Serum Autoantibodies and Their Clinical Associations in Patients with Childhood- and Adult-Onset Linear Scleroderma. A Single-Center Study

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ABSTRACT. ***Objective.*** To determine the frequency of selected serum autoantibodies and their clinical associations in patients with childhood-onset (ChO) or adult-onset (AO) linear scleroderma (LiScl) evaluated at a single institution.

Methods. Seventy-two patients (ChO = 40, AO = 32), including 12 with *en coup de sabre*, were studied. All ChO patients had disease onset before age 16 years. Clinical features (extent of cutaneous disease, activity, and joint contractures) were recorded. Antinuclear antibodies (ANA) were identified by indirect immunofluorescence (HEp-2 cells), and anti-single-stranded DNA (anti-ssDNA), antihistone (AHA), and antichromatin (AChA) autoantibodies were detected by ELISA.

Results. There were no significant differences between groups in regard to gender, proportion with LiScl/E, or clinical features except joint contractures (ChO > AO; $p = 0.04$). There were no differences in the frequency of ANA or other autoantibodies between the groups except for AHA (ChO > AO). AHA was more frequently found with anti-ssDNA ($p < 0.0001$). LiScl patients with positive anti-ssDNA and/or AHA had more extensive cutaneous involvement and more often had joint contractures ($p < 0.05$). Anti-ssDNA was present more frequently in AO than in ChO patients with active lesions ($p = 0.04$). ANA and AChA were not associated with any clinical features. Both AHA and anti-ssDNA levels showed good correlation with disease severity.

Conclusion. Over two-thirds of LiScl patients had ANA. Patients with ChO were similar to those with AO with regard to the frequency of selected serum autoantibodies. Anti-ssDNA and AHA were frequently found together and both were associated with more extensive skin disease with joint contractures. LiScl disease severity correlated with the serum levels of both these antibodies. (First Release Nov 1 2008; J Rheumatol 2008;35:2439–44; doi:10.3899/jrheum.080098)

Key Indexing Terms:
LINEAR SCLERODERMA AUTOANTIBODIES CLINICAL ASSOCIATIONS

Localized scleroderma (LS) has distinctive cutaneous features that are quite different from those of systemic sclerosis (SSc). Although both are recognized to be autoimmune diseases, LS does not affect internal organs. Its incidence was estimated to be 2.7 per 100,000 in a population-based study, similar to the estimated incidence of SSc of 20 new cases per million population annually[1,2]. Children are afflicted 9–10 times more frequently with LS than SSc, while childhood-onset (ChO) SSc is rare[3]. Classification of LS is descriptive. Based on morphologic appearance, 3 main subtypes are recognized: circumscribed morphea, generalized morphea, and linear scleroderma (LiScl)[4,5]. LiScl is the most common form of LS reported in children (65%–67%)[2,6]. It is characterized by one or more linear or band-like areas of abnormal skin. LiScl includes "*en coup de sabre*," which consists of linear lesions affecting the face or scalp. The consequences of LS include localized growth failure, joint contractures, cosmetic issues, and psychological disturbances[2,6,7]. These complications occur more commonly in LiScl, as it frequently involves underlying subcutaneous tissue, skeletal muscle, and, rarely, bone[7].

Although the pathogenesis of LS remains unclear, immunologic abnormalities are common, as evidenced by the presence of lymphocytes and plasma cells in skin lesions. Serum autoantibodies, including antinuclear antibodies (ANA; 46%–63% using HEp-2 cells as substrate),

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anti-single-stranded DNA (anti-ssDNA), antihistone antibody (AHA), antitopoisomerase IIa, anti-nucleosome antibodies, and rheumatoid factor are frequently detected[8-18]. These reports included all subtypes of LS and most had small numbers of patients.

We examined the frequencies of serum antibodies, including ANA, anti-ssDNA, and AHA, and their clinical associations in a large, single-center cohort of patients with LiScl. Since the most common ANA staining pattern found in patients with LS is homogeneous, we also examined antichromatin antibody (AchA)[9,19-21].

MATERIALS AND METHODS

Patients. Patients with LS seen at the University of Pittsburgh adult and pediatric scleroderma clinics or entered into the National Registry for Childhood Onset Scleroderma between November 1991 and December 2005, and whose serum was available, were included. Patients with disease onset before age 16 years were classified as having childhood onset (ChO). Serum samples were obtained from all patients at their first evaluation after written informed consent/assent was obtained. The study was approved by the University of Pittsburgh Institutional Review Board.

Disease activity and extent of skin involvement. All patients were examined by one or both of the study rheumatologists (TAM, TA). An "active" lesion was defined as one having any of the following characteristics: new lesion developing within the previous 6 months; enlargement of a prior lesion within the last 6 months; or a lesion with an erythematous border. Contracture was defined as limited range of motion of a joint secondary to skin and subcutaneous tissue involvement, but not due to arthritis.

In assessing the extent of LiScl lesions, we divided the skin surface into 14 sites (head, neck, chest, abdomen, upper back, lower back, right and left upper arms, right and left forearms/hands/fingers, right and left buttocks/thighs, and right and left legs/feet/toes). The total number of sites affected in each patient was recorded.

Localized Scleroderma Severity Score (LSS). In order to demonstrate changes of disease severity (activity plus damage) in relation to changes in autoantibody levels over time, we used a recently published skin severity scoring system[22] based on assessment of the extent (surface area) and intensity (erythema, skin thickness) of LS lesions as well as new lesion development and enlargement of existing lesions. The score includes 4 elements, as follows.

(1) Surface Area Score (SA): 14 surface anatomic sites (see above) were graded as 0: no involvement; 1: ≤ 1/3 of the surface area affected; 2: > 1/3 to 2/3; or 3: > 2/3 affected.

(2) Erythema Score (ES): the color of a lesion's border was graded as 0: normal or postinflammatory hyper/hypopigmentation; 1: slight erythema/pink; 2: red; and 3: dark red or violaceous.

(3) Skin Thickness Score (ST): each individual lesion was graded using palpation: 0: normal skin thickness; 1: mild increase in thickness (skin firm but mobile); 2: moderate thickness (skin difficult to move); 3: marked thickness (skin impossible to move).

(4) New Lesion/Extension of Existing Lesion (N/E): any extension of a preexisting lesion or new lesion developing within the previous 6 months was given a score of 3.

This scoring method was applied to the most representative lesion in a given cutaneous site. For each surface anatomic site, the SA, ES, ST, and N/E were recorded and then combined. Thus each site could have a maximum score of 12, and the maximum total body score would be 14 × 12 or 168. For example, a patient developed 2 new lesions during the past 4 months on the right lower back and abdomen (2 surface areas). Less than half of the lower back (SA = 1), but almost the entire abdomen (SA = 3) was involved. There was 3+ skin thickness in the back and 2+ in the abdomen, with faint erythema (ES = 1) on the back and definite erythema (ES = 2) on the abdomen. In summary, both sites had new lesions (N/E = 3 for lower back and N/E = 3 for abdomen). These lesions have severity scores of 8 for the lower back and 10 for the abdomen, giving a total LSS of 18. This severity index was determined by one author (TA).

Serologic studies. Sera were stored at –80°C. ANA was detected by indirect immunofluorescence on HEp-2 cell substrate (HEp-2000®; Immunoconcepts, Sacramento, CA, USA), as described[8,9]. A dilution ≥ 1:40 was considered positive. Anti-ssDNA, AHA, and AChA were determined by ELISA kits (Inova Diagnostics, San Diego, CA, USA) according to the manufacturer's directions. Anti-ssDNA levels > 69 U, AHA > 1 U, and AChA > 20 U were considered positive. In serial anti-ssDNA measurements, we used 2 different ELISA kits (Inova Diagnostics; Bio-Rad Laboratories, Hercules, CA, USA) for 2 patients; in both instances a level > 20 U was considered positive.

Statistical analysis. Means and standard deviations were used to describe continuous data. Chi-square and Fisher's exact tests were used for differences between proportions. The Kolmogorov-Smirnov test was used to determine the normality of data. Student's t test was used for differences between means. A p value < 0.05 was considered to be significant. All statistical procedures were performed with Stata software v.8 (Stata Corp., College Station, TX, USA) and SPSS v.15 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic and clinical features. Table 1 summarizes the demographic and clinical characteristics of the patients. Seventy-two patients with LiScl were included. Over 80% of all patients were female. Forty (56%) of the 72 patients had childhood-onset (ChO) disease. Six ChO and 6 adult-onset (AO) patients had LS *en coup de sabre*. The mean ages at onset of ChO and AO were 8.8 ± 3.9 and 32.9 ± 14.5 years, respectively. The mean ages at the time of sera collection were 14.4 ± 9.3 years for ChO and 36.0 ± 14.0 years for AO. About half of LiScl patients (44%) had more than 2 anatomic sites involved, and AO patients significantly less frequently had > 2 cutaneous sites affected (31% in AO vs 55% in ChO; p = 0.04). Each onset group had a similar proportion with active disease at first visit. ChO patients had a significantly greater frequency of joint contracture (40% vs 19%; p = 0.04).

Thirty-two patients were not taking any medication; 13 were using topical corticosteroids only, 1 topical corticosteroids and calcipotriol, 1 topical tacrolimus and calcipotriol, 6 D-penicillamine (D-pen), 1 D-penicillamine and methotrexate (MTX), 4 D-penicillamine and prednisone, 3 prednisone only, 3 MTX only, 3 MTX and prednisone, 1 penicillin, 1 hydroxychloroquine, 1 MTX and hydroxychloroquine, and 2 PUVA. The proportions of LiScl patients with joint contractures or extensive cutaneous disease were not different when patients were dichotomized according to duration from onset to diagnosis (n = 66; ≤ 12 months vs > 12 months; p = 0.848 and p = 0.860), disease duration (≤ 2 years vs > 2 years; p = 0.072 and p = 0.635), any of the above types of therapy (p = 0.204 and p = 0.205), and durations of treatment (≤ 2 years vs > 2 years; p = 0.449 and p = 0.778), respectively.

Autoantibody frequency. Table 2 shows the prevalence of serum autoantibodies in this LiScl patient population. ANA

Table 1. Demographic and clinical features.

	Childhood Onset (ChO), n = 40	Adult Onset (AO), n = 32	Total, n = 72	ChO vs AO
En coup de sabre, n (%)	6 (15)	6 (19)	12 (17)	NS
Female, n (%)	31 (78)	27 (84)	58 (81)	NS
Age at onset, yrs ± SD	8.8 ± 3.9	32.9 ± 14.5		
Disease duration, yrs, median (range)	2.5 (0.3–33.0)	2.0 (0.3–15.0)	2.1 (0.3–33.0)	
> 2 sites affected, n (%)	22 (55)	10 (31)	32 (44)	0.04
Active disease, n (%)	23 (58)	20 (63)	43 (60)	NS
Contractures, n (%)	16 (40)	6 (19)	22 (31)	0.04

NS: not significant.

Table 2. Autoantibody prevalence.

	Childhood Onset (ChO), n = 40	Adult Onset (AO), n = 32	Total, n = 72	ChO vs AO
ANA, n (%)	27 (68)	22 (69)	49 (68)	NS
Anti-ssDNA*, n (%)	14/39 (36)	6/31 (19)	20/70 (29)	NS
Antihistone*, n (%)	19/39 (49)	8/31 (26)	27/70 (39)	0.05
Antichromatin*, n (%)	5/38 (13)	3/31 (10)	8/69 (12)	NS
Anti-ssDNA and antihistone, n (%)	12/39 (31)	4/31 (13)	16/70 (23)	NS

* Denominator indicates patients tested for the variable. NS: not significant.

was detected in 49 (68%) of all LiScl patients combined, similar in the ChO and AO groups. A homogeneous nuclear staining pattern was found more frequently than speckled or nucleolar patterns (63%, 33%, and 4%, respectively), and no sample had centromeric staining. One-third of patients had anti-ssDNA, which was found more often in ChO than AO patients (36% vs 19%; p = 0.13). AHA was detected more frequently in ChO than AO patients (49% vs 26%; p = 0.05). AChA was infrequently found. Anti-ssDNA was found more frequently than AHA (p < 0.001). Sixteen patients (23%) had both anti-ssDNA and AHA and 39/70 (56%) had neither of these antibodies. Half of the ANA-positive patients (54%) had none of the other serum autoantibodies. Of 12 patients having *en coup de sabre*, 11 (92%) had a positive ANA but only one-third or fewer had anti-ssDNA, AHA, or AchA.

Autoantibodies and clinical associations. Table 3 summarizes the clinical associations of serum autoantibodies in this patient population. ANA was not associated with extent of cutaneous LiScl, disease activity, or joint contractures. Anti-ssDNA, AHA, and AChA were all associated with involvement of more than 2 cutaneous sites. None of the antibodies was associated with LiScl disease activity. The presence of anti-ssDNA and AHA was associated with joint contractures in ChO but not in AO patients. In ChO patients, AChA was also associated with joint contractures (p = 0.032). LiScl patients with both anti-ssDNA and AHA had more extensive disease and a greater frequency of joint contractures compared to patients with neither of these serum autoantibodies (p < 0.01).

Autoantibody levels and clinical characteristics. Serum levels of autoantibodies and their clinical associations are illustrated in Figure 1. Significantly higher mean anti-ssDNA levels were detected in LiScl patients with more extensive skin involvement (103.50 ± 116.17 U vs 47.47 ± 59.81 U; p

Table 3. Presence of autoantibodies and their clinical associations.

	No. of Sites Affected*			Disease Activity			Contractures		
Autoantibodies	ChO	AO	Total	ChO	AO	Total	ChO	AO	Total
ANA	NS	NS	NS	NS	NS	NS	NS	NS	NS
Anti-ssDNA	NS	0.004	0.001	NS	NS	NS	0.013	NS	0.012
Antihistone	NS	NS	0.007	NS	NS	NS	0.015	NS	0.004
Anti-ssDNA and antihistone**			0.002			NS			0.006
Antichromatin	NS	NS	0.044	NS	NS	NS	0.032	NS	NS

* Comparison between ≤ 2 and > 2 sites. ** Comparison between anti-ssDNA and antihistone both positive vs both negative. ChO: childhood onset; AO: adult onset. NS: not significant.

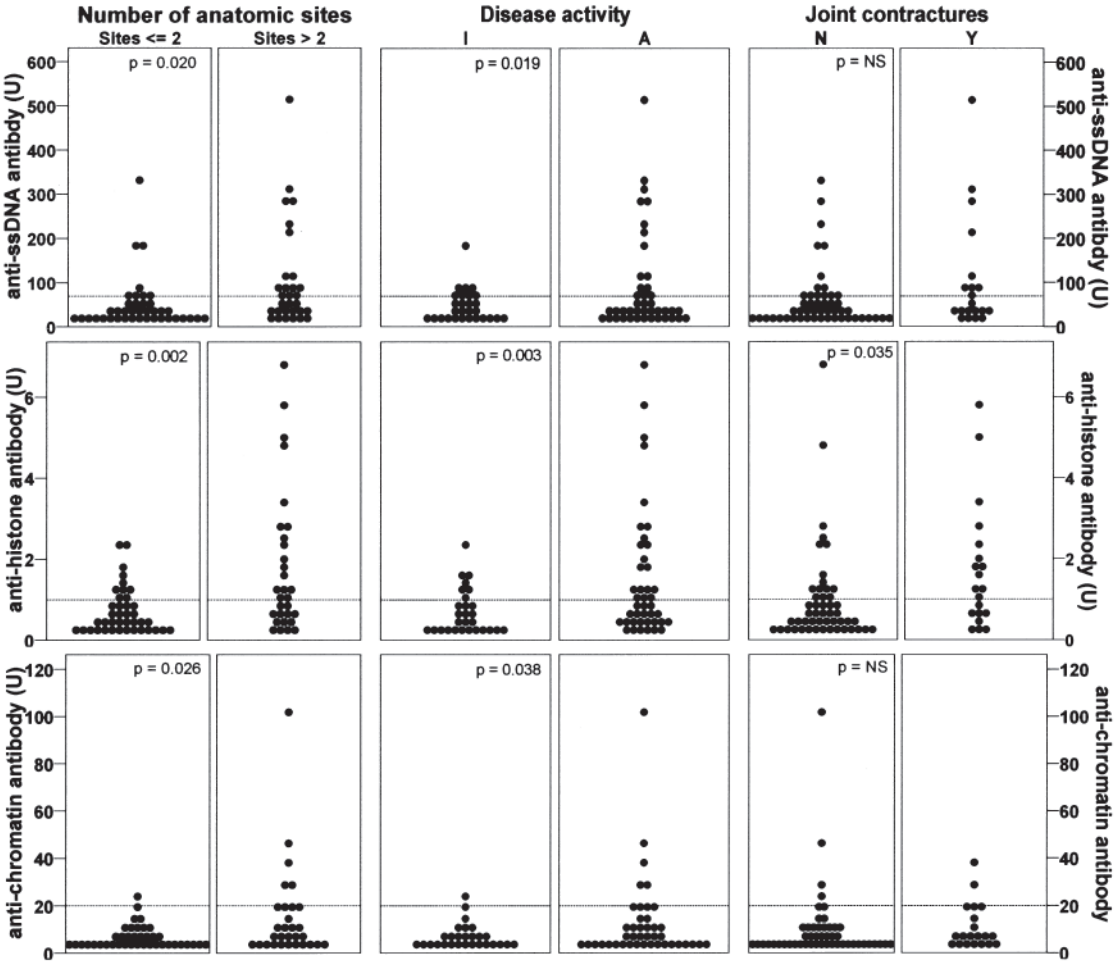

Figure 1. Serum autoantibody levels by ELISA and their clinical association in patients with linear scleroderma. Anti-ssDNA, antihistone, and antichromatin antibody levels are shown in the top, middle, and bottom panels, respectively. Broken lines indicate cutoff values. Number of affected sites (≤ 2 vs > 2), disease activity (I: inactive, A: active), and joint contractures (N: no, Y: yes) are shown on the left, middle, and right.

= 0.020) and with active disease (89.42 ± 112.22 U vs 44.58 ± 35.83 U; p = 0.019). Mean AHA levels were significantly higher in patients with more skin areas involved (1.8 ± 1.77 U vs 0.71 ± 0.56 U; p = 0.002), active disease (1.5 ± 1.59 U vs 0.69 ± 0.54 U; p = 0.003), and joint contractures (1.7 ± 1.54 U vs 0.96 ± 1.20 U; p = 0.035). Although AChA was found less frequently in this LiScl patient cohort, patients with more skin surface-area involvement and active disease tended to have higher levels of this antibody.

Changes of skin severity scores and autoantibody levels with therapy. We followed 3 patients with new LiScl (not included among the 72 patients presented in Tables 1, 2, 3 or Figure 1) from whom serial blood samples had been obtained. Their anti-ssDNA and AHA levels over time are illustrated in Figure 2. Followup duration ranged from 7 to 21 weeks. Patients A and C had LiScl affecting the trunk and/or limbs. Patient B had facial involvement (*en coup de sabre*). Patient A had no active lesions at her first visit (Week 0) and was not treated until Week 16, when she developed a new lesion. Then oral prednisone and MTX were started. Patients B and C started taking prednisone and MTX at Week 0, when the diagnosis of LiScl was made. The localized scleroderma severity index (LoSSI) was recorded. Patients A and B had anti-ssDNA but not AHA. Patient C had both autoantibodies. As shown in Figure 1, levels of anti-ssDNA and AHA paralleled disease severity. Patient A did not receive systemic therapy until Week 16, when the LoSSI score increased from 4 to 10 and anti-ssDNA increased from 66 to 133 U. After initiation of systemic corticosteroid and MTX therapy, both the LoSSI scores and anti-ssDNA levels improved.

DISCUSSION

An autoimmune pathogenesis of localized scleroderma has been postulated based on reports of histologic abnormalities, serum autoantibodies, and coexisting personal or familial autoimmune disorders[21]. The detection of serum autoantibodies could play a role in disease classification and pro-

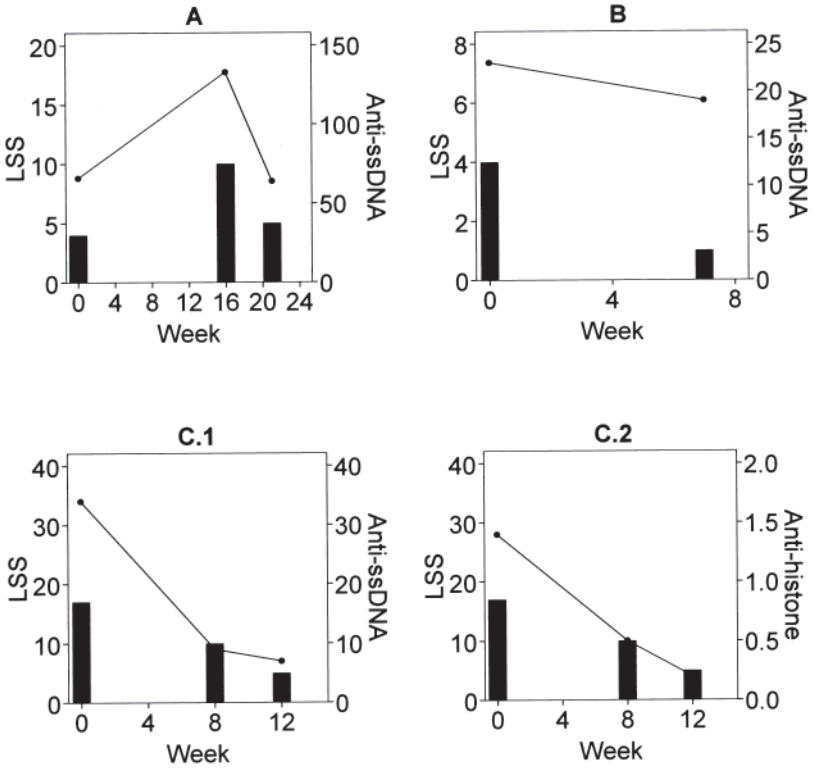

Figure 2. Serial measurement of anti-ssDNA and/or antihistone antibody and localized scleroderma severity score (LSS) in 2 LiScl patients (A and C) and one patient with *en coup de sabre* (B). Scales for LSS (bars) and anti-ssDNA or antihistone antibody levels (•) are shown on the left and right of each graph, respectively.

gression and in identifying subgroups of patients potentially responsive to therapy.

This is the largest cohort of patients with linear scleroderma evaluated at a single US center. We observed that there was no difference in the frequency of serum autoantibodies (ANA, anti-ssDNA, AHA, and AChA) between patients with childhood-onset and those with adult-onset disease. These autoantibodies have been reported to have prognostic value, as they have been associated with extent of disease and disability. Takehara, *et al* first observed the presence of ANA in patients with LS in 1983, using cultured human cell substrates[20]. Their findings were confirmed by others, with the prevalence of ANA in LS patients ranging from 46% to 63% using similar methods[8-11,13-15,17,18]. Our LiScl-only cohort was found to have ANA more frequently than those reported from Japan (68% vs 42%–50%, respectively). We also observed a higher frequency of ANA in our patients with *en coup de sabre* (11/12). A homogeneous nuclear staining pattern was the most frequent, similar to other reports[21].

An increased frequency of AHA in LS was first noted by Sato, *et al* in 1993, by immunoblotting and ELISA[17]. We detected AHA in 39% of LiScl patients, nearly twice as frequently in the ChO (49%) compared with the AO (26%) patients (p = not significant). The presence of AHA did not distinguish active from inactive skin disease, but we observed significantly higher AHA levels by ELISA in LiScl patients with more extensive cutaneous involvement, active disease, and joint contractures. Sato, *et al* reported that AHA levels correlated with bilateral LS distribution, the number of lesions, and muscle involvement in a mixed group of patients with LS[15].

Falanga, *et al* described high-titer anti-ssDNA antibody in 7 LiScl patients using a radioimmunoassay technique[8]. A subsequent study by the same investigators showed that 50% of 53 LiScl patients with either LiScl alone or with LiScl plus morphea had anti-ssDNA[9]. This antibody was found more frequently in patients with 2 or more linear lesions, prolonged (> 2 yrs) active disease, and contractures[9]. In our LiScl patient cohort, anti-ssDNA was detected in over one-third of patients with ChO (36%) versus 19% with AO disease. Anti-ssDNA antibody also was correlated with the extent of disease (> 2 anatomic sites involved). The discrepancy of anti-ssDNA frequency between the current study and our previous report[8] can be explained by the different antibody detection methods (ELISA vs radioimmunoassay) and a somewhat different study population (the present study did not include patients with both LiScl and morphea). In the present study, the proportion of patients with anti-ssDNA antibody did not correlate with LiScl dis-

ease activity, perhaps in part because we used different criteria to define active disease. However, higher anti-ssDNA levels were correlated both with more than 2 anatomic sites involved and with active disease ($p < 0.05$).

AChA has not been evaluated in LS. We found this autoantibody in 12% of LiScl patients, with no difference between groups with different age at disease onset. AChA was associated with extent of disease, but not with disease activity or contracture. However, higher levels of AChA were noted in patients with more extensive and active cutaneous disease.

Anti-ssDNA levels fluctuated with disease activity in skin and skeletal muscle, according to studies by Takehara, *et al*[21]. Both anti-ssDNA and AHA titers have been shown to decrease over time in response to therapy[9,23]. We chose to use the LoSSI to reflect LiScl disease severity as our preliminary study showed that this measure was highly reliable[22]. Levels of serum antibodies were found to correlate well with this severity score in several LiScl patients (Figure 2).

The presence of anti-ssDNA and AHA in LiScl was strongly correlated, confirming their 25% coexistence in Japanese patients with LiScl[17]. Coexistence of these autoantibodies was associated with more extensive disease and increased frequency of joint contractures in LiScl patients, regardless of the age of onset.

The limitations of our study are the relatively small number of patients once they are divided into subgroups, and its cross-sectional design. In our cohort, time from disease onset to diagnosis, disease duration, therapy regimens, and duration of therapy had no significant association with either presence of joint contractures or extent of cutaneous disease. We found higher frequencies of certain clinical features with the presence of anti-ssDNA and AHA. These antibodies may help identify subgroups of LiScl patients with more severe, active disease who may be candidates for aggressive treatment. Although these antibodies can be tested in commercial laboratories, there is no standardization between laboratories, making interpretation of results difficult in office practice. Before they are routinely used, these autoantibodies should be examined in a large, prospectively followed group of patients with LiScl and their correlations with disease activity confirmed.

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