Musculoskeletal Involvement in SSc Is Associated with Worse Scores on Short Form-36 and Scleroderma Health Assessment Questionnaire and Lower Tumor Necrosis Factor- α Gene Expression in Peripheral Blood Mononuclear Cells

Eliza R. Pelrine, BA · Marie-Dominique Ah-Kioon, PhD · Meng Zhang, PhD · Franck J. Barrat, PhD · Robert F. Spiera, MD · Jessica K. Gordon, MD

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Abstract *Background:* Systemic sclerosis (SSc) is characterized by a wide variety of symptoms and disease manifestations including joint pain, gastrointestinal dysfunction, interstitial lung disease, and cardiomyopathy. *Questions/Purposes:* Using the Scleroderma Health Assessment Questionnaire (SHAQ) and Short Form-36 (SF-36) we explored how patient-reported physical health, mental health, and functional status related to these clinical characteristics and to cytokine levels utilizing the Hospital for Special Surgery Scleroderma Registry. *Methods:* In a cross-sectional study of 185 patients meeting the 2013 ACR/EULAR criteria for SSc, we compared disease features and patient-reported outcomes (PROs). Interleukin-6 (IL-6), interleukin-1 β (IL1 β), and tumor necrosis factor- α (TNF α) levels were

assessed by luminex and ELISA assays in a subset of 32 patients. The Pearson correlation coefficient, Spearman correlation coefficient, two-sample *t* test or Wilcoxon rank sum test, ANOVA or Kuskal-Wallis test, and Pearson chi-squared or Fisher's exact test were performed as applicable to detect the association between disease manifestations, PROs, and blood biomarkers. *Results:* The modified Rodnan skin score (MRSS) was positively correlated with SHAQ scores. Patients who had musculoskeletal involvement scored worse on both the SHAQ and SF-36. Lower levels of TNF α expression in PBMCs were also correlated with musculoskeletal involvement. No other significant correlations were found between clinical factors, PROs, and cytokine data. *Conclusion:* Musculoskeletal outcomes are a major determinant of quality of life and function in patients with SSc. These results emphasize the importance of musculoskeletal outcomes in clinical studies of SSc.

Level of Evidence: Prognostic Study: Level II

All work was performed at Hospital for Special Surgery.

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E. R. Pelrine, BA (✉) · R. F. Spiera, MD · J. K. Gordon, MD
Department of Medicine, Hospital for Special Surgery,
535 East 70th Street,
New York, NY 10021, USA
e-mail: eliza.pelrine@gmail.com

M. Zhang, PhD
Department of Biostatistics, Hospital for Special Surgery,
New York, NY, USA

M.-D. Ah-Kioon, PhD · F. J. Barrat, PhD
Department of Research, Hospital for Special Surgery,
New York, NY, USA

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Introduction

Systemic sclerosis (SSc) is a heterogeneous multisystem disease characterized by autoimmunity, fibrosis, and vasculopathy [9]. Of the rheumatic diseases, SSc has the highest case-specific mortality rate, with half of cases dying as a result of pulmonary or cardiac-related causes [2]. SSc has multiple additional clinical manifestations including Raynaud's phenomenon, arthropathy/arthritis, myopathy, gastrointestinal dysfunction, and renal disease, which also contribute to morbidity and mortality [16].

While much of the pathogenesis of SSc is unknown, many cytokines have been implicated in its fibrosis and

vasculopathy. Serum levels of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF α), both pro-inflammatory cytokines, are elevated in SSc patients. IL-6, specifically, has been linked with early disease and presence of pulmonary fibrosis and has correlated with the modified Rodnan skin score (mRSS) [4, 5, 15]. In the skin, TNF α , IL-6, as well as CXCL8, another pro-inflammatory protein which is regulated by interleukin 1 β (IL1 β), are differentially expressed in SSc patients as compared to healthy controls [8, 10]. Many of these cytokines have been the target of potential therapies, though none have been shown to be disease modifying to date.

Current clinical trials tend to focus on improving skin, lung, and vascular outcomes and use the modified Rodnan skin score (MRSS), the forced vital capacity (FVC), or the digital ulcer count as objective clinical outcome measures. This focus comes from the high morbidity and mortality associated with these manifestations [2, 6, 7, 21]. Musculoskeletal outcomes (like the Hand Mobility in Scleroderma assessment) have only rarely been used in scleroderma studies. Patient-reported outcomes (PROs) are commonly used as secondary outcomes. The Scleroderma Health Assessment Questionnaire (SHAQ) and Short Form-36 (SF-36) have both been validated in SSc patients as measures of quality of life (QoL) [11, 14]. The goals of this pilot, hypothesis-generating study were to examine the relationships between the clinical features of SSc and PROs to determine which disease manifestations impact patient-reported QoL and to determine whether any of the clinical features or PROs were correlated with blood biomarkers.

Patients and Methods

This was a cross-sectional, exploratory pilot study of patients enrolled in the SSc Registry and Repository at the Hospital for Special Surgery (HSS) between July 2006 and March 2015. This registry has been reviewed annually and approved by the HSS Institutional Review Board since July 25, 2006. To be enrolled in the registry, participants had to be 18 years of age or older and diagnosed with SSc and provide written informed consent ($n=276$). Registry participation involved completing questionnaires, being assessed by a rheumatologist (see below for details), and an optional blood draw for biomarker studies that occurred at regularly scheduled office visits every 6–12 months. To be included in our analysis, participants had to have been enrolled in the registry as well as completed both the SHAQ and the SF-36 and had clinical and demographic data available ($n=185$). The average age was 51.5 ± 13.9 years and 82.2% of participants were female (Table 1).

Each patient was assessed by a rheumatologist as part of the registry for the presence of various disease manifestations (Tables 1 and 2). For patients who had multiple registry visits, the data from the initial visit was used. Gastrointestinal involvement was defined as presence of GERD, GAVE, small intestinal bacterial overgrowth, and/or esophageal

Table 1 Patient demographics

	Whole cohort ($n=185$)	Cytokine sub-analysis ($n=32$)
Age (avg. $\pm$ SD)	51.51 ± 13.9	51.51 ± 11.1
Gender (% female)	82.2	84.4
Race (%)		
White	67.2	58.6
Black	14	31
Hispanic	9.7	3.4
Asian	4.7	6.9
Other	2.1	0.0
Disease type (%)		
Diffuse	65.4	71.4
Limited	29.7	21.4
Other	4.9	7.1
Disease duration (years, avg. $\pm$ SD)	5.96 ± 6.1	6.77 ± 5.3

dysmotility. Cardiac involvement was defined as the presence of cardiomyopathy, pericardial effusion, and/or arrhythmia. Musculoskeletal (MSK) involvement was defined as the presence of myalgias, myositis, arthritis, and/or joint pain. SF-36 questionnaires are scored from 0–100, with 100 being the best score and 0 being the worst. SHAQs are scored from 0–3 with 0 being the best score and 3 being the worst.

An additional cytokine analysis had been performed on samples from 32 of 185 participants as part of a sub-study. Eligibility criteria were the same for these patients as for the registry as a whole, but participation was determined on willingness to consent to the optional blood draw. Serum interleukin-6 (IL-6) and tumor necrosis factor- α (TNF α) levels were measured by Luminex (25-Plex Panel, Life Technologies) and ELISA. Concentrations of interleukin-1 β (IL1 β), TNF α , and IL-6 released by peripheral blood mononuclear cells (PBMCs) after 24 h of culture were also measured by Luminex (25-Plex Panel, Life Technologies). Gene expression of these cytokines was measured by quantitative PCR (qPCR) with results expressed as the relative Ct compared to expression of the ubiquitin gene.

Demographic and clinical data were summarized using descriptive statistics. Clinical characteristics and cytokine data were analyzed for correlations with the SHAQ and SF-36 scores. The Pearson correlation coefficient or Spearman correlation coefficient were used to see whether there is correlation between two continuous variables. The two-sample t test or Wilcoxon rank sum test were used to detect the difference in the mean or median for a continuous variable between the two categories of a categorical variable. The ANOVA or Kruskal-Wallis test were used to see whether there is a difference in the mean or median for a continuous variables among three or more categories of a categorical variable. Assessment for association between diffuse SSc (dcSSc) and limited SSc (lcSSc) in organ involvement was done using Pearson's chi-squared and Fisher's exact tests. All tests were two-sided with a significance level of 0.05. All analyses were performed using SAS for Windows 9.3 (SAS Institute Inc., Cary, NC).

Results

Among all participants, 65.4% had dcSSc, 29.7% had lcSSc, and 4.9% was classified under another disease type (mixed, overlap, sine). The average disease duration was 5.96 ± 6.1 years (Table 1). Patients with dcSSc had a significantly higher prevalence of ILD and ulcers than lcSSc ($p < 0.0001$ and $p = 0.003$, respectively; Table 2). No other significant differences were seen between disease types in our cohort or between the whole cohort and the smaller cytokine sub-analysis.

MSK involvement correlated with lower (worse) SF-36 Mental Component scores (95% CI 50.8–60.0, $\chi^2 = 11.32$, $p = 0.0008$, Fig. 1), lower SF-36 Physical Component scores (95% CI 43.9–53.0, $\chi^2 = 19.60$, $p < 0.0001$), and higher (worse) SHAQ scores (95% CI 0.81–1.14, $\chi^2 = 11.54$, $p = 0.0007$, Fig. 1). Worse SHAQ scores also correlated with high (worse) modified Rodnan skin scores (mRSS) ($\rho = 0.21$, $p = 0.01$, Table 3). SF-36 and SHAQ scores were also compared by age, gender, race, disease duration, disease type, and other organ system involvement, and no significant differences between the groups were observed (Table 3). No adjustments were made for multiple comparisons in this analysis.

Lower TNF α expression in peripheral blood mononuclear cells (PBMC) correlated with MSK involvement (95% CI 177.8–560.8, $\chi^2 = 103.21$, $p = 0.0002$, Fig. 2) SF-36 Mental Health component scores positively correlated with IL-6 levels in PBMC ($\rho = 0.81$, $p = 0.03$). Disease duration negatively correlated with TNF α expression in PBMC ($\rho = -0.9$, $p = 0.04$). Presence of ILD correlated with higher TNF α levels in serum (95% CI 803.8–4851.1, $\chi^2 = 5.83$, $p = 0.03$). Presence of PAH positively correlated with IL1 β concentration in PBMCs (95% CI –1.57–5.22, $\chi^2 = 6.4$, $p = 0.03$). No other significant associations were seen between clinical characteristics and cytokine levels (Table 4). After using a Bonferroni correction to account for the 112 comparisons made in the cytokine analysis, only

Fig. 1. Mean $\pm$ SD SF-36 Mental Component scores in MSK(+) and MSK(–) patients (a), mean $\pm$ SD SF-36 Physical Component scores in MSK(+) and MSK(–) patients (b), and mean $\pm$ SD SHAQ scores in MSK(+) and MSK(–) patients (c). * $p = 0.0008$, ** $p < 0.0001$, *** $p = 0.0007$.

Table 2 Clinical characteristics

	Diffuse	Limited	
mRSS (avg. $\pm$ SD)	22.5 $\pm$ 10.8	6.0 $\pm$ 4.3	
Disease manifestations—% (out of <i>n</i>)			<i>p</i> value
ILD	64.9 (111)	17.6 (51)	<0.0001
PAH	12.2 (115)	15.1 (53)	0.602
GI	76.8 (142)	71.9 (64)	0.452
SRC	4.4 (113)	0 (48)	0.323
Ulcers	52.8 (123)	28.3 (53)	0.003
Raynaud's	89.3 (122)	94 (50)	0.403
Cardiac edema	11.1 (99)	10 (50)	1
Pericardial effusion	6.2 (97)	0 (47)	0.177
Arrhythmia	5.1 (99)	8.2 (49)	0.479
Myalgias	25.8 (89)	23.6 (55)	0.766
Myositis	0 (67)	2.2 (45)	0.402
Arthritis	17.3 (52)	8.3 (24)	0.486
Joint pain	50.6 (81)	52 (50)	0.878

Values that have been italicized are considered statistically significant

the correlation between MSK involvement and TNF α expression in PBMC remained significant.

Discussion

In our cross-sectional study of 185 SSc patients, the single domain of organ system involvement which correlated with measures of patient-reported quality of life was MSK. SSc patients from our cohort with MSK involvement reported worse scores on the SHAQ and SF-36. Joint involvement in SSc contributes to disability while synovitis, joint contractures, and tendon friction rubs have all been associated with more severe disease [1, 13, 18]. This, in combination with our data, supports MSK involvement as an important factor in the management of SSc.

The major limitations of this study were related to our small sample size. While our registry had almost

Table 3 Clinical correlations

	SF-36 mental		SF-36 physical		SHAQ	
	Mean $\pm$ SD	<i>p</i> value	Mean $\pm$ SD	<i>p</i> value	Mean $\pm$ SD	<i>p</i> value
Gender						
Male	54.7 $\pm$ 26.8, 24	0.76	46.8 $\pm$ 25.8, 27	0.62	0.99 $\pm$ 0.91, 27	0.92
Female	56.9 $\pm$ 22.4, 155		48.6 $\pm$ 23.7, 158		0.97 $\pm$ 0.85, 148	
Race						
White	59 $\pm$ 22.6, 114	0.08	51 $\pm$ 23.9, 117	0.08	0.92 $\pm$ 0.79, 112	0.83
African American	55.5 $\pm$ 21.8, 22		53.5 $\pm$ 24.9, 23		0.86 $\pm$ 0.91, 22	
Hispanic	43.9 $\pm$ 24.6, 12		36.1 $\pm$ 21.1, 12		1.25 $\pm$ 1.04, 12	
Asian	49.2 $\pm$ 21, 12		39.3 $\pm$ 23.3, 13		1.17 $\pm$ 0.95, 13	
Mixed/other	72 $\pm$ 18.6, 4		58.5 $\pm$ 22.4, 4		1.01 $\pm$ 0.52, 4	
Disease type						
Limited	58.3 $\pm$ 25.8, 59	0.63	51.1 $\pm$ 25.2, 59	0.18	0.92 $\pm$ 0.85, 56	0.13
Diffuse	55.1 $\pm$ 22.1, 92		48.4 $\pm$ 23, 97		1.06 $\pm$ 0.84, 92	
Other	57.4 $\pm$ 19.0, 9		36.4 $\pm$ 23.9, 9		0.53 $\pm$ 0.47, 7	
ILD						
Present	52.4 $\pm$ 23.3, 33	0.26	43.5 $\pm$ 21.3, 34	0.11	1.07 $\pm$ 0.94, 36	0.53
Not present	57.7 $\pm$ 23.8, 82		51.8 $\pm$ 26.3, 84		0.92 $\pm$ 0.86, 76	
PAH						
Present	51.8 $\pm$ 22.3, 18	0.38	42.1 $\pm$ 21.3, 19	0.26	1.28 $\pm$ 1.09, 18	0.36
Not present	56.3 $\pm$ 23.5, 104		49.8 $\pm$ 25.2, 106		0.96 $\pm$ 0.79, 99	
GI						
Involved	55.7 $\pm$ 22.8, 116	0.6	47.5 $\pm$ 24, 117	0.3	1.07 $\pm$ 0.89, 107	0.12
Uninvolved	57.1 $\pm$ 25.2, 39		51.2 $\pm$ 23.8, 39		0.83 $\pm$ 0.76, 42	
Cardiac						
Involved	51.3 $\pm$ 23.9, 25	0.44	40 $\pm$ 24, 26	0.05	1.34 $\pm$ 1.09, 21	0.1
Uninvolved	54.9 $\pm$ 23.7, 94		50.4 $\pm$ 24.5, 96		0.89 $\pm$ 0.77, 97	
Musculoskeletal						
Involved	50.2 $\pm$ 23.8, 76	0.0008	41.9 $\pm$ 23.3, 77	<0.0001	1.18 $\pm$ 0.94, 74	0.0007
Uninvolved	67.7 $\pm$ 21.5, 32		63.3 $\pm$ 21.2, 34		0.52 $\pm$ 0.48, 34	

Values that have been italicized are considered statistically significant

300 patients enrolled at the time of analysis, less than 200 had sufficient data available to be included. A repeat study in the future when more patients have been enrolled could strengthen our findings and allow for

more in-depth analyses of race, ethnicity, and, particularly, cytokine levels. With only 32 total patients, the blood biomarker analyses were substantially underpowered. However, as a hypothesis-generating study, we find these limitations to be reasonable.

The leading causes of mortality in SSc are ILD, SSc renal crisis, and cardiac involvement [6]. These manifestations greatly impact general health, and it is surprising that our study did not find them to have significant impacts on patient-reported quality of life (QoL). In other studies, other organ systems were noted to have significant effect on QoL. For example, Khanna et al. reported GI functional status correlated with patient-reported depressive symptoms while Nietert et al. in the Scleroderma Lung Study found that lung disease correlated with worse scores on the SF-36 [7, 12]. We classified patients with organ system involvement even if the degree of involvement was mild, and this may account for this finding. However, the same holds true for all organ systems we surveyed, meaning that even patients with mild MSK involvement were classified as such. The clear importance of MSK involvement in patient QoL that our results suggest highlights the need for MSK outcomes in both clinical and research settings. Measures like tender and swollen joint counts, the Cochin questionnaire, and the Hand Mobility

Fig. 2. Mean $\pm$ SD TNF- α gene expression levels of PBMCs in MSK(+) and MSK(-) patients. **** p = 0.0002.

Table 4 Cytokine correlations

	TNF α (serum)		IL6 (serum)		TNF α (PBMC)		IL6 (PBMC)		IL1b (PBMC)		TNF α (gene-PBMC)		IL6 (gene-PBMC)		IL1b (Gene-PBMC)	
	ρ	p value	ρ	p value	ρ	p value	ρ	p value	ρ	p value	ρ	p value	ρ	p value	ρ	p value
SF-36 mental	-0.01	0.97	-0.08	0.78	0.22	0.64	0.81	0.03	0.2	0.66	0.26	0.62	0.23	0.38	0.41	0.32
SF-36 physical	0.06	0.83	-0.04	0.87	0.11	0.8	0.36	0.38	0.33	0.43	0.6	0.21	-0.17	0.5	0.48	0.23
SHAQ	-0.48	0.06	-0.39	0.14	-0.18	0.67	-0.15	0.71	-0.66	0.07	-0.2	0.7	0.2	0.42	-0.17	0.69
Age	-0.03	0.87	0.04	0.82	-0.37	0.17	0.07	0.79	-0.22	0.42	-0.12	0.73	0.11	0.58	-0.38	0.22
Disease duration	0.04	0.87	0.14	0.58	-0.26	0.53	0.11	0.8	-0.41	0.3	-0.9	0.04	0.13	0.63	0.26	0.62
mRSS	-0.2	0.48	-0.11	0.71	-0.2	0.7	-0.14	0.79	-0.44	0.38	-0.48	0.33	-0.15	0.57	0.33	0.42
Gender	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value	χ^2	p value
Race	0.86	0.35	1.17	0.28	1.78	0.18	1.19	0.27	1.62	0.2	0.41	0.52	1.72	0.19	0.97	0.32
Disease type	2.59	0.46	2.7	0.44	6.26	0.1	6.21	0.1	4.2	0.24	2.42	0.3	5.55	0.13	0.5	0.48
Musculoskeletal	0.2	0.9	0.22	0.9	1.26	0.53	1.04	0.59	2.92	0.23	0.04	0.84	0.11	0.94	1.18	0.55
Cardiac	0.09	0.76	0.0005	0.98	0.37	0.56	1.43	0.26	2.8	0.13	103.21	0.0002	1.29	0.27	0.12	0.74
ILD	0.2	0.66	0.25	0.62	0.54	0.48	2.19	0.17	0.8	0.39	0.44	0.54	0.13	0.72	0.24	0.64
PAH	5.83	0.03	3.08	0.09	0.02	0.89	0.3	0.59	0.82	0.39	0.02	0.9	0.69	0.42	1.07	0.35
GI	0.27	0.61	0.17	0.69	0.0009	0.98	1.88	0.21	6.4	0.03	2.75	0.19	0.2	0.66	0.41	0.55
	0.58	0.45	0.11	0.74	2.46	0.15	0.37	0.56	0.54	0.48	0.03	0.87	0.33	0.57	1.37	0.27

Values that have been italicized are considered statistically significant

in Scleroderma assessment can provide objective assessments of morbidity associated with MSK involvement that subjective QoL assessments like the SHAQ and SF-36 cannot. While skin and lung outcomes are important measures in disease activity, patient quality of life should not be overlooked while designing studies. Future work should focus on optimizing MSK outcomes for clinical trials.

In our cohort, ILD and ulcers were more common in dcSSc than in lcSSc. ILD has previously been linked with anti-Scl70 antibody positivity and negatively correlated with anti-centromere antibody positivity [17, 19]. These antibodies are, respectively, associated with dcSSc and lcSSc which supports the discordance in ILD prevalence observed in our cohort [21]. However, ischemic digital ulcers have been reported to be more common in lcSSc than dcSSc [3]. The fact that we see more digital ulcers in the patients with dcSSc in this study gives an indication of the relatively severe disease experienced by participants in this cohort. Therefore, it is possible that our results may overrepresent severe disease types and not be generalizable to SSc patients as a whole.

TNF α expression in PBMCs was significantly lower in our patients with MSK involvement as compared to those with no MSK involvement. These results are of particular interest because previous literature suggests that MSK involvement in SSc is correlated with *increased* expression of TNF α in myopathies [20]. It should be noted that the literature reports on TNF α levels in the serum while our study reports on the gene expression in PBMC. These contradicting results suggest the need for further study of TNF α in SSc, particularly on whether the presence of certain other clinical features could have skewed our results.

In summary, we found the musculoskeletal domain to be highly important in patient-reported QoL. While lung, cardiac, and renal involvement are serious complications of SSc, patients in our cohort did not find them to be significant determinants of QoL. This data supports further studies in the MSK domain and should guide hypotheses of how to improve outcomes and QoL in patients with SSc.

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Compliance with Ethical Standards

Conflict of Interest: Eliza R. Pelrine, BA, Marie-Dominique Ah-Kioon, PhD, Meng Zhang, PhD, Franck J. Barrat, PhD, and Robert F. Spiera, MD have declared that they have no conflict of interest. Jessica K. Gordon, MD, reports grants from the HSS Clinician Scientist Development Award through the Kellen Foundation, during the conduct of the study.

Human/Animal Rights: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Informed Consent: Informed consent was obtained from all patients for being included in the study.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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