1 **The European Scleroderma Trials and Research group (EUSTAR) Task Force for the**

2 **Development of Revised Activity Criteria For Systemic Sclerosis: derivation and**

3 **validation of a preliminarily revised EUSTAR activity index**

4

5 **Gabriele Valentini[1], Michele Iudici[1], Ulrich A. Walker[2], Veronika K. Jaeger[2], Murray**

6 **Baron[3], Patricia Carreira[4], László Czirják[5],Christopher P. Denton[6], Oliver Distler[7],**

7 **Eric Hachulla[8], Arianne Herrick[9], Otylia Kowal-Bielecka[10], Janet Pope[11], Ulf Müller-**

8 **Ladner[12], Gabriela Riemekasten[13], Jerome Avouac[14],Marc Frerix[12], Suzana**

9 **Jordan[7],Tünde Minier[5], Elise Siegert[14],Voon H. Ong[6], Serena Vettori[1] and**

10 **YannickAllanore[15]**

11

12 **Affiliations**

13 1 Department of Clinical and Experimental Medicine, Rheumatology Section, Second

14 University of Naples, Naples, Italy

15 2 Department of Rheumatology, Basel University, Basel, Switzerland

16 3 Division of Rheumatology, Jewish General Hospital, McGill University, Montreal, Quebec

17 H3T 1E2, Canada

18 4Department of Rheumatology, 12 de Octubre University Hospital, Madrid, Spain

19 5 Department of Rheumatology and Immunology, University of Pécs, Medical Centre,

20 Pécs, Hungary

21 6 Centre for Rheumatology and Connective Tissue Disease, Division of Medicine, Royal

22 Free Campus, University College London, London NW3 2QG, UK

23 7 Division of Rheumatology, University Hospital Zurich, Zurich, Switzerland

24 8 Internal Medicine Department, Claude Huriez Hospital, Lille University, Lille, France

25 9 Centre for Musculoskeletal Research and NIHR Manchester Musculoskeletal Biomedical

26 Research Unit, The University of Manchester, Manchester Academic Health Science

27 Centre, Manchester, UK

28 10 Department of Rheumatology and Internal Medicine, Medical University of Bialystok,

29 Bialystok, Poland

30 11Department of Medicine, St. Joseph's Health Care, University of Western Ontario,

31 London, Ontario N6A 4V2, Canada

32 12 Justus-Liebig University Giessen, Department of Rheumatology and Clinical

33 Immunology, Kerckhoff-Klinik, Bad Neuheim, Germany

34 13 Clinic for Rheumatology, University of Lübeck, Lübeck, Germany

35 14 Department of Rheumatology and Clinical Immunology, University Hospital Charité,

36 Berlin, Germany

37 15 Cochin Institute, Paris Descartes University, INSERM U1016 and CNRS UMR8104,

38 Paris, France

39

43

44 Address Correspondence to Prof. Gabriele Valentini, Department of Clinical and

45 Experimental Medicine, Rheumatology Section, Second University of Naples, Naples,

46 Italy. II Policlinico, Edificio 3, Via Pansini 5, 80131 Napoli. Italia.

47 **Tel** +390815464487- **Fax** +390815666747- **E mail** gabriele.valentini@unina2.it

48

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

Abstract

Background European Scleroderma Study Group (EScSG) activity indexis currently used to assess disease activity in Systemic Sclerosis (SSc). Its validity has been criticized.

Methods Three investigators assigned an activity score on a 0-10 scale for 97 clinical charts. The median score served as gold standard. Two other investigators labelled the disease as inactive/moderately active or active/very active. Univariate-multivariate linear regression analyses were used to define variables predicting the "gold standard", their weight and derive an activity index. The cut-off point of the index best separating active-very active from inactive-moderately active disease was identified by a Receiver Operating Curve analysis. The index was validated on a second set of 60 charts assessed by three different investigators on a 0-10 scale and defined as inactive/moderately active or active/very active by other 2 investigators. One hundred and twenty-three were investigated for changes over time in the index and their relationships with those in the summed Medsger severity score.

Results A weighted 10-point activity index was identified and validated: Δ-skin=1.5 (Δ =patient assessed worsening during the previous month), modified Rodnan skin score>18=1.5; digital ulcers=1.5; tendon friction rubs=2.25; C reactive protein>1mg/dl=2.25; diffusing capacity of the lung for CO <70%=1.0. A cut-off ≥2.5 was found to identify patients with active disease .Changes of the index paralleled those of Medsger's summed severity score (p= 0.0001).

Conclusions A preliminarily revised SSc activity index has been developed and validated, providing a valuable tool for clinical practice and observational studies.

Introduction

The assessment of patients with systemic sclerosis (SSc) should address different disease aspects: diagnosis and fulfilment of classification criteria, extent of organ involvement, activity (the reversible part of the disease process), damage (the irreversible part of the disease process), prognosis prediction, outcome, and response to treatment.[1] Defining disease activity in SSc cannot be done using a single variable and it is challenging for a number of reasons: first, patients can present with an indolent course, irrespective of whether or not they belong to either of the two disease subsets, i.e., diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc);[2-6] second, SSc flares can be difficult to separate from quiescent disease;[1]third, the two main morphological manifestations of the disease (interstitial fibrosis and vascular occlusion) may reflect both activity and damage and finally, validated biological markers reflecting disease activity are still lacking.[7]

In 2001, the European Scleroderma Study Group (EScSG) in an analysis of the clinical charts of 290 patients from 19 European SSc centres, identified 11 disease activity variables and developed a preliminary activity index.[8,9] The construct validity of the index was first verified by the jackknife technique (i.e., assessing the dispersion of the correlation coefficients calculated by removing out 1 patient at a time), [10] then confirmed by calculating the correlations between the index and the rank of disease activity assigned by 4 experts to 30 charts, selected to represent different degrees of disease activity.[11]

This index was subsequently endorsed by EUSTAR and has been used to assess disease activity in 149 studies.[12]Its criterion validity is supported by its correlation with the physician global assessment of activity of the Canadian Scleroderma Research group (CSRG), [13] its association with anti-topoisomerase1 titre,[14] and its role as the main predictor of the scleroderma phenotype (presenting a higher procollagen transcription) of skin fibroblasts from SSc patients.[15]

However, it has some limitations due to the procedure underlying its development. In fact, it most patients had a long disease duration and the number of missing values was high. Moreover,

4

the face validity of hypocomplemaentemia has been questioned since the complement fixation is not thought to be important in SSc. [16,17]. Finally, it was not validated on an independent cohort. Here, we present the results of a EUSTAR study devoted to revise the original activity index in order to improve and validate it.

Material and Methods

Derivation study

The Study Coordinator selected 97 clinical charts from patients included in the EUSTAR database.[5]The selection was carried out in order to identify patients fulfilling the 1980 ACR criteria for the classification of SSc;[18] followed in SSc referral centres in order to reduce the number of missing values; and representing one of the following disease subgroups: early dcSSc, late dcSSc, early lcSSc, and late lcSSc. Early disease was defined a disease duration ≤ 3 years from the onset of the first non-Raynaud symptom.[19]

Three clinical investigators (YA, CD and OK-B) from centres other than those from which the patients charts were derived, assigned a disease activity score on a 0-10 scale to each chart. The reliability of this scoring system was assessed by the evaluation of the Interclass Correlation Coefficient (ICC). The median disease activity score was used as the "gold standard" to identify items significantly associated to disease activity.

To this aim, the study coordinator selected from the items listed in the EUSTAR chart,[5]the following 18 thought to have face validity as activity variables 1)*anamnestic*:Δ-skin, Δ- vascular, Δ-heart/lung worsening (i.e. worsening, as evaluated by the patient during the month before enrolment, of skin induration, Raynaud's phenomenon and/or digital ischemic ulcers and dyspnea and/or palpitations, respectively); 2) *clinical*: active digital ulcers; modified Rodnan skin score(mRss); tendon friction rubs(TFR); muscle weakness; arthritis; 3) *laboratory*: C-Reactive Protein (CRP) elevation; erythrocyte sedimentation rate (ESR)/h value; hypocomplementemia; creatin-kinase(CK) elevation; proteinuria; 4) *functional and imaging* : systolic Pulmonary Arterial Pressure(sPAP) and pericardial effusion at echocardiography; ground glass and lung fibrosis at

lung High Resolution Computed Tomography (HRCT); Forced Vital Capacity(FVC); Diffusing Lung capacity for carbon monoxide-single breath (DLCO).

Subsequently, we performed an univariate linear regression analysis to search for significant associations between each of the selected items and the median disease activity score given by the 3 experts. Cut-off values for sPAP, FVC and DLCO were derived from literature.[20-22]

The items significantly associated with gold standard in univariate analysis, were all entered in a multivariate linear regression analysis to identify the set of variables independently associated with the "gold standard". As far as the remaining 2 continuous variables (mRss and ESR) , we made a number of attempts devoted to both identify the cut off point most significantly associated with the gold standard (highest R^2; lowest p)to construct a model with the highest sum of sensitivity and specificity .Each variable found to be significantly associated in multivariate analysis was assigned a weight corresponding to beta coefficient adjusted in order to construct a 10-point weighted activity index.

Two other investigators (LC and GV), who were unaware of the values assigned on the 0-10 scale, evaluated each of the 97 charts as inactive (corresponding to no need to change treatment and requiring a follow-up after six months- 1 year), or moderately active (corresponding to no need to change treatment and a three-six-monthly follow-up), or active (needing treatment intensification and one-three-monthly follow-up), or very active (requiring hospitalization for active disease).The reliability of this system was assessed by the evaluation of Cohen's K. The charts that had received a discordant evaluation by the 2 investigators were resent to them for a reassessment devoted to find an agreement.

For each patient the overall disease activity was calculated summing the scores of the new index. The cut-off value presenting the highest sum of sensitivity and specificity in separating patients with active-very active disease from those with inactive-moderately active condition was identified by a receiver operating (ROC) curve.

Validation study

The new index was validated on 60 patients recruited from the same database and selected in order to satisfy the following aspects: 1) fulfilling 2013 ACR/EULAR criteria for the classification of SSc;[23]2) belonging to patients recruited at SSc centres with SSc expertise in whom capillaroscopy had been performed and the pattern defined according to Cutolo et al.;[24] 3) being representative of one of the following disease subgroups, as described above: early dcSSc, late dcSSc, early lcSSc, and late lcSSc. The 60 charts were assessed for disease activity on a 0-10 scale by three different investigators (PC,AH, JP) and defined as inactive, moderately active, active, and very active by other two investigators (MB and EH), all of whom unaware of the derived index. The reliability of the two scoring systems was assessed by ICC and K statistics respectively.

Changes of the index over time and its relationships to summed Medsger severity score

In order to furtherly validate the index, we assessed the changes in the activity index detected in patients from either derivation or validation cohorts in a follow-up visit at least 6 months apart and compared it with the changes in the summed Medsger severity score[25], that is a validated measure of disease severity in observational studies.[26] We undertook this approach by considering that severity reflects both activity and damage and its change, being damage irreversible, can only depend on changes in the activity part of the disease process.

Results

Derivation study

Table 1 lists the main epidemiological, serological and clinical features of the 97 patients considered in the derivation part of this study. All of the patients also satisfied 2013 ACR/ EULAR criteria. The ICC among the activity scores given by the three clinical experts was 0.786 indicating that either the median or the mean value could be considered consistent measures of disease activity and supporting the use of one of them as a gold standard.

TABLE 1

Epidemiological, serological, capillaroscopic and clinical features of the 97 SSc patients

Patients	Whole cohort N=97	Early dcSSc N=25	Late dcSSc N=24	Early lcSSc N=23	Late lcSSc N=25
Sex (F/M)	77/20	15/10	21/3	18/5	23/2
Age (years), median; range	55 (21-89)	54 (21-70)	55 (25-75)	52 (23-89)	58 (41-75)
Disease duration (from the first non-Raynaud's manifestation):years (median; range)	3 (0.5-47)	1 (0.5-3)	6 (3.1-18)	2 (0.5-3)	9 (4-47)
Antinuclear antibodies positive°	95 (98)	24	24	23	24
Anti-Scl-70 antibody positive°°	50 (51%)	15	14	11	10
Anti-centromere antibody positive°	17 (17%)	0	2	5	10
Anti-RNA polymerase III antibody positive°°	6 (6%)	2	3	0	1
Anti-U1RNP antibody positive°°	1 (1%)	0	0	1	0
Raynaud's phenomenon	97 (100%)	25	24	23	25
Active Digital Ulcers*	16 (16%)	4	7	2	3
Arthritis**	15 (15%)	5	4	0	6
Proximal muscle weakness***	29 (30%)	9	6	6	8
Tendon Friction Rubs(TFR)****	14 (14%)	6	3	4	1
Skin sclerosis******	92 (95%)	25	24	20	23
Esophageal, stomach and/or intestinal involvement^	66 (68%)	16	15	15	20
Interstitial lung disease (CT)^^	53/89 (59%)	13/25	17/24	10/18	13/22
FVC^^^, % of the predicted value (mean±SD)	87.5±18.9	89.4±17.3	78.7±20.0	90.8±12.8	91.0±22.3
DLCO^^^, % of the predicted value(mean±SD)	65.7±21.5	65.9±20.1	56.5±19.9	69.9±16.4	70.6±26.3
Estimated systolic pulmonary artery pressure (sPAP)>30 mmHg^^^^	22 (23%)	4	5	6	7
Heart disease^^^^^	45 (46%)	9	14	7	15
Scleroderma renal crisis (previous)°	3 (3%)	2	1	0	0

209

210 ° By IF on Hep-2 cells; °° By ELISA on ANA positive sera; * ranging from small infarcts of the
211 digital tips to digital gangrene; ** symmetric swelling and tenderness of the peripheral joints; *** as
212 detected at physical examination; **** perception of leathery crepitus during motion of hands,
213 wrists,elbows,shoulders, knees, ankles (both at anterior and posterior aspects) ***** thickening and
214 induration of the skin as detected by physical examination; ^dysphagia and/or heartburn and/or
215 bloating and/or vomiting and/or diarrhea and/or constipation; ^^either ground glass or interstitial
216 fibrosis as detected at lung high resolution computed tomography;^^^ Forced vital capacity
217 ^^^^diffusing lung for Carbon monoxide (single breath), ^^^^as assessed by B-MODE Doppler
218 echocardiography; ^^^^^ diastolic dysfunction and/or cardiac blocks and/or palpitations and/or left
219 ventricular Ejection Fraction< 55% as assessed by EKG and B-MODE Doppler echocardiography,
220 °rapidly deteriorating kidney failure with or without accelerated/malignant hypertension..
221 --

222 Table 2 lists the items, out of the 19 selected, that resulted to be associated to the median value of
223 the three 0-10 scores,in univariate linear regression analysis.
224 **TABLE 2**

Associations between each potential activity parameter and the gold standard in Univariate Regression Analysis

Item	R^2*	P
ESR	0.441	**0.0001**
Digital ulcers	0.294	**0.0001**
CRP> 1 mg/dl	0.256	**0.0001**
mRss	0.238	**0.0001**
Δ-skin worsened	0.180	**0.0001**
Δ- vascular worsened	0.164	**0.0001**
Δ-heart/lung worsened	0.160	**0.0001**
CK elevation	0.127	**0.0003**
TFR	0.126	**0.0004**
FVC<80 % of predicted	0.114	**0.0007**
Muscle weakness	0.094	**0.002**
DLCO<70% of predicted	0.085	**0.003**
Dyspnea and/or palpitations	0.057	**0.017**
sPAP>30 mmHg	0.052	**0.023**
Arthritis	0.029	0.094
Lung fibrosis at lung HRCT	0.017	0.223
Hypocomplementemia	0.005	0.478
Ground glass at lung HRCT	0.0005	0.835

*R-squared coefficient ;ESR. erythrocyte sedimentation rate;CRP. C-reactive protein; mRss. modified Rodnan skin score; CK. creatin kinase; TFR. tendon friction rubs; FVC. forced vital capacity; DLCO. diffusing lung for Carbon monoxide (single breath), HRCT. high resolution computed tomography;

It is notable that no finding detected at high resolution computed tomography of the lung was associated to the gold standard. However, the extent of lung involvement is not defined in the EUSTAR chart.

With single exceptions (e.g. tendon friction rubs for lcSSc), the same items were also associated with the gold standard in each of the 2 subsets as well as in early and late disease (data not shown).After a number of attempts, we identified a mRss> 18 and a ESR > 50 mm/h as the cut off points most significantly associated with the gold standard (highest R2; lowest p), i.e. the value corresponding to the highest association of the variable in univariate analysis. These values were entered in multivariate analysis along with the other items resulted to be associated with the gold standard.

242   Table 3 lists the items resulted to be associated to the gold standard in multiple regression analysis

243   and the respective weight that was assigned depending on the β values of the regression model, in

244   order to construct a 10-point index.

245   **Table 3**

246   **Items found to be associated with the activity gold standard in multiple linear regression**
247   **analysis and the resulting 2016 preliminary activity index**

Items	beta regression coefficient (SE)	p	Weight
Δ-skin	1.00 (0.313)	0.001	1.5
Digital ulcers	1.078 (0.373)	0.004	1.5
Modified Rodnan skin score > 18	1.040 (0.319)	0.001	1.5
Tendon friction rubs	1.408 (0.317)	0.0001	2.25
C-reactive protein > 1 mg/dl	1.401 (0.285)	0.0001	2.25
DLCO < 70% of the predicted value	0.527 (0.259)	0.044	1.0

248

249   *SE.* standard error; **DLCO.** Diffusing lung capacity for carbon monoxyde

250

251   Since amRss lower than 18 can also reflect active disease, in the final index we considered a

252   formulain which the highest possible value that was associated with the cut off point found to be

253   most significantly associated with the gold standard. In detail, each mRss score lower than 18 can

254   contribute to the overall activity score according to the following formula: mRss score x 0.084.

255   **TABLE 4**

256   **REVISED EUSTAR INDEX**

ITEM	WEIGHT
Δ-skin	1.5
Digital ulcers	1.5
Modified Rodnan skin score > 18	1.5
or for	
Modified Rodnan skin score up to 18	Scorex 0.084
Tendon friction rubs	2.25
C-reactive protein > 1 mg/dl	2.25
DLCO < 70% of the predicted value	1.0

257

258   The assessment of disease activity on the Likert scale was the same (i.e. either

259   inactive/moderately active or active/very active) in 91 patients; differed in 6 (Cohen's K=0.851). In

these 6 patients an agreement was reached after a re-evaluation of each chart that had given a discrepant evaluation. Out of the 97 SSc patients, 57 were considered to be inactive to moderately active; 40 active to very active.

Figure 1 shows the ROC curve exploring the best cut off value discriminating between inactive-moderately active disease (no treatment change needed) and active-very active disease. A value≥2.5 resulted to have the maximal sum of sensitivity (80.0% ;95%CI 64.4-90.9) and specificity (91.2%;95%CI 80.7-97.1) and was used to validate the index in the validation cohort

Validation

Table 5 shows the epidemiological, serological, capillaroscopic and clinical features of the additional set of 60 patients selected for the validation cohort. Out of the 60 patients,47 also satisfied 1980 ACR criteria for the classification of the disease.

The scores given by the three raters were significantly correlated (ICC=0.749). Moreover, their median values were significantly correlated to the respective calculated indices (Rho=0.772, 95%CI 0.644-0.857; p<0.0001).The early capillaroscopic pattern was associated with the gold standard (R^2=0.07; p=0.029). Nevertheless, adding it to the other items (Δ-skin; digital ulcers; modified Rodnan skin score >18; tendon friction rubs; C-reactive protein >1 mg/dl; DLCO < 70% of the predicted value) in multivariate regression analysis, did not improve the performance of the index

The evaluations on the Likert scale were consistent (i.e. either inactive/moderately active or active/very active) in 46 patients; differed in 14 (Cohen's K=0.525). In these 14 patients an agreement was reached. Out of the 60 patients, 37 were considered to be inactive to moderately active, 23 active to very active. An index ≥2.5 identified active/very active disease as defined by MB and EH with a 73.9% (95%CI 51.6-89.8) sensitivity and 78.3% (95%CI 61.8-90.2) specificity.Performing the validation process in the 47 patients also satisfying 1980 classification criteria, gave very similar results. In this cohort, a EScSG activity index ≥3 identified active disease with a 52.2% sensitivity and 89.1% specificity.

TABLE 5
Epidemiological, serological, capillaroscopic and clinical features of the 60SSc patients
analyzed in the validation cohort

Patients	Whole series n=60	Early dcSSc n=16	Late dcSSc n=14	Early lcSSc n=16	Late lcSSc n=14
Sex (F/M)	50/10	12/4	13/1	15/1	10/4
Age (years), median; range	56 (24-81)	51.5 (24-75)	59.5 (33-68)	56 (25-80)	58 (44-81)
Disease duration from 1st non-Raynaud manifestation (years), median; range	2 (0-35)	1.5 (0-3)	13.5 (4-35)	1 (0-2)	8.5 (4-35)
Antinuclear antibodies positive	58 (97%)	15	13	16	14
Anti-Scl-70 antibody positive	24 (40%)	8	8	5	3
Anti-centromere antibody positive	15 (25%)	0	2	6	7
Anti-RNA polymerase I-III antibody positive	9/56 (16%)	3/13	3/13	2	1
Anti-U1RNP antibody positive	1/55 (2%)	0/13	0/12	0/16	1
Scleroderma pattern on nailfold capillaroscopy					
Early	13	1	1	6	5
Active	23	9	3	5	6
Late	19	3	8	5	3
Raynauds' phenomenon	59 (98%)	16	14	15	14
Active Digital Ulcers	14 (23%)	4	5	3	2
Arthritis	13 (21%)	3	2	4	4
Proximal muscle weakness	6 (10%)	1	3	1	1
Tendon Friction Rubs (TFR)	4 (7%)	2	1	1	0
Skin fibrosis	51 (85%)	16	13	11	11
Esophageal, stomach and/or intestinal involvement	40 (67%)	12	11	11	6
Interstitial lung disease (CT)	38/55 (69%)	10/14	9/12	6	6/13
Estimated sPAP>30 mmHg	9/52 (17%)	3/12	1/13	4/13	1
Heart disease	26 (43%)	4	7	8	7
Scleroderma renal crisis (previous)	1 (2%)	0	0	0	1

290

291 *CT. computed tomography. sPAP. systolic pulmonary arterial pressure*

292

293 *Changes of the index over time and its relationships to summed Medsger severity score*

294 A follow-up visit made after 6-38 months (median 13) was available in 123 out of the 157 patients

295 from either derivation and validation cohorts. The calculated index unchanged in 36 patients,

296 decreased in 59, increased in 28.The changes in the activity index resulted to be significantly

297 correlated to those in the Medsger severity score in the 123 patients with a follow-up visit (Rho=

298 0.330; 95%CI 0.162-0.479, p=0.0002), pointing out a significant relationship between the index and

299 the course of disease severity. In particular, at baseline, 43 out of the 123 patients had an activity

300 index ≥ 2.5. Twenty-two resulted to have an activity index < 2.5 at the end of follow-up; out of

301 them, 18 experienced a decrease (≥1 point), 4 a stable severity score. On the other hand, among

302 the remaining 80 patients with a baseline activity index < 2.5, 8 resulted to develop an activity

303 index ≥ 2.5 at the end of follow-up;out of them, 5 experienced an increase (≥1 point), 3 a stable

304 severity score.

305

306 **Discussion**

307 Using the multinational EUSTAR database, we have identified a preliminarily revised set of

308 weighted items correlated to disease activity in patients with SSc.The 2001 EScSG study [8, 9]

309 was based on the analysis of 290 patients, most of whom with longstanding disease and was

310 affected by a high number of missing values ensuing in a low number of patients evaluable for

311 most items. In order to overcome these limitations, we only relied on charts from centres with a

312 large and scientifically supported expertise and included a high proportion of patients with early

313 disease.

314   The 97 patients selected for the derivation cohort present some aspects that deserve to be

315 discussed. First, 21 out the 48 patients with lcSSc were anti-Scl-70 positive. Differences in the

316 prevalence of anti-Scl-70 positivity among patients from different geographical regions have long

317 been known: 29% of French patients with lcSSc vs 15% of American patients. [27]. Since all our

318 patients came from European centres, this is an expected result. Secondly, 2 dcSSc patients were

319 anticentromere antibody positive. However, this figure does not differ from the 5% prevalence of

320 ACA in dcSSc reported by Steen et al. [28].Thirdly, 5 lcSSc patients presented tendon friction rubs.

321 Again, tendon friction rubs have been detected in 5% of lcSSc patients supporting the absence of

322 any derived generalizability issues.[29]

323   The revised EUSTAR activity index differs from the original EScSG index in several aspects.

324 Hypocomplementemia and arthritis were not associated with disease activity in the present study,

325 even in univariate analysis. The role of hypocomplementemia in assessing SSc activity has been

largely debated. [16,17] Hudson et al. [30] investigated 321 patients from the Canadian Scleroderma Research Group Registry, and found that hypocomplementemia was significantly associated with inflammatory myositis and vasculitis, and concluded that it may identify a subgroup of SSc patients who have overlap disease. These data suggest that some patients enrolled in the EScSG study [31] were affected by SSc (all of them satisfied the 1980 ACR criteria)[18] in overlap with other autoimmune systemic rheumatic diseases. This aspect might also justify the exclusion of arthritis.

The revised EUSTAR index contains tendon friction rubs and increased serum CRP. Tendon friction rubs were associated with diffuse and reduced survival in 1301 SSc patients.[32] This item was predictive of worsening of skin fibrosis and scleroderma renal crisis in the EUSTAR cohort.[32] CRP levels were increased in early disease and were associated with activity, skin, lung, kidney disease and poor survival in 1043 SSc patients from the CSRG Registry.[33]

Similarly to the EScSG activity index, the revised EUSTAR index contains mRSS, digital ulcers, and DLCO. mRSS reflects the degree of skin sclerosis and has long been considered a measure of disease activity in SSc. [34] One could argue that a decreasing mRss (e.g. from 24 to 18) might represent a reduced disease activity. Nevertheless, the persistence of defined skin sclerosis is not consistent with inactive disease. Digital ulcers are clearly related to vascular disease activity and have been recently found to predict the occurrence of new digital ulcers during follow-up and to be associated with cardiovascular morbidity and decreased survival.[35] A decreased DLCO can depend on both vascular and interstitial lung disease. In the absence of pulmonary hypertension, however, it has been found to provide the best overall estimate of HRCT-measured lung fibrosis. [36]

Similarly to the EScSG activity index, the revised EUSTAR index contains ∆-factors (namely ∆-skin). ∆-items had been criticized because they can fail to capture persistent activity and are influenced by depression.[16] Recently, however, patient assessment has been reported to be significantly correlated to mRSS, the Short Form 36 health survey physical component and skin involvement in the last month. [37] In any case, the present index is less influenced by ∆ items, which represented 45% of the 2001 index with respect to the 15% of the present one.

14

In the present study, three patients of the derivation cohort and 1 of the validation cohort had previously presented with scleroderma renal crisis, preventing the use of the revised EUSTAR activity index in that context.

Following the publication of the EScSG activity criteria, several attempts have been made to identify a set of criteria with an improved performance. Diaconu et al. [38] asked 6 SSc experts to evaluate 40 charts completed by clinical investigators from Nijmegen; 20 patients had early disease, not yet satisfying 1980 ACR classification criteria [18] and 20 had established disease. They derived an eight-unweighted item index (scleroderma, mRSS, fatigue, exertional dyspnoea, DLCO, musculoskeletal symptoms, ESR and digital ulcers), performing similarly to the EScSG activity index [9] in patients with either early or late disease. Furthermore, Minier et al. [39] identified two activity indexes (a 12-point extended index including Δ variables and a simplified 8.5-point devoid of them) by investigating 131 consecutive patients at enrolment and 1 year later. These patients were assessed using a standardised protocol including high-resolution computed tomography of the lung and echocardiography. The authors confirmed the good construct validity of the original EScSG activity index and found a very good correlation both at baseline and after 1 year between both the extended and the simplified score and the original EScSG activity.

The SSc activity reported herein represents a step forward with respect to the EScSG activity index.[9]. First of all, unlike the EScSG activity index, it was validated on an independent cohort..Moreover, , the lower number and value of Δ-factors as well as the exclusion of disputable items like hypocomplementemia give it a greater face validity. In addition, , the greater sensitivity detected in the validation cohort make it valuable in better charachterising the series investigated in observational studies.Finally, the revised EUSTAR activity index was found to parallel Medsger severity score over time.

Our study has some limitations.

First, the evaluation of predefined EUSTAR charts did not allow to capture either the extent of lung involvement, which has been found to be related to disease activity [40] or any change in laboratory, physical or physiologic or radiological parameter, preventing any consideration of the changes of parameters like FVC/DLCO, Δ-fibrosis at lung HRCT or acute phase reactants. This

aspect can have prevented the inclusion of these items. In that regard, however, one should consider the possible unavailability of some previous values and the need to assess disease activity at the first patient visit. Secondly, no relevant biomarker was investigated. This limitation could be approached in the future by a collaborative multicentre study including the assessment of parameters not included in the EUSTAR chart..Finally, the lower specificity with respect to the EScSG activity index in the validation cohort requires a careful evaluation in the clinical setting e.g. the patient with a respiratory infection presenting high CRP and low DLCO, who would be considered active according to the index, but is suffering from an unrelated condition..

In conclusion, the revised EUSTAR activity index is feasible, presents face, construct and content validity and represents a step forward to the so far widely used EScSG activity index.Future collaborative, prospective studies are needed to further improve its performance.

Contributors

Design of the study: GV, YA**; Acquisition of data:** GV, MI, UW, VKJ, PC, LC, CD, OD, EH, AH, OKB, JP, UML, GR, JA, MF, SJ, TM, ES, VO, SV, YA**. Data interpretation and analysis:** GV, MI, UW, VKJ, MB, PC, LC, CD, OD, EH, AH, OKB, JP, UML, GR, YA**. Drafting and revisiting the manuscript:**GV, MI, UW, VKJ, PC, LC, CD, OD, EH, AH, OKB, JP, UML, GR, YA**. Final approval of the manuscript:** GV, MI, UW, VKJ, MB, PC, LC, CD, OD, EH, AH, OKB, JP, UML, GR, JA, MF, SJ, TM, ES, VO, SV, YA.

Competing interests

GVhas received research funding in the area of systemic sclerosis from Abbvie, Actelion, Bayer, BMS, Merck SD, Pfizer, Roche. CD has been a consultant to Roche, GSK, Actelion, Inventiva,

16

References

1) Valentini G. The assessment of the patient with Systemic Sclerosis. *Autoimmunity Rev* 2003;2:370-6

2) Ferri C, Valentini G, Cozzi F, *et al.* Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients.*Medicine (Baltimore)* 2002 ;81:139-53

3) Hachulla E, Gressin V, GuillevinL,*et al.* Early detection of pulmonary arterial hypertension in systemic sclerosis: a French nationwide prospective multicenter study. *Arthritis Rheum.* 2005;52:3792-800

4) Hunzelmann N, Genth E, Krieg T, *et al.* The registry of the German Network for Systemic Scleroderma: frequency of disease subsets and patterns of organ involvement.*Rheumatology (Oxford)* 2008;47:1185-92

5) Walker UA, Tyndall A, Czirják L, *et al.* Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials And Research group database. *Ann Rheum Dis.* 2007;66:754-63

6) Nihtyanova SI, Schreiber BE, Ong VH, *et al.* Prediction of pulmonary complications and long-term survival in systemic sclerosis. *Arthritis Rheumatol* 2014;66:1625-35

7) Castro SV, Jimenez SA. Biomarkers in systemic sclerosis.*Biomark Med.* 2010;4:133-47

8) Valentini G, Della Rossa A, Bombardieri S, *et al.* European multicentre study to define disease activity criteria for systemic sclerosis. II. Identification of disease activity variables and development of preliminary activity indexes.*Ann Rheum Dis* 2001;60:592-8

9) Valentini G, D'Angelo S, Della Rossa A,*et al.*European Scleroderma Study Group to define disease activity criteria for systemic sclerosis. IV. Assessment of skin thickening by modified Rodnan skin score.*Ann Rheum Dis* 2003 ;62:904-5

10) Shao J, Tu D. *The jackknife and the bootstroop.* Springer. New York. 1995

11) Valentini G, Bencivelli W, Bombardieri S, *et al.*European Scleroderma Study Group to define disease activity criteria for systemic sclerosis. III. Assessment of the construct validity of the preliminary activity criteria.*AnnRheumDis.* 2003 ;62:901-3

451   12)http://scholar.google.it/citationsview_op=view_citation&hl=it&user=3z2zFpUAAAAJ&citation_for

452   _view=3z2zFpUAAAAJ:9yKSN-GCB0IC. Accessed on September 2015

453   13) Fan X, J Pope, The Canadian Scleroderma Research Group *et al.* What is the relationship

454   between disease activity, severity and damage in a large Canadian systemic sclerosis cohort?

455   Results from the Canadian Scleroderma Research Group (CSRG). *Rheumatol Int* 2010, 30:1205-

456   10

457   14) Hanke K, Dahnrich C, Bruchner CS, *et al.*Diagnostic value of anti-topoisomerase I antibodies in

458   a large monocentric cohort. *Arthritis Res Therapy* 2009; 11: R28

459   15) Qi Q, Guo Q, Tan G,*et al.* Predictors of the scleroderma phenotype in fibroblasts from systemic

460   sclerosis patients. *J Eur Acad Dermatol Venereol* 2009; 23:160-8

461   16) Hudson M, Steele R; Canadian Scleroderma Research Group (CSRG) *et al.*Update on indices

462   of disease activity in systemic sclerosis.*Semin Arthritis Rheum* 2007;37:93-8

463   17) Foocharoen C, Distler O, Becker M, *et al.*Clinical correlations of hypocomplementaemia in

464   systemic sclerosis: an analysis of the EULAR Scleroderma Trial and Research group (EUSTAR)

465   database.*Scand J Rheumatol* 2012;41:243-6

466   18) Masi AT, Rodnan GP, Medsger TA, *et al.* Preliminary criteria for classification ofsystemic

467   sclerosis (scleroderma). *Arthritis Rheum* 1980;23:581–90

468   19) Furst DE, Clements PJ, Steen VD, *et al.*The modified Rodnan skin score is an accurate

469   reflection of skin biopsy thickness in systemic sclerosis.*J Rheumatol* 1998;25:84-8

470   20) D'Andrea A, Naeije R, Grunig E, Caso P, D'Alto M, Di Palma E, et al. Echocardiography of the

471   pulmonary circulation and right ventricular function: exploring the physiologic spectrum in 1480

472   normal subjects. Chest 2014;145:1071-78.

473   21) American Thoracic Society. Standardization of Spirometry 1994 update. Am J Respir Crit Care

474   Med 1995; 152: 1107-36

475   22) American Thoracic Society. Single breath Carbon monoxide diffusing capacity (transfer

476   Factor). Reccomendation of a standard technique-1995 update. Am J Respir Crit Care Med 1995;

477   152: 2185-98

478

479   23) van den Hoogen, Khanna D,Fransen J, et al. Classification Criteria for Systemic Sclerosis: An

480   American College of Rheumatology-European League Against Rheumatism Collaborative

481   Initiative. Arthritis Rheum 2013;65:2737-47.

482   24) Cutolo M, Sulli A, Pizzorni C, et al. Nailfold videocapillaroscopy assessment of microvascular

483   damage in systemic sclerosis. J Rheumatol 2000;27:155-60

484   25) Medsger TA, Bombardieri S, Czirjak L, Scorza R, Della Rossa A, Bencivelli W. Assessment of

485   disease severity and prognosis. Clin Exp Rheumatol 2003; 21 (Suppl. 29):S42-S4623)

486   26)   Harel D, Hudson M, Iliescu A, Baron
487   M; Canadian Scleroderma Research Group, Steele R.
488   <u>Summed and Weighted Summary Scores for the Medsger Disease Severity Scale Compared with</u>
489   <u>the Physician's Global Assessment of Disease Severity in Systemic Sclerosis.</u> J Rheumatol. 2016
490   Jun 15. pii: jrheum.151440. [Epub ahead of print]
491

492   27) Meyer OC, Fertig N, Lucas M, Somogyi N, Medsger TA. Disease subsets, antinuclear antibody

493   profile, and clinical features in 127 French and 247 US adult patients with systemic sclerosis. *J*

494   *Rheumatol.* 2007;34:104-9.

495   28) Steen VD. Autoantibodies in systemic sclerosis. Semin Arthritis Rheum. 2005;35:35-42.

496   29) Steen VD, Medsger TA jr. The palpable tendon friction rub: an Important physical examination

497   finding in patients with Systemic Sclerosis. *Arthritis Rheum* 1997;40:1146-51

498   30) Hudson M, Walker JG, Fritzler M, *et al*. Hypocomplementemia in systemic sclerosis--clinical

499   and serological correlations. *J Rheumatol.*2007;34:2218-23 15.

500   31) Della Rossa A, Valentini G, Bombardieri S, *et al*. European multicentre study to define disease

501   activity criteria for systemic sclerosis. I. Clinical and epidemiological features of 290 patients from

502   19 centres.*Ann Rheum Dis* 2001;60:585-91

503   32) Avouac J, Walker UA, Hachulla E, *et al*.Joint and tendon involvement predict disease

504   progression in systemic sclerosis: a EUSTAR prospective study.*Ann Rheum Dis*. 2014 Aug 27

505 33) Muangchan C, Harding S, Khimdas S, *et al*. Association of C-reactive protein with high disease

506 activity in systemic sclerosis: results from the Canadian Scleroderma Research Group.*Arthritis*

507 *Care Res (Hoboken)* 2012 ;64: 1405-14

508 34) Walker JG, Steele RJ, Schnitzler M*et al*. The association between disease activity and duration

509 in systemic sclerosis. *J Rheumatol* 2010;37:2299–306

510 35) Mihai C, Landewé R, van der Heijde D. Digital ulcers predict a worse disease course in

511 patients with systemic sclerosis.*Ann Rheum Dis.* 2015 Feb 16. doi:10.1136/annrheumdis-2014-

512 205897 [Epub ahead of print]

513 36) Tashkin DP, Volkmann ER, Tseng CH*et al*. Relationship between quantitative radiographic

514 assessments of interstitial lung disease and physiological and clinical features of systemic

515 sclerosis. *Ann Rheum Dis.* 2014 Dec 1. doi: 10.1136/annrheumdis-2014-206076. [Epub ahead of

516 print]

517 37) Wiese AB, Berrocal VJ, Furst DE *et al*. Correlates and responsiveness to change of measures

518 of skin and musculoskeletal disease in early diffuse systemic sclerosis. *Arthritis Care Res*

519 *(Hoboken)* 2014;66:1731-9

520 38) Diaconu D, Vonk M, Denton C , *et al*. Disease activity criteria for patients with early systemic

521 sclerosis [abstract]. *Ann Rheum Dis* 2009;68(Suppl 3):P275

522 39) Minier T, Nagy Z, Balint Z, *et al*. Construct validity of the European Scleroderma Study Group

523 activity index and investigation of possible new disease activity markers in systemic sclerosis.

524 *Rheumatology* 2010; 49:1133–45.

525 40)Goh NS, Desai SR, Veerarghavan S, *et al*. Interstitial lung disease in systemic sclerosis: a

526 simple staging system. *Am J Respir Crit Care Med* 2008;177:1248-54

527

528
529
530
531
532
533

534

535

536

537

538

539

540 **Figure Legends.**

541

542   **Figure 1.** Receiver Operating Curve showing the relation between the value of the

543 calculated 2016 EUSTAR activity index and the presence of active disease in the

544 derivation cohort.

545

546   To be deleted

547   **Figure 2.** Receiver Operating Curve showing the relation between a EScSG activity

548 index ≥3 and the presence of active disease.

549

550

551