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

Ph.D. thesis

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I. INTRODUCTION

Systemic sclerosis (systemic scleroderma, SSc) is a connective tissue disease with complex pathomechanism. 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, however, compared to the other rheumatic diseases, the prognosis of SSc is unfavourable.

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.

The aim of our research 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 SSc, a leading cause of morbidity and mortality.

II. BACKGROUND

General characteristics of systemic sclerosis

Systemic sclerosis is an autoimmune disease involving multiple internal organs, and is characterized by vasculopathy, fibrosis of the skin and internal organs, and the activation of the immune system. Consequently, the clinical presentation of the disease is very heterogeneous, the severity and disease course can differ substantially among patients. Despite this pronounced heterogeneity, patients can be divided into two subsets based on the distribution of skin involvement, internal organ involvement, and autoantibody positivity: limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) systemic sclerosis subsets.

The pathogenesis of systemic sclerosis

There are three major pathways which play role in the appearance of skin and internal organ involvement. The first is the vasculopathy: it is based on the pathologically increased vasospasm of small arteries, which is provoked by external environmental or internal immunopathological effects. The consequence is ischemic and reperfusional damage, and this

results in endothelial cell damage. In genetically susceptible organs these induce autoimmune inflammation and increased fibrosis, leading to the development of systemic sclerosis. The inflammation starts with perivascular infiltration of lymphocytes and macrophages, later more types of inflammatory cells and cytokines will be also involved. The last process is the progressive fibrosis, which is the major hallmark of this disease. Due to the increased production and deposition of collagen and other extracellular matrix components the normal tissue structures will be impaired and the internal organs suffer irreversible damage.

II.1. Investigation of disease activity 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. 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.

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, and **laboratory parameters** indicating the inflammatory activity, ongoing fibrosis, and vascular changes during the disease course.

The assessment of skin thickening in dcSSc proved to be a useful marker in the evaluation of disease activity, severity and mortality. Improvement in skin score was associated with a more favourable outcome. The modified Rodnan skin score (MRSS) is used for the assessment of skin thickening in the majority of the clinical trials, as in multicenter clinical trials it was proved to be a simple, reliable and valid outcome measure which also showed sensitivity to change.

Further clinical parameters, that could be useful in disease activity assessment, are the **appearance or worsening of ulcers;** the **worsening in musculoskeletal status;** the **appearance of gastrointestinal or renal symptoms**; and the **appearance or worsening of interstitial or vascular pulmonary involvement**.

Although MRSS is a 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. 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 evaluation of skin thickening.

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.

The European Scleroderma Study Group (EScSG) has developed a disease activity index (EScSG activity index) for systemic sclerosis patients based on a multicenter study involving 19 European Centers, including also our center. This is the only available composite activity index for the moment. This index appears simple and easy to use as it evaluates clinical items (MRSS, carbon monoxide diffusion capacity /DLCO/, presence of scleredema, arthritis and digital ulcers), some basic laboratory investigations (erythrocyte sedimentation rate /ESR/ hypocomplementaemia) and patients' complaints regarding worsening in skin status, vascular symptoms and cardiopulmonary symptoms. However, these criteria await for a full validation, as the construct validity has been confirmed only on a small cohort of SSc patients and also the responsiveness of the index has not been proved yet.

II.1.1. Potential biomarkers for disease activity assessment in systemic sclerosis

Along the parameters of clinical investigations and changes in different symptoms reported by patients, some relative easily reachable laboratory parameters (ESR, hypocomplementaemia) are also included into the EScSG activity index. However, no biomarkers, reflecting the pathomechanism of the disease (e.g. endothelial cell injury, activation of the immune system, collagen deposition resulting from activated fibroblasts) were included. The identification and introduction into the EScSG activity index of some biomarkers related to the pathomechanism of disease could play a crucial role in the more accurate assessment of activity and its follow-up.

Many biomarkers related to activation of the immune system, ongoing fibrosis and vasculopathy have been associated with disease activity in previous studies.

The **von Willebrand factor** (vWF) is marker of endothelial cell activation and its level correlated with the extent of internal organ involvement in SSc. P-selectin and E-selectin play role in the adhesion of leukocytes. The level of the **soluble** form of **E-selectin** (sE-selectin) was found to be elevated in SSc. **P-selectin glycoprotein ligand-1** (PSGL-1) is a high affinity ligand for P-selectin. 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.

The overproduction of **vascular endothelial growth factor** (VEGF), a proangiogenetic factor was also demonstrated in systemic sclerosis. This could be essential in a paradox in the decreased angiogenetic activity that is characteristic to this disease.

From the markers of fibrosis, the level of **procollagen Type I N-Terminal Propeptide**

(PINP) correlated with the change in the MRSS. The cross-linked **collagen I carboxiterminal telopeptide** (serum crosslaps, CTX-1), a marker of collagen degradation, correlated with the extent of skin involvement (MRSS), the acute-phase proteins and indicators of decreased pulmonary function (DLCO<75%). The concentration of **procollagen type III N terminal propeptid** (PIIINP) was found to be also in direct proportion with the extension of skin involvement in different studies, and indicated the prognosis of the disease. The PIIINP negatively correlated with DLCO.

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 unfavourable prognosis. Several studies including ours showed that the value of ESR was significantly higher in patients with dcSSc than those with lcSSc.

The **KL-6 (**Kerbs von Lungren 6 antigen), produced by type II alveolar epithelial cells, is marker of pulmonary fibrosis/involvement. Circulating KL-6 concentration strongly correlated with the severity of interstitial lung disease (ILD), and also with disease activity in one study.

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) were raised in scleroderma associated with interstitial lung involvement. The levels of SP-A and SP-D also correlated with the activity of disease.

The **B-cell activation factor** (BAFF) is one of the markers reflecting the activation of the immune system. A recent study demonstrated elevated BAFF levels, and correlation of BAFF levels with skin fibrosis in patients with SSc. **A PRoliferation-Inducing Ligand** (APRIL) is also a tumor necrosis factor (TNF) superfamily member with close homology to BAFF. 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).

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.

The **Soluble CD40 Ligand** (sCD40L) is released from activated CD4+ T cells. The CD40/CD40L interactions activate B cells, upregulate endothelial adhesion molecules, and induce fibrosis. A recent 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.

II.2. 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. During the course of disease different types of severe internal organ involvements can occur (e.g. cardiac, lung, renal, gastrointestinal involvement, digital ulcers). These organ-based complications produce the high case-specific mortality rate observed among 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.

II.2.1. Socio-economic impact of disease activity and severity in systemic sclerosis

The positive correlation between disease activity and cost-of-illness in rheumatoid arthritis (RA) and psoriatic arthritis (PsA) was already proved in previous Hungarian multicenter studies in which also our Clinic was involved. There were no available Hungarian data on the factors that influence the cost-of-illness in systemic sclerosis (e.g. disease activity, severe internal organ involvement, musculoskeletal status).

II.2.2. Evaluation of different types of cardiopulmonary vasculopathy in SSc

In present, pulmonary disease produces the highest mortality in terms of SSc-related problems, but cardiovascular deaths are consistently reported as being responsible for 20-30% of all premature deaths.

Cardiac involvement in systemic sclerosis includes coronary artery disease (CAD), pulmonary arterial hypertension (PAH) related right ventricular changes and microvascular disease (MVD). The clinical presentation of these conditions is often atypical, making their distinction difficult. 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.

III. AIMS OF THE STUDY

III.1. 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 perhaps 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 a 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.

III.2. 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 and 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).

IV. PATIENTS AND METHODS

IV.1. Investigation of disease activity in systemic sclerosis

131 consecutive, unselected patients with SSc from the Department of Rheumatology and Immunology, University of Pécs, Hungary were included prospectively and re-investigated 12±1.3 month later. On the basis of a predefined standard protocol we have recorded the main clinical parameters (MRSS, presence of ulcers, number of contractures, hand anatomic index (HAI), spirometry values with DLCO, presence of PAH diagnosed by right heart catheterisation, left ventricular ejection fraction (LVEF), chest X-ray, barium swallow), the EScSG activity index, and Disease Severity Scale (DSS).

The patients also filled out the skin self assessment questionnaire, developed by our research team and the validation based on the OMERACT filter also was part of the study. The patients also filled out the Scleroderma Health Assessment Questionnaire (S-HAQ), validated also to Hungarian language. Markers of **collagen metabolism** (PINP, PIIINP, CTX-1), **endothelial cell activation** (sE-selectin, vWF, sPSGL-1), **proangiogenetic factors** (VEGF), 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 were determined from the serum and plasma samples collected at the time of investigations by ELISA and RIA techniques.

The project was approved by the local Research Ethics Committee (Approval No. 2720/2006).

IV.2. 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

IV.2.1. For the investigation of the socio-economic impact of disease activity and severity in systemic sclerosis 80 consecutive SSc inpatients were enrolled prospectively into the cross-sectional survey in 2007. We have recorded the clinical and laboratory parameters by a standard protocol, and the patients filled out the S-HAQ disability questionnaire, the EQ-5D general health questionnaire and a special questionnaire about the SSc related health care resource utilisations, developed by a rheumatologist and a health-economic specialist. Data collection was carried out at the Dept. of Rheumatology and Immunology Clinic, University of Pécs, and data analysis was performed at the Health Economics and Technology Assessment Research Centre, Corvinus University of Budapest. Costs were divided into **direct medical**, **direct non-medical** and **indirect costs**. Costs of drugs, diagnostic procedures, aids and devices, hospitalization related to SSc, GP and specialist visits were sorted to direct medical costs. Direct non-medical costs were the costs of home remodelling, transportation and informal care. Indirect costs were comprised of costs related to loss of productivity because of disability retirement and costs of sick leave because of SSc. Hungarian official price, tariffs and reimbursement lists of 2006 were used for cost calculation. Authorization by the local Research Ethics Committee was also obtained (No. 2896/2007).

IV.2.2. For the evaluation of different types of cardiopulmonary vasculopathy in SSc a total of 120 consecutive SSc cases attending our Department were enrolled in the study. 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. We have recorded the parameters of physical examination and clinical investigations, medication and laboratory parameters at the Dept. of Rheumatology and Immunology. 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).

The trial protocol was approved by the Medical Research Council Scientific and Ethical Committee.

Statistical analyses were performed using the Statistical Package of Social Sciences, versions 14.0 and 15.0 (SPSS Inc., Chicago, IL, USA).

V. RESULTS

V.1. Investigation of disease activity in systemic sclerosis

V.1.1 Validation of the skin self assessment questionnaire

We have examined the validity of domains regarding the **skin thickness, tethering** and **thinning** of the self assessment questionnaire based on the OMERACT filter. As reported in a previous study, we have also demonstrated, that the examiner cannot completely differentiate between the skin thickened because of disease activity and the skin tethering, which appears after long disease duration and reflects the damage. We have observed the same phenomenon at the patients' self assessment about skin thickening and tethering (the two parameters correlated highly with each other). From this reason we have validated only the **skin thickness domain** of the questionnaire.

We have validated the questionnaire regarding face validity, content validity, criterion validity, construct validity, classification capacity, reliability and feasibility. However, we could not validate the responsiveness of the questionnaire, probably because of the long disease duration and low MRSS in the majority of the patients.

V.1.2. Clinical characteristics of the SSc patients and the results of biomarker determinations

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/. 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.

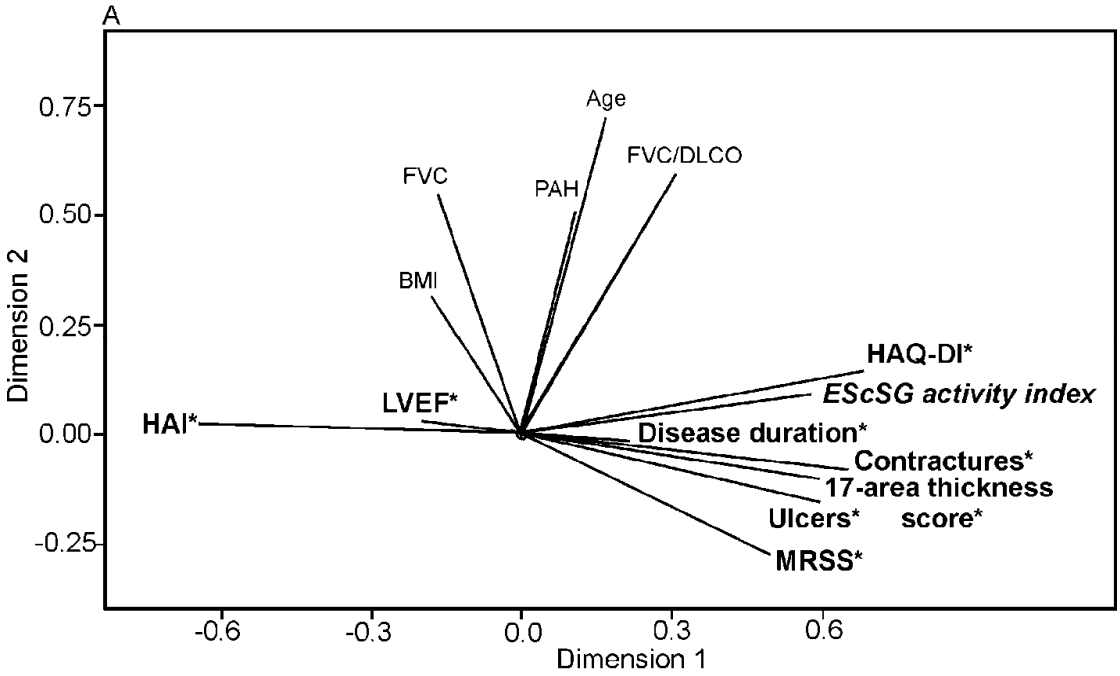
The comparison of biomarker levels among systemic sclerosis patients, patients with primary Raynaud's phenomenon (PRP) and healthy controls is shown in Table 1.

Table 1. Median (percentiles) values of the investigated laboratory parameters in SSc patients, patients with primary Raynaud phenomenon and healthy controls

	SSc baseline (n=131)	**SSc 1 year (n=123)**	**Healthy controls (n=30)**	**Primary Raynaud's phenomenon (n=51)**
	Median (Percentiles)	Median (Percentiles)	Median (Percentiles)	Median (Percentiles)
PIIINP[§§] (μg/l)	3.8 (3.1;4.5)	**4.3*** [††] (3.8;5.2)	4.0 (3.7;4.4)	3.6 (3.4;4.2)
PINP (μg/l)	**45.0**** [††] (34;65)	**44.5*** [††] (30;60.1)	33.5 (28.3;44.5)	33.0 (25.7;37.8)
CTX-1 (ng/ml)	**0.4**** [††] (0.2;0.6)	**0.3**** [††] (0.2;0.6)	0.2 (0.2;0.3)	0.2 (0.1;0.3)
SPD (ng/ml)	**1997.4**** [††] (1367.1;3736.8)	**1961.4**** [††] (1218.8;3709.2)	1238.9 (648.6;1445.5)	1199.6 (734;1727.3)
vWF[§§] (μg/ml)	**30.0**[††] (21.9;38.6)	33.4 (25.8;41.3)	28.6 (22.6;40)	37.8 (28.1;48.8)
sPSGL-1[§§] (U/ml)	**241.8**** [††] (170.6;298.4)	**262.3**** [††] (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[§] (ng/ml)	**34.4**[††] (25.5;46)	**34.4**[††] (24.7;47.8)	32.1 (21.3;41.3)	89.2 (34.7;226.7)
KL-6 (U/ml)	**802.2**** [††] (534.3;1246.7)	**935.3**** [††] (583.8;1323.3)	516.3 (316.5;644.4)	625.7 (387.7;744.8)
BAFF[§§](pg/ml)	413 (338.2;561.3)	**556.9**** [††] (439;690.4)	383.7 (327.5;435.4)	440.4 (385.1;564.7)
APRIL[§§] (U/ml)	**9.8**** [††] (7.2;11.5)	3.2 (1.5;6.9)	2.9 (2.2;4.8)	4 (2.1;7.7)
sCD40L[§§] (U/ml)	**2.0*** [††] (1.4;2.6)	**1.7**[††] (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; [†] p<0.05, [††] p<0.01 between the SSc patients and those with primary Raynaud phenomenon; [§] p<0.05, [§§] 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.*

Figure 1. Categorical principal component analysis with the clinical data and the EScSG activity index of 131 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.*

V.1.3. 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 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 1).

V.1.4. Development of a new 12 point activity index

As the lung-related parameters were independent from the EScSG activity index (Figure 1.), 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 (ΔMRSS: 3-7.5 point based on the baseline MRSS values, ΔDLCO: 9-10%, ΔHAQ-DI: 0.2-0.25). 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.

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. The weighting of items in the newly constructed activity index is presented in Table 2. For the characterisation of cardiopulmonary involvement we have introduced the change in DLCO≥9% over one year and FVC/DLCO>1.8. As an objectively measurable parameter of change in skin status, we introduced the change in MRSS≥3-7.5 points at one year follow-up. 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. For the assessment of musculoskeletal involvement we have introduced the HAQ-DI≥1 parameter (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≥0.2 point at one year assessment. Thus the total score of the newly generated activity index was 12.0 points (Table 2).

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. 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.

Table 2. 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[e]	0.5
	Scleredema	0.5		Scleredema	0.5
	ΔSkin[a] (Patient)	2.0		ΔSkin[a] (Patient)	1.0
				ΔMRSS≥3-7.5[f]	1.0
Vascular	Digital ulcers	0.5	Vascular	Digital ulcers	0.5
	ΔVascular[b] (Patient)	0.5		ΔVascular[b] (Patient)	0.5
				ΔUlcer score[g]	0.5
Joints	Arthritis	0.5	Joints	Arthritis	0.5
				HAQ-DI≥1	0.5
				ΔHAQ-DI≥0.2[h]	0.5
Lung/heart	DLCO<80%	0.5	Lung/heart	DLCO<80%	0.5
	ΔLung/heart[c] (Patient)	2.0		ΔLung/heart[c] (Patient)	1.0
				ΔDLCO≥9%[i]	0.5
				FVC/DLCO>1.8	1.0
Laboratory	ESR>30mm/h	1.5	Laboratory	ESR>30mm/h	1.5
	Hypocomplementaemia[d]	1.0		Hypocomplementaemia	1.0
Total score		**10.0**			**12.0**

(Patient) – reported by the patients.
[a]change in skin symptoms during last month, [b]change in vascular symptoms during last month, [c]change in cardiopulmonary symptoms, [d]C3, C4 or total complement decreased, [e]change in patient reported 17-area thickness score over one year, [f] change in modified Rodnan skin score over one year, [g]appearance of ulcers and/or increase in severity of ulcers over one-year reinvestigation, [h]change in Health Assessment Questionnaire Disability Index over one year, [i]change in DLCO during one year.

V.1.5. 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 PRP, the 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 1.).

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.

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.

V.1.6. 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.

CRP, serum albumin, VEGF, vWF and **sPSGL-1** and 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 the **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-topo I titer**, **KL-6**, **SP-D**, **PINP** and **PIIINP**. 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 (ΔMRSS, ΔUlcer score, ΔHAQ-DI, ΔDLCO), 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). 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.

V.2. 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

V.2.1. Socio-economic impact of disease activity and severity in systemic sclerosis

Evaluating the costs of SSc in the field of rheumatic diseases within the same country, SSc related costs exceeded the costs of RA and also of PsA in Hungary (9619 euro/patient/year in SSc, 6868 euro/patient/year in RA, and 5574 euro/patient/year in psoriatic arthritis). 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) in systemic sclerosis.

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 Disease Severity Scale, S-HAQ and HAQ-DI) and patients' perception on health status (VAS) correlated significantly only with direct costs.

The high costs of illness were mostly determined by the costs of productivity loss.

V.2.2. Evaluation of different types of cardiopulmonary vasculopathy in SSc

The left and right heart catheterisation performed to accurately assess the cardiopulmonary vasculopathy found PAH in the "suspected PAH" group in 12/20 cases and in the "suspected coronary artery disease" group in 2/10 cases. Coronary angiography was positive in 9 cases in the ''suspected PAH'' group, and in 6 cases in the "suspected CAD" group. Severely reduced CFR was found in seven cases in the ''suspected PAH'' and in three patients in the ''suspected CAD'' group. The PAH, CAD and decreased CFR showed significant overlap in SSc patients.

VI. CONCLUSIONS

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**. Based on the questionnaire, the patient reported skin thickness score ('17-area thickness score') can be calculated, which correlated consistently with the MRSS and also with the EScSG activity index.

At the moment in the assessment of disease activity in systemic sclerosis we have at disposal only the EScSG activity index, which is a weighted composite index. The validity of

this index was studied only on a relatively small patient group when the index was developed.

We evaluated the EScSG activity index on a large, unselected, consecutive SSc cohort. We confirmed that the construct validity of the index is good and it is capable to assess disease activity, as it both reflects the peripheral vascular and fibrotic phenomena of this disease.

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. Therefore the usefulness of these two clinical parameters as disease activity markers should also be evaluated in forthcoming studies.

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 on categorical principal component analysis (Figure 1). 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. Further studies 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.

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. In our study we found increased median values of the level of **PINP** and **CTX-1** (markers of type I collagen metabolism) in SSc patients compared to controls. The levels of **SP-D** and **KL-6** were also raised in SSc patients. These markers were previously also demonstrated by our research group and others that may be useful diagnostic markers and indicators of disease activity/damage in patients with pulmonary interstitial diseases. 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. 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. 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 in systemic sclerosis. 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**. 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.

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 (9619 euro/patient/year in SSc, 6868 euro/patient/year in RA, and 5574 euro/patient/year in psoriatic arthritis. A more expanded use of rather expensive biological drugs for the treatment of RA in the past years has presumably decreased the difference between costs of RA and SSc. 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. The high costs of illness were mostly determined by the costs of productivity loss. Productivity loss of the SSc patients was remarkable in our patient sample, 80% of working age patients 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. ''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 non-invasive screening methods are not sensitive enough for distinguishing CAD and PAH in patients with SSc. A more invasive approach, such as systematic coronary angiography even at the first cardiac catheterisation, may be necessary to properly characterise the cardiopulmonary vasculopathy and consequent heart involvement in SSc.

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VIII. LIST OF PUBLICATIONS RELATED TO THE SUBJECTS INCLUDED IN THE THESIS

Papers

1. **Minier T**, Nagy Z, Bálint Z, Farkas H, Radics J, Kumánovics G, Czömpöly T, Simon D, Varjú C, Németh P, Czirják L. Construct validity evaluation of the European Scleroderma Study Group activity index, and investigation of possible new disease activity markers in systemic sclerosis. Rheumatology(Oxford) 2010;49(6):1133-45. If: **4.236**
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