Construct validity evaluation of the European Scleroderma Study Group activity index, and investigation of possible new disease activity markers in systemic sclerosis

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Abstract

Objectives. To evaluate the construct validity of the European Scleroderma Study Group (EScSG) activity index and to propose modifications if necessary.

Methods. One hundred and thirty-one consecutive patients were investigated and re-evaluated 1 year later. Modified Rodnan skin score (MRSS), skin ulcers and joint contracture numbers, hand anatomic index (HAI), BMI, spirometry, carbon monoxide diffusing capacity (DLCO), left ventricular ejection fraction, pulmonary arterial hypertension, HAQ Disability Index (HAQ-DI), patient skin self-assessment questionnaire and several biomarkers were recorded, in addition to the data required for the EScSG activity index. Statistical analysis was performed by categorical principal component analysis (CATPCA).

Results. The EScSG activity index appeared in the same dimension as the HAQ-DI, ulcer score and joint contractures, MRSS, patient-reported skin score and HAI by CATPCA. Parameters of lung involvement appeared in another dimension. We constructed a 12-point activity index that was equally associated with both dimensions, by adding the forced vital capacity/DLCO, change in DLCO, change in the ulcer scores, HAQ-DI and patient-reported skin score. Biomarkers including vascular endothelial growth factor, soluble P-selectin glycoprotein ligand-1, CRP and albumin were related to both the EScSG and the 12-point index, though they did not improve the total variance of the model.

Conclusion. The construct validity of the EScSG activity index is good, though the lung-related disease activity may not be sufficiently represented. Further validation steps may be required for both the EScSG and our 12-point activity index.

Key words: Systemic sclerosis, Scleroderma, Disease activity, Systemic sclerosis activity index, Self-assessment questionnaire, Biomarkers, Skin ulcers, Categorical principal component analysis.

Introduction

SSc is a multisystem autoimmune disease, with complex pathogenesis resulting in obliterative vasculopathy, tissue injury, fibrosis, remodelling and atrophy. The European Scleroderma Study Group (EScSG) developed preliminary disease activity indices to be used with SSc patients [1–4]. The EScSG activity index is a simple and feasible instrument that evaluates both clinical items and certain laboratory values, including the modified Rodnan skin score (MRSS), carbon monoxide diffusing capacity (DLCO),...
presence of sclerodema, digital ulcers, arthritis, ESR, hypocomplementaemia, and patient-reported worsening of skin, vascular and cardiopulmonary symptoms [3, 4]. However, these criteria await further validation, as construct validity has only been confirmed on a small cohort of 30 SSC patients, and further work is also requested to prove the responsiveness of the index. 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.

The key points in the disease process are endothelial cell injury, inflammation, immune activation and collagen deposition by activated fibroblasts. Theoretically, disease activity assessment should reflect all these important factors, and also their impact on the function of different organ systems.

Several biomarkers of immune activation, ongoing fibrosis and vascular injury have been published as potential indicators of disease activity in SSC [5, 6]. The endothelial cell activation marker, von Willebrand factor antigen (vWFAG), seems to correlate with the extent of internal organ involvement [7, 8]. P- and E-selectin contribute to the adhesion and activation of leucocytes. The level of the soluble form of E-selectin (sE-selectin) was found to be elevated in SSC [9]. 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 soluble PSGL-1 (sPSGL-1) were found to be associated with a lower frequency and severity of lung fibrosis [10]. The angiogenic factor vascular endothelial growth factor (VEGF), is permanently up-regulated in SSC, and this could be essential in a paradoxic manner to the decreased angiogenetic activity that characterizes this particular disease [11].

With regard to markers of fibrosis, procollagen Type I N-terminal propeptide (PINC) level correlated with changes in the MRSS [12]. The cross-linked collagen I carboxy-terminal telopeptide (serum cross-laps; CTX-1), a marker of collagen degradation, was found to be correlated with the extent of skin involvement (MRSS), acute-phase protein levels and indicators of decreased pulmonary function (DLCO < 75%) [13]. The serum level of procollagen Type III N-terminal propeptide (PINC) correlated with the extension of skin involvement and also indicated the prognosis of the disease. PINC was found to correlate positively with MRSS and inversely with DLCO [14].

Markers of inflammation include elevated ESR and CRP level, which may be signs of increased disease activity and unfavourable prognosis [14–16]. Several studies including our own [14–16] showed that the value of ESR was significantly higher in patients with dcSSC than in those with lcSSC.

Markers of pulmonary fibrosis/involvement include Krebs von Lungrten 6 antigen (KL-6), which is a mucin-like protein produced by Type II alveolar epithelial cells. Circulating KL-6 concentration strongly correlated with the severity of interstitial lung disease (ILD), and also with disease activity in one study [17–20]. Type II alveolar epithelial cells also produce surfactant proteins, including surfactant proteins A and D (SP-A and -D), which are raised in scleroderma-associated ILD. The levels of SP-A and SP-D correlated with the activity of the disease [18, 21].

Markers of immune activation include the B-cell activation factor (BAFF). A recent study demonstrated elevated BAFF level and correlation of BAFF level with skin fibrosis in patients with SSC [22]. The proliferation-inducing ligand (APRIL) is a TNF superfamily member with close homology to BAFF. In a recent study, serum APRIL levels tended to be higher in patients with dcSSC when compared with those with lcSSC, and SSC patients with elevated APRIL levels had significantly higher incidence of pulmonary fibrosis and decreased vital capacity [23].

The soluble CD40 ligand (sCD40L) is released from activated CD4+ T cells. CD40–CD40L interactions activate B cells, up-regulate endothelial adhesion molecules and induce fibrosis. A recent study showed that patients with dcSSC exhibited relatively persistent elevations of sCD40L concentration, whereas temporary elevations were observed in lcSSC patients during follow-up [24]. Another study reported the association of plasma sCD40L concentrations with the presence of digital ulcers in SSC patients. Concentrations of plasma sCD40L were also significantly higher in patients with pulmonary arterial hypertension (PAH) [25]. With regard to the 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 [26, 27].

In this study, we further confirmed the content and construct validity of the ESoCG activity index in a large cohort of SSC patients, and we have explored possible new activity markers. Additionally, we also made attempts to improve the existing activity index by adding some new disease activity-related items, in spite of the fact that the numerous biomarkers that were studied showed somewhat controversial results.

**Patients and methods**

**Study groups**

In our prospective study, 131 consecutive, unselected patients with SSC were included and re-investigated 12 (1.3) months later. A further 3-year re-investigation is also planned. Diagnosis of scleroderma and classification into dcSSC or lcSSC subgroups were performed at entry based on the criteria proposed by LeRoy et al. [28]. The female/male ratio was 9.9 : 1 (17 : 1 in lcSSC group and 4.8 : 1 in dcSSC group, respectively), mean (s.d.) age at entry into the study was 55.9 (11.6) years [57.4 (10.3) years in the lcSSC group and 52.6 (13.8) years in the dcSSC group, respectively]. Disease duration was defined as the period of time in years, from the date of onset of the first non-RP symptom until the patient’s first and second...
inclusion during the study. The mean (s.d.) disease duration was 8.1 (7.2) years [8.6 (7.5) years in the lcSSc group and 7.0 (6.3) years in the dcSSc group, respectively]. One hundred and twenty-three patients appeared for the 1-year re-investigation, five patients died during those 12 months and three were lost to follow-up.

Demographic, clinical and laboratory items were recorded by our standard protocol [14, 16]. Patients underwent echocardiography, spirometry, Dlco measurement and high-resolution CT, if necessary. PAH was defined by right heart catheterization [29, 30]. Oesophageal involvement was established with barium swallow or oesophago-gastroscopy. Scleroderma renal crisis was recorded as kidney involvement. Musculoskeletal involvement was defined as a decrease of >25% of the normal range of muscle strength. Symmetrical muscle weakness/bilateral quadriceps muscle strength (flexion contractures on hands, arthralgia, arthritis, symmetrical muscle weakness/bilateral quadriceps muscle strength ≤3 on a 1–5 scale) and presence of myositis were also evaluated.

MRSS was assessed according to the standard method [31]. Three investigators, unaware of the clinical presentation of the particular patients, performed a parallel investigation of each case. Previously, the examiners [Z.B., H.F., G.K., C.V. and L.C.] underwent a MRSS assessment validation process [29, 32]. 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. Patients also filled out our newly developed and validated skin self-assessment questionnaire [33]. This particular questionnaire contained questions about skin thickness (in regions identical with those evaluated by MRSS), which were summarized in the ‘17-area thickness score’ variable.

The Hungarian validated version of the Scleroderma HAQ was used [34], and the HAQ Disability Index (HAQ-DI) was calculated, corrected with aids and devices. Medsger Disease Severity Scale [35] and ESCSG activity index [3, 4] 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) [36]. The presence of joint contractures was blindly evaluated by an experienced physical therapist [Z.B. or H.F.]. The ‘number of contractures’ variable was counted as the sum of contractures of the following bilateral joints (by summarizing the presence of contractures in all joint axes): shoulder, elbow, wrist, the MCP joint of the second and third fingers, the PIP joint of the second and third fingers, hip, knee and ankle (range 0–30). Joint contracture was defined as a decrease of >25% of the normal range of motion in at least one joint axis.

Laboratory tests included blood cell counts, ESR, CRP, haemoglobin, haematocrit, serum creatinine kinase (CK), lactate dehydrogenase (LDH), total protein, serum albumin and complement (C3, C4). Clinical data of the enrolled patients are depicted in Table 1. More detailed data about these patients were previously published elsewhere [33].

### ELISA and 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-topo I antibody titre (Hycoy 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 determined as described [37]. Levels of sE-selectin, sPSGL-1 (Bender MedSystems GmbH, Vienna, Austria), vWF (United States Biological, Swampscott, MA, USA), were determined from plasma samples, using commercial ELISA kits according to the manufacturer’s instructions. Serum PIIINP and PINP concentrations were determined by RIA using RIA kits from Orion Diagnostica (Espoo, Finland).

As controls for these particular tests, the sera of 51 patients with primary RP (PRP) 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 of the University of Pécs, Hungary (Approval No. 2720/2006).

### Statistical analysis

The normality of distribution for the laboratory and clinical parameters was investigated by the Kolmogorov–Smirnov test. As the majority of these parameters had non-normal distribution, median values were determined and non-parametric tests (Mann–Whitney U-test and Wilcoxon test, respectively) were used to compare patient and control subgroups, or baseline and 1-year re-investigation 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 direction and magnitude of correlations between numerous pairs of differently scaled variables in a simple (i.e. 2D) 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 of <45° with the vector of the reference variable (namely, the ESCSG activity index) are considered related to it [38]. In the 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 variance contained by the axes of dimensions. When no remarkable increase in the total variance 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, Chicago, IL, USA) program was used for all analyses.

Results

Clinical characteristics and biomarker results in the SSc patients

The clinical parameters of the patients are depicted in Table 1. As we compared the median values of biomarker levels of the SSc patient group with healthy controls and patients with PRP, PIP, CTX-1, SP-D, and KL-6 were found to be significantly higher in the SSc group, both at baseline and 1-year re-investigation compared with both control groups (P < 0.01).

The sCD40L titre in the SSc group was elevated only compared with 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 with the SSc patients and healthy controls (Table 2).

Comparing the lcSSc and dcSSc subsets, CTX-1, SP-D and KL-6 also differed significantly between the two SSc subsets at baseline investigation, with higher median values in dcSSc patients. At 1-year re-investigation, 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 3).

When the changes in the median biomarker levels at 1-year follow-up were studied, PIIINP and BAFF levels increased significantly in the SSc group. Their levels at the time of re-investigation were significantly higher in the lcSSc and dcSSc groups; however, the difference between the ct values in the two SSc subsets disappeared (Table 2).

Relationship of the EScSG activity index to the clinical parameters

We evaluated the relationship between the EScSG activity index and certain clinical parameters including MRSS, 17-area thickness score, disease duration, HAQ-DI,
Disease activity evaluation in SSc

The parameters of pulmonary involvement (FVC, FVC/DLCO) loaded in the second dimension and the 1-year follow-up data showed the same settings (Fig. 1).

Development of a new 12-point activity index
As the lung-related parameters were independent of the ESSG activity index (Fig. 1), we generated a new index that reflects lung-related disease activity somewhat better. The selection of variables was based on clinical judgement 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 skin self-assessment, skin ulcer score, HAQ-DI, FVC, FVC/DLCO, left ventricular ejection fraction (LVEF), presence of PAH, HAI of the dominant side, BMI, ulcer score and number of joint contractures and age at 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 led to an increase of ~10% in 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 2D model.

The ESSG activity index loaded in the first dimension and showed association with the HAQ-DI, ulcer score, MRSS, 17-area thickness score and number of contractures, and showed inverse correlation with the HAII (decrease in HAI meaning the worsening of hand function).

### Table 2: Median (percentiles) values of the investigated laboratory parameters in SSc patients, patients with PRP and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>SSc baseline (n = 131)</th>
<th>SSc 1 year (n = 123)</th>
<th>Healthy controls (n = 30)</th>
<th>PRP (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIINP(^{55}), (\mu g/l)</td>
<td>3.8 (3.1–4.5)</td>
<td>4.3(^{\uparrow}) (3.8–5.2)</td>
<td>4.0 (3.7–4.4)</td>
<td>3.6 (3.4–4.2)</td>
</tr>
<tr>
<td>PINP, (\mu g/l)</td>
<td>45.0(^{11}) (34–65)</td>
<td>44.5(^{11}) (30–60.1)</td>
<td>33.5 (28.3–44.5)</td>
<td>33.0 (25.7–37.8)</td>
</tr>
<tr>
<td>CTX-1, ng/ml</td>
<td>0.4(^{11}) (0.2–0.6)</td>
<td>0.3(^{11}) (0.2–0.6)</td>
<td>0.2 (0.2–0.3)</td>
<td>0.2 (0.1–0.3)</td>
</tr>
<tr>
<td>SPD, ng/ml</td>
<td>1997.4(^{11}) (1367.1–3736.8)</td>
<td>1961.4(^{11}) (1218.8–3709.2)</td>
<td>1239.8 (648.6–1445.5)</td>
<td>1199.8 (734–1727.3)</td>
</tr>
<tr>
<td>vWF(^{55}), (\mu g/ml)</td>
<td>30.0(^{11}) (21.9–38.6)</td>
<td>33.4 (25.8–41.3)</td>
<td>28.6 (22.6–40)</td>
<td>37.8 (28.1–48.8)</td>
</tr>
<tr>
<td>sPSG-1(^{\uparrow}), U/ml</td>
<td>241.8(^{11}) (170.6–298.4)</td>
<td>262.3(^{11}) (214–314.3)</td>
<td>324.2 (273.6–361.8)</td>
<td>322.9 (288.5–388.5)</td>
</tr>
<tr>
<td>VEGF, pg/ml</td>
<td>122.1 (80.2–192.5)</td>
<td>129.2 (88.7–201.8)</td>
<td>93.8 (60–164.2)</td>
<td>111.7 (78.4–227.9)</td>
</tr>
<tr>
<td>sE-selectin(^{3}), ng/ml</td>
<td>34.4(^{11}) (25.5–46)</td>
<td>34.4(^{11}) (24.7–47.8)</td>
<td>32.1 (21.3–41.3)</td>
<td>89.2 (34.7–226.7)</td>
</tr>
<tr>
<td>KL-6, U/ml</td>
<td>802.2(^{11}) (534.3–1246.7)</td>
<td>935.3(^{11}) (583.8–1323.3)</td>
<td>516.3 (316.5–644.4)</td>
<td>625.7 (387.7–744.8)</td>
</tr>
<tr>
<td>BAFF(^{55}), pg/ml</td>
<td>413 (338.2–561.3)</td>
<td>556.9(^{11}) (439–690.4)</td>
<td>383.7 (327.5–435.4)</td>
<td>440.4 (385.1–564.7)</td>
</tr>
<tr>
<td>APRIL(^{55}), U/ml</td>
<td>9.8(^{11}) (7.2–11.5)</td>
<td>3.2 (1.5–6.9)</td>
<td>2.9 (2.2–4.8)</td>
<td>4 (2.1–7.7)</td>
</tr>
<tr>
<td>sCD40L(^{55}), U/ml</td>
<td>2.0(^{11}) (1.4–2.6)</td>
<td>1.7(^{11}) (1.3–2.2)</td>
<td>1.4 (1–2.3)</td>
<td>1.1 (0.6–1.7)</td>
</tr>
</tbody>
</table>

\(^{\uparrow}\)P < 0.05, \(^{\uparrow\uparrow}\)P < 0.01 between the SSc and healthy control groups; \(^{1}\)P < 0.05, \(^{11}\)P < 0.01 between the SSc patients and those with PRP; \(^{55}\)P < 0.05, \(^{555}\)P < 0.01 between the baseline and 1-year follow-up levels of laboratory parameters in SSc patients. Bold characters represent significant differences between the subgroups.

### Table 3: Median (percentiles) values of the laboratory parameters in the SSc subsets during the baseline and 1-year re-investigation

<table>
<thead>
<tr>
<th></th>
<th>lcSSc baseline (n = 90)</th>
<th>dcSSc baseline (n = 41)</th>
<th>lcSSc 1 year (n = 87)</th>
<th>dcSSc 1 year (n = 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIINP, (\mu g/l)</td>
<td>3.8 (3–4.5)</td>
<td>3.7 (2.9–5)</td>
<td>4.3 (3.8–5.1)</td>
<td>4.2 (3.7–6.5)</td>
</tr>
<tr>
<td>PINP, (\mu g/l)</td>
<td>45.2 (36.2–67.3)</td>
<td>41 (31.1–64.5)</td>
<td>45.5 (31.4–59.3)</td>
<td>42.1 (28.3–63)</td>
</tr>
<tr>
<td>CTX-1, ng/ml</td>
<td>0.4 (0.2–0.5)</td>
<td>0.5 (0.3–0.7)</td>
<td>0.3 (0.2–0.6)</td>
<td>0.3 (0.2–0.5)</td>
</tr>
<tr>
<td>SPD(^{10}), (\mu g/ml)</td>
<td>1829.6 (1198.6–3175.6)</td>
<td>2899 (1762.8–5123.1)</td>
<td>1737.2 (1089.4–3277.7)</td>
<td>2799.7 (1815.4–5763.4)</td>
</tr>
<tr>
<td>vWF, (\mu g/ml)</td>
<td>29.1 (22.6–36.3)</td>
<td>32.7 (19.3–43.1)</td>
<td>33.2 (25.5–39.8)</td>
<td>35 (27.6–50.1)</td>
</tr>
<tr>
<td>sPSG-1, U/ml</td>
<td>239.0 (172.9–297.7)</td>
<td>250.7 (164.8–303.4)</td>
<td>271.4 (213.4–317)</td>
<td>248.3 (216–294.3)</td>
</tr>
<tr>
<td>VEGF, pg/ml</td>
<td>118.1 (80–190.6)</td>
<td>144.0 (81.7–225.7)</td>
<td>124.7 (87–197.8)</td>
<td>142.6 (93.1–231.7)</td>
</tr>
<tr>
<td>sE-selectin, ng/ml</td>
<td>33.2 (24.9–45.8)</td>
<td>37.0 (26.1–51)</td>
<td>36.2 (24–50.4)</td>
<td>33.9 (25.6–44.7)</td>
</tr>
<tr>
<td>KL-6, (\mu g/ml)</td>
<td>760.2 (464.7–1076)</td>
<td>1045.5 (676.4–1884.4)</td>
<td>844.2 (533–1190.2)</td>
<td>1176.3 (690.3–2345.7)</td>
</tr>
<tr>
<td>BAFF, pg/ml</td>
<td>413.2 (344.9–510.8)</td>
<td>413.0 (311.4–691.4)</td>
<td>569.3 (465.9–711.2)</td>
<td>545.7 (424–683.2)</td>
</tr>
<tr>
<td>APRIL, U/ml</td>
<td>9.6 (7–11.6)</td>
<td>9.8 (8.6–11.1)</td>
<td>3.8 (2.1–10)</td>
<td>2.5 (0.8–5.9)</td>
</tr>
<tr>
<td>sCD40L, U/ml</td>
<td>2.1 (1.5–2.5)</td>
<td>1.9 (1.3–2.8)</td>
<td>1.8 (1.3–2.4)</td>
<td>1.7 (1.2–2)</td>
</tr>
</tbody>
</table>

\(^{\uparrow}\)P < 0.05, \(^{\uparrow\uparrow}\)P < 0.01 between the lcSSc and dcSSc subgroups at baseline evaluation; \(^{1}\)P < 0.05, \(^{11}\)P < 0.01 between the lcSSc and dcSSc subgroups at 1-year re-investigation. Bold characters represent significant differences between the subgroups.
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 points based on the baseline MRSS values, ΔDLCO: 9–10%, ΔHAQ-DI: 0.2–0.25) defined on a Delphi exercise [39]. In general, these values are higher compared with the ‘minimally important difference’ values (ΔMRSS: 3.2–5.3 and ΔHAQ-DI: 0.1–0.14) [40].

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 (Fig. 2). 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 (Fig. 2). The newly constructed activity index contained the following new variables: the change in DLCO > 9% [39] over 1 year with 0.5 point value and FVC/DLCO > 1.8 [41] 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 ulcer score’ variable, which scored the appearance of new ulcers on patients who had none at baseline investigation and also the increase in the ulcer score at 1-year follow-up. This variable counted as an additional 0.5 point. Another half a point was added to the index if the HAQ-DI > 1 (which means moderate or severe disability that may be caused...
by extensive skin involvement, characteristic of early diffuse scleroderma) and change in HAQ-DI ≥ 0.2 point at 1-year assessment was also scored with 0.5 point. Thus, the total score of the newly generated activity index was 12.0 points (Table 4).

The relationship of the 12-point activity index to the clinical parameters of 1-year re-investigation was also examined by CATPCA. The disease activity-related clinical parameters were distributed into two dimensions, identical to those seen in the previous CATPCAs (Fig. 2). 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

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**TABLE 4** The differences between the structure of the EScSG activity index and the newly generated 12-point activity index in SSc

<table>
<thead>
<tr>
<th>Domain</th>
<th>Item</th>
<th>Point</th>
<th>Domain</th>
<th>Item</th>
<th>Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>MRSS &gt; 14</td>
<td>1.0</td>
<td>Skin</td>
<td>MRSS &gt; 14</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Scleredema</td>
<td>0.5</td>
<td></td>
<td>Scleredema</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>ΔSkin&lt;sup&gt;a&lt;/sup&gt; (patient)</td>
<td>2.0</td>
<td></td>
<td>ΔSkin&lt;sup&gt;a&lt;/sup&gt; (patient)</td>
<td>1.0</td>
</tr>
<tr>
<td>Vascular</td>
<td>Digital ulcers</td>
<td>0.5</td>
<td>Vascular</td>
<td>Digital ulcers</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>ΔVascular&lt;sup&gt;b&lt;/sup&gt; (patient)</td>
<td>0.5</td>
<td></td>
<td>ΔVascular&lt;sup&gt;b&lt;/sup&gt; (patient)</td>
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</tr>
<tr>
<td>Joints</td>
<td>Arthritis</td>
<td>0.5</td>
<td>Joints</td>
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<tr>
<td></td>
<td>HAQ-DI</td>
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<td>HAQ-DI</td>
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<td>ΔHAQ-DI ≥ 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>ΔHAQ-DI ≥ 0.2&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Lung/heart</td>
<td>DL&lt;sub&gt;CO&lt;/sub&gt; &gt; 80%</td>
<td>0.5</td>
<td>Lung/heart</td>
<td>DL&lt;sub&gt;CO&lt;/sub&gt; &gt; 80%</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>ΔLung/heart&lt;sup&gt;d&lt;/sup&gt; (patient)</td>
<td>2.0</td>
<td></td>
<td>ΔLung/heart&lt;sup&gt;d&lt;/sup&gt; (patient)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>FVC/DL&lt;sub&gt;CO&lt;/sub&gt; &gt; 1.8</td>
<td>1.0</td>
<td></td>
<td>FVC/DL&lt;sub&gt;CO&lt;/sub&gt; &gt; 1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Laboratory</td>
<td>ESR &gt; 30 mm/h</td>
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<td>ESR &gt; 30 mm/h</td>
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</tr>
<tr>
<td></td>
<td>Hypocomplementaemia&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
<td>Hypocomplementaemia&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Total score</td>
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<td>10.0</td>
<td></td>
<td></td>
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</table>

<sup>a</sup>Change in patient-reported 17-area thickness score over 1 year; <sup>b</sup>change in skin symptoms during last month; <sup>c</sup>change in MRSS over 1 year; <sup>d</sup>change in vascular symptoms during last month; <sup>e</sup>appearance of ulcers or increase in severity of ulcers over 1-year re-investigation; <sup>f</sup>change in HAQ-DI over 1 year; <sup>g</sup>change in cardiopulmonary symptoms; <sup>h</sup>change in DL<sub>CO</sub> during 1 year. (patient): reported by the patients; <sup>i</sup>C3, C4 or total complement decreased. For details, see ‘Patients and methods’ section,
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, having been associated with it both at baseline and at 1-year re-investigation (Fig. 3). Serum albumin, VEGF, vWF, sPSGL-1 and CRP were found to be in consistent correlation with the EScSG activity index at both investigations.

Fig. 3 Relationships between the EScSG activity index, clinical data and various laboratory parameters in SSc patients using CATPCA. (A) Baseline data of 131 consecutive SSc patients. (B) 1-year follow-up data of 123 consecutive SSc patients. Parameters that were found to be correlated with the EScSG activity index by CATPCA are indicated in bold type marked by an asterisk. For details, see ‘Patients and methods’ section.
We also examined the relationship between these laboratory parameters and the 12-point activity index. 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 with the anti-topo I titre, KL-6, SP-D, PINP and PIIINP (Fig. 4). The total variance of this model was similar to the original (28 vs 26.1% in the original EScSG model).

As the 12-point activity index proposed by our research team cannot be used at the first visit of the patient to 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 1-year re-investigation (Spearman’s $r = 0.911$, $P < 0.001$ and $r = 0.831$, $P < 0.001$, respectively), 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 1-year re-investigation (data not shown).

**Conclusions**

The EScSG activity index developed in a European multicentre study is the only available instrument for the assessment of disease activity in SSc. Its validity has only been tested on a relatively small unselected patient cohort [3]. A recent study examined a simple preliminary model of eight variables. Similar to our findings, in this particular model, the authors also included musculoskeletal symptoms and an additional variable of pulmonary involvement (exertional dyspnoea) besides scleredema, MRSS, fatigue, DLCO, ESR and digital ulcer. This model was found to perform similarly to the existing EScSG disease activity score in early and late SSc [42].

MRSS assessment plays a central role in disease activity assessment in patients with early dcSSc, because these patients are potential candidates for therapeutic trials. However, a new subgroup of patients with low MRSS, but severe internal organ involvement comparable with high MRSS patients was also identified recently [43]. Still, the majority of SSc patients have relatively long disease duration and a low MRSS. We therefore think that
the evaluation of ongoing oblitative vasculopathy, inflammatory phenomena and organ-specific activity markers might be appropriate tools for the assessment of disease activity in unselected SSc patients in clinical practice.

The EScSG activity index is a very useful and appropriate tool to assess disease activity in SSc, although some domains of this particular index may still be a topic of debate [44, 45]. In our present study, we evaluated the EScSG activity index on a large, unselected, consecutive SSc cohort, containing both lcSSc and dcSSc patients, as seen in the multicentre study performed by the EScSG [1–4]. 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 (Fig. 1). 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 also provide valid and reliable data regarding their skin involvement; therefore, we have recently developed and validated a skin self-assessment questionnaire. This particular patient-reported ‘17-area thickness score’ correlated with the MRSS and also with the EScSG activity index [33]. Furthermore, our CATPCA analysis revealed that the EScSG activity index might not reflect sufficiently the pulmonary interstitial and vascular involvement of the disease, as the lung-related parameters were sorted into a separate dimension by CATPCA analysis (Fig. 1).

The 12-point index constructed by us includes some new, feasible parameters like patient-reported 17-area thickness score, HAQ-DI, change in MRSS, HAQ-DI and DLCO at 1-year follow-up, the change in ulcer score and the FVC/DLCO ratio. Like the EScSG activity index, our newly developed index also does not sufficiently include gastrointestinal and kidney involvement of scleroderma patients.

This particular new index appropriately reflects the pulmonary interstitial and vascular involvement 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%).

In our attempt to enhance the information referring to objectively measurable change in organ involvement, we introduced some parameters of change (e.g. in DLCO, MRSS, HAQ-DI and ulcer score). 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-year re-investigation of our cohort is also planned.

At the first examination of the patient these particular changes cannot be assessed. Omitting the new parameters for assessing the changes, 8.5 points remain which sufficiently reflect disease activity. The 8.5-point activity index is highly correlated with the original EScSG activity index and may be used for the first investigation.
There is a need to find 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. Numerous biomarkers have been studied, but the results seem to be somewhat controversial. 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 with controls, as also seen in other clinical investigations [13]. We also confirmed that SP-D and KL-6 may be useful diagnostic markers and indicators of disease activity and also damage in patients with pulmonary interstitial diseases [17, 46]. On the contrary, lower serum levels of sPSGL-1 were found in the SSc patients compared with the healthy controls and PRP patients, potentially indicating a protective role against pulmonary involvement [10].

A previous study found higher vWF levels in patients with RP and SSc compared with healthy controls [47], and the levels in the Raynaud group were slightly higher than in scleroderma patients [48]. The elevated levels of vWF and sE-selectin levels in our PRP patients might reflect that the endothelial cell activity in these patients with shorter disease duration might be more pronounced compared with the SSc group with relatively longer disease duration (mean 8 years).

The CATPCA, performed both with the baseline and 1-year re-investigation data, revealed the relationship of five markers, namely CRP, albumin, VEGF, sPSGL-1 and vWF to the EScSG activity index. Our group has already found that the increase in CRP influenced the prognosis of scleroderma [13]. The quantification of gastrointestinal involvement in SSc is difficult because part of it can be involved. The relationship of albumin with both activity indices might be a reflection of the malabsorption associated with a more active stage of disease, thus may reflect additional organ involvement. The decrease in albumin can also be considered as a sign of inflammation. The elevated markers of endothelial cell activation (vWF) and angiogenesis (VEGF) have also previously been found to be signs of an ongoing pathological disease process [8, 48, 50]. 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). sPSGL-1 was identified in a study as a 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 index (Figs 3 and 4). The 12-point index also reflected the pulmonary involvement as well as the vascular and fibrotic component of disease activation. Therefore, those laboratory markers that 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-topo I titre, KL-6, SP-D, PINP and PIIINP, which might reflect pathological processes that were under-represented in the original index.

In summary, our findings confirm that the EScSG activity index is a good composite index with good construct validity. Its face, content and construct validity have been previously proved, but demonstration of responsiveness is still lacking. However, our results also indicated that this index may not represent sufficiently the ongoing pulmonary interstitial and vascular involvement; therefore, further validation steps are required in the future. In spite of the fact that the biomarkers investigated in this study did not perform adequately to our expectations in the CATPCA model, we identified some clinical assessment tools and laboratory markers that deserve further attention in future studies, and we also constructed a new 12-point activity index that may better reflect the interstitial and vascular lung activity of the disease.

The evaluation of skin ulcers and contractures, CRP, serum albumin level, VEGF, vWF and sPSGL-1 are potential candidates for future studies. If the 12-point activity index proves to be a reliable, more sensitive index of disease activity, the role of KL-6, SP-D, anti-topo I antibody, PINP and PIIINP would also be worth considering for further evaluation.

### Rheumatology key messages

- The construct validity of the EScSG scleroderma activity index is good.
- The newly introduced 12-point activity index encompasses lung-related disease activity in SSc better.

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