Clinical and Serological Heterogeneity in Patients with Anticentromere Antibodies

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ABSTRACT. ***Objective.*** To evaluate the clinical and serological heterogeneity in patients with anticentromere antibodies (ACA).

Methods. One hundred twenty patients with ACA were analyzed retrospectively. ACA were detected initially on the basis of indirect immunofluorescence on HEp-2 cells, and then antibodies to CENP-B were measured by ELISA. Antibodies to other nuclear antigens were also detected by double immunodiffusion and/or ELISA.

Results. Eighty-four patients (70.0%) had systemic sclerosis (SSc; scleroderma) and 36 patients (30.0%) had other rheumatic diseases or miscellaneous disorders. Among patients with SSc, 35 patients (41.7%) had SSc in overlap mostly with Sjögren's syndrome (SS), in part with rheumatoid arthritis and/or primary biliary cirrhosis (PBC). Five of 36 patients (13.9%) without SSc also had overlap syndrome of more than 2 rheumatic diseases or PBC. All CREST features (calcinosis, Raynaud's, esophageal dysmotility, sclerodactyly, telangiectasias) were found significantly more in SSc than in other diseases. A combination of RST was the most frequently seen, followed by CREST and CRST in the SSc group. In contrast, 22 of 36 patients (61.1%) without SSc had no CREST features, and the rest had only Raynaud's phenomenon and/or telangiectasia. Twenty-five of 75 patients (33.3%) with SSc and 6 of 25 patients (24.0%) with other diseases had a slight elevation of creatine phosphokinase concentration with no apparent myositis signs and/or skin lesions, suggesting a new additional sign of patients with ACA. Seventy-two patients (60.0%) had ACA alone and 48 patients (40%) had ACA mixed with other disease marker antinuclear antibodies (ANA). ACA alone occurred more frequently in patients with SSc and in the non-overlap group, whereas patients with ACA mixed with other ANA were more frequently found in the other disease and the overlap syndrome groups. Anti-CENP-B ELISA levels of the SSc group were significantly higher than those of other disease groups in all patients, in patients with ACA alone, and in patients having ACA together with other ANA. The most frequently concurrent ANA were anti-SSA/Ro antibodies; and the other ANA, including anti-SSB/La, RNP, topoisomerase-I, Jo-1, Ku, and dsDNA antibodies, were also positive alone or combined with more than 2 ANA in patients with ACA. Five patients with CREST syndrome having ACA and anti-RNP antibodies had clinical manifestations compatible with mixed connective tissue disease. SS was found in 37.0% of patients who had higher anti-CENP-B ELISA levels and higher coincidence of anti-SSA/Ro antibodies than the patients without SS.

Conclusion. ACA were positive mostly in patients with SSc with CREST features and partly in other rheumatic disorders. The high levels of ACA may be necessary for the development of CREST features, and frequent concurrence of other disease marker ANA may contribute to the development of heterogeneous clinical characteristics, including overlap syndrome, in patients with ACA. (J Rheumatol 2005;32:1488–94)

Key Indexing Terms:
ANTICENTROMERE ANTIBODIES ANTI-CENP-B ANTIBODIES SYSTEMIC SCLEROSIS
CREST SYNDROME OVERLAP SYNDROME SJÖGREN'S SYNDROME

Patients with rheumatic diseases are characterized by the presence of a variety of antinuclear antibodies (ANA) in their sera. Some ANA have such a close association with a certain disease that they are considered a disease marker ANA. As one of these ANA, anticentromere antibodies (ACA) are found in a high proportion of patients with CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), a benign variant of systemic sclerosis (SSc; scleroderma)[1-3], which has been classified into a limited form SSc[4]. However, the term CREST syndrome is still widely used clinically, because not all patients with limited SSc have the features of CREST, and the patients with CREST syndrome have an association with ACA compared to other types of limited SSc.

ACA have also been recognized in patients with diffuse form of SSc[2,3], Sjögren's syndrome (SS)[5-8], primary biliary

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Accepted for publication March 4, 2005.

cirrhosis (PBC)[9,10], systemic lupus erythematosus (SLE)[2,11-13], rheumatoid arthritis (RA)[14,15], and Raynaud's phenomenon (RP) without definite rheumatic disorders[2,11,15,16]. Since the disease distribution of patients with ACA is thus heterogeneous, it is necessary to reevaluate whether a close relationship is still found between ACA and CREST syndrome, and what features of CREST are found in ACA-positive patients with or without SSc. In addition, although other disease marker ANA including SSA/Ro, SSB/La, RNP, Sm, topoisomerase-I, and Jo-1 and double-stranded DNA antibodies have occasionally been found in patients with ACA, clinical characteristics of these patients have not been fully clarified.

We retrospectively reevaluated clinical and serological heterogeneity in patients with ACA.

MATERIALS AND METHODS

Patients. During the routine screening of indirect immunofluorescence (IIF) ANA tests over a period of 10 years, ACA were identified in sera of 120 patients. Further clinical and serological evaluations of all patients with ACA were then performed by experienced rheumatologists in our hospital in order to avoid chart review, which has its inherent limitations.

Patients who had at least RP and sclerodactyly were considered as having SSc with CREST features according to the classification proposed by LeRoy, *et al*[4] that sclerodactyly is suggestive of a spectrum of SSc associated conditions. Calcinosis was detected by hand radiography, RP was determined by case history, esophageal dysmotility was detected using barium swallow radiography, and sclerodactyly and telangiectasia were detected on examination by a rheumatologist. Forty of 108 ACA-positive patients examined had sicca features of SS according to the criteria of the Japan Health and Welfare service[17]. All patients with SS had more than one focus of mononuclear cell infiltrations in minor salivary gland biopsies without fibrotic change.

Antibody determination. ANA were detected using IIF with commercially prepared HEp-2 cell lines as substrate at an initial serum dilution of 1:40 using polyvalent secondary antibody (Fluoro Hepana test kit, Medical Biological Laboratories Co., Ltd., Nagoya, Japan). ACA were identified by their typical discrete speckled pattern in interphase and metaphase nuclei. ACA-positive sera were stored at –20°C for further immunological examinations. Antibodies to the major centromeric protein, CENP-B, in all ACA-positive sera on the basis of IIF were then measured by ELISA (anti-CENP-B ELISA) using the Mesacup CENP-B test kit (Medical Biological Laboratories). Antibodies to saline extractable nuclear antigens (SSA/Ro, SSB/La, RNP, Sm, topoisomerase-I, and Jo-1) were detected by double immunodiffusion using reference sera of each known antibody specificity, the titers of which were measured by ELISA using the Mesacup SSA/Ro, SSB/La, RNP, Sm, topoisomerase-I, Jo-1 kits (Medical Biological Laboratories). The secondary antibody of all ELISA kits used in this study was polyvalent. Anti-double-stranded DNA (dsDNA) antibodies were measured by Farr assay using radioimmunoassay. Anti-Ku antibodies[18] were detected by double immunodiffusion.

Statistical analysis. Differences in the prevalence of the antibodies between the 2 groups were analyzed by Fisher's exact test. Mean antibody levels in the different groups were compared by Student t test. A p value less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

ACA were positive in 120 patients with connective tissue diseases and other disorders. There were 117 women and 3 men aged 33 to 77 years at diagnosis, with a mean age of 54.5 years in SSc and 53.6 years in other diseases. As shown in Table 1, 84 patients (70.0%) had SSc and 36 (30.0%) had other rheumatic diseases or miscellaneous disorders.

Among 84 patients with SSc, 49 patients (58.3%) had only SSc. The other 35 patients (41.7%) had SSc in overlap; 25 had SSc and SS; 4 each had SSc and RA, SSc and PBC, respectively; one with SSc had RA and SS; one with SSc had PBC and SS.

Among 36 patients without SSc, 25 patients had pure rheumatic diseases including 3 with RA, 4 with SLE, 2 with discoid lupus erythematosus, 3 with dermatomyositis (DM), one with polymyositis (PM), 10 with primary SS, and 2 with Raynaud's disease. Six patients had no apparent rheumatic disease: 2 had PBC, one each had pulmonary fibrosis, chronic thyroiditis, polyneuritis, and type C hepatitis. The remaining 5 patients (13.9%) had overlap syndrome including one each with RA and SS, SLE and SS, PM and RA, DM and PBC, SS and PBC, respectively.

Thus, a total of 40 patients (33.3%) had overlap syndrome of more than 2 rheumatic diseases or PBC, and 80 patients (66.7%) had non-overlap features. Overall incidence of SSc was 84, RA 10, PBC 9, and SS 40.

Table 2 presents CREST features of 120 patients with

Table 1. Clinical features of 120 patients with anticentromere antibodies.

Disease	No.		Total (%)
Systemic sclerosis (SSc)			84 (70.0)
SSc alone		49	
SSc in overlap		35	
SSc + RA	4		
SSc + SS	25		
SSc + PBC	4		
SSc + RA + SS	1		
SSc + PBC + SS	1		
Other diseases			36 (30.0)
Other diseases alone		31	
RA	3		
SLE	4		
Discoid lupus erythematosus	2		
DM	3		
PM	1		
Primary SS	10		
Raynaud's disease	2		
PBC	2		
Pulmonary fibrosis	1		
Chronic thyroiditis	1		
Polyneuritis	1		
C-type hepatitis	1		
Other diseases in overlap		5	
RA + SS	1		
SLE + SS	1		
PM + RA	1		
DM + PBC	1		
SS + PBC	1		

SSc: systemic sclerosis, RA: rheumatoid arthritis, SS: Sjögren's syndrome, SLE: systemic lupus erythematosus, DM: dermatomyositis, PM: polymyositis, PBC: primary biliary cirrhosis.

Table 2. CREST features of 120 patients with anticentromere antibodies.

Symptoms	Systemic Sclerosis, n = 84 (%)	Other Diseases, n = 36 (%)
Calcinosis (C)	43 (51.2)	0
Raynaud's phenomenon (R)	84 (100)	11 (30.6)
Esophageal dysmotility (E)	29 (34.5)	0
Sclerodactyly (S)	84 (100)	0
Telangiectasia (T)	65 (77.4)	5 (13.9)
Combinations of CREST features		
CREST	18 (21.4)	0
CRST	17 (20.2)	0
CRES	1 (1.2)	0
CRS	7 (8.3)	0
REST	4 (4.8)	0
RES	6 (7.1)	0
RST	27 (32.1)	0
RS	4 (4.8)	0
RT	0	2 (5.6)
R	0	9 (25.0)
T	0	3 (8.3)
None	0	22 (61.1)
Slight elevation of serum CPK*	25/75 (33.3)	6/25 (24.0)

* Elevation within 3 times normal upper ranges excluding 6 patients with PM or DM.

ACA. In the SSc group all patients had RP and sclerodactyly. In addition, 43 patients (51.2%) had calcinosis, 29 (34.5%) had esophageal dysmotility, and 65 (77.4%) had telangiectasia. In the other disease groups, however, no CREST features were found, except RP (30.6%) and telangiectasia (13.9%). Combinations of more than RP and sclerodactyly of the CREST features were found in all patients with SSc. A combination of RST (32.1%) was the most frequent, followed by CREST (21.4%) and CRST (20.2%) in the SSc group. In other disease groups, 14 patients (38.9%) had only RP and/or telangiectasia, and the remaining 22 patients (61.1%) had no features of CREST. Six patients in the SSc group who had initially had primary SS and only RP had developed CREST syndrome during followup periods.

A slight elevation of serum creatine phosphokinase (CPK) concentrations within 3 times the normal upper limit was observed in 31 of 100 patients with ACA, excluding 6 patients with DM or PM, 25 of 75 (33.3%) with SSc, and 6 of 25 (24.0%) with other disease. They were lacking apparent myositis signs and/or skin lesions and did not develop DM or PM during the followup period. The elevations of CPK were sustained constantly or occasionally from their first visit to our hospital, and isozyme analyses revealed increases of CPK-MM.

The characteristics of ACA and concurrence of other disease marker ANA are shown in Table 3 and Figure 1. Seventy-two patients (60.0%) had ACA alone and 48 (40%) had ACA mixed with other ANA. The incidence of ACA alone was significantly higher in the SSc group than in other disease groups ($p < 0.05$), whereas ACA mixed with other ANA was found more frequently in the other disease group

Table 3. Characteristics of anticentromere antibodies (ACA) and concurrent antinuclear antibodies (ANA).

Antinuclear Antibodies	Systemic Sclerosis, n = 84 (%)	Other Diseases, n = 36 (%)
ACA alone	55 (65.5)*	17 (47.2)
ACA with other ANA	29 (34.5)	19 (52.8)***
Concurrent antibodies to		
SSA/Ro	19 (22.6)	9 (25.0)
SSA/Ro + SSB/La	1 (1.2)	3 (8.3)
SSA/Ro + dsDNA	0	2 (5.6)
RNP	2 (2.4)	0
RNP + SSA/Ro	3 (3.6)	0
RNP + SSA/Ro + SSB/La	1 (1.2)	0
RNP + SSA/Ro + dsDNA	0	1 (2.8)
Topo-I	1 (1.2)	0
Topo-I + SSA/Ro	1 (1.2)	0
Jo-1	0	1 (2.8)
Ku	0	1 (2.8)
dsDNA	1 (1.2)	2 (5.6)
Overall incidence of concurrent antibodies to		
SSA/Ro	23 (27.4)	12 (33.3)
SSA/Ro + SSB/La	2 (2.4)	3 (8.3)
RNP	6 (7.1)	1 (2.8)
Topo-I	2 (2.4)	0
Jo-1	0	1 (2.8)
Ku	0	1 (2.8)
dsDNA	1 (1.2)	5 (13.9)**

* $p < 0.05$ versus other diseases, ** $p < 0.01$, *** $p < 0.05$ versus SSc. Topo-I: topoisomerase-I, dsDNA: double-stranded DNA.

($p < 0.05$). All ACA-positive sera by IIF had antibodies to CENP-B by ELISA. Anti-CENP-B ELISA levels of the SSc group were significantly higher than those of the other disease group in single patients ($p < 0.01$; Figure 1A), in patients with ACA alone ($p < 0.01$; Figure 1B), and in patients with ACA and other ANA ($p < 0.05$; Figure 1C).

ACA alone also occurred more frequently in patients of the non-overlap group than in patients in the overlap syndrome group [58 of 80 patients (72.5%) vs 14 of 40 patients (35.0%); $p < 0.01$]. In contrast, ACA mixed with other ANA occurred more frequently in the overlap syndrome group than in the non-overlap group [26 of 40 patients (65.0%) vs 22 of 80 patients (27.5%); $p < 0.01$]. There was, however, no significant difference of anti-CENP-B ELISA levels between the overlap and non-overlap group.

Anti-SSA/Ro antibodies were the most frequently coexisting ANA — the overall incidence was 23 patients (27.4%) in SSc and 12 patients (33.3%) in the other disease group. Two patients with SSc and 3 in the other disease group had anti-SSA/Ro+SSB/La antibodies. Anti-RNP antibodies were positive in 6 patients (7.1%) with SSc and one patient (2.8%) with other diseases. Among these, 5 patients with SSc (2 with CREST, each one with CRST, CRES, RES) fulfilled the criteria[19] for the classification of mixed connective tissue disease (MCTD). Simultaneous occurrence of anti-topoisomerase-I antibodies was found in 2 patients who had

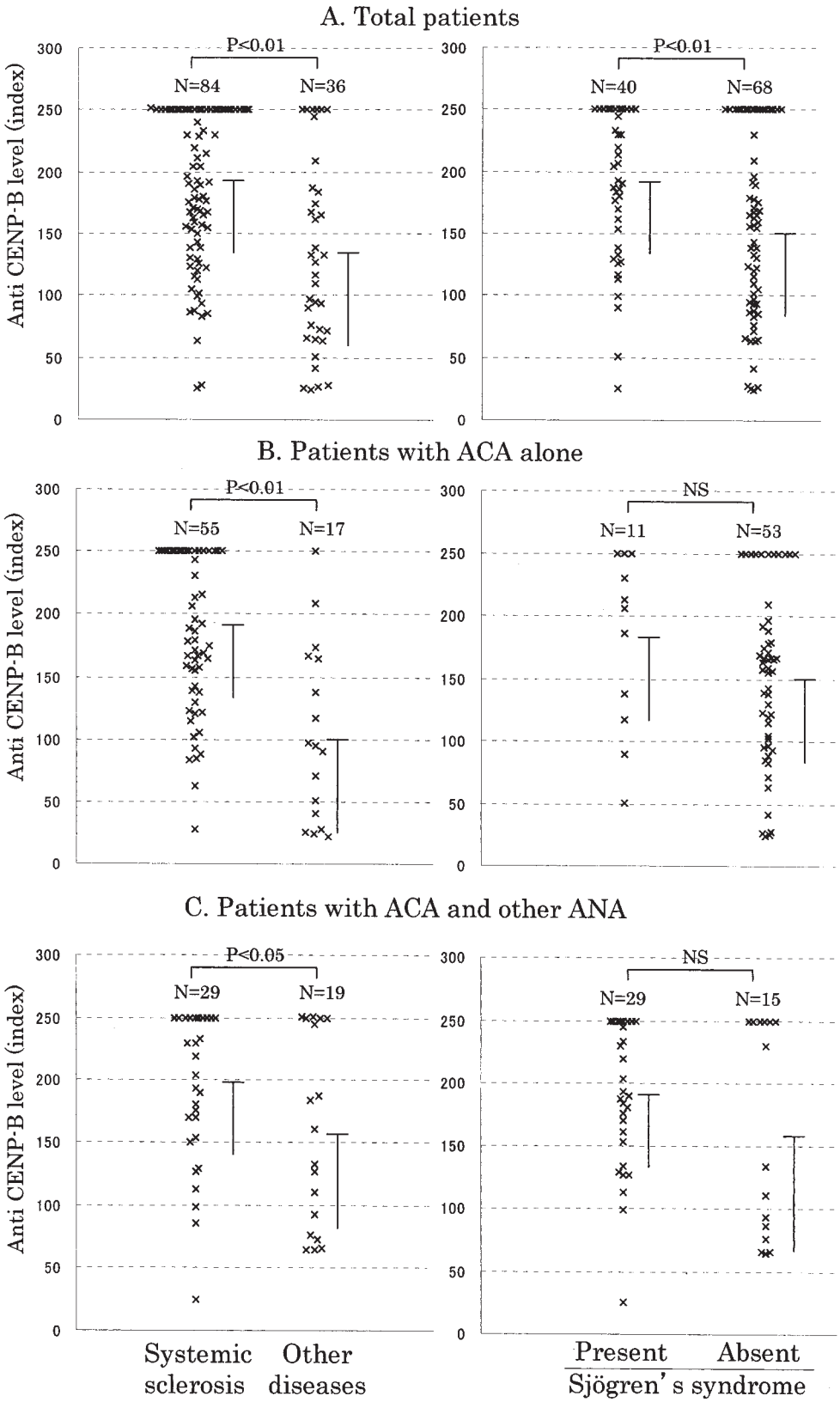

Figure 1. Comparison of anti-CENP-B ELISA levels between patients with SSc and other diseases, and between patients with and without sicca features of SS, in all patients (A), in patients with ACA (B), and in patients with ACA and other ANA (C). Horizontal lines show mean values and vertical lines indicate SD. The cutoff index of anti-CENP-B ELISA is 16.

clinical characteristics of diffuse SSc in addition to CREST features. Anti-Jo-1 antibodies were positive in a patient with PM, and anti-Ku antibodies were present in a patient with DM. Anti-dsDNA antibodies occurred significantly more in the other disease group than in the SSc group ($p < 0.01$) who had clinical features of SLE. All these ANA were positive alone or combined with more than 2 ANA in patients having ACA, as shown in Table 3.

Table 4 and Figure 1 give characteristics of ACA and concurrent disease marker ANA in patients with or without sicca features of SS. SS was found in 40 of 108 patients (37.0%) examined, including 10 with primary SS and 30 with secondary SS (27 with SSc and 3 with other diseases). ACA together with other ANA were found more frequently in patients with SS ($p < 0.01$), whereas ACA alone frequently occurred in patients without SS ($p < 0.01$). Anti-CENP-B ELISA levels were significantly higher in patients with SS than in patients without SS ($p < 0.01$; Figure 1A). However, no significant difference of anti-CENP-B ELISA levels between patients with and without SS was observed in patients with ACA alone (Figure 1B), or in patients with ACA and other ANA (Figure 1C). Among a variety of ANA coexisting with ACA the overall incidence of anti-SSA/Ro antibodies was significantly higher in patients with SS than in patients without SS ($p < 0.01$). The anti-SSB/La antibodies accompanied by SSA/Ro antibodies were found in 4 patients with SS and in one patient without SS.

DISCUSSION

Early studies[1-3] emphasized that ACA had such a close association with CREST syndrome that they were considered disease marker antibodies. However, later studies[6,7,12,15] did not confirm a close relation between ACA and CREST syndrome, because of their occurrence in other rheumatic and in nonrheumatic diseases. In our study, however, 84 of 120 patients (70%) with ACA were classifiable as having SSc with CREST features. Thus, a fairly clear clinical characteristic was confirmed when a patient with more than RP and sclerodactyly was considered as having SSc with CREST features according to the classification proposed by LeRoy, *et al*[4] that sclerodactyly is suggestive of a spectrum of SSc associated conditions. A diminished specificity between ACA and CREST syndrome in later studies may be explained by using different definitions for CREST syndrome. Moreover, clinical data in previous reports were frequently obtained from chart reviews, in which symptoms and signs of SSc or CREST syndrome may have been missed in patients seen by non-rheumatologists, whereas clinical and serological evaluations of all patients with ACA in our study were performed by experienced rheumatologists in our hospital. In addition, since 41.7% of patients with SSc had overlap syndrome of more than 2 rheumatic diseases or PBC, as clarified for the first time in this study, symptoms or signs of SSc may have been overlooked by clinical features of overlapping diseases.

All CREST features were found significantly more in SSc than in other diseases. The combination of RST was the most frequently seen, followed by CREST and CRST, in the SSc group. In contrast, 61.1% of patients without SSc had none of the CREST features, and the remainder had only RP and/or telangiectasia. Since anti-CENP-B ELISA levels of the SSc group were significantly higher than those of other disease groups, the high levels of ACA may be necessary for the development of SSc with CREST features. It is also reported that CREST syndrome is not established within a short period, and that patients initially having only RP tend to develop CREST syndrome later[2,16]. Six patients in our study who had primary SS and RP at first examination developed CREST syndrome during followup periods.

The conspicuous finding in this study was that 33.3% of patients with SSc and 24.0% of patients with other diseases had a slight elevation of CPK levels, within 3 times the normal upper limit, excluding 6 patients with DM or PM who had high CPK levels. All patients lacked apparent signs of myositis and/or skin lesions at diagnosis, and none developed DM or PM during followup periods. The slight elevations of CPK were sustained constantly or occasionally from their first visit to our hospital, and isozyme analyses revealed increases of CPK-MM. These findings suggested subclinical myositis similar to "simple myopathy" in patients with SSc reported by Clements, *et al*[20]. Nineteen of 24 patients with SSc in their study had "simple myopathy," 7 of whom had an abnormal CPK level with a mean of 137% ± 47% of normal; 18 had myogenic changes on electromyography (EMG). They did not find signs of inflammation on 3 muscle biopsies, and their patients demonstrated no significant change in CPK levels without corticosteroid or immunosuppressive therapy. One of their SSc patients with elevated CPK had CREST syndrome, but the presence of

Table 4. Characteristics of anticentromere antibodies (ACA) and concurrent antinuclear antibodies (ANA) in patients with or without sicca features of Sjögren's syndrome (SS).

Antinuclear Antibodies	Sicca Features of SS	
	Present n = 40 (%)	Absent n = 68 (%)
ACA alone	11 (27.5)	53 (77.9)**
ACA with other ANA	29 (72.5)*	15 (22.1)
Overall incidence of concurrent antibodies to		
SSA/Ro	23 (57.5)*	8 (11.8)
SSA/Ro + SSB/La	4 (10.0)	1 (1.5)
RNP	4 (10.0)	3 (4.4)
Topo-I	1 (2.5)	1 (1.5)
Jo-1	0	1 (1.5)
Ku	0	1 (1.5)
dsDNA	2 (5.0)	3 (4.4)

* $p < 0.01$ versus "absent," ** $p < 0.01$ versus "present." Topo-I: topoisomerase-I, dsDNA: double stranded DNA.

ACA was not clarified at that time. Although further information including findings on EMG and muscle biopsies is necessary, the slight elevation of CPK found in our study has not been reported previously to our knowledge, suggesting a new additional sign of patients with ACA.

All ACA-positive sera by IIF had antibodies to CENP-B by ELISA. This finding is consistent with the report by Earnshaw, *et al*[21] that all test sera from patients with CREST syndrome reacted with the CENP-B protein, and also supports the report by Conrad, *et al*[22] that the CENP-B protein was the major target of the IIF ACA response in diseases other than SSc. Sixty percent of patients had pure ACA and 40% had ACA together with other disease marker ANA. The frequency of ACA alone was significantly higher in patients with SSc with CREST features and in the non-overlap group, whereas ACA together with other ANA occurred more frequently in the other disease and overlap syndrome groups. Anti-CENP-B ELISA levels of the SSc group were, however, significantly higher than those of the other disease group in single patients, in patients with ACA alone, and in patients having ACA together with other ANA. There was no difference in anti-CENP-B ELISA levels between the overlap and non-overlap groups. These findings suggest that high levels of ACA may be necessary for the development of SSc with CREST features, and overlap syndrome in patients with ACA may be derived from the concurrence of other disease marker ANA. Zimmermann, *et al*[14] have reported 3 interesting "overlap" patients with longstanding RA and consecutive evolution of incomplete CREST syndrome who had ACA crossreacting with anti-hnRNP (A2/RA33) antigens, an ANA marker antigen for RA.

The most frequently coinciding disease marker ANA were anti-SSA/Ro antibodies: 27.4% in the SSc group and 33.3% in the other disease group. Their prevalence was the highest compared to previous reports (2% to 13%)[6,7,11]. Kuwana, *et al*[23] reported that anti-RNA polymerase II and anti-SSA/Ro antibodies were present together with anti-topoisomerase I antibodies more frequently in sera of Japanese SSc patients than in sera of Caucasian patients, indicating an influence of ethnic background linked to genetic and environmental factors. Although they did not describe the coexistence of anti-SSA/Ro antibodies and ACA, the high prevalence of anti-SSA/Ro antibodies in our study may also be derived from racial differences.

Anti-RNP antibodies were positive in 6 patients (7.1%) with SSc and one patient (2.8%) with other diseases, the prevalence of which was almost the same as that in Danish SSc patients (6.5%) reported by Jacobsen, *et al*[24]. Five of 6 patients with SSc (2 with CREST, one each with CRST, CRES, and RES) fulfilled the criteria for the classification of MCTD[19], although the differentiation between SSc and MCTD was difficult. The coexistence of anti-RNP antibodies and ACA appears in 3 previous reports. Fritzler, *et al*[2] reported one ACA-positive patient who had anti-RNP antibody and clinical features consistent with MCTD. Sato, *et al*[25] reported a case of an SSc patient with ACA, anti-topoisomerase I, and anti-RNP antibodies. Vlachoyiannopoulos, *et al*[6] reported 2 patients with ACA and anti-RNP antibodies without describing clinical features suggestive of MCTD.

Anti-dsDNA antibodies together with ACA were found significantly in SLE patients, with the same prevalence reported by Caramaschi, *et al*[7] and McCarty, *et al*[11]. The simultaneous occurrence of anti-topoisomerase I antibodies and ACA was observed in 2 of our patients with almost the same frequency as previous reports[5,6,15]. All these patients had clinical features of CREST syndrome and proximal SSc. However, the concurrences of anti-Jo-1 antibodies in a patient with PM and anti-Ku antibodies in a patient with DM have not been reported previously.

Sicca features of SS were observed in 40 of 108 patients (37.0%) examined, including 10 with primary SS and 30 with secondary SS, with almost the same incidence (33.3%) as reported by Tramposch, *et al*[5], but a higher incidence than reported by Vlachoyiannopoulos, *et al*[6] (17%) and by Caramaschi, *et al*[7] (13.6%). Since all patients with SS in our study had more than one focus of mononuclear cell infiltrations in minor salivary gland biopsies without fibrotic change, the sicca features appearing in SSc and in other diseases may be derived not from a glandular fibrotic process, but from an overlap process of SS. It has also been clarified that the patients with SS had higher anti-CENP-B ELISA levels and higher concurrence of anti-SSA/Ro antibodies than the patients without SS.

We have reconfirmed a fairly close relationship between ACA and SSc with CREST features. The levels of ACA and frequent concurrence of other disease marker ANA may participate in development of heterogeneous clinical characteristics including overlap syndrome in patients with ACA.

REFERENCES

1. Tan EM, Rodnan GP, Garcia I, Moroi Y, Fritzler MJ, Peebles C. Diversity of antinuclear antibodies in progressive systemic sclerosis. Anti-centromere antibody and its relation to CREST syndrome. Arthritis Rheum 1980;23:617-25.
2. Fritzler MJ, Kinsella TD, Garbutt E. The CREST syndrome: A distinct serologic entity with anticentromere antibodies. Am J Med 1980;69:520-6.
3. Weiner ES, Earnshaw WC, Senecal J-L, Bordwell B, Johnson P, Rothfield N. Clinical associations of anticentromere antibodies and antibodies to topoisomerase I. A study of 355 patients. Arthritis Rheum 1988;31:378-85.
4. LeRoy EC, Krieg T, Black C, et al. Scleroderma (systemic sclerosis): Classification, subsets and pathogenesis. J Rheumatol 1988;15:202-4.
5. Tramposch HD, Smith D, Senecal J-L, Rothfield N. A long-term longitudinal study of anticentromere antibodies. Arthritis Rheum 1984;27:121-4.
6. Vlachoyiannopoulos PG, Drosos AA, Wiik A, Moutsopoulos HM. Patients with anticentromere antibodies, clinical features, diagnoses and evolution. Br J Rheumatol 1993;32:297-301.
7. Caramaschi P, Biasi D, Manzo T, Carletto A, Poli F, Bambara LM. Anticentromere antibody-clinical associations. A study of 44

patients. Rheumatol Int 1995;14:253-5.
8. Katano K, Kawano M, Koni I, Sugai S, Muro Y. Clinical and laboratory features of anticentromere antibody positive primary Sjögren's syndrome. J Rheumatol 2001;28:2238-44.
9. Bernstein RM, Callender ME, Neuberger JM, Hughes GRV. Anticentromere antibody in primary biliary cirrhosis. Ann Rheum Dis 1982;41:612-4.
10. Makinen D, Fritzler M, Davis P, Sherlock S. Anticentromere antibodies in primary biliary cirrhosis. Arthritis Rheum 1983;26:914-7.
11. McCarty GA, Rice JR, Bembe ML, Barada FA Jr. Anticentromere antibody. Clinical correlations and association with favorable prognosis in patients with scleroderma variants. Arthritis Rheum 1983;26:1-7.
12. Soma Y, Takehara K, Ishibashi Y. Clinical distribution of anticentromere antibody in Japanese patients. Dermatologica 1989;178:16-9.
13. Nakano M, Ohuchi Y, Hasegawa H, Kuroda T, Ito S, Gejyo F. Clinical significance of anticentromere antibodies in patients with systemic lupus erythematosus. J Rheumatol 2000;27:1403-7.
14. Zimmermann C, Steiner G, Skriner K, Hassfeld W, Petera P, Smolen JS. The concurrence of rheumatoid arthritis and limited systemic sclerosis. Clinical and serological characteristics of overlap syndrome. Arthritis Rheum 1998;41:1938-45.
15. Zuber M, Gotzen R, Filler I. Clinical correlation of anticentromere antibodies. Clin Rheumatol 1994;13:427-32.
16. Kallenberg CG, Pastoor GW, Wouda AA, The TH. Antinuclear antibodies in patients with Raynaud's phenomenon. Clinical significance of anticentromere antibodies. Ann Rheum Dis 1982;41:282-7.
17. Miyawaki S, Tojo T. Classification criteria for Sjögren's syndrome in Japan. In: Homma M, Sugai S, editors. Sjögren's syndrome — State of the art. Amsterdam/New York: Kugler Publications; 1994:77-9.
18. Mimori T, Akizuki M, Yamagata H, Inada S, Yosida S, Homma M. Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap. J Clin Invest 1981;68:611-20.
19. Kasukawa R, Tojo T, Miyawaki S. Preliminary diagnostic criteria for classification of mixed connective tissue disease. In: Kasukawa R, Sharp GC, editors. Mixed connective tissue diseases and antinuclear antibodies. Amsterdam: Elsevier; 1987:41-7.
20. Clements PJ, Furst DE, Campion DS, et al. Muscle disease in progressive systemic sclerosis. Diagnostic and therapeutic consideration. Arthritis Rheum 1978;21:62-71.
21. Earnshaw W, Bordwell B, Marino C, Rothfield N. Three human chromosomal autoantigens are recognized by sera from patients with anti-centromere antibodies. J Clin Invest 1986;77:426-30.
22. Conrad K, Stahnke G, Liedvogel B, et al. Anti-CENP-B response in sera of uranium miners exposed to quartz dust and patients with possible development of systemic sclerosis (scleroderma). J Rheumatol 1995;22:1286-94.
23. Kuwana M, Kaburaki J, Arnett FC, Howard RF, Medsger TA Jr, Wright TM. Influence of ethnic background on clinical and serological features in patients with systemic sclerosis and anti-DNA topoisomerase I antibody. Arthritis Rheum 1999;42:465-74.
24. Jacobsen S, Halberg P, Ullman S, et al. Clinical features and serum antinuclear antibodies in 230 Danish patients with systemic sclerosis. Br J Rheumatol 1998;37:39-45.
25. Sato S, Ihn H, Soma Y, Shimozuma M, Shishiba T, Takehara K. A case of systemic sclerosis with anticentromere, antitopoisomerase I, and anti-U1RNP antibodies. J Rheumatol 1993;20:1961-3.