Anticentromere-A and Anticentromere-B Antibodies Show High Concordance and Similar Clinical Associations in Patients with Systemic Sclerosis

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ABSTRACT. ***Objective.*** To determine the diagnostic sensitivity and specificity and the clinical usefulness of parallel anticentromere-A and anticentromere-B antibody (anti-CENP-A and anti-CENP-B) testing in patients with systemic sclerosis (SSc).

Methods. Sera from 280 consecutive patients with SSc and 259 controls were tested for the presence of anti-CENP-A and anti-CENP-B antibodies by a monospecific line immunoblot assay (LIA) with recombinant human centromere proteins A and B as well as by indirect immunofluorescence (IIF). Crossreactivity and possible associations with clinical manifestations were studied.

Results. Both antibodies revealed a diagnostic sensitivity of 36.8% and a specificity of > 97% for SSc, with a high concordance rate of 94.3% despite different amino acid sequences of the antigens and absence of crossreactivity. There was a significant correlation of the antibody levels measured by LIA. Both antibodies were associated with similar clinical manifestations and identified patients with limited disease and rather mild skin sclerosis.

Conclusion. Detected by LIA, anti-CENP-A and anti-CENP-B antibodies show high concordance in patients with SSc and share significant associations to clinical manifestations, but are not completely identical. Detection of both antibodies in parallel may slightly increase the diagnostic sensitivity for SSc. (First Release Oct 1 2010; J Rheumatol 2010;37:2548–52; doi:10.3899/jrheum.100402)

Key Indexing Terms:
SYSTEMIC SCLEROSIS ANTICENTROMERE ANTIBODIES

Systemic sclerosis (SSc) is a rare and heterogeneous connective tissue disease characterized by fibrosis, vascular pathology, and autoimmune inflammation. Its outcome may vary from mild to very severe and life-threatening. The detection of autoantibodies is an important part in the diagnostic process for prognosis and risk stratification.

Anticentromere antibodies (ACA) belong to the typical and highly specific autoantibodies in SSc first described by Moroi, *et al* in 1980[1]. These are a heterogeneous group of antibodies directed against different antigens clustered around the kinetochore, for example CENP-A (17 kDa), CENP-B (80 kDa), CENP-C (140 kDa), CENP-D (50 kDa), CENP-E (312 kDa), CENP-F (400 kDa), CENP-G (95 kDa), and CENP-O (38 kDa)[2,3,4,5]. For serological detection, CENP-A and CENP-B are available as purified recombinant antigens. With a sensitivity of 20%–30% for SSc, anti-CENP-B autoantibodies seem to have the greatest relevance for clinical practice among the various ACA[6,7,8,9].

There is debate about the diagnostic value of simultaneous detection of anti-CENP-A and anti-CENP-B autoantibodies in patients with SSc. Previous studies presumed a similar sensitivity and specificity for SSc of anti-CENP-A and anti-CENP-B autoantibodies by using ELISA[6,10]. A recent study presented evidence that there might be a higher specificity of anti-CENP-A antibodies for SSc[11]. We studied the diagnostic value of both antibodies in unselected sera from consecutive patients and tried to determine whether a simultaneous detection may provide additional information, especially concerning possible clinical associations. Line immunoblot assays (LIA) can detect various antibodies without loss of sensitivity and specificity, compared to other assays[12].

MATERIALS AND METHODS

Sera from 280 consecutive patients with SSc assessed from 2004 to 2007 were tested for the existence of anti-CENP-A and anti-CENP-B antibodies by a monospecific LIA with recombinant human centromere protein B

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(full-length) and recombinant human centromere protein A (full-length), provided by Euroimmun AG, Lübeck, Germany. Further, all sera were analyzed for their antibody staining pattern by indirect immunofluorescence (IIF). In immunoadsorption experiments, sera containing both autoantibodies were preadsorbed with either CENP-A or CENP-B antigens and afterward their reactivity in LIA was tested.

All analyses were conducted according to the manufacturer's instructions and carried out by staff unaware of the diagnosis and clinical characteristics of the patients.

The assessment and clinical characterization of patients with SSc was strictly realized according to the criteria of the German Network for Systemic Sclerosis and the European Scleroderma Trial and Research Network and conducted at the same time as the antibody detection. Patients were divided into different subsets depending on the extent of organ involvement as described[12,13,14,15]. Our study included 113 patients with limited disease (lcSSc), 96 patients with diffuse scleroderma (dcSSc), 51 patients with SSc overlap syndrome (including mixed connective tissue diseases), 16 patients with undifferentiated connective tissue disease (UCTD), and 4 patients with SSc without scleroderma skin features. As controls, serum samples from patients with systemic lupus erythematosus (SLE; n = 72), Sjögren's syndrome (SS; n = 49), and rheumatoid arthritis (RA; n = 88) as well as from healthy blood donors (n = 50) were included.

For evaluation of fibrotic skin changes, the modified Rodnan Skin Score (mRSS) was used[16]. Pulmonary fibrosis was defined as bibasilar fibrosis on chest radiograph and/or high resolution computer tomography scans. Pulmonary arterial hypertension (PAH) was defined as a mean pulmonary artery pressure above 25 mm Hg at rest or 30 mm Hg during exercise by right-heart catheterization or as a systolic pulmonary arterial pressure > 40 mm Hg by echocardiography. Lung function was assessed as predicted forced vital capacity (FVC) and predicted diffusion capacity (DLCO) in a single-breath method. The presence of 2 or more of the following symptoms was defined as cardiac involvement: diastolic dysfunction, conduction abnormalities, cardiomyopathy, reduced left ventricular ejection fraction, valvular changes, or pericarditis not explained by another cause than SSc. Renal involvement was defined as creatinine elevation, proteinuria, renal-caused hypertension, and present or past renal crisis due to SSc.

For statistical analysis, the SPSS V 15.0 statistical package and the Microsoft calculation software, Excel V 12 (2007), were used. To identify associations between SSc symptoms and the occurrence of ACA, chi-squared tests, Fisher's exact tests, Mann-Whitney U tests, OR, and Spearman's rank correlation were used[12,15]. P values < 0.05 were considered statistically significant.

The study was approved by the local ethical committee (EA1/013/705). Written informed consent was obtained from each patient.

RESULTS

Within the assessed cohort, anti-CENP-A and anti-CENP-B antibodies were the most frequent antibodies, with 103 positively tested sera, providing a sensitivity of 36.8% each (95% CI 31.2-42.5%). In the control groups, anti-CENP-B antibodies were found in 1 patient with SS (2%), in 1 patient with SLE (1.4%), and in 3 patients with RA (3.4%), and hence revealed a diagnostic specificity for SSc of 98.1% (95% CI 96.4-99.8%). In comparison, anti-CENP-A antibodies were found in 5 patients with SLE (6.9%), in 1 patient with RA (1.1%), and in 1 patient with SS (2%), providing a diagnostic specificity of 97.3% (95% CI 95.6-99.4%) for SSc. When both antibodies were considered in parallel, the diagnostic sensitivity for SSc increased to 37.9% (95% CI 32.2-43.6%) and the diagnostic specificity decreased to 96.1% (95% CI 93.8-98.5%). One hundred of the ACA-positive tested sera were double-positive for anti-CENP-A as well as for anti-CENP-B antibodies. Only 6 out of 280 (2.1%) patients with SSc showed a positive reaction to only 1 of both studied ACA. As a result, there was a concordance rate of 94.3% in the assessed cohort. Discordance of the antibodies' distribution could be detected in 2 patients with lcSSc and 1 patient with dcSSc, overlap syndrome, UCTD, and SSc without scleroderma, respectively. However, there was a significant correlation between the measured antibody levels irrespective of the underlying SSc subsets ($p = 0.522$, $p < 0.0005$; Figure 1A). In immunoadsorption experiments using CENP-A and CENP-B antigens, the reactivity of the opposite antibody was not affected and no crossreactivity was found (Figure 1B).

Comparison of the antibody staining pattern in IIF, in particular the occurrence of the centromere pattern, with the ACA positivity measured by LIA also revealed a high concordance. Only 1 serum revealing a centromere staining pattern in IIF was negatively tested in LIA when both ACA were considered. Three sera having either anti-CENP-A or -B antibodies did not have a centromere pattern in IIF. Two of these sera had low ACA levels in LIA and revealed a discrete speckled pattern. The third serum was additionally positive for anti-RNP antibodies and revealed a homogeneous speckled pattern in IIF. In summary, there is a concordance rate of centromere pattern in IIF with anti-CENP-B positivity by LIA of 97.5%, and with anti-CENP-A positivity, 98.2%. When both ACA were considered, the concordance rate of the centromere pattern in IIF was 98.6%.

Both ACA specificities characterize patients with lcSSc. Eighty-four of the 103 anti-CENP-B antibody-positive patients had lcSSc; this is related to 74.3% of all patients with lcSSc. With regard to anti-CENP-A antibody-positive patients, 82 had lcSSc (72.6%). Only 6 and 7 of the 96 patients with dcSSc were positively tested for anti-CENP-B or anti-CENP-A antibodies, respectively. If there was a positive test result for either anti-CENP-B or anti-CENP-A antibodies, the odds for having lcSSc increased to 22.6-fold (95% CI 11.9-42.7) and 18.4-fold (95% CI 9.9-34.1), respectively. The OR for dcSSc was 0.06 (95% CI 0.025-0.144) for both of the tested ACA.

Clinical associations related to the presence of ACA are shown in Table 1. There were only minor differences in the statistical results for both antibodies. Patients positive for anti-CENP-B or anti-CENP-A antibodies are characterized by a milder disease manifestation and less fibrosis and skin sclerosis. Only 13.6% of the anti-CENP-B (anti-CENP-A 15.5%) antibody-positive patients had lung fibrosis, in comparison to 47.5% of ACA-negative patients. The degree of skin fibrosis and sclerosis (assessed by mRSS) was also significantly lower in the ACA-positive patients. Further, ACA-positive patients had digital ulcers less frequently than the ACA-negative patients ($p = 0.043$). The proportion of

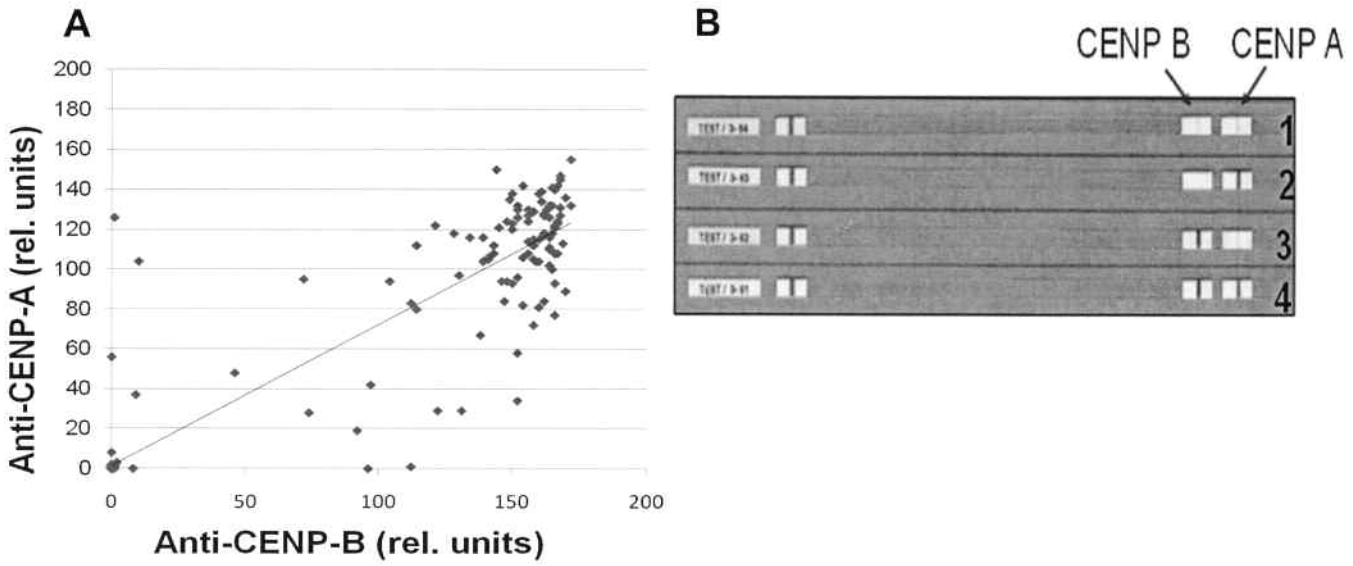

Figure 1. A. Levels of anticentromere-A antibodies and anticentromere-B antibodies measured by line immunoblot assay (LIA; relative units); Spearman's rank correlation p = 0.522; p < 0.0005. B. Example from immunoadsorption test experiments. A representative serum containing anticentromere (CENP)-A and -B antibodies was incubated with CENP-A or CENP-B antigens and subsequently incubated in an LIA coated with recombinant, purified centromere antigens. For example, strip no. 3 shows that the CENP-A antigen complexes only anti-CENP-A antibodies in the serum. Anti-CENP-B antibodies remain unbound; they still can bind to the coated CENP-B antigen on the LIA. Strip 1 shows serum adsorbed with CENP-A and CENP-B antigen. Strip 2 shows serum adsorbed with CENP-B antigen. Strip 4 shows serum without adsorption.

Table 1. Overview of the proportion of different disease manifestations in anticentromere-B antibody-positive patients (n = 103), anticentromere-A antibody-positive patients (n = 103), and anticentromere antibody-negative patients (n = 177). P values were calculated by chi-squared tests comparing anticentromere antibody-positive and negative patients.

Disease Manifestation	Anti-CENP-B+, n = 103	Anti-CENP-A+, n = 103	ACA–, n = 177	p, Anti-CENP-B+ vs ACA–	p, Anti-CENP-A+ vs ACA–
Lung fibrosis, %	13.6	15.5	47.5	< 0.0005*	< 0.0005*
PAH, %	24.3	25.2	19.8	0.231	0.290
mRSS, median (IQR)	4.0 (4.0)	4.0 (4.0)	7.0 (12.0)	< 0.0005*	< 0.0005*
Digital ulcers, %	32.0	32.0	44.6	0.043*	0.043*
Gastrointestinal involvement, %	77.7	75.7	76.8	1.000	0.900
Musculoskeletal involvement, %	87.4	87.4	93.2	0.127	0.127
Cardiac involvement, %	29.1	30.1	46.9	0.007*	0.008*
Renal involvement, %	17.5	17.5	21.5	0.443	0.443
Nervous system involvement, %	12.6	11.7	18.1	0.244	0.133
Sicca syndrome, %	68.9	69.9	68.9	1.000	1.000

* Significant. ACA: anticentromere antibodies; CENP: centromere; PAH: pulmonary arterial hypertension; mRSS: modified Rodnan skin score; IQR: interquartile range.

patients with cardiac involvement was also significantly smaller in the ACA-positive group. The occurrence of PAH was slightly more frequent in the ACA-positive patients but with no significant difference compared to ACA-negative patients. No correlations were found between the antibody levels and the mRSS, the DLCO, and the FVC.

Although a higher frequency of PAH and a lower frequency of cardiac involvement, lung fibrosis, and creatine kinase (CK) elevation were detectable at first glance in the anti-CENP-A or -B antibody-positive patients in the lcSSc subset (Table 2), these clinical associations could not be statistically confirmed. This lack of confirmation could be due to a low number of cases. In addition, a slightly higher frequency of PAH, sicca syndrome, and tendon friction rubs were seen in the ACA-positive patients with dcSSc. Further, lung fibrosis and kidney involvement had a lower frequency in those patients (data not shown). Due to the small number of patients with dcSSc who did not have ACA, no appropriate statistical analysis could be performed.

DISCUSSION

Anticentromere antibodies belong to the most prevalent and

Table 2. Overview of the proportion of different disease manifestations in the subset of limited systemic sclerosis (n = 113), based on anticentromere antibody status. P values calculated by chi-squared tests, except in the case of mRSS, where Mann-Whitney U tests were used.

Disease Manifestation	Anti-CENP-B+, n = 84	Anti-CENP-B–, n = 29	p*	Anti-CENP-A+, n = 82	Anti-CENP-A–, n = 31	p**
mRSS, median (IQR)	4.0 (3.75)	5.0 (5.0)	0.759	4.0 (3.25)	5.0 (6.0)	0.816
Digital ulcers, %	33.3	34.5	1.000	34.1	32.3	1.000
Lung fibrosis, %	13.1	17.2	0.552	13.4	16.1	0.765
PAH, %	22.6	10.3	0.183	22.0	12.9	0.425
Cardiac involvement, %	27.4	41.4	0.170	25.6	45.2	0.067
Gastrointestinal involvement, %	78.6	75.9	0.798	78.0	77.4	1.000
Renal involvement, %	19.0	20.7	1.000	19.5	19.4	1.000
Renal crisis, %	2.4	3.6	1.000	2.4	3.3	1.000
Musculoskeletal involvement, %	90.5	89.7	1.000	90.2	90.3	1.000
Joint contractures, %	51.2	65.5	0.201	52.4	61.3	0.526
CK elevation, %	3.6	10.3	0.175	3.7	9.7	0.343
Nervous system involvement, %	15.5	24.1	0.397	14.6	25.8	0.177
Sicca syndrome, %	65.5	65.5	1.000	65.9	64.5	1.000

* Calculated between anti-CENP-B antibody-positive and negative patients. ** Calculated between anti-CENP-A antibody-positive and negative patients. mRSS: modified Rodnan skin score; IQR: interquartile range; PAH: pulmonary arterial hypertension; CK: creatine kinase.

highly specific antibodies in patients with SSc, characterizing patients with limited disease and less fibrosis. In our study, a well characterized single-center cohort was analyzed regarding the prevalence and clinical associations of 2 ACA subtypes, anti-CENP-A and anti-CENP-B.

The detected frequencies of 36.8% in patients with SSc and of about 74.3% for lcSSc in the cohort are similar to previous study results, which revealed a prevalence of anti-CENP-B antibodies in patients with SSc between 20% and 47.5% in general and between 50% and 70% specifically for patients with lcSSc[8,9,13,14].

Irrespective of the underlying SSc subset, a high concordance of positive reactivity of 94.3% to either anti-CENP-A or -B antibodies could be detected. Similar results using ELISA were published by Russo, *et al*, who could also show a high concurrence of anti-CENP-A and -B antibodies in 45 sera selected by their positive centromere pattern in IIF[10]. Additionally, there was a high concordance rate of the ACA positivity detected by the newly developed LIA and the other test systems (ELISA, IIF) that were used. The slight discordance of the antibody distribution between the LIA and ELISA or IIF may be caused by a lack of 100% sensitivity of each test system and partially due to measured titers slightly below or above the cutoff values. Anticentromere-A or -B antibody positivity by ELISA with concurrent absence of a typical centromere pattern in IIF was seen in a recently published study as well, and was interpreted as the described nuclear speckled pattern 1[11].

Because of the significant concordance rate, there is also a high congruence regarding clinical associations. To analyze whether the frequencies and high concordance within the assessed cohort are possibly based on crossreactivity between both antibodies, immunoadsorption tests were performed and no crossreactivity was found — at least *in vitro*. Alignment analyses also revealed completely different amino acid sequences (data not shown).

The observed differences of the clinical manifestations associated with either ACA positivity or negativity in the whole cohort are similar to those previously published, and may be partially explained by the high proportion of lcSSc within the ACA-positive patient subset[11,14]. However, the distribution of clinical manifestations reflects the typical characteristics of dcSSc and lcSSc[8,12,14]. Analyzing the subsets of lcSSc and dcSSc separately, no statistically significant differences of clinical associations between ACA-positive and ACA-negative patients could be detected. Hence, there was a tendency toward a higher frequency of PAH and a lower frequency of cardiac involvement, lung fibrosis, and CK elevation in the ACA-positive lcSSc patients. No differences in skin involvement or digital ulcers could be seen. The observed differences of clinical manifestations of the ACA-positive and negative patients in the dcSSc group have to be interpreted cautiously because of the low number of only 6 ACA-positive patients out of 96 patients with dcSSc.

In the study cohort, a very high proportion of patients had pulmonary arterial hypertension. In the group of ACA-positive patients, PAH was slightly more frequent than in ACA-negative patients, but there was no statistically significant difference between the 2 groups. Several reasons may explain the high proportion of PAH in the cohort. All patients were regularly screened either by echocardiography or right-heart catheter as described, but defining PAH by echocardiography tends to overestimate the true incidence of PAH[17]. In addition, we have analyzed data from a wide variety of SSc subsets, and these subsets may have a higher incidence of PAH compared to the classical lcSSc and dcSSc subsets[18]. However, we could not confirm that ACA positivity is associated with a significantly higher frequency

of PAH, but the number of patients may be too small for the detection of significant differences. Further, the number of patients with renal and cardiac involvement seemed to be comparatively large, which may be the result of a selection bias toward more severe cases in our tertiary referral center[14].

Interestingly, anti-CENP-A antibodies showed a higher frequency among the patients with SLE (6.9%) than the anti-CENP-B antibodies did (1.4%), suggesting that these antibodies may play some role in SLE as well. Further studies are necessary to verify these findings.

As a result of the almost identical prevalence and high concordance of the tested ACA, their diagnostic value seems to be almost identical when analyzed independently. But there was a slight increase of the diagnostic sensitivity for SSc when both antibodies were detected simultaneously. In contrast to others' findings, a diagnostic predominance of either anti-CENP-A or -B antibodies for SSc could not be detected, at least not in the assessed cohort[11]. The distinct role of both antibodies in the limited subset of SSc remains to be further investigated, because differences in the frequencies of certain clinical manifestations between ACA-positive and negative patients with lcSSc could be detected.

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