Anti-PM-Scl antibody in patients with systemic sclerosis

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ABSTRACT

Objectives. *To compare systemic sclerosis (SSc) patients with and without anti-PM-Scl antibody.*

Methods. *We reviewed the medical records of 76 anti-PM-Scl antibody positive SSc patients and 2349 anti-PM-Scl negative SSc patients first evaluated during 1980-2004. Patients were included if they had a clinical diagnosis of SSc either alone or in overlap with another connective tissue disease. Anti-PM-Scl antibody was screened for by indirect immunofluorescence and tested by Ouchterlony double immunodiffusion.*

Results. *Anti-PM-Scl antibody positive patients had a significantly higher frequency of a positive ANA with nucleolar staining (87% vs. 32%, p<0.0001) and were younger at both symptom onset (p=0.004) and first physician diagnosis of SSc (p<0.001). They were classified more often as having overlap with another connective tissue disease, particularly polymyositis-dermatomyositis, and more frequently had limited cutaneous involvement (72% vs. 52%, p=0.001). Maximal skin thickening was less in anti-PM-Scl antibody patients (mean modified Rodnan total skin score 6.0±6.3 vs. 15.9±14.2, p<0.001). Anti-PM-Scl antibody positive patients less frequently had peripheral vascular (91% vs. 98%, p=0.0002) and gastrointestinal (52% vs. 79%, p=0.0001) disease. Lung involvement overall had a similar distribution between both groups. However, radiographic evidence of pulmonary fibrosis was more frequent in anti-PM-Scl antibody positive patients (50% vs. 37%, p=0.05) and pulmonary arterial hypertension was less often detected (5% vs. 15%, p<0.04). Skeletal muscle involvement (51% vs. 14%, p<0.0001) and subcutaneous calcinosis (p<0.003) were both significantly more often observed in anti-PM-Scl antibody positive patients. Joint, heart, and kidney involvement were similar in both groups. Overall survival was significantly better for anti-PM-Scl antibody positive patients (10 year cumulative survival rate 91% vs. 65%, p=0.0002). After adjustment for age, sex and limited vs. diffuse cutaneous involvement, patients with anti-PM-Scl antibody were significantly less likely to die (HR=0.32, 95% CI, [0.14, 0.72] p=0.006).*

Conclusion. *SSc patients with anti-PM-Scl antibody are younger and significantly more often have limited cutaneous involvement, skeletal muscle disease, pulmonary fibrosis and calcinosis compared to anti-PM-Scl antibody negative SSc patients. Ten-year cumulative survival is significantly better in anti-PM-Scl antibody positive SSc patients.*

Introduction

Systemic sclerosis (SSc) is a chronic connective tissue disease whose hallmark symptoms are thickening of the skin (scleroderma), Raynaud phenomenon, and involvement of various internal organs (1). SSc can be divided into two major variants based on the extent of skin involvement: limited cutaneous involvement (lcSSc), with skin thickening only distal to the elbows and knees and generally less serious internal organ involvement, and diffuse cutaneous involvement (dcSSc), where skin thickening is more widespread and internal organ involvement is more frequent and more severe (1). SSc occasionally occurs as an overlap syndrome, with features of another connective tissue disease such as polymyositis (PM), dermatomyositis (DM), systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA).

One of eight SSc-associated serum autoantibodies is found in the sera of over 90% of patients with SSc (2). These antibodies are important markers of clinical subsets and help to predict disease course. Their role in the pathogenesis of the disease is unknown. Patients with lcSSc tend to have anticentromere, anti-Th/To or anti-Ku antibodies, while those with dcSSc more often have anti-

Scl 70 (topoisomerase I), anti-RNA polymerase III or anti-U3RNP antibodies. Anti-PM-Scl and anti-U1RNP antibodies are most common in patients with overlap syndromes (2).

The purpose of this study was to use data collected in the Pittsburgh Scleroderma Databank to describe the characteristics of SSc patients positive *vs.* negative for anti-PM-Scl antibody. We examined their demographic features, disease classification characteristics, clinical and laboratory data, including organ system involvement, natural history of disease and survival.

Materials and methods
Patients
Patients first evaluated at the University of Pittsburgh between 1980 and 2004 with a confirmed diagnosis of SSc were eligible for inclusion in the study. All patients gave informed consent for the use of their clinical information and serum samples as required by the Institutional Review Board of the University of Pittsburgh. All available medical records, including initial and follow-up visit notes, were reviewed. For organ system involvement, data reported include manifestations recorded at any time in the disease course which were considered to be due to systemic sclerosis. The maximal recorded skin thickness score was used. Death information was obtained from available medical records and through the Social Security Death Index. Disease onset was defined as the time of the first symptom attributable to SSc.

Disease classification
Patients were classified as having diffuse or limited cutaneous involvement based on the degree and extent of skin involvement during the disease course. A patient who had skin thickening proximal to the elbows or knees (upper arms, thighs, trunk) at any time was considered to have diffuse cutaneous involvement.

The designation of "overlap" was made by the attending Scleroderma Clinic physician. Admittedly, this decision is arbitrary. For the most part, overlap patients also satisfied the Bohan and Peter diagnostic criteria for polymyo-sitis/dermatomyositis (3) or the ACR classification criteria for systemic lupus erythematosus (4) or rheumatoid arthritis (5).

Laboratory methods
All patients had serum tested for anti-PM-Scl autoantibody. Serologic studies were performed using standard techniques. They included tests for anticentromere antibodies (ACA) on a HEp-2 cell substrate; antibodies against topoisomerase I , U1RNP, and PM-Scl by double immunodiffusion; and antibodies to Th/To, RNA polymerase III, and U3RNP by immunoprecipitation, as previously reported (6-8). Calf thymus extract was used as antigen for the anti-U1RNP antibody assay, and rabbit thymus extract was used as antigen for the anti-topoisomerase I antibody assay. A concentrated calf thymus extract was prepared using a 30–60% ammonium sulfate precipitation of crude calf thymus acetone extract for anti-PM-Scl antibody. Sera were diluted 1:40 for ACA. No dilutions were performed for anti-topoisomerase I, anti-U1RNP or anti-PM-Scl.

Definitions of organ involvement
SSc organ involvement at any time during the course of the disease was considered present if the following predefined criteria were satisfied: 1) peripheral vascular (Raynaud phenomenon or any one of digital pitting scars, digital tip ulceration, digital gangrene, or abnormal nailfold capillaries); 2) cutaneous (any skin thickening using the modified Rodnan method) (9); 3) articular (any one of joint swelling, carpal tunnel syndrome, palpable tendon or bursal friction rubs, joint space narrowing or erosion on radiograph, or finger joint contractures with third fingertip to palm distance in full flexion 2.0 cm or greater); 4) muscular (proximal muscle weakness on physical examination plus any one of an elevated serum creatine kinase level, myopathic changes on electromyogram, or abnormal muscle biopsy compatible with myopathy); 5) gastrointestinal tract (any one of distal esophageal dysmotility or stricture (by esophagram or motility study), hypomotility of the duodenum or small in-testine, malabsorption syndrome, bacterial overgrowth in the small intestine, wide-mouthed colonic sacculations or death due to SSc-related gastrointestinal disease); 6) pulmonary (any one of restrictive lung disease (forced vital capacity [FVC] <70% predicted plus forced expiratory volume in one second/FVC >80%), pulmonary fibrosis on chest radiograph, diffusing capacity for carbon monoxide <65% predicted, intrinsic pulmonary hypertension defined as either an estimated pulmonary artery systolic pressure >40 mm Hg by echocardiogram or a mean pulmonary artery pressure of >25 mm Hg on right heart catheterisation, pleuritic chest pain plus a pleural friction rub or pleural effusion, or death due to SSc-related lung disease; 7) cardiac (any one of estimated left ventricular ejection fraction <45% or clinical evidence of left-sided congestive heart failure, pericarditis (pericardial pain and either a pericardial friction rub or pericardial effusion), arrhythmia requiring treatment, complete heart block, or death due to SSc-related heart disease; 8) renal (clinical evidence of "scleroderma renal crisis", defined as the abrupt onset of accelerated arterial hypertension with or without rapidly progressive oliguric renal failure, or death due to SSc-related renal disease). If there were other more likely causes of these organ system involvements, they were not attributed to SSc.

Sjögren's syndrome (SS) was considered present if a patient had dry eyes or dry mouth plus any one of the following: positive Schirmer test, ophthalmologist confirmed reduced tearing, abnormal lip biopsy, or positive serum anti-SSA or anti-SSB antibody. Calcinosis was confirmed either on physical examination or radiographically.

Statistical analysis
Student's *t*-test was used to detect significant differences between distributions (means) of continuous data. Chi-square analysis was used to determine significant differences between sets of categorical data, with Fisher's exact test when appropriate. Survival was measured from the time of first physician diagnosis of SSc and survival

curves were compared using Kaplan-Meier and Mantel-Haenszel tests. The Cox proportional hazards method was used to adjust for factors which could confound the interpretation of survival data.

Results

Demographic features

Compared to the anti-PM-Scl antibody negative group, anti-PM-Scl antibody positive patients were significantly younger at symptom onset (37.6±17.7 vs. 42.7±15.3 years), at diagnosis (40.9 vs. 47.3 years) and at first Pittsburgh evaluation (44 vs. 50 years). They were more frequently Caucasian (97% vs. 91%, p=0.08). Disease duration from first symptom to first physician diagnosis of SSc did not differ between the groups.

Disease classification

Of the 76 anti-PM-Scl antibody positive patients, 40 had SSc alone (12 dcSSc, 28 lcSSc) as shown in Table I. The frequency of lcSSc was significantly greater in anti-PM-Scl antibody positive patients (p=0.0139). The mean maximum modified Rodnan skin score was significantly lower in PM-Scl patients, as expected (6.0±6.3 vs. 15.9±14.2, p<0.001). Thirty six of the patients had SSc in overlap with another connective tissue disease (33 with PM/DM, 2 with both PM/DM and SLE and 1 with RA). This proportion with SSc in overlap (47%) was significantly higher than the 8% in the anti-PM-Scl negative patient group (p=0.0001).

Organ system involvement

A summary of organ system involvement is shown in Table II. Peripheral vascular disease was significantly less common in the anti-PM-Scl positive group (91% vs. 98%, p=0.0001), primarily due to differences in the frequency of Raynaud phenomenon. There were no differences in the frequencies of features which would suggest severity of peripheral vascular disease such as digital pitting scars, digital tip ulcers or digital gangrene. Anti-PM-Scl antibody positive dcSSc patients had a significantly lower mean total skin score than anti-PM-Scl negative dcSSc patients.

Table I. Classification of systemic sclerosis (SSc) according to presence or absence of anti-PM-Scl antibody.

Diagnosis	Anti-PM-Scl positive	Anti-PM-Scl negative	p-value
SSc alone (not in overlap)	n=40	n=2152	0.01
limited cutaneous SSc	28 (70%)	1081 (50%)	
diffuse cutaneous SSc	12 (30%)	1065 (49%)	
unclassified	0	6 (1%)	
SSc in overlap	n=36	n=197	0.0006
with PM/DM*	33 (92%)	101 (50%)	
with PM/DM + SLE†	2 (6%)	21 (11%)	
with RA‡	1 (3%)	12 (6%)	
with SLE	0	59 (30%)	
with PM/DM +RA	0	3 (2%)	
with SLE + RA	0	1	

*PM/DM: polymyositis/dermatomyositis; †SLE: systemic lupus erythematosus; ‡RA: rheumatoid arthritis.

Table II. Organ system involvement in anti-PM-Scl antibody positive vs. negative patients.

Organ system	Anti-PM-Scl positive n=76	Anti-PM-Scl negative n=2349	p-value
Peripheral vessels	68 (91%)	2303 (98%)	<0.0002
Joints/tendons	64 (84%)	1844 (79%)	NS
Skeletal muscles	39 (51%)	327 (14%)	<0.0001
Gastrointestinal tract	24/46 (52%)	1436/1819 (79%)	0.0001
Lung (overall)	45 (59%)	1352 (58%)	NS
Pulmonary fibrosis	31/62 (50%)	760/2031 (37%)	0.04
PAH*	3/59 (5%)	247/1688 (15%)	0.05
Heart	6/54 (11%)	377/1876 (20%)	NS
Kidney	6 (8%)	234 (10 %)	NS

Denominators are shown when not all patients were evaluated.
*PAH: pulmonary arterial hypertension, not secondary to pulmonary fibrosis.

Skeletal myopathy was significantly more frequent in the PM-Scl patients than in the comparison group (51% vs. 14%, p<0.0001), as expected, given the high frequency of overlap with myositis in anti-PM-Scl antibody positive patients. There were no significant differences in the frequencies of individual features which characterise muscle involvement such as serum muscle enzyme levels. Gastrointestinal tract involvement was significantly less common in the anti-PM-Scl antibody positive patients (52% vs. 79%, p=0.0001). As with skeletal muscle involvement, no distinguishing gastrointestinal features were identified such as the frequencies of esophageal or small intestinal disease.

Although overall lung involvement was found with equal frequency in the two groups, pulmonary fibrosis was more often detected in anti-PM-Scl positive patients (50% vs. 37%, p<0.0442). In contrast, the severity of pulmonary fibrosis was significantly lower in anti-PM-Scl patients. Using the Medsger et

al. severity index (10), the proportion of patients with severe or end stage interstitial lung disease was 9 of 31 (29%) in anti-PM-Scl antibody positive patients and 417/760 (55%) in the anti-PM-Scl negative comparison group (p=0.00818). Pulmonary arterial hypertension not secondary to pulmonary fibrosis was significantly less common in the anti-PM-Scl positive group (5% vs. 15%, p=0.0395).

There were no significant differences in other organ system involvements. Intracutaneous or subcutaneous calcinosis was more common in anti-PM-Scl antibody positive patients than in the comparison group (34% vs. 17%, p=0.0026). As expected, calcinosis was more frequent in DM than in PM SSc overlap patients (26% vs. 18%, NS). The frequency of Sjögren's syndrome was similar in the two groups.

ANA testing

Among the anti-PM-Scl antibody patients, 66 (87%) had a positive ANA

with nucleolar staining. In contrast, among anti-PM-Scl antibody negative patients, this proportion was 32%, *p*<0.0001. Two SSc patients with anti-PM-Scl antibody had another SSc-associated antibody (1 had anti-U1RNP and 1 had anti-U3RNP).

Causes of death

There were only 6 disease-related deaths among the anti-PM-Scl positive patients, including 3 from pulmonary fibrosis and 2 from pulmonary hypertension.

Survival

Survival was significantly better in anti-PM-Scl antibody positive patients than in the SSc comparison group. Five years after the first physician diagnosis of SSc, all 76 anti-PM-Scl antibody positive patients were living. In contrast, in the anti-PM-Scl negative patient group there was an 80% survival at 5 years. The 10-year cumulative survival rate (CSR) from the first physician diagnosis of SSc for anti-PM-Scl antibody positive patients was over 91%, as depicted in Figure 1, and the 20-year CSR was 66% (*p*<0.0001). After adjustment for age, gender and diffuse cutaneous involvement, patients with PM-Scl antibody were 68% less likely to die at 10 years than those without this antibody (HR=0.32, *p*=0.006), as depicted in Table III.

Discussion

Anti-PM1 antibody was first described by Wolfe *et al.* in 1977 and was proposed to be a marker of polymyositis (11). It was present in 61% of 28 patients with PM or DM and in 7 of 8 patients with a myositis-SSc overlap. Later, it was determined that this antibody was directed against a distinct nuclear antigen in the human exosome. This antigen exists both in the nucleus and, at higher concentrations, in the nucleolus (12, 13). This likely accounts for ANA staining which is predominantly, but not exclusively, nucleolar. The antibody was renamed anti-PM-Scl in recognition of its frequent occurrence in patients with clinical features of SSc or PM/DM or an overlap of these conditions (14). Our goal was to determine if
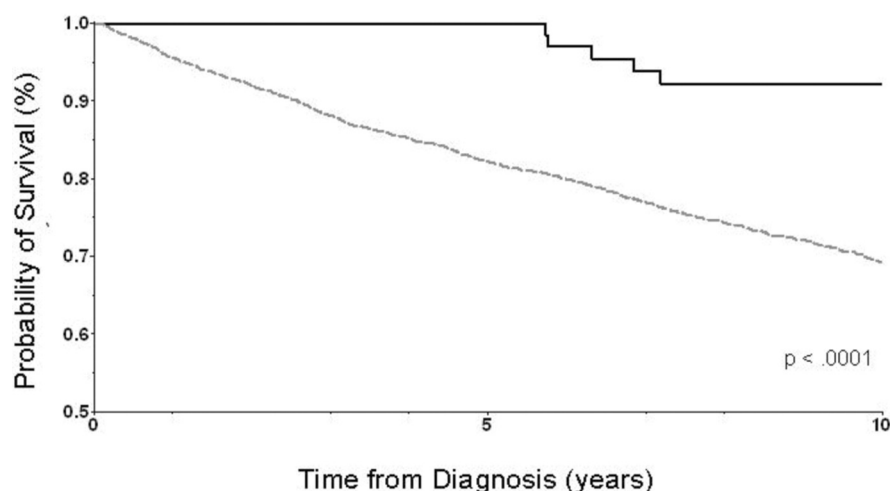

Fig. 1. Cumulative 10-year survival from first physician diagnosis of systemic sclerosis (SSc) in anti-PM-Scl positive and anti-PM-Scl negative SSc patients.
——— Anti-PM-Scl positive (n=76).
– – – PM-Scl negative (n=2349).

Table III. Effect of anti-PM-Scl antibody on mortality after adjustment for age, gender and diffuse cutaneous disease.

	Hazard ratio	95% Confidence interval	*p*-value
Age	1.05	1.04 – 1.06	<0.0001
Female	1.53	1.29 – 1.81	<0.0001
Diffuse skin thickening	1.41	1.22 – 1.65	<0.0001
Anti-PM-Scl antibody	0.32	0.14 – 0.73	0.006

anti-PM-Scl antibody identifies a distinct subset of patients with SSc.

Anti-PM-Scl antibodies are typically found in a small proportion of SSc patients, usually 2–5% (2, 15-17). However, in a recently published study by Hanke *et al.*, anti-PM-Scl testing was performed using a line immunoblast assay in 280 German SSc patients, of whom 35 (13%) were positive (18). There was also a higher frequency of other SSc-associated serum autoantibodies in anti-PM-Scl positive patients in this population, notably anti-topoisomerase I. It is possible that ethnic variations among populations or non-specific reactivity to PM-Scl or topoisomerase I antigens account for these results (19).

A younger age at onset is consistent with anti-PM-Scl antibody being disproportionately frequent in childhood onset SSc and declining in relative frequency with increasing age (20). We found that anti-PM-Scl antibody is strongly associated with SSc with limited cutaneous involvement in overlap with PM/DM as did other authors.

In comparison with SSc patients without this antibody, our anti-PM-Scl positive patients had a reduced frequency of peripheral vascular disease, pulmonary arterial hypertension and gastrointestinal tract involvement. Hanke *et al.* described an increased proportion of patients with digital ulcers (18). This finding could be due to inclusion of ulcers over the proximal interphalangeal joints (traumatic, related to joint contractures) in addition to digital tip ulcers (ischaemic). Although anti-PM-Scl antibody positive patients had a somewhat increased frequency of pulmonary fibrosis, the severity of interstitial lung disease was reduced in these patients, as described in *Results*.

In our Pittsburgh experience, anti-PM-Scl antibody positive patients have the best survival of all SSc-related serologic subsets (2, 16). Most studies in the medical literature combine all anti-PM-Scl patients together, regardless of their clinical classification (SSc alone, SSc with PM/DM, PM/DM alone or other). Three such reports confirmed an excellent prognosis in anti-PM-Scl

antibody positive patients, with a combined 55 of 58 (95%) patients found to be living after 5–8 years of follow-up (10-12). Younger age at onset with fewer co-morbid conditions may contribute to improved survival in these patients. If a patient with SSc has anti-PM-Scl antibody, the managing physician should focus on the activity and severity of both myositis and interstitial lung disease, which can be treated with anti-inflammatory/immunosuppressive therapy to improve functional status or halt disease progression.

In conclusion, we believe that SSc patients with anti-PM-Scl antibody comprise a group of younger patients with prominent skeletal muscle involvement and an excellent prognosis. Testing for this autoantibody should be performed in all SSc patients with a positive nucleolar staining ANA.

References

1. SILVER RM, MEDSGER TA JR, BOLSTER MB: Systemic sclerosis and scleroderma variants: Clinical aspects. *In*: KOOPMAN WJ and MORELAND LW (Eds.): *Arthritis and Allied Conditions: A Textbook of Rheumatology*, 15th edition. Philadelphia, Lippincott Williams & Wilkins, 2005: 1633-80.
2. STEEN V: Autoantibodies in systemic sclerosis. *Semin Arthritis Rheum* 2005; 35: 35-42.
3. BOHAN A, PETER JB: Polymyositis and dermatomyositis (parts 1 and 2). *N Engl J Med* 1975; 292: 344-7, 403-7.
4. HOCHBERG MC: Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
5. ARNETT FC, EDWORTHY SM, BLOCH DA *et al.*: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
6. OKANO Y, STEEN VD, MEDSGER TA: Autoantibody reactive with RNA polymerase III in systemic sclerosis. *Ann Intern Med* 1993; 119: 1005-13.
7. KAO AH, LACOMIS D, LUCAS M, FERTIG N, ODDIS CV: Anti-signal recognition particle autoantibody in patients with and patients without idiopathic inflammatory myopathy. *Arthritis Rheum* 2004; 50: 209-1.
8. POORMOGHIM H, LUCAS M, FERTIG N, MEDSGER TA JR: Systemic sclerosis sine scleroderma: demographic, clinical, and serologic features and survival in forty-eight patients. *Arthritis Rheum* 2000; 43: 444-51.
9. BRENNAN P, SILMAN A, BLACK C *et al.*: Reliability of skin involvement measures in scleroderma. *Br J Rheumatol* 1992; 31: 467-70.
10. MEDSGER TA JR, BOMBARDIERI S, CZIRJAK L, SCORZA R, DELLA ROSA A, BENCIVELLI W: Assessment of disease severity and prognosis. *Clin Exp Rheumatol* 2003; 21: 542-6.
11. WOLFE JF: Antinuclear antibody with distinct specificity for polymyositis. *J Clin Invest* 1977; 59: 176-8.
12. REICHLIN M, MADDISON PJ *et al.*: Antibodies to a nuclear/nucleolar antigen in patients with polymyositis overlap syndromes. *J Clin Immunol* 1984; 4: 40-4.
13. BROUWER R, PRUIJN G, VENROOIJ W: The human exosome: an autoantigenic complex of exoribonucleases in myositis and scleroderma. *Arthritis Res* 2001; 3: 102-6.
14. ODDIS CV, TARGOFF IN: PM-Scl Autoantibodies. *In* PETER JB and SHOENFELD Y (Eds.): *Autoantibodies*. Amsterdam, Elsevier 1996: 642-50.
15. MARGUERIE C *et al.*: The clinical and immunogenetic features of patients with autoantibodies to the nucleolar antigen PM-Scl. *Medicine* 1992; 71: 327-36.
16. VANDERGHYNST F, OCMANT A *et al.*: Anti-PM/Scl antibodies in connective tissue disease: clinical and biological assessment of 14 patients. *Clin Exp Rheumatol* 2006; 24: 129-33.
17. GAUDIN C, FORTENFANT F, ADOUE D, BEYNE-RAUZY O: Anti-PM-Scl autoantibody. Study of prevalence and significance. *Rev Med Interne* 2006; 27: 665-70.
18. HANKE K, BRUCKNER CS, DAHNRICH C *et al.*: Antibodies against PM/Scl-75 and PM/Scl-100 are independent markers for different subsets of systemic sclerosis patients. *Arthritis Res Ther* 2009; 11: R22.
19. MAHLER M, FRITZLER MJ: The changing landscape of the clinical value of the PM/Scl autoantibody system. *Arthritis Res Ther* 2009; 11: 106.
20. SCALAPINO K, ARKACHAISRI T, LUCAS M *et al.*: Childhood onset systemic sclerosis: classification, clinical and serologic features and survival in comparison with adult onset disease. *J Rheumatol* 2006; 33: 1004-13.
21. STEEN V, MEDSGER TA: Changes in causes of death in systemic sclerosis, 1972-2002. *Ann Rheum Dis* 2007; 66: 940-4.