	28 (31.1%)
Pericarditis	3 (2.5%)	0 (0.0%)	0 (0.0%)	3 (3.3%)
Renal involvement	2 (1.7%)	0 (0.0%)	0 (0.0%)	2 (2.2%)
Esophageal involvement	63 (52.9%)	9 (50.0%)	2 (20.0%)	47 (52.2%)
Interstitial pulmonary involvement	78 (65.0%)	12 (60.0%)	8 (80.0%)	58 (64.4%)

Abbreviations: PAH: pulmonary arterial hypertension, CAD: coronary artery disease, lcSSc: limited cutaneous systemic sclerosis, dcSSC: diffuse cutaneous Systemic sclerosis, HAQ-DI: Health assessment questionnaire disability index, BMI: body mass index, NYHA: New York Heart Association,

\*:  $p < 0.05$  \*\* $p < 0.001$  unpaired *t* test or Fisher's exact test as appropriate.

Cardiac catheterisation was performed in 30 cases. In all, 20 patients were included in the ‘‘suspected PAH’’, and 10 cases in the ‘‘suspected CAD’’ group. Among the 120 patients with SSc, the prevalence of PAH was 11.6% (14/120), while the prevalence of verified CAD and of severe CFR reduction were 12.5% (15/120) and 8.3% (10/120), respectively. Normal coronary vessels and pulmonary pressure, as well as preserved CFR, were found in eight cases. There was a considerable overlap among these groups. In all, 12 patients in the ‘‘suspected PAH’’ group and 2 in the ‘‘suspected CAD’’ group had PAH. Coronary angiography was positive in 9 cases in the ‘‘suspected PAH’’ group. Severely reduced CFR was found in seven cases in the ‘‘suspected PAH’’ and in three patients in the ‘‘suspected PAH’’ group (Figure 8). Of the 15 patients with coronary lesions detected by morphological and functional assessment, 8 underwent revascularization (1 bypass grafting, 7 percutaneous coronary stent implantations).

**Figure 8.** Catheterisation findings in 30 systemic sclerosis patients. Panel A displays the frequency of coronary artery disease (CAD) and pulmonary hypertension as the indications for catheterisation. Panel B illustrates the considerable overlap found among the abnormalities of the pulmonary circulation as well as the coronary micro and macrovasculature.



*Abbreviations: PAH: pulmonary arterial hypertension, CAD: coronary artery disease, MVD: microvascular disease*

## CONCLUSIONS

The **EScSG activity index**, developed in a European multicenter study, is a very useful and appropriate tool to assess the disease activity in SSc, although some domains of this particular index may be still a topic of debate [152, 153]. Furthermore, its validity has only been tested on a relatively small unselected patient cohort [72]. In this present study, we evaluated the EScSG activity index on a large, unselected, consecutive SSc cohort, containing both lcSSc and dcSSc patients. We confirmed that the construct validity of the EScSG activity index is good. Additionally we have found that two feasible physical examinations, namely the **ulcer score** and **number of contractures**, were also strongly correlated with the EScSG activity index (Figure 4). Therefore, we recommend that the usefulness of these two clinical parameters as disease activity markers should also be evaluated in forthcoming studies.

We previously assumed that patients could provide valid and reliable data regarding their skin involvement, and therefore we have developed and validated a **skin self assessment questionnaire**. The '17-area thickness score', reported by the patient correlated with the MRSS [69] and also with the EScSG activity index [154].

Based on the statistical analysis we have concluded that the EScSG activity index might not reflect sufficiently the pulmonary interstitial and vascular involvements of the disease, as the lung-related parameters were sorted into a separate dimension (Figure 4). To improve the sensitivity of the existing activity index, we included some new, feasible parameters including the patient reported **17-area thickness score, the HAQ-DI, change in MRSS, HAQ-DI, and DLCO at one year follow-up, the change in ulcer score** and the **FVC/DLCO ratio**, and constructed the **12 point activity index**. This particular new index appropriately reflects the pulmonary interstitial and vascular involvements of the disease, but the total variance of this newly generated instrument was not significantly higher than that of the EScSG activity index (32% vs. 30%). As the EScSG activity index, our newly developed index does also not sufficiently include the gastrointestinal, and kidney involvement of the scleroderma patients. Further studies and validation steps will be required to clarify the usefulness of the 12 point activity index for the follow-up of SSc patients.

A further 3 years re-investigation of our cohort is also underway.

We also constructed a simplified activity index (the **8.5 point activity index**) that can be used at the first visit of the patient, when no parameters of change are available. This index sufficiently reflects the disease activity, and this simplified activity index is highly correlated with the original EScSG activity index.

There is a need for finding **biomarkers** that can reliably reflect either the overall or the organ specific disease activity, as in many cases there is a lack of evident clinical signs and

symptoms in the progression of a certain organ involvement. We have selected several promising biomarkers to test their usefulness on our large consecutive patient cohort. We found increased median values of the level of **PINP**, and type I collagen degradation marker, the **CTX-1** was also increased in our SSc patients compared to controls, as also seen in other clinical investigations [93]. We also confirmed that the **SP-D** and **KL-6** may be useful diagnostic markers and indicators of disease activity/damage in patients with pulmonary interstitial diseases [94, 99]. On the contrary, lower serum levels of **sPSGL-1** were found in the SSc patients, compared to the healthy controls and PRP patients potentially indicating a protective role against pulmonary involvement [89].

The elevated levels of **vWF** and **sE-selectin** levels in our patients with primary Raynaud's phenomenon might reflect that the endothelial cell activity in these patients with shorter disease duration might be more pronounced compared to the SSc group with relative long disease duration (mean 8 years).

The statistical analysis performed both with the baseline and one-year reinvestigation data revealed the relationship of five markers, namely the **CRP**, **albumin**, **VEGF**, **sPSGL-1** and **vWF**, to the EScSG activity index. Our group has previously already found that the increase in CRP influenced the prognosis of scleroderma [52]. The quantification of gastrointestinal involvement in SSc is difficult, because every part of it can be involved. The relationship of the albumin with both activity indices might be the reflection of the malabsorption, associated with a more active stage of disease, thus may reflect additional organ involvement. The decrease in albumin can be also considered as sign of inflammation. The elevated markers of endothelial cell activation (vWF) and angiogenesis (VEGF) were previously also found to be signs of ongoing pathologic disease process [83, 90, 155]. Endothelial cell activation is one of the primary events in the pathogenesis. Moreover, the altered angiogenesis and tissue hypoxia cause many of the characteristic symptoms of the disease (e.g. presence of digital ulcers, capillary abnormalities, teleangiectasia). The sPSGL-1 was identified in a study as possible protective marker against pulmonary fibrosis, its role in disease activity should be further evaluated.

Four out of these aforementioned five laboratory markers (**VEGF**, **albumin**, **CRP**, **sPSGL-1**) identified by CATPCA were found to be associated with both the **EScSG activity index** and the **12 point activity index** (Figures 6 and 7). The 12 point index reflected also the pulmonary involvement as well as the vascular and fibrotic component of disease activation. Therefore those laboratory markers which were related to the 12 point activity index may also be potential candidates for further investigations in the search for further valuable activity markers in scleroderma. These are the **anti-topoisomerase I titer**, **KL-6**, **SP-D**, **PINP** and

**PIIINP**, which might reflect the pathologic processes, which were underrepresented in the original index.

Regarding the costs of illness in systemic sclerosis, our collaborative study provides data on health care utilisation and costs of patients with SSc in Hungary based on a cross-sectional survey performed in 2007. Total costs amounted to mean **9619 euros/patient/year**. The rate of direct costs was 44% and the proportion of disability pension and hospitalisation were the highest among the cost items (55.2% and 28.3%, respectively).

Costs of patients with dcSSc were higher than with lcSSc. Disease activity had significant impact on both direct and indirect costs whilst disease severity, disability (measured by DSS, S-HAQ and HAQ-DI) and patients' perception on health status (VAS) correlated significantly only with direct costs.

Evaluating the costs of SSc in the field of rheumatic diseases within the same country, SSc related costs exceeded the costs of rheumatoid arthritis and also of psoriatic arthritis in Hungary, as also seen in a Canadian study [117, 156]. Based on these two comparisons, costs of SSc seem to be higher than of RA although a more expanded use of rather expensive biological drugs for the treatment of RA in the past years has presumably decreased the difference. Productivity loss of the SSc patients was remarkable in our patient sample. The rate of disability pension seems to be higher in Hungary than in other countries [114, 157]. Among the 48 patients of working age, 9 (18.8%) were in employment (one of them was on permanent sick leave) and 39 (81.2%) were on disability pension.

The study performed on the evaluation of different types of cardiopulmonary vasculopathy in SSc demonstrated that coronary artery disease may mimic, and can appear in combination with PAH in patients with SSc. Patients with SSc with reduced physical capacity and exertional dyspnoea showed a considerable overlap between PAH and CAD. The incidence of PAH in our collaborative study was similar to that observed previously [126, 149]. Intriguingly, in patients who showed signs of PAH by non-invasive investigations, the prevalence of CAD and PAH was comparable. Patients with suspected CAD also showed similar overlap, although this was less prominent. "Pure" PAH without coronary disease was a rather rare finding in our cohort, only affecting 10% of the cases. These findings suggest that the current screening methods are not ideal for distinguishing CAD and PAH in patients with SSc. A more invasive approach, such as coronary angiography, may be necessary to properly characterise heart involvement in SSc. Interestingly, a reasonable explanation for findings suggestive of cardiopulmonary involvement was lacking in a remarkably high number of patients (8/30) with normal RHC values, normal coronaries and without severe pulmonary fibrosis.

## SUMMARY AND NEW RESULTS

In our study we proposed to further evaluate the psychometric properties (in particular the construct validity) of the EScSG activity index using our prospectively enrolled large patient cohort. We also looked for possible additional clinical parameters and biomarkers, which could be used in the assessment of disease activity. The modified Rodnan skin score is a feasible examination, which is an indicator of disease activity in scleroderma. We considered that patients can also reliably appreciate their skin status, therefore we developed and validated a new skin self assessment questionnaire.

As there is only little data about the socio-economic impact of disease activity and severity in systemic sclerosis, we also surveyed our scleroderma cohort from this point of view. Our results probably reflect the situation in the Central European countries.

The clinical presentation of cardiopulmonary vasculopathies, one of the major causes of mortality in systemic sclerosis, is often atypical, making their distinction difficult. Our invasive cardiac examination in SSc patients indicates that a more complex approach should be developed for the evaluation of cardiopulmonary involvement in SSc, as in selected patients, coronary revascularization successfully eases symptoms and improves physical capacity.

### A. Investigation of disease activity in systemic sclerosis

- The **construct validity** of the **EScSG activity index** was found to be good in our study carried out on a large, prospectively enrolled patient cohort, and reflected both the vascular and fibrotic phenomena of the disease.
- We have found that the number of **ulcers** and **contractures**, two physical parameters that can be easily assessed, showed consequent relationship with the EScSG activity index. We propose the further investigation of these two parameters, to evaluate their use in the assessment of disease activity.
- We developed and validated a **patient reported self assessment questionnaire** that provides reliable, valid data for the assessment of skin thickness.
- The patient reported skin thickness score correlated also with the EScSG activity index on CATPCA.
- We have also shown, that the EScSG activity index **does not reflect sufficiently** the interstitial and vascular component of **pulmonary involvement**.
- Therefore we have developed a **12-point activity index**, which reflects better the interstitial and vascular component of lung involvement in systemic sclerosis.

- We have identified **five biomarkers** that consequently correlated to the EScSG activity index based on CATPCA during both the baseline and 1 year investigations: the **CRP, albumin, VEGF, sPSGL-1**, and the **vWF**.
- **Four** out of the five previously listed biomarkers (**CRP, albumin, VEGF**, and the **sPSGL-1**) also correlated to the **12-point activity index** by CATPCA.
- If the use of the **12-point activity index** in the assessment of disease activity will be demonstrated, we consider worthy to further investigate those biomarkers (**anti-DNA topoisomerase-I antibody, KL-6, SP-D, PINP, PIIINP**) that also correlated with the newly developed activity index.

**B. Investigations of the socio-economic impact of disease activity and severity in systemic sclerosis, and characterisation of cardiopulmonary vasculopathy in systemic sclerosis, a leading cause of morbidity and mortality, by invasive techniques**

- To our knowledge, our study is the first that offers data both on direct and indirect costs of SSc in Europe.
- The investigation of **costs-of-illness** showed, that disease activity had significant impact on both direct and indirect costs whilst disease severity, disability (measured by DSS, S-HAQ and HAQ-DI) and patients' perception on health status (VAS) correlated significantly only with direct costs.
- Productivity loss of the SSc patients was remarkable in our patient sample, 80% of working age patients were on disability pension.
- The costs in systemic sclerosis overseeded the cost of rheumatoid arthritis and also the costs of psoriatic arthritis (**9619 Euros/patient/year in SSc**, 6868 Euros/patient/year in rheumatoid arthritis and 5574 Euros/patient/year in psoriatic arthritis).
- Based on the results of the left- and right heart catheterisations we have demonstrated, that patients with SSc with reduced physical capacity and exertional dyspnoea show a considerable **overlap** between **pulmonary arterial hypertension** and **coronary artery disease**.
- These findings suggest that the current screening methods are not ideal for distinguishing pulmonary arterial hypertension and coronary artery disease in patients with SSc. A **more invasive** attitude should therefore be implemented in SSc for the assessment and adequate treatment of consequent cardiopathy, which comprises also the systematic coronarography even at the first heart catheterisation.



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