The role of inflammatory markers in assessment of disease activity in systemic sclerosis

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ABSTRACT

Objective. *The role of the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) in the assessment of disease activity in systemic sclerosis (SSc) remains controversial. We sought to evaluate the relationship between clinical features of SSc and raised inflammatory markers and to determine if changes in ESR and CRP reflect changes in other disease features over time.*

Methods. *One thousand, five hundred and forty-five patients enrolled in the Australian Scleroderma Cohort Study were observed over a mean 3.52±2.91 years and assessed at 6,119 study visits. Generalised estimating equations were used to determine the relationship between ESR≥20mm/hr and CRP≥5mg/L and features of disease. The associations between change in inflammatory markers and change in skin scores and respiratory function tests were analysed.*

Results. *Overall, there was a significant association between raised ESR and forced vital capacity (FVC)<80% predicted, diffusing capacity of the lung (DLCO)<80% predicted, pulmonary arterial hypertension (PAH), body mass index (BMI), proximal muscle strength, anaemia, and hypocomplementaemia ($p<0.05$). Raised CRP was significantly associated with modified Rodnan Skin Score>20, FVC<80%, DLCO<80%, PAH, digital ulcers, BMI, synovitis, tendon friction rub, anaemia, and hypocomplementaemia ($p<0.05$). A significant deterioration in respiratory function tests (RFTs) was associated with a 2-fold increase in both ESR and CRP ($p<0.05$).*

Conclusion. *Raised inflammatory markers are associated with pulmonary, cutaneous and musculoskeletal manifestations of SSc. Rising inflammatory markers are correlated with declining respiratory function tests. This suggests inflammatory markers have a role in the assessment of SSc disease activity.*

Introduction

Systemic sclerosis (SSc) is a connective tissue disease of unknown aetiology and is characterised by fibrosis of the skin and internal organs. The clinical course of SSc is often one of persistent activity leading to exponential accrual of organ damage. Measuring disease activity is challenging as end organ dysfunction results from inflammatory, vasculopathic and fibrotic processes (1-3). However, many features of SSc are thought to have an early 'inflammatory phase' preceding fibrotic and vasculopathic changes (4).

Disease activity in many rheumatic diseases is reflected by a systemic inflammatory response, quantified by inflammatory markers; erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). The relationship between ESR and CRP and disease activity in SSc remains contentious and their role, if any, in measurement of disease activity is poorly defined. As was highlighted in a recent review, though many potential SSc biomarkers have been shown to be associated with particular disease manifestations, there is a marked lack of validated biomarkers to predict the development of disease manifestations or potential therapeutic responses (5). Further, there is a paucity of validated biomarkers of disease activity in SSc.

Raised inflammatory markers in early disease are a poor prognostic factor associated with increased mortality (8-12) and physician-rated high disease activity. A study of 529 Italian patients that evaluated risk factors for mortality in SSc demonstrated that an ESR

≥25mm/hr at the time of SSc diagnosis was associated with a hazard ratio of 1.93 for death (13). This association with mortality and physician-rated disease activity has meant that elevated inflammatory markers have been included in the European Scleroderma Trials and Research Group (EUSTAR) Activity Index (8, 14, 15).

Previous studies have examined the relationship between raised inflammatory markers and SSc-disease manifestations. Cross-sectional analysis of the Canadian Scleroderma Research Group (CSRG) Cohort Study revealed that the modified Rodnan skin score (mRSS), total lung capacity <80% and serum creatinine were predictors of elevated CRP (8). This study found no association between raised CRP and the presence of inflammatory arthritis. In contrast, analysis of the EUSTAR cohort showed the presence of synovitis had the strongest association with elevated inflammatory markers, leading the authors to suggest that inflammatory arthritis may account for the systemic inflammatory response in SSc (16). It has been shown that patients with a raised ESR have a higher risk of presenting with digital ulcers (6) and elevated ESR was included by Manfredi *et al*. in a prognostic model of SSc-associated digital ulcers (7).

It remains to be established whether raised inflammatory markers are associated with disease manifestations throughout the course of SSc and whether change in inflammatory markers is associated with changes in other features of SSc. As such, using generalised estimating equations (GEE) to permit analysis of associations at each study visit over time, we evaluated the relationship of raised ESR and CRP to clinical manifestations of SSc in the Australian Scleroderma Cohort Study (ASCS). Additionally, we sought to evaluate whether changes in inflammatory markers are reflected in changes in other features of SSc. This study was performed to inform the development of the Scleroderma Clinical Trials Consortium (SCTC) Activity Index and to ascertain whether there is a rationale for the inclusion of inflammatory markers in an activity index.

Methods

Patients

Patients were recruited from the ASCS, a multi-centre cohort study of risk and prognostic factors in SSc. Patients were recruited from centres specialising in the care of SSc (St Vincent's Hospital, Melbourne and Monash Health, Victoria; Royal Prince Alfred Hospital, St George Hospital, Royal North Shore Hospital, Liverpool Hospital and John Hunter Hospital, New South Wales; Canberra Hospital, Australian Capital Territory; Sunshine Coast Rheumatology and Prince Charles Hospital, Queensland; Royal Adelaide Hospital and The Queen Elizabeth Hospital, South Australia; Fiona Stanley Hospital, Western Australia; Menzies Institute for Medical Research, Tasmania). The study was carried out in accordance with the National Statement on Ethical Conduct Involving Humans (May 2015) (17) and was approved by the human research ethics committees of each centre. Written informed consent was obtained from all patients prior to collection of data.

All patients included fulfilled 2013 ACR/EULAR criteria for the diagnosis of SSc (18) and had information available to define their disease subset according to LeRoy criteria (19).

Data collection

Demographic and disease-related data were collected prospectively as per a standardised protocol at recruitment and annual reviews. Demographic data included gender, age and race. Disease related variables included disease subtype (19), disease duration (defined as date of onset of first non-Raynaud's manifestation), autoantibody profile, length of follow-up and disease manifestations at each visit. An early disease subgroup was defined as patients recruited within 2 years of disease onset. mRSS was calculated at each visit (20). The presence of Raynaud's phenomenon, digital ulcers, gastro-oesophageal reflux, diarrhoea, post-prandial bloating, faecal incontinence, tendon friction rubs, synovitis, proximal muscle strength on manual muscle testing were based on physician assessment at each study visit.

Respiratory function tests (RFTs) measured were forced vital capacity (FVC) and diffusing capacity of the lung for carbon monoxide corrected for haemoglobin (DLCO), recorded as percent predicted. High resolution computed tomography (HRCT) scan of the chest was performed if lung disease was suspected on the basis of abnormal RFTs or if respiratory crackles were present on examination. Typical findings of pulmonary fibrosis on HRCT were used to define the presence of interstitial lung disease (ILD). Honeycomb pattern ILD changes were recorded as present if honeycombing was documented in the final radiologist's report. Ground glass pattern changes are not recorded in the ASCS. Patients suspected of having pulmonary arterial hypertension (PAH) on the basis of systolic pulmonary artery pressure ≥40mmHg on transthoracic echocardiography (TTE) or DLCO<50% with preserved lung volumes were referred for right heart catheterisation (RHC). PAH was defined by a mean pulmonary artery pressure ≥25mmHg and pulmonary arterial wedge pressure ≤15mmHg on RHC. Myocardial disease was defined by suggestive changes on endomyocardial biopsy or cardiac magnetic resonance imaging (MRI) or suspected on the basis of arrhythmia or conduction defect on electrocardiograph, left ventricular dysfunction and/or diastolic dysfunction attributed to SSc on TTE. Non-trivial pericardial effusions on TTE were recorded. Renal crisis was defined by the presence of at least two of; new onset hypertension in the absence of alternate aetiology, rising creatinine and new onset microangiopathic haemolytic anaemia. Gastric antral vascular ectasia was diagnosed on endoscopy. Myositis was defined by a positive muscle biopsy or suspected on the basis of elevated creatine kinase, electromyographic or MRI findings consistent with myositis.

Raised inflammatory markers

Raised inflammatory markers were defined as ESR ≥20mm/hr and CRP ≥5mg/L, as per the upper limit of normal of the local laboratory. Preliminary analyses were also conducted using

higher values of ESR ≥38mm/hr and CRP ≥12mg/L (values of the 90th percentile of values for the SSc cohort at baseline) to determine if higher cut-offs correlated more closely with features of disease activity. Higher cut-off values did not significantly alter the multivariable analyses therefore analyses using the lower cut-off values are presented.

Statistical analysis

Descriptive statistics (mean ± standard deviation, median, and number (percent)) were used to describe the characteristics of the patient cohort. Univariable and multivariable analyses were conducted using GEE to account for the expected correlation that arises when repeated measurements are taken from the same individual at multiple visits over time (21). GEE enabled the analysis of the relationship between raised ESR and CRP and clinical variables at each visit. Analyses using generalised linear modelling were performed to evaluate the relationship between ESR and CRP as continuous variables (data not shown). The results of this analysis were consistent with the results of GEE presented. The relationships between ESR and CRP as dichotomous variables was felt to be more clinically relevant, hence we have presented the findings of the GEE analyses.

Subgroup analyses were conducted in patients with diffuse cutaneous disease (dcSSc) and those with early disease. Raised ESR and CRP were the explanatory variable in univariable analyses. Statistically significant variables from the univariable analyses were included in multivariable models in which raised ESR and CRP were the outcome variable. Statistical significance was defined as $p<0.05$.

Univariable analysis of change in inflammatory markers and change in mRSS and RFTs was performed. Another subgroup of patients with ILD diagnosed by HRCT was included in these analyses. Change in inflammatory markers was defined as a 2-fold increase in both ESR and CRP between each study visit. An increase of 25% in mRSS has been used in previous studies as a clinically meaningful change

Table I. Patient characteristics (n=1545).

Characteristic	% or mean ± SD
Age at recruitment, years	57.34 ± 12.57
Duration of disease at recruitment, years	10.95 ± 10.14
Disease duration ≤2 years at recruitment	18.15%
Follow-up duration, years	3.52 ± 2.91
Death (during follow-up)	16.65%
Sex	
Female	86.02%
Race	
Caucasian	92.71%
Asian	4.39%
Aboriginal/Torres Strait Islander	1.15%
Hispanic	0.74%
Other	1.01%
Disease subtype	
Diffuse cutaneous	25.44%
Limited cutaneous	74.56%
Serology	
ANA centromere pattern	47.29%
Scl70 positive	14.69%
RNA polymerase III positive	13.19%*
Disease manifestations ever during follow-up	
Raynaud phenomenon	95.18%
Digital ulcers	39.10%
mRSS>20	17.02%
Interstitial lung disease	23.95%
Pulmonary arterial hypertension	9.97%
F VC<80% predicted	27.96%
DLCO<80% predicted (corrected for haemoglobin)	71.97%
Myocardial disease	7.12%
Gastro-oesophageal reflux	82.06%
Oesophageal stricture	11.39%
Gastric antral vascular ectasia	8.48%
Pseudo-obstruction	3.85%
Faecal incontinence	27.64%
Lowest Body Mass Index	24.93±5.41
Renal crisis	2.85%
Tendon friction rub	8.86%
Synovitis	30.72%
Raised creatine kinase	24.47%
Myositis	5.57%
Erectile dysfunction¥	43.14%
Anaemia•	35.02%
Thrombocytosis‖	5.95%
ESR ≥20mm/hr	48.80%
CRP ≥5mg/L	53.66%
Treatment ever during follow-up	
Prednisolone	26.30%
Disease modifying agents¶	32.30%
Biologic therapy••	1.36%
Cyclophosphamide	2.27%
Intravenous immunoglobulin	0.39%
Pulmonary arterial hypertension therapy$	9.58%

*% calculated from total of 963 patients who have had RNAPIII Ab testing. This test is not available at all ASCS centres.

¥% calculated from total number of male patients with data on erectile dysfunction recorded.

•Anaemia = Male Hb <135g/L Female Hb <120g/L.

‖Platelet count >400 x$10^{9/L}$.

¶Disease modifying agents: includes treatment at study visit with any or combination of: azathioprine, cyclosporine, hydroxychloroquine, leflunomide, methotrexate, mycophenolate, penacillamine.

••Biologic therapy: includes treatment at a study visit with any or combination of: tumour necrosis alpha inhibitors, rituximab, tocilizumab, abatacept.

$Pulmonary arterial hypertension therapy: includes treatment at a study visit with any or combination of: ambrisentan, macitentan, bosentan, epoprostinol, riociguat, sildenafil, tadalafil, selexipag, sitaxsentan, inhaled iloprost in patients diagnosed with pulmonary arterial hypertension.

ANA: anti-nuclear antibody; DLCO: diffusing capacity of the lung for carbon monoxide; FVC: forced vital capacity; mRSS: modified Rodnan skin score; Scl70: anti-scleroderma-70 antibodies, SD: standard deviation; SSc: systemic sclerosis.

in skin score (22). A 10% decrease in FVC and 15% decrease in DLCO can identify patients at increased risk of poor pulmonary outcome (23).
All statistical analyses were performed using STATA 14.2 software (StataCorp, College Station, TX, USA).

Results

Patient characteristics

This study included 1,545 patients enrolled in the ASCS from 1st January 2007 to 21st March 2017. One thousand, three hundred and twenty-nine (86.02%) patients were female with a mean age of 57.34±12.57 years and mean disease duration of 10.95±10.14 years at recruitment. The mean follow-up for the whole cohort was 3.52±2.91 years and patients with dcSSc were followed for a mean 3.82±3.01 years. Eighteen percent (18.15%) were recruited within 2-years of the first non-Raynaud's symptom and these patients were followed for mean 3.37±2.69 years. One hundred and three (42.04%) of the early disease cohort had dcSSc.
Analyses were based on 6,119 visits with mean 3.98±2.60 visits per patient, and a mean interval of 1.12 years between study visits. There were 4,998 ESR and 4,971 CRP results recorded, and 49% and 53.66% of patients recorded an elevated ESR and CRP respectively throughout the study. At baseline, the mean ESR was 18.83±18.18mm/hr and mean CRP 6.80±15.02mg/L for the whole cohort. Patients with dcSSc and early disease had mean baseline ESR of 21.64±18.84mm/hr and 19.84±17.65mm/hr and CRP of 10.00±20.48mg/L and 9.60±25.89mg/L, respectively. Disease characteristics of the study participants are outlined in Table I.

Univariable analysis

Results of univariable analyses are detailed in Table II. In summary, for the whole cohort, raised ESR was associated with mRSS>20 (OR 1.49, $p<0.001$), ILD (OR 1.76, $p<0.001$), PAH (OR 1.98, $p<0.001$) and abnormal RFTs (FVC<80% OR 1.62, $p<0.001$, DLCO<80% OR 1.58, $p<0.001$). One hundred and forty-eight patients ever recorded a honeycomb pattern of interstitial pulmonary changes on HRCT. When considering this radiographic pattern of ILD, raised ESR continued to be significantly associated with honeycomb-pattern ILD (OR 2.28, $p<0.001$). Musculoskeletal manifestations of tendon friction rub (OR 1.37, $p=0.032$) was positively associated, and full muscle strength (OR 0.71, $p<0.001$) was inversely associated with elevated ESR.
Raised CRP was also associated with cutaneous and respiratory manifestations of disease (mRSS>20 OR 1.67, $p<0.001$, ILD OR 1.52, $p<0.001$, PAH OR 2.87, $p<0.001$) synovitis (OR 1.23, $p=0.014$), tendon friction rub (OR 1.92, $p<0.001$) and digital ulcers (OR 1.30, $p=0.001$). As with raised ESR, raised CRP was significantly associated with honeycomb pattern of ILD on HRCT (OR 1.56, $p=0.001$). Anaemia (ESR OR 2.59, $p<0.001$; CRP OR 1.73, $p<0.001$) and thrombocytosis (ESR OR 2.72, $p<0.001$, CRP OR 2.36, $p<0.001$) were both associated with raised inflammatory markers.
There were no clear patterns of association between raised inflammatory markers and cardiac, gastrointestinal or renal manifestations of SSc.

Analyses with alternate FVC and DLCO cut-off values

We evaluated the relationship between inflammatory markers and more stringent RFT cut off values. The associations seen between raised inflammatory markers and reduced FVC and DLCO were similar for cut-off values <70% and <60% predicted (see supplementary index). Use of lower RFT cut-off values did not significantly alter the multivariable models and potentially excluded a proportion of patients with 'active' respiratory disease who do not yet have significant organ impairment. For this reason, we chose to present multivariable models using FVC<80% and DLCO<80% cut off values.

Multivariable analysis

Multivariable analyses of the association with raised ESR and CRP were performed for each subgroup separately, shown in Tables III and IV. Erectile dysfunction was excluded from the multivariable models because of low patient visits available for analysis (n=601 visits).
Raised ESR correlated with respiratory manifestations of disease, in particular PAH (whole population: OR 1.57, $p=0.008$; early disease: OR 6.31, $p=0.002$) and DLCO<80% (whole population: OR 1.50, $p<0.001$; dcSSc: OR 1.73, $p=0.004$; early disease: OR 2.39, $p<0.001$). Raised CRP correlated with cutaneous, articular, vascular and respiratory manifestations across all groups analysed. mRSS>20 was significantly associated with raised CRP (whole population: OR 1.98, $p<0.001$; dcSSc: OR 1.70, $p<0.001$; early disease: OR 1.85, $p=0.005$). Synovitis and tendon friction rubs were significantly associated with raised CRP in the whole population (OR 1.24, $p=0.029$ and OR 2.14, $p<0.001$, respectively) and the early disease subgroup (OR 1.70, $p=0.012$ and OR 3.86, $p<0.001$, respectively). PAH (OR 2.12, $p<0.001$), digital ulcers (OR 1.32, $p=0.005$) and FVC<80% (OR 1.37, $p=0.003$) were all associated with raised CRP in the whole population.

Analysis of raised inflammatory markers and treatment

Univariable and multivariable analyses of the association between raised ESR and CRP and treatment were performed (see supplementary index). Evaluation of the associations between raised ESR and CRP and therapy were not the main aim of the study. Therefore, the final multivariable models presented in the main article do not include therapy. The relationship of raised ESR and CRP to therapies is difficult to interpret in an observational study, as treatment decisions are made according to the treating physician's discretion and are not blinded to results, including ESR and CRP. It is highly likely that therapeutic decisions made by the physician were significantly influenced by the presence of elevated inflammatory markers.

Change in inflammatory markers and disease manifestations over time

To further investigate the relationship between raised inflammatory markers and skin and respiratory disease, we

Table II. Univariable association of raised inflammatory markers and disease activity.

	Whole SSc cohort n=1,545 patients (6,119 visits)				Diffuse cutaneous disease n=393 patients (1,629 visits)				Disease duration ≤2 years at recruitment n=295 patients (963 visits)			
	ESR ≥20mm/hr		CRP ≥5mg/L		ESR ≥20mm/hr		CRP ≥5mg/L		ESR ≥20mm/hr		CRP ≥5mg/L	
Variable	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Cutaneous manifestations												
mRSS>20	1.49 (1.22-1.81)	<0.001	1.67 (1.35-2.06)	<0.001	1.01 (1.00-1.03)	0.039	1.49 (1.16-1.92)	0.002	1.62 (1.12-2.34)	0.010	1.90 (1.31-2.78)	0.001
Digital ulcers	1.04 (0.90-1.21)	0.610	1.30 (1.11-1.52)	0.001	1.03 (0.81-1.30)	0.810	1.23 (0.95-1.58)	0.116	1.26 (0.85-1.88)	0.246	1.32 (0.88-2.00)	0.183
Cardiac manifestations												
Arrhythmias	1.10 (0.96-1.27)	0.166	1.10 (0.94-1.28)	0.225	1.00 (0.75-1.32)	0.974	1.10 (0.82-1.48)	0.529	1.19 (0.82-1.73)	0.369	1.27 (0.86-1.89)	0.225
Conduction abnormalities	0.98 (0.69-1.39)	0.907	0.86 (0.60-1.28)	0.408	1.10 (0.55-2.21)	0.792	0.88 (0.44-1.78)	0.722	0.97 (0.37-2.59)	0.958	0.77 (0.30-1.98)	0.588
Diastolic dysfunction	1.07 (0.77-1.48)	0.686	0.84 (0.60-1.18)	0.322	0.68 (0.36-1.28)	0.230	1.63 (0.80-3.31)	0.181	0.64 (0.26-1.57)	0.328	0.80 (0.33-1.94)	0.620
LV systolic dysfunction	1.14 (0.98-1.33)	0.087	1.24 (1.05-1.46)	0.010	0.99 (0.73-1.36)	0.966	1.53 (1.09-2.15)	0.015	1.19 (0.81-1.75)	0.370	1.33 (0.89-1.99)	0.159
Pericardial effusion	1.21 (0.93-1.57)	0.163	1.58 (1.20-2.07)	0.001	1.24 (0.77-2.01)	0.373	1.43 (0.85-2.42)	0.176	1.04 (0.43-2.51)	0.931	2.27 (0.97-5.28)	0.058
Pulmonary manifestations												
Interstitial lung disease	1.76 (1.46-2.12)	<0.001	1.52 (1.27-1.83)	<0.001	1.75 (1.28-2.40)	<0.001	1.30 (0.97-1.76)	0.083	1.35 (0.88-2.08)	0.171	1.09 (0.70-1.68)	0.708
Honeycomb pattern on HRCT	2.28 (1.74-2.99)	<0.001	1.56 (1.20-2.03)	0.001	1.92 (1.27-2.91)	0.002	1.24 (0.84-1.82)	0.284	2.44 (1.20-4.93)	0.013	0.67 (0.31-1.44)	0.306
Pulmonary arterial hypertension	1.98 (1.51-2.61)	<0.001	2.87 (2.20-3.75)	<0.001	2.16 (1.23-3.79)	0.008	3.67 (2.03-6.63)	<0.001	6.49 (2.72-15.48)	<0.001	2.97 (1.36-6.46)	0.006
FVC<80%	1.62 (1.35-1.91)	<0.001	1.67 (1.41-1.98)	<0.001	1.69 (1.30-2.19)	<0.001	1.48 (1.13-1.95)	0.004	1.80 (1.26-2.57)	0.001	2.01 (1.40-2.90)	<0.001
DLCO<80%	1.58 (1.34-1.87)	<0.001	1.57 (1.32-1.87)	<0.001	1.91 (1.34-2.71)	<0.001	1.71 (1.21-2.41)	0.003	2.81 (1.82-4.32)	<0.001	1.83 (1.21-2.76)	0.004
Gastrointestinal manifestations												
Reflux	0.93 (0.81-1.05)	0.242	1.07 (0.93-1.23)	0.363	0.90 (0.70-1.15)	0.385	1.24 (0.95-1.62)	0.112	0.85 (0.58-1.26)	0.426	1.41 (0.96-2.08)	0.078
Diarrhoea	1.08 (0.95-1.22)	0.239	1.08 (0.95-1.23)	0.258	1.31 (1.03-1.67)	0.030	1.31 (1.01-1.71)	0.043	0.91 (0.66-1.27)	0.594	1.48 (1.06-2.07)	0.021
Post prandial bloating	1.06 (0.94-1.19)	0.360	1.09 (0.96-1.24)	0.166	1.19 (0.95-1.48)	0.122	1.19 (0.94-1.51)	0.155	0.89 (0.65-1.21)	0.440	1.31 (0.96-1.80)	0.094
Body mass index	1.03 (1.01-1.04)	0.001	1.05 (1.03-1.07)	<0.001	1.01 (0.98-1.04)	0.582	1.05 (1.02-1.08)	0.001	1.01 (0.97-1.04)	0.706	1.06 (1.02-1.10)	0.001
Renal manifestations												
Serum creatinine	1.00 (0.99-1.00)	0.083	1.003 (1.00-1.01)	0.002	1.00 (1.00-1.01)	0.077	1.00 (0.99-1.00)	0.768	1.00 (0.99-1.01)	0.204	1.01 (0.99-1.01)	0.104
Systolic blood pressure	1.00 (0.99-1.00)	0.519	1.00 (0.99-1.00)	0.209	1.00 (0.99-1.01)	0.535	1.00 (0.99-1.01)	0.771	1.00 (0.99-1.01)	0.575	0.99 (0.99-1.01)	0.882
Diastolic blood pressure	0.99 (0.99-1.00)	0.234	1.00 (0.99-1.00)	0.798	0.99 (0.99-1.00)	0.814	1.01 (0.99-1.02)	0.110	0.98 (0.96-0.99)	0.004	0.99 (0.97-1.01)	0.275
Musculoskeletal manifestations												
Synovitis	1.09 (0.94-1.27)	0.270	1.23 (1.04-1.45)	0.014	1.35 (1.01-1.80)	0.040	1.86 (1.36-2.53)	<0.001	1.34 (0.95-1.91)	0.098	1.69 (1.19-2.42)	0.004
Tendon friction rub	1.37 (1.03-1.82)	0.032	1.92 (1.41-2.62)	<0.001	1.46 (1.01-2.13)	0.047	2.14 (1.40-3.27)	<0.001	2.40 (1.33-4.34)	0.004	3.24 (1.74-6.02)	<0.001
Proximal power	0.71 (0.60-0.84)	<0.001	0.74 (0.62-0.88)	0.001	0.69 (0.52-0.92)	0.011	0.80 (0.59-1.07)	0.129	0.60 (0.40-0.90)	0.014	0.84 (0.57-1.24)	0.380
Elevated CK	0.97 (0.82-1.15)	0.733	1.04 (0.88-1.24)	0.630	1.25 (0.93-1.67)	0.144	1.05 (0.77-1.44)	0.747	1.55 (1.05-2.30)	0.028	1.08 (0.72-1.61)	0.717
Microvascular manifestations												
Raynaud phenomenon	0.93 (0.80-1.10)	0.406	0.97 (0.81-1.15)	0.708	1.08 (0.79-1.48)	0.629	1.21 (0.85-1.70)	0.303	0.80 (0.50-1.27)	0.343	1.15 (0.69-1.90)	0.596
Erectile dysfunction	2.06 (1.35-3.14)	0.001	1.36 (0.93-1.99)	0.116	2.82 (1.50-5.30)	0.001	2.18 (1.21-3.95)	0.010	1.15 (0.47-2.83)	0.754	2.27 (0.96-5.34)	0.061
Serological manifestations												
Anaemia	2.59 (2.25-2.96)	<0.001	1.73 (1.50-1.99)	<0.001	2.96 (2.32-3.77)	<0.001	1.67 (1.31-2.18)	<0.001	3.36 (2.40-4.71)	<0.001	2.81 (1.99-3.97)	<0.001
Thrombocytosis	2.72 (1.93-3.83)	<0.001	2.36 (1.69-3.31)	<0.001	1.78 (1.02-3.09)	0.041	3.16 (1.80-5.54)	<0.001	1.82 (0.97-3.40)	0.062	4.48 (2.37-8.47)	<0.001
Hypocomplementaemia	0.76 (0.65-0.90)	0.001	0.65 (0.54-0.77)	<0.001	0.84 (0.62-1.16)	0.291	0.63 (0.44-0.89)	0.009	0.85 (0.58-1.25)	0.402	0.56 (0.37-0.87)	0.009
CRP≥5mg/L	2.67 (2.37-3.02)	<0.001			2.27 (2.18-3.40)	<0.001			3.04 (2.23-4.12)	<0.001		
ESR≥20mm/hr			3.22 (2.81-3.68)	<0.001			3.54 (2.74-4.57)	<0.001			3.37 (2.43-4.67)	<0.001

CI: confidence interval; CK: creatine kinase; CRP: C-reactive protein; DLCO: diffusing capacity of the lung for carbon monoxide; ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; GEE: generalised estimating equations; HRCT: high resolution computed tomography of the chest; LV: left ventricle; mRSS: modified Rodnan skin score; OR: odds ratio; SSc: systemic sclerosis.

conducted a GEE analysis of a two-fold increase in both ESR and CRP between visits and worsening of mRSS and RFTs. (see Table V).

A two-fold increase in ESR and CRP between visits was recorded on 172 occasions and was significantly associated with a 10% decrease in FVC between corresponding visits in the whole cohort (OR 1.60, p=0.032), dcSSc subgroup (OR 3.07, p=0.004), early disease subgroup (OR 3.15, p=0.015) and patients who had ILD on HRCT (OR 2.31, p=0.012). A two-fold rise in inflammatory markers was significantly associated with a decrease in DLCO between visits in patients with dcSSc (OR 2.90, p=0.008) and those who had ILD on HRCT (OR 2.05, p=0.041). Analysis of patients with limited disease (lcSSc) showed no significant relationship between change in inflammatory markers and RFTs (see supplementary index). There was no significant relationship between rise in inflammatory markers and change in mRSS in any patient group.

Table III. Multivariable analysis: clinical associations with ESR≥20mm/hr.

Variable	OR (95% CI)	p-value
Whole SSc cohort n=1,545 patients (6,119 visits)		
FVC<80%	1.32 (1.10-1.62)	0.006
DLCO<80%	1.50 (1.25-1.82)	<0.001
PAH	1.57 (1.12-2.20)	0.008
Body mass index	1.04 (1.03-1.06)	<0.001
Proximal power	0.68 (0.55-0.84)	<0.001
Anaemia	2.77 (2.36-3.26)	<0.001
Hypocomplementaemia	0.76 (0.62-0.92)	0.006
Diffuse cutaneous disease n=393 patients (1,629 visits)		
FVC<80%	1.48 (1.10-1.99)	0.009
DLCO<80%	1.73 (1.19-2.50)	0.004
Anaemia	3.24 (2.42-4.32)	<0.001
Disease duration≤ 2 year n=245 patients (963 visits)		
DLCO<80%	2.39 (1.51-3.79)	<0.001
PAH	6.31 (1.95-20.40)	0.002
Tendon friction rub	2.45 (1.19-5.06)	0.015
Anaemia	3.08 (2.05-4.62)	<0.001

CI: confidence interval; DLCO: diffusing capacity of the lung for carbon monoxide; ESR: erythrocyte sedimentation rate; FVC: forced vital capacity; ILD: interstitial lung disease; OR: odds ratio; PAH: pulmonary arterial hypertension; SSc: systemic sclerosis.

Table IV. Multivariable analysis: clinical associations with CRP≥ 5mg/L.

Variable	OR (95% CI)	p-value
Whole SSc cohort n=1,545 patients (6,119 visits)		
mRSS>20	1.98 (1.54-2.54)	<0.001
FVC<80%	1.37 (1.12-1.68)	0.003
DLCO<80%	1.31 (1.08-1.59)	0.007
PAH	2.12 (1.50-2.96)	<0.001
Digital ulcers	1.32 (1.09-1.60)	0.005
Body mass index	1.06 (1.05-1.08)	<0.001
Synovitis	1.24 (1.02-1.51)	0.029
Tendon friction rub	2.14 (1.43-3.22)	<0.001
Anaemia	1.60 (1.34-1.90)	<0.001
Hypocomplementaemia	0.71 (0.58-0.89)	0.002
Diffuse cutaneous disease n=393 patients (1,629 visits)		
mRSS>20	1.70 (1.27-2.28)	<0.001
DLCO<80%	1.79 (1.23-2.62)	0.003
Body mass index	1.07 (1.03-1.11)	<0.001
Tendon friction rub	3.08 (1.79-5.32)	<0.001
Anaemia	1.89 (1.38-2.61)	<0.001
Disease duration ≤2 years n=245 patients (963 visits)		
mRSS>20	1.85 (1.20-2.84)	0.005
Diarrhoea	1.53 (1.04-2.26)	0.031
Body mass index	1.10 (1.06-1.15)	<0.001
Synovitis	1.70 (1.13-2.57)	0.012
Tendon friction rub	3.86 (1.83-8.17)	<0.001
Anaemia	2.75 (1.83-4.12)	<0.001
Hypocomplementaemia	0.59 (0.36-0.97)	0.039

CI: confidence interval; CRP: C-reactive protein; DLCO: diffusing capacity of the lung for carbon monoxide; FVC: forced vital capacity; mRSS: modified Rodnan Skin Score; OR: odds ratio; PAH: pulmonary arterial hypertension; SSc: systemic sclerosis.

Discussion

Raised inflammatory markers correspond not only with features of disease traditionally considered 'inflammatory' such as arthritis but also with fibrotic and vasculopathic disease manifestations such as ILD, PAH and digital ulcers. Our analysis has shown a relationship between PAH and a measurable inflammatory response. PAH in SSc is thought to be a consequence of small vessel endothelial dysfunction with minimal inflammation and immunosuppression has no role in the management of SSc-PAH (24, 25). This is unlike other connective tissue diseases where response of PAH to immunosuppressive therapy is consistent with inflammatory lesions contributing to the pathogenesis (26, 27).

A previous study demonstrated that an elevated CRP at baseline predicts a decline in FVC and progression of ILD in patients with early SSc (12). In this study, raised inflammatory markers were associated with FVC<80% and increasing inflammatory markers were associated with declining lung volumes throughout the duration of the study. This was observed across all subgroups analysed except in lcSSc, suggesting that the association in the whole cohort is accounted for by patients with dcSSc. Two-fold increase in ESR and CRP between visits showed a strong relationship to declining DLCO as well as FVC in patients with dcSSc and ILD on HRCT. A reduced DLCO is a risk factor for development of PAH (24) and decreasing FVC and DLCO have been shown to predict for adverse

Table V. Association between increasing ESR & CRP and change in mRSS and RFTs.

Variable	Whole SSc cohort n=1,545 patients (6,119 visits)		Diffuse cutaneous disease n=393 patients (1,629 visits)		Disease duration ≤ 2 years n=245 patients (963 visits)		ILD diagnosed on HRCT n=370 (1,717 visits)	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
25% increase in mRSS	0.83 (0.53-1.28)	0.393	1.39 (0.59-3.27)	0.450	1.95 (0.82-4.63)	0.129	1.30 (0.65-2.59)	0.456
10% decrease in FVC	1.60 (1.04-2.46)	0.032	3.07 (1.44-6.55)	0.004	3.15 (1.24-7.97)	0.015	2.31 (1.20-4.45)	0.012
15% decrease in DLCO	1.37 (0.87-2.16)	0.175	2.90 (1.33-6.33)	0.008	2.11 (0.84-5.29)	0.112	2.05 (1.03-4.07)	0.041

CI: confidence interval; DLCO: diffusing capacity of the lung for carbon monoxide; FVC: forced vital capacity; mRSS: modified Rodnan skin score; OR: odds ratio; RFT: respiratory function test; SSc: systemic sclerosis.

respiratory outcome (23). Our findings suggest that elevated inflammatory markers correlate with the presence of respiratory disease and the association of rising inflammatory markers with worsening respiratory function point to a possible inflammatory component of progressive lung disease. An alternate explanation for this association is that infection is driving the systemic inflammatory response in patients with ILD given this group's high risk of infection. However, infection is unlikely to solely account for the association demonstrated in this study as analyses were performed to examine associations at each annual visit throughout the patients' total study participation and it is unlikely that patients would present each year, at the time of testing, with active infection.

Raised inflammatory markers are more commonly associated with dcSSc compared to lcSSc (8, 12, 28). We found an association between raised CRP and high mRSS across all groups studied. However, there was not a relationship between rising inflammatory markers and change in mRSS. Even in patients with early disease, when skin score changes can be expected to be most pronounced (29, 30), there was no association between worsening mRSS and increasing inflammatory markers, consistent with the finding of the CSRG study (8).

Synovitis and myositis are inflammatory manifestations of SSc, yet there are inconsistent reports regarding a corresponding measurable inflammatory response. The CSRG found no association between elevated CRP levels and synovitis or myositis (8). In contrast, analysis of the EUSTAR cohort showed a strong association between raised inflammatory markers, synovitis and worsening joint contractures (16). Our results show that elevated CRP is consistently associated with articular manifestations of disease; synovitis and tendon friction rubs are significantly associated with elevated CRP. Raised ESR was only significantly associated with tendon friction rub in patients with early disease and not with synovitis in any group studied. Myositis, indicated by impaired proximal muscle strength, was associated with raised ESR in the whole cohort only. Although muscle involvement in SSc is often inflammatory, it is typically mild and non-specific, so this may account for the lack of a consistent measurable systemic inflammatory response in this study.

Initial inflammatory injury followed by fibrotic change and damage is thought to lead to gastrointestinal and cardiac manifestations of disease (31-34). This study did not show a clear relationship between raised inflammatory markers and gastrointestinal or cardiac features of disease. This may indicate that development of these disease manifestations is not generally accompanied by a measurable systemic inflammatory response. Alternatively, the lack of association seen may be due to the imperfect measures of involvement used in these organ systems and indicate that clinical tools are insufficiently sensitive to detect the early inflammatory component of these organ manifestations.

Anaemia can indicate SSc-organ involvement, particularly of the gastrointestinal tract. This study demonstrated an association between anaemia and raised inflammatory markers. Anaemia of chronic disease may account for the anaemia seen in this patient population and this association may reflect a more 'inflamed state' of those patients who have chronically active SSc.

We found an inverse correlation between raised inflammatory markers and hypocomplementaemia. The role of complement in measuring SSc-disease activity is controversial. Hypocomplementaemia has been found to identify patients with SSc-overlap disease and be associated with periods of disease activity in this subgroup (35, 36). Analysis of a large group of patients with SSc, including those with overlap syndromes, found hypocomplementaemia was associated with digital ulcers, joint contractures and proteinuria on univariable analysis only (37). Digital ulcers were only associated with elevated CRP in this study. Given that complement levels are generally elevated in states of inflammation, this study may reflect that for patients without overlap syndromes, hypocomplementaemia does not correlate with high disease activity.

Elevated inflammatory markers across each patient group showed a significant relationship with higher BMI values. This is somewhat unexpected as lower BMI values can indicate more severe SSc and raised inflammatory markers appear to be associated with other manifestations of more severe disease. However, obesity has been associated with both increased ESR and CRP, perhaps accounting for the finding of the association between higher BMI and raised inflammatory markers (38).

With its longitudinal design and use of GEE for analysis of associations in 6,119 visits among 1,545 patients, we believe this to be largest study to

date of associations of inflammatory markers in SSc. However, the results need to be interpreted within the limitations of this study and its observational design. Data, specific to SSc, are collected on patients annually and no information about concurrent medical conditions, particularly infection, are collected. Data are collected in an ambulatory care setting when patients are most likely to be well at the time of review. This aims to minimise the confounding effect of concurrent illnesses on the disease and treatment data collected. Whilst it is not possible to account for non-SSc related causes of raised inflammatory markers, concurrent acute illnesses are unlikely to account for all associations seen by the nature and context in which the study data are collected. Also, given the large number of visits analysed throughout the study, even if some patients had a concurrent illness at the time of review, infection can not solely account for the relationships observed between raised inflammatory markers and SSc-disease features. It is possible that changes to therapy may have had an effect on ESR and CRP results. As exact start and stop dates of medications and dosages of medications are not captured at study visits, it was not possible to directly evaluate the effects of change in therapy on ESR and CRP. There is a variable length of follow-up of patients in this study making it possible that raised inflammatory markers were detected by chance alone and were not necessarily persistently elevated in periods of increased disease activity. By using GEE for statistical analysis, we have attempted to overcome these limitations by analysing the associations of raised ESR and CRP with other disease manifestations at each study visit over time.

Conclusions

We have shown that approximately 50% of SSc patients have raised inflammatory markers throughout their disease course. There is an association between raised inflammatory markers and cardiopulmonary disease manifestations and there is an important association between rising inflammatory markers and deteriorating RFTs. Elevated CRP is associated with both synovitis and tendon friction rub as well as digital ulcers. This study shows that raised inflammatory markers are associated with vasculopathic and fibrotic, as well as inflammatory disease manifestations, indicating a role for ESR or CRP in assessing SSc-disease activity.

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