Patients with Antibodies to Both PmScl and dsDNA

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ABSTRACT. ***Objective.*** To determine the significance of dsDNA antibodies in patients with antibodies to PmScl.
Methods. All patients testing positive for PmScl and/or dsDNA antibodies at an academic medical center between 1977 and 2002 were identified. Charts for the PmScl-positive patients were reviewed for manifestations of lupus, scleroderma, or polymyositis/dermatomyositis. Patients with antibodies to dsDNA were matched to each of the double-positive PmScl+/dsDNA+ patients on the basis of sex, race, age, and date of autoantibody testing. Standard classification criteria for lupus, scleroderma, and myositis were used (excluding dsDNA, PmScl, or antinuclear antibodies as criteria), and the number of subjects meeting classification criteria was recorded.
Results. Records were available for 38 out of 47 patients who were identified as PmScl-positive. The prevalence of dsDNA antibodies in this group was 42% (16/38). Patients with PmScl and dsDNA antibodies had a higher prevalence of systemic lupus erythematosus (8/16 vs 2/22; p = 0.008) and a lower rate of scleroderma or myositis (1/16 vs 9/22; p = 0.025) than dsDNA-negative patients with PmScl antibodies. The prevalence of systemic lupus erythematosus, myositis, and scleroderma in patients with PmScl and dsDNA antibodies was not different from the prevalences of these diseases in a matched cohort of patients who were dsDNA-positive.
Conclusion. Antibodies to PmScl are associated with scleroderma and myositis when dsDNA antibodies are not present. In the presence of dsDNA antibodies, PmScl antibodies do not appear to have clinical relevance. (J Rheumatol 2004;31:2169–74)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
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The human PmScl complex is a group of nuclear proteins involved in the processing of ribosomal RNA that is homologous to the yeast exosome protein complex[1]. Proteins of the PmScl complex are targets of autoimmunity, and anti-PmScl autoantibodies have been associated with polymyositis and dermatomyositis (myositis), progressive systemic sclerosis (SSc, scleroderma), or an overlap between the 2[2,3]. However, in several clinical reports describing the association of anti-PmScl with these clinical syndromes, the study populations specifically excluded patients with lupus[4,5]. Moreover, in other studies in which lupus patients were included, PmScl antibodies were associated with systemic lupus erythematosus (SLE) at a detectable frequency[6,7].

Recent data suggest that antibodies to topoisomerase I, which had previously been believed to be highly specific for scleroderma, were in fact present in a substantial number of patients with lupus in conjunction with anti-dsDNA antibodies[8]. Given that dsDNA antibodies have high specificity for the diagnosis of lupus[9], and that PmScl antibodies have been thought to have specificity for scleroderma/myositis, we sought to examine the frequency with which antibodies to these 2 autoantigens coexisted and to characterize the disease phenotype of patients expressing these antibodies.

We analyzed all inpatients and outpatients from the University of Missouri Medical Center and the Harry S Truman Memorial VA Hospital with sera sent to the University of Missouri Antinuclear Antibody (ANA) Testing Laboratory between 1977 and 2002, a period of time over which consistent standardized assays for both PmScl and dsDNA were performed[3]. We identified 47 total patients with antibodies to PmScl, of whom 38 had available clinical information. Of this group, 16/38 (42%) patients also had antibodies to dsDNA. When we collected clinical information on patients with PmScl antibodies, we found that the subgroup of PmScl-positive patients with dsDNA antibodies was distinct from the PmScl single-positive group in having a high prevalence of lupus and a low prevalence of scleroderma/myositis. Rather, these PmScl+/dsDNA+ patients were clinically similar to a matched group of dsDNA single-positive patients. These results provide further insight into the interpretation of positive PmScl laboratory studies, and into the pathogenesis of PmScl-associated autoimmune syndromes.

MATERIALS AND METHODS

Patients. All laboratory and clinical data were collected following institutional review board protocols. Patients seen at the University of Missouri Medical Center or the Harry S Truman Memorial VA Hospital who had

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positive tests for PmScl and/or dsDNA performed at the University of Missouri Antinuclear Antibody Testing Laboratory between 1977 and 2002 were identified from a computer database as described[10]. Since it was standard practice at our centers to order these tests as part of an autoantibody profile, in almost every case, both tests were performed. Structured retrospective chart reviews were then performed to abstract data regarding the clinical disease manifestations present for each patient with PmScl antibodies, and for matched patients with dsDNA antibodies. The chart review gathered all clinical and laboratory data relevant to the classification criteria for the diagnoses of SLE [using the 1997 revision of the 1982 American College of Rheumatology (ACR) criteria[11,12]], scleroderma (using the 1980 preliminary ACR criteria[13]), and myositis (using the Bohan and Peter criteria for definite polymyositis and dermatomyositis[14,15]). This explicitly included clinical data from before the laboratory studies were performed, concurrent data, and data from any subsequent followup. The classification schemes were applied to each patient, excluding the use of dsDNA and PmScl positivity as diagnostic criteria. Since PmScl could cause ANA positivity, ANA were also not considered as a diagnostic criterion. For the initial phase of chart review with the dsDNA+ patients, only sex, race, age at autoantibody testing, and date of autoantibody testing were obtained; for subjects identified as matches to members of the PmScl+ cohort, further information was gathered as in the PmScl+ patients by an investigator blinded to the matching. For analysis of anti-RNP-positive patients, the classification criteria of Alarcon-Segovia for mixed connective tissue disease (MCTD) were also applied[16].

Autoantibody assays. Autoantibodies were measured in the University of Missouri ANA Testing Laboratory using consistent procedures instituted and monitored by Dr. Gordon Sharp, the Director or Emeritus Director of the Laboratory for the duration of the study. Antibodies to PmScl were determined by double immunodiffusion, using a calf thymus nuclear extract antigen source and prototype PmScl-positive antisera controls, as described[3]. Double-stranded DNA antibodies were assayed by immunofluorescence using *Crithidia lucillae*, as described[17]. Consistent with the clinical results reporting-threshold for the University of Missouri ANA Testing Laboratory, patients ever found to have dsDNA titers of at least 1:10 were designated positive; all others were designated negative for dsDNA antibodies. ANA patterns were determined by immunofluorescence on a HEp-2 substrate, as described[3]. RNP antibodies were determined by hemagglutination, immunodiffusion, and immunoblotting, as described[10].

Statistics. Comparisons between group proportions were performed using Fisher's exact test with Prism 3.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Between 1977 and 2002, a total of 9021 patients at the University of Missouri Medical Center and the Harry S Truman Memorial VA Hospital underwent assays for dsDNA and PmScl antibodies. An additional 284 patients had assays for dsDNA but not PmScl, and 12 patients had assays for PmScl but not dsDNA. Out of this total population, 47 patients (0.5%) were found to be positive for antibodies to PmScl, and 421 patients (4.5%) were found to have dsDNA antibodies.

Clinical data were available to evaluate for 38/47 PmScl-positive patients; dsDNA antibodies had been checked in each of these cases. Of these, 22/38 (58%) were negative for antibodies to dsDNA, but 16/38 (42%) had dsDNA antibodies at a significant titer (Table 1). Of the PmScl-positive patients with clinical information available, 10/38 met diagnostic criteria for scleroderma and/or myositis (Table 2); 9/10 (90%) of these scleroderma/myositis patients were in the dsDNA-negative subgroup (Fisher's exact test, $p = 0.025$). On the other hand, out of the overall group of PmScl-positive patients, 10/38 met diagnostic criteria for SLE (Tables 1 and 2); 8/10 (80%) of these lupus patients were in the dsDNA positive subgroup (Fisher's exact test, $p = 0.008$). The lupus cases in the PmScl+/dsDNA+ subgroup experienced major manifestations including neuropsychiatric lupus in 3 cases (of which one case also had glomerulonephritis), and biopsy-proven glomerulonephritis in 3 additional cases (Table 1). Neuropsychiatric or renal manifestations were not present in either dsDNA-negative SLE case.

A matched cohort of one dsDNA-positive patient with available clinical information was selected for each of the 16 PmScl-positive patients studied. Matching was performed strictly on the basis of sex, race, age, and (when possible) date of autoantibody assays out of the pool of 421 dsDNA-positive patients from our center (Table 3). We were able to match every PmScl+/dsDNA+ subject with a dsDNA+ patient of the same sex and race. In most cases, we were also able to match to patients of similar age who had undergone autoantibody testing at a similar time. The age match and timespans between autoantibody testing for matched patients who were male, non-white, or in older age groups were less close in some instances, due to the relative paucity of such subjects in our dsDNA+ cohort. Only one of the PmScl+/dsDNA+ patients (Patient 5) was from the Harry S Truman Memorial VA Hospital; a matched patient for this case could only be found at the University of Missouri Medical Center. For each matching subject that was selected, we found that PmScl had been tested, although this was not used as a criterion in the matching process.

As a test of the robustness of our matching procedure, we compared the dsDNA titers of the PmScl+/dsDNA+ patients to their matches (Table 3). In 8/16 cases, the dsDNA titers were within 2 dilutions of each other; in 6 cases the PmScl+/dsDNA+ patients had significantly lower dsDNA titers than their matches, while in 2 cases the PmScl+/dsDNA+ patients had significantly higher dsDNA titers than their counterparts. The absence of a strong bias of dsDNA titers in favor of either group suggested that the 2 groups were well matched.

Comparing the clinical manifestations of the dsDNA single-positive patients to the patients double-positive for dsDNA and PmScl antibodies, we observed a similarly high frequency of SLE and low frequency of scleroderma/myositis in both groups (Tables 4 and 5). Out of the PmScl+/dsDNA+ patients, 8/16 met criteria for SLE, but only 1/16 met criteria for either scleroderma or myositis. Similarly, out of the matched dsDNA+ cohort, 7/16 subjects met criteria for SLE, and only 1/16 met criteria for scleroderma or myositis. Thus, in these patients with dsDNA antibodies, the presence or absence of PmScl antibodies did not appear to affect the likelihood of having scleroderma, myositis, or lupus.

Table 1. dsDNA status and rheumatic disease manifestations of a cohort of 38 patients with PmScl antibodies.

Patient	PmScl	dsDNA	SSc Manifestations*	PM Manifestations**	SLE Manifestations†
1	+	+			***Ma, Ph, A, Se, Re, H***
2	+	+	RP		***Ma, D, Ph, U, A, Se, Re, N, H***
3	+	+			
4	+	+	RP, Pu		***Ma, Ph, U, A, Re, H***
5	+	+			***Ma, Ph, U, A, Se, N, H***
6	+	+	Sc,RP	DM	Ph, U, A
7	+	+	Sc, RP		Ma, A
8	+	+			A, Se, H
9	+	+			Ma, Ph, A
10	+	+	Sc, RP		
11	+	+			***D, Ph, U, A***
12	+	+			
13	+	+	***Sc, RP, Pu***	We, DM	***Ma, Ph, A, Se, H***
14	+	+	RP		***Ma, Ph,U, Re, H***
15	+	+			H
16	+	+		We	***Ma, Ph, N, H***
17	+	–			
18	+	–			Di, Ph, N
19	+	–	***Sc, RP, Pu***		A, Se, Re
20	+	–	RP		***Ma, Ph, U, Se, I***
21	+	–			Re
22	+	–	***Sc, RP, DP***	We, DM	
23	+	–	***Sc, RP, Pu***	DM	Ma, A, Se
24	+	–	Sc, RP	We, DM	
25	+	–	RP		Se, Re, H
26	+	–	Sc, RP	***We, Bx, My, DM***	
27	+	–	Pu	***We, Bx, My, DM***	U, A
28	+	–			
29	+	–	RP	DM	Ph, U, Se
30	+	–	Sc, RP		***Ph, A, H, I***
31	+	–	***Sc, RP, DP***	DM	U
32	+	–			N
33	+	–	Sc, RP	We, Bx	A
34	+	–			A
35	+	–	Sc	***We, Bx, DM***	
36	+	–	RP	***We, Bx, DM***	
37	+	–	***Sc, RP, DP***	We	A
38	+	–	RP		U

* ACR criteria[13]; ** Bohan and Peter criteria[14,15]; † ACR criteria[11,12]. PmScl: anti-PmScl antibodies detected; dsDNA: anti-doublestranded DNA antibodies detected; Sc: sclerodactyly; RP: Raynaud's phenomenon; DP: digital pits; Pu: pulmonary fibrosis; We: proximal muscle weakness; Bx: muscle biopsy consistent with myositis; My: electromyogram consistent with myositis; DM: classic dermatomyositis rash; Ma: malar rash; D: discoid rash; Ph: photosensitivity; U: oral ulcers; A: arthritis; Se: serositis; Re: glomerulonephritis; N: neuropsychiatric lupus; I: immunologic abnormalities (excluding ANA and dsDNA). Bold and italicized entries show that classification criteria for the indicated diagnosis were met.

In a secondary analysis, when we compared the PmScl+/dsDNA+ patients to the PmScl+/dsDNA– patients based on individual clinical manifestations of disease, trends toward the same patterns observed for the overall diseases were also present. The largest differences in prevalence between individual clinical characteristics between the groups were for malar rash and for manifestations satisfying the "hematologic disease" classification criterion for SLE (each present in 9/16 PmScl+/dsDNA+ vs 2/22 PmScl+/dsDNA– patients; Fisher exact test, p = 0.0029). These differences approached but did not reach statistical significance after the Bonferroni correction for multiple comparisons was applied. The traits with the greatest increase in prevalence in the PmScl+/dsDNA– group compared to the PmScl+/dsDNA+ group were dermatomyositis rash (9/22 vs 2/16 patients; Fisher exact test, p = 0.08) and sclerodactyly (10/22 vs 4/16 patients; Fisher exact text, p = 0.3).

When we examined the ANA staining patterns reported for patients in each group, we found that only one of 9 PmScl+/dsDNA+ patients in whom this information was available had nucleolar staining, while 8 of 16

Table 2. Associations of dsDNA antibody status and diagnoses in patients with PmScl antibodies.

	Meets Criteria for SLE	Meets Criteria for SSc or Myositis
PmScl+/dsDNA+	8/16*	1/16
PmScl+/dsDNA–	2/22	9/22†

PmScl+/dsDNA+: patients with antibodies to both PmScl and dsDNA; PmScl+/dsDNA–: patients with antibodies to PmScl but not dsDNA antibodies. * Fisher's exact p = 0.008 versus PmScl+/dsDNA– patients. † Fisher's exact p = 0.025 versus PmScl+/dsDNA+ patients.

PmScl+/dsDNA– patients with available data had this classical PmScl-associated pattern (Fisher exact test, p = 0.0875)[4]. Notably, the one PmScl+/dsDNA+ patient who did have a nucleolar pattern had been diagnosed with scleroderma/myositis overlap syndrome clinically, although this patient failed to meet formal classification criteria for SLE, scleroderma, or myositis. In comparison, of the 8 PmScl+/dsDNA– patients with the nucleolar pattern, 7 had been clinically diagnosed with scleroderma, myositis, or both; 6 met formal classification criteria for either scleroderma (3 patients) or myositis (3 additional patients), and none met criteria for SLE.

Table 3. Matching of a cohort of dsDNA+ patients to the PmScl+/dsDNA+ patients.

PmScl Patient*	Sex	Race	Age at Test, yrs	dsDNA Titer	Match Patient**	Sex	Race	Age at Test, yrs	dsDNA Titer	Years Between†
1	F	W	36	1:160	39	F	W	38	1:4000	2
2	F	B	35	1:10	40	F	B	39	1:160	10
3	F	B	33	1:16	41	F	B	35	1:16	8
4	F	W	39	1:80	42	F	W	36	1:10	1
5	M	W	27	1:80	43	M	W	28	1:5120	20
6	F	W	22	1:40	44	F	W	21	1:128	10
7	F	W	32	1:20	45	F	W	35	1:1280	1
8	F	W	73	1:20	46	F	W	77	1:20	1
9	F	W	62	1:160	47	F	W	62	1:40	1
10	F	W	52	1:10	48	F	W	53	1:160	5
11	M	W	64	1:32	49	M	W	60	1:40	8
12	M	W	27	1:16	50	M	W	23	1:320	1
13	F	W	31	1:10	51	F	W	34	1:16	1
14	M	W	44	1:20	52	M	W	59	1:10	9
15	F	O	66	1:32	53	F	O	74	1:40	8
16	F	W	60	1:320	54	F	W	59	1:40	0

* Patient number as in Table 1. Age at test: age when dsDNA antibody testing was performed. ** Patient number assigned to the dsDNA+ patient from our center matched to the corresponding PmScl+ patient (matching done strictly on the basis of sex, race, age at test, and when possible year of testing). † Years between the time that dsDNA testing was performed for the matched patients. W: white; B: black; O: other.

Table 4. Clinical characteristics of dsDNA+ matched cohort.

Patient	PmScl	dsDNA	SSc Manifestations	PM Manifestations	SLE Manifestations
39	–	+	RP	We	***Ma, U, A, Se, Re, H***
40	–	+	RP	We	***Ma, Se, Re, H***
41	–	+			N
42	–	+	Pu		A
43	–	+			U, Se, N
44	–	+			***Ma, U, Se, Re, N, H***
45	–	+			***Ma, Ph, U, A, Se, Re, H***
46	–	+		We	Ph, A
47	–	+		We	A
48	–	+	RP		Ma, U, N
49	–	+			***Ph, U, A, Re, N***
50	–	+	RP	We, My	***Ma, Ph, U, A, Re, H, I***
51	–	+	RP	We, My	***Ma, Ph, A, Se***
52	–	+			A
53	–	+	***Sc, RP, Pu***		Se, H
54	–	+			A

Abbreviations as in Table 1; patient numbers as in Table 3. Bold and italicized entries show that classification criteria for the indicated diagnosis were met.

Table 5. Lack of associations of PmScl antibody status with diagnosis in patients with dsDNA antibodies.

	Meets Criteria for SLE	Meets Criteria for SSc or Myositis
PmScl+/dsDNA+	8/16	1/16
Matched dsDNA+	7/16	1/16

Matched dsDNA+: cohort of patients with dsDNA antibodies matched to the PmScl+/dsDNA+ patients on the basis of sex, race, age, and date of testing (see Table 3).

Anti-RNP antibodies were present in 2/16 PmScl+/dsDNA+ patients, both of whom met classification criteria for SLE and MCTD. Anti-RNP antibodies were present in 5/22 PmScl+/dsDNA– patients, one of whom met classification criteria for both SLE and MCTD, one of whom met criteria for SLE only, and one of whom met criteria for MCTD only. The additional 2 RNP+/PmScl+/dsDNA– patients did not meet criteria for any specific rheumatic diagnosis. Anti-RNP antibodies were present in 1/16 patients in the matched PmScl–/dsDNA+ cohort of patients; this patient met criteria for SLE only. It thus appears that RNP antibodies occur at a measurable but low rate in PmScl+ patients, and that these antibodies occur at similar rates in dsDNA+ and dsDNA– subgroups.

We considered the possibility that PmScl+/dsDNA+ patients might have an increased tendency to develop "overlap syndrome" manifestations of scleroderma and/or myositis to a greater extent than their matched dsDNA+ counterparts, even if such patients did not completely fulfill scleroderma or myositis diagnostic criteria. However, there was no significantly increased incidence of any scleroderma or myositis manifestation recorded for this study in the PmScl+/dsDNA+ patients compared to the matched dsDNA+ group (Tables 1 and 4).

DISCUSSION

This study describes the clinical manifestations of one of the largest cohorts of PmScl-positive patients reported to date. Unlike previous studies, this report also assessed patients for anti-dsDNA antibodies, and collected clinical data relevant to the classification criteria for SLE as well as for scleroderma and myositis. We discovered that a substantial proportion of PmScl-positive patients at our center also had dsDNA antibodies, and that the presence of dsDNA antibodies was as predictive of a diagnosis of lupus in PmScl-positive patients as in a matched cohort of patients that were single-positive for antibodies to dsDNA alone.

These results echo those of Gussin, *et al* with regard to anti-topoisomerase I antibodies and anti-dsDNA[8]. In both studies, antibodies typically thought to be specific for scleroderma occurred in patients with lupus, but only in the context of dsDNA antibodies. The implications of these results with regard to PmScl testing include the following. Clinically, patients with anti-PmScl but without anti-dsDNA are likely to have scleroderma/myositis overlap, and are unlikely to have lupus. Conversely, lupus should be strongly considered in dsDNA+ patients, regardless of their PmScl antibody status.

Immunologically, further studies will be needed to determine whether PmScl antibody expression (and topoisomerase I antibody expression) in lupus is due to nonspecific polyclonal activation of autoantibody-secreting B cells, or to an antigen-driven phenomenon. It is notable that these scleroderma-associated autoantibodies appear to develop particularly in the minority of lupus patients who are dsDNA-positive. If antigen-driven, our results suggest that the immunogens that drive the development of PmScl and topoisomerase I immunity in scleroderma/myositis are distinct from the lupus immunogen(s) that lead to these antibodies, in that the scleroderma-associated immunogens do not contain antigenic dsDNA. The variation in the ANA patterns observed in PmScl+/dsDNA+ patient sera from the nucleolar pattern classically associated with PmScl antibodies also suggests that alternative forms of PmScl antigens are being targeted.

Limitations of this study include its retrospective design, and the reliance on clinical data from only a single medical center and its affiliated veterans hospital, potentially introducing both reporting bias and referral bias. Despite the relatively large size of the PmScl+ cohort, group sizes were not large enough to identify differences in the prevalence of individual disease manifestations between PmScl+ patients with and those without dsDNA antibodies. We speculate that the increased prevalence of PmScl+/dsDNA+ patients at our center compared to other reports may be due to the inclusion of PmScl testing in the routine panel of autoantibody assays ordered for a wide variety of indications. This may have led to a reduced pretest probability of a diagnosis of scleroderma/myositis compared to cohorts assembled at centers where PmScl testing needed to be specially ordered. On the other hand, other reports of PmScl+ cohorts have also described PmScl-positive SLE patients[6,7], in whom dsDNA antibodies were present when dsDNA testing was performed.

Additionally, the components of the PmScl complex with which antibodies reacted in individual patients were not identified in this study. It has been reported that the majority of scleroderma/myositis patients with PmScl antibodies have reactivity with a small number of epitopes on the 100 kDa protein subunit of PmScl[18]. However, multiple components of the PmScl/exosome complex have also been documented to be human autoantigens[5], and these or other PmScl-associated molecules or PmScl cross-reactive molecules could be the targets of autoimmunity in the PmScl+/dsDNA+ patients. It is an open question whether fine mapping of the anti-PmScl epitopes of PmScl+/dsDNA+ patients would find different epitope-targeting than in isolated PmScl+ patients. It will also be of

interest to determine in future studies whether patients double-positive for antibodies to PmScl and dsDNA have the same strong HLA-DR3 association reported for PmScl antibodies[4,7]. The absence of increased scleroderma or myositis overlap symptoms in the dsDNA+/PmScl+ patients seen in our study suggests that the PmScl antibodies arising in these patients do not themselves mediate scleroderma or myositis pathogenesis. If these antibodies prove to be indistinguishable from those seen in isolated PmScl+ patients, this would suggest that PmScl antibodies in general are a marker rather than a mediator of disease.

In summary, a significant subset of patients with PmScl antibodies also have antibodies to double-stranded DNA. The presence of dsDNA antibodies as well as PmScl antibodies in the same patient is associated with SLE, while the classic myositis/scleroderma syndrome seen with PmScl antibodies is associated with the absence of dsDNA antibodies.

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