British Journal of Dermatology (1986) **115**, 393–401.

Clinical and Laboratory Investigations

Scl 70 antibody—a specific marker of systemic sclerosis

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Accepted for publication 21 March 1986

SUMMARY

Scl 70 antibodies were tested for in 107 patients with systemic sclerosis: 68 with acrosclerosis and 39 with diffuse scleroderma. Anticentromere antibodies (ACA) and other antinuclear antibodies (ANA) were tested for by indirect immunofluorescence on HEp-2 cells. Positive results for Scl 70 antibodies were obtained in 77% of cases of diffuse scleroderma and 44% of acrosclerosis. ACA and Scl 70 antibodies were found to be mutually exclusive. If acrosclerosis cases positive for anticentromere antibodies are excluded, the percentage of acrosclerosis cases positive for Scl 70 was 63%. ACA were found to be a marker of a benign, abortive subset of acrosclerosis with almost no cutaneous involvement (CREST), whereas Scl 70 did not discriminate between acrosclerosis and diffuse scleroderma. On HEp-2 cells Scl 70 positive sera gave a characteristic, fine speckled, almost homogeneous nuclear staining pattern.

Antinuclear antibodies (ANA) are detectable in the majority of cases of systemic sclerosis (Jordon *et al.*, 1977; Tan *et al.*, 1980). In our series of 298 cases followed up for several (usually more than 10) years, the frequency of ANA-positive tests on tissue substrates in repeated studies ranged from 80% in acrosclerosis to 94% in diffuse scleroderma (Jablonska *et al.*, 1986). In a proportion of acrosclerosis cases with negative ANA findings on tissue subtrates, the test performed on the human cell line HEp-2 disclosed anticentromere antibodies (ACA) (Chorzelski *et al.*, 1985). These are considered to be a marker of CREST (Tan *et al.*, 1980; Moroi *et al.*, 1980; Fritzler, Kinsella & Garbutt, 1980; Kallenberg *et al.*, 1982; Cattogio *et al.*, 1983; Tuffanelli *et al.*, 1983; Steen *et al.*, 1984; Chorzelski *et al.*, 1985). In our series, ACA were invariably absent in patients with diffuse scleroderma.

Another antibody, Scl 70, detected by immunodiffusion, has been found mainly in patients with diffuse scleroderma (Tan *et al.*, 1980). Scl 70 antigen, prepared from rabbit thymus, is a basic non-histone protein located on the chromosomes of the cells in mitosis (Douvas, Achten & Tan, 1979). In patients with diffuse scleroderma, antibodies to Scl 70 were found in 23% (6 of

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26) by Tan *et al.* (1980) and in 34% (10 of 29) by Cattogio *et al.* (1983). In CREST (acrosclerosis) Scl 70 antibodies were reported in 13% of patients: 3 of 24 patients reported by Tan *et al.* (1980) and 6 of 46 patients reported by Cattogio *et al.* (1983).

The aim of the present study was to evaluate the specificity of Scl 70 antibody for systemic sclerosis compared with that of ACA, and to measure the frequency of occurrence of Scl 70 in acrosclerosis and diffuse scleroderma, and its association with ANA and ACA.

METHODS

Patients

The studies were performed in 107 patients with systemic sclerosis: 68 with acrosclerosis and 39 with diffuse scleroderma (Table 1). Controls comprised 90 patients with other connective tissue diseases, 10 with mixed connective tissue disease (MCTD), 37 with systemic lupus erythematosus (SLE), 22 with other collagen diseases and 18 with chronic glomerulonephritis (Table 2).

The criteria for diagnosis of acrosclerosis were: a protracted and relatively benign course, limited skin changes (usually confined to the hands, forearms and face, with telangiectases and, frequently, calcium deposits), and pronounced Raynaud's phenomenon preceding indurations and visceral involvement by several years (Jablonska, 1975; Rodnan, Jablonska & Medsger, 1979). Diffuse scleroderma was classified according to the preliminary ARA criteria of Masi *et al.* (1980) and according to Jablonska (1975).

TABLE 1. Frequency of Scl 70 antibody in patients with systemic scleroderma

Diagnosis	No. of cases	No. positive (%)
Acrosclerosis	68	30 (44)
Diffuse scleroderma	39	30 (77)
Total	107	30 (56)

TABLE 2. Incidence of Scl 70 in controls

Diagnosis	No. of cases	Scl 70 no. positive
MCTD	13	2
SLE	37	0
Other collagen diseases*	22	0
Chronic glomerulonephritis without symptoms of collagenoses	18	0
Total	90	2

* Dermatomyositis, 3; DLE and DDLE, 4; morphea, 6; suspected collagenoses, 9.

Patients were classified as having diffuse scleroderma if indurations of the skin involved the face, extremities and the trunk ('central cutaneous involvement'), often with symmetrical hyper- and hypopigmentation. The course was usually more rapid and severe, with a short period between the onset of Raynaud's phenomenon and the onset of indurations (Jablonska, 1975; Masi *et al.*, 1980).

Patients were considered to have lung involvement if they had any one of the following findings:

Bibasilar pulmonary interstitial fibrosis on chest X-ray;
vital capacity, forced expiratory volume for first second, mid-expiratory flow, or thoracic gas volume less than 75% of predicted normal.

Patients were considered to have heart involvement if they had any one of the following findings on electrocardiography, ECHO-cardiography and/or X-ray: cardiac arrhythmias, conduction disturbances, right heart failure secondary to pulmonary hypertension, or pericarditis. Patients were considered to have kidney involvement if they had abnormal clearance and/or changes in urinalysis, and were considered to have muscle involvement if they had any one of the following findings: primary muscle involvement on electromyography, increased levels of muscle enzymes: creatine-phosphokinase, aldolase and transaminases, or morphological changes in muscle biopsy.

MCTD was diagnosed according to the clinical and serological criteria of Sharp *et al.* (1979) and SLE according to the revised ARA criteria (Tan *et al.*, 1982).

Antibody testing

Scl 70 antigen was prepared from calf thymus according to the method of Tan *et al.* (1980), with certain modifications by Kumar *et al.* (1984). The antigen is very unstable, and antigenicity is lost if it is stored for prolonged periods at 4°C. 50 g calf thymus freshly obtained from the slaughter house was cut into small cubes and homogenized in a Waring blender with 100 ml of phosphate buffered saline containing 1 mM of mercaptoethanol and 0·1 mM of phenylmethylsulphonyl fluoride. The homogenate was stirred for 2 h at 4°C and then centrifuged at 14 000 ***g*** for 1 h. The supernatant, which contained primarily RNP and Sm activity, was discarded. The pellet was suspended in phosphate buffered saline containing 0·5 M NaCl and mixed for 1 h. The supernatant obtained upon centrifugation of this homogenate (at 68 000 ***g*** for 2 h) contained Scl 70 activity and was extensively dialysed against distilled water and freeze dried. The freeze dried preparation was dissolved in 10 mM Tris-HCl buffer pH 7·5 to give a final protein concentration of 200 mg/ml.

Immunodiffusion was performed according to the method of Tan *et al.* (1980), as standardized for the detection of antibodies to RNP and Sm by Kumar *et al.* (1984).

All sera were also tested on HEp-2 cells for ACA and other ANA, using the method described previously (Beutner *et al.*, 1985). The conjugate used was fluorescein labelled goat anti-human IgG (Heintel, Vienna, Austria), with a fluorescein to protein ratio of 2·2, 16 units/ml. This was diluted 1:128 to contain $\frac{1}{8}$ units/ml.

RESULTS

Scl 70 antibody appeared significantly more frequently in diffuse scleroderma (77% of patients) than in acrosclerosis (44%) ($P < 0.05$; Table 1). The controls were negative, with the exception of two patients with MCTD (Table 2), both falling within the spectrum of systemic sclerosis (pronounced Raynaud's phenomenon with indurative oedema of the hands, mask-like facies

with thinning of the lips and, in one, diminished oesophageal motility). Thus, Scl 70 appears to be highly specific of systemic sclerosis. Patients with acrosclerosis positive for ACA were invariably negative for Scl 70 (Table 3). Conversely, patients with acrosclerosis negative for ACA were found to be positive for Scl 70 in 63% cases, whereas in acrosclerosis patients as a whole Scl 70 antibody was present in 44% of the cases (Table 1).

It should be emphasized that among 39 cases of diffuse scleroderma, 16 observed by us for more than 10 years were classified initially as severe acrosclerosis or transitional form, acrosclerosis-diffuse scleroderma. The frequency of Scl 70 antibody in this group was 12 of 16 (75%), i.e. essentially the same as in typical cases of diffuse scleroderma. Therefore, the transitional form was grouped with diffuse scleroderma.

Immunofluorescence studies on HEp-2 showed a characteristic pattern with all sera positive in the immunodiffusion test for Scl 70 antibody. The fluorescence pattern was almost homogeneous due to diffuse fine, densely packed speckles associated, in most cases, with weak nucleolar staining (Fig. 1).

If patients are classified according to the varieties of systemic sclerosis and presence or absence of Scl 70 antibody, slight differences can be seen (Tables 4 and 5). There were no

TABLE 3. Occurrence of Scl 70 antibody with anticentromere antibody in patients with acrosclerosis

Diagnosis	No. of cases	Scl 70 no. positive (%)
ACA-negative	48	30 (63)
ACA-positive	20	0

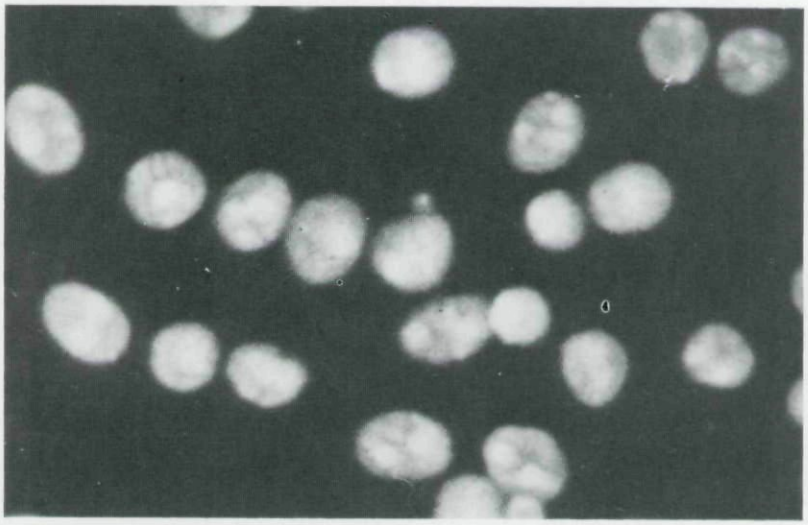

FIGURE 1. Immunofluorescence pattern of Scl 70 antibody on HEp-2 cells: fine granular, almost homogeneous staining with visible nucleoli (original × 400).

TABLE 4. Clinical and laboratory findings in Scl 70 positive and Scl 70 negative patients with acrosclerosis

Clinical and laboratory findings	Scl 70 positive (30 cases)	Scl 70 negative[1] (38 cases)	Scl 70 negative ACA-negative[2] (18 cases)
Mean age (years)	49·8	47·5	44·8
Sex ratio F:M	28:2	34:4	17:1
Mean duration of Raynauds phenomenon (years)	18·3	12·3	13·2
Mean duration of sclerosis (years)	11·4	5·2	6·2
Mean period between appearance of Raynauds phenomenon and indurations (years)	6·9	6·2	7·0
Indurative oedema and/or indurations confined to the digits	18/28 (64%)	32/36 (89%)*	14 (82%)
Sclerodactyly with contractures and immobilization of fingers ('grip')	12/28 (43%)	5/36 (14%)**	5/17 (29%)
Digital scars and/or acrolysis	23/28 (82%)	22/36 (61%)	10/17 (56%)
Cutaneous involvement extending up to one third of forearm	11/28 (39%)	7/36 (9%)	7/17 (41%)
Calcinosis	6/28 (21%)	17/36 (47%)*	8/17 (47%)
Telangiectasia	22/28 (78%)	30/36 (83%)	14/17 (82%)
Central cutaneous involvement[3]	6/28 (21%)	0/36**	0/17*
Arthralgia	24/28 (86%)	31/36 (86%)	15/17 (88%)
Decreased oesophageal motility	14/28 (50%)	22/36 (61%)	11/17 (65%)
Heart involvement	9/26 (35%)	24/36 (67%)*	10/17 (59%)
Kidney involvement	0/28	0/36	0/17
Lung involvement	13/28 (46%)	15/36 (42%)	6/17 (35%)
Muscle involvement and/or EMG abnormalities	3/28 (11%)	7/36 (31%)	4/17 (24%)
Immunological findings			
ANA other than ACA—on HEp-2	23/23 (100%)	9/18 (50%)**	9/18 (50%)**
Positive at a titre ⩾ 1:80—on monkey oesophagus	19/30 (63%)	10/38 (26%)**	9/18 (50%)
Exclusively nucleolar pattern	0/30	3/38 (8%)	3/18 (17%)*
RNP positive	0/30	4/38 (11%)	4/18 (22%)*

[1] This group includes ACA-positive and ACA-negative cases.

[2] In this group only cases negative for ACA are included; cases positive for ACA have been described in detail previously (Chorzelski *et al.*, 1985).

[3] Some indurations and/or atrophy, mainly on the thorax.

Significantly different from SCL 70 positive patients (fraction test) $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

differences in major clinical characteristics, i.e. age, sex, and time between appearance of Raynaud's phenomenon and skin indurations, between the SCL 70 positive and negative groups.

As can be seen in Table 4, skin indurations confined to the digits, and calcinosis were more frequent in acrosclerosis cases negative for Scl 70, whereas immobilization and contractures of the fingers were more frequent in patients positive for Scl 70. However, if only cases negative for ACA are evaluated, these differences were not statistically significant. Visceral involvement was comparable in the two groups, with the exception of somewhat more frequent heart changes in Scl 70 negative cases. It should be stressed that central cutaneous involvement was invariably absent in patients negative for Scl 70, whereas slight changes on the thorax were noticed in 20% of cases positive for Scl 70.

TABLE 5. Clinical and laboratory findings in Scl 70 positive and Scl 70 negative cases with diffuse scleroderma

Clinical and laboratory findings	Scl 70 positive (30 cases)	Scl 70 negative (9 cases)
Mean age (years)	45·3	39·7
Sex ratio F:M	27:3	8:1
Mean duration of Raynauds phenomenon (years)	7·8	7·4
Mean duration of sclerosis (years)	3·9	4·0
Mean period between appearance of Raynauds phenomenon and indurations (years)	3·9	3·4
Indurative oedema and/or indurations confined to the digits	19/27 (70%)	7/9 (78%)
Sclerodactyly with contractures and immobilization of fingers ('grip')	14/27 (52%)	2/9 (23%)
Digital scars and/or acrolysis	26/27 (96%)	5/9 (55%)**
Cutaneous involvement extending up to one third of the forearm	25/27 (93%)	8/9 (89%)
Calcinosis	12/27 (44%)	1/8 (13%)
Telangiectasia	17/27 (63%)	1/9 (11%)*
Central cutaneous involvement	26/27 (96%)	7/9 (78%)
Arthralgia	27/27 (100%)	8/9 (89%)
Decreased oesophageal motility	20/27 (74%)	4/9 (44%)
Heart involvement	19/27 (70%)	8/9 (89%)
Lung involvement	21/27 (78%)	2/9 (22%)**
Kidney involvement	7/27 (26%)	2/9 (22%)
Muscle involvement and/or EMG abnormalities	11/27 (41%)	5/9 (56%)
ANA-positive at a titre ⩾ 1:80—on HEp-2	25/25 (100%)	5/8 (63%)**
—on monkey oesophagus	27/30 (90%)	5/9 (55%)*
Nucleolar pattern	0/30	3/9 (33%)**
RNP positive	0/30	1/9 (11%)

Significantly different from Scl 70 positive group $*P < 0{\cdot}05$, $**P < 0{\cdot}01$ compared using a fraction test.

As can be seen in Table 5, digital scars and acrolysis, telangiectases and lung involvement were more frequent in cases of diffuse scleroderma positive for Scl 70.

Antinuclear antibodies, as detected on HEp-2 cells and monkey oesophagus, were found in a higher percentage of cases of acrosclerosis and diffuse scleroderma positive for Scl 70 (Tables 4 and 5).

RNP antibodies were found in five cases negative for Scl 70, four cases of acrosclerosis and one of diffuse scleroderma. Nucleolar staining without any associated patterns was found in five cases, four of acrosclerosis and one of diffuse scleroderma, all negative for Scl 70.

DISCUSSION

Scl 70 antibody was found to be a specific serological marker of systemic scleroderma. In our series it was detected at a much higher frequency than reported in previous publications (Tan *et al.*, 1980; Catoggio *et al.*, 1983), but comparable results have been reported recently by Takehara, Moroi and Ishibashi (1985). The higher detection rates of Scl 70 in our study, 77% in

diffuse scleroderma and 44% in acrosclerosis, may be related to differences in the type of cases studied and, at least in part, to the technique of antigen preparation (Kumar *et al.*, unpublished observations).

On HEp-2, sera positive for Scl 70 showed a very characteristic, fine speckled, almost homogeneous immunofluorescence pattern, which could be recognized easily. This pattern corresponds to the fine speckles described by Tan *et al.* (1980). If the titre of ANA with this pattern on HEp-2 was higher than 1:80, it was invariably associated with a positive immunodiffusion test for Scl 70. Since immunodiffusion is less sensitive than immunofluorescence, it is to be expected that Scl 70 antibody, demonstrated in lower titres in the indirect IF test, would not be detectable by immunodiffusion. Thus, it is conceivable that Scl 70 antibody occurs even more frequently than detected in our study.

In contrast to ACA antibody, which is a marker of an abortive subset of acrosclerosis with almost no cutaneous indurations (Chorzelski *et al.*, 1985), Scl 70 is a marker of systemic sclerosis as a whole, with prevalence in diffuse scleroderma, but present also in a high proportion of cases of acrosclerosis. Thus, it does not discriminate between the subsets of systemic scleroderma. There were only slight clinical differences between cases positive and negative for Scl 70. In patients with acrosclerosis negative for Scl 70, the indurations were confined more frequently to the fingers and there were fewer contractures of the fingers; calcinosis was found in a somewhat larger proportion of the patients, and there was no induration or atrophy on the trunk; the disease was thus less severe than in patients positive for Scl 70 antibody.

In patients with diffuse scleroderma the clinical differences between patients positive and those negative for Scl 70 were less conspicuous.

Antinuclear antibodies were found at titres above 1:80 on HEp-2 in all patients positive for Scl 70. They were significantly less frequent in both acrosclerosis and diffuse scleroderma patients negative for Scl 70. On monkey oesophagus, ANA at titres above 1:80 were not found in all Scl 70 positive cases, but they also occurred significantly more frequently in acrosclerosis and diffuse scleroderma cases positive for Scl 70 (Table 5).

ACA positive cases of acrosclerosis were, in our study, invariably negative for Scl 70 antibody. Only two exceptional cases positive for Scl 70 and ACA antibodies have been reported by Ruffatti *et al.* (1985), and the authors stress that this is a rare finding.

Scl 70 was not detected in other collagen diseases, with the exception of two overlap cases, both with some cutaneous symptoms of scleroderma (MCTD within the spectrum of scleroderma).

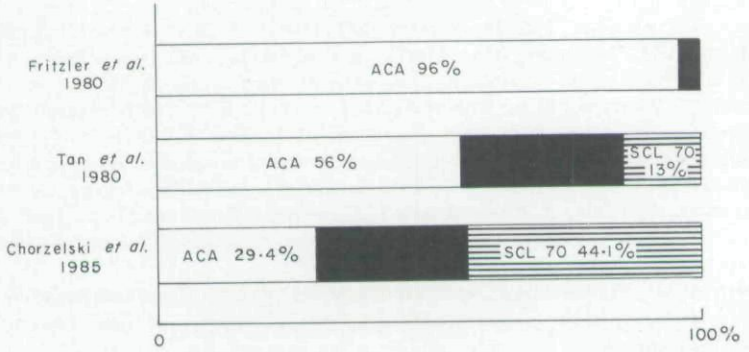

FIGURE 2. Published incidence of ACA and Scl 70 in acrosclerosis. □: Percentage of patients positive for ACA, and ▩: for SCL 70. ■: Percentage of patients negative for both ACA and SCL 70.

As shown in Figure 2, the gap between the frequency of ACA and of Scl 70 will probably be narrowed in future studies either by increasing the sensitivity of the methods or by detection of other, as yet unidentified antibodies. In our series, this gap is filled, in a part, by the RNP and anti-nucleolar antibodies.

The value of tests for Scl 70 antibody and ACA may well prove to lie also in their prognostic role, in predicting whether patients with acrosclerosis will, in time, develop into true diffuse scleroderma or remain as cases of acrosclerosis with minimal cutaneous involvement. This is supported by more than 10 years of retrospective study on 16 cases of diffuse scleroderma, diagnosed originally as acrosclerosis, and later as transitional form; 12 of these 16 (75%) were positive for Scl 70 antibody. Follow-up studies are needed to determine whether cases of acrosclerosis that are positive for Scl 70 antibodies are at greater risk of developing frank diffuse scleroderma.

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