EXTENDED REPORT

Distinct recognition of antibodies to centromere proteins in primary Sjögren's syndrome compared with limited scleroderma

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Background: Anticentromere antibodies are characteristically observed in scleroderma but have recently been reported in other autoimmune rheumatic disorders, including Sjögren's syndrome. It is not known whether distinct centromere proteins (CENP) are targeted in primary Sjögren's syndrome (pSS) and scleroderma.

Objective: To determine whether antibodies to CENP-B and CENP-C are present in these two disorders.

Methods: Sera from 45 patients with pSS and 33 with limited scleroderma were studied. All patients met classification criteria for pSS and scleroderma, respectively. Sera were used to immunoprecipitate in vitro translated CENP-B and CENP-C. The proportions recognising CENP-B or CENP-C were compared.

Results: 10 of 45 patients (22%) with pSS and 18 of 33 (55%) with scleroderma had antibodies recognising CENPs ($p=0.004$). Seven of 10 (70%) CENP positive patients with pSS recognised CENP-C alone, compared with one of 18 (6%) CENP positive patients with scleroderma (odds ratio (OR) = 40 (95% confidence interval (CI), 3.5 to 450) ($p=0.003$). In contrast, the majority (15 of 18 (83%)) of CENP positive scleroderma sera recognised both CENP-B and CENP-C, compared with none of 10 pSS sera (OR = 93 (95% CI, 4.4 to 1979) ($p=0.0001$).

Conclusions: The pattern of CENP recognition differs markedly in pSS and limited scleroderma. While patients with pSS predominantly recognise CENP-C alone, dual recognition of CENP-B and CENP-C is most frequent in scleroderma. These findings suggest that obtaining antibodies to specific centromere antigens is useful diagnostically, and imply that distinct mechanisms underlie the unique patterns of centromere autoreactivity in pSS and scleroderma.

Anticentromere antibodies are recognised as a distinct feature in scleroderma.¹ The strong association of these antibodies with the limited subset of scleroderma, including the CREST variant (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia),² provides a valuable diagnostic tool in the clinical evaluation of patients presenting with Raynaud's phenomenon.^{3,4} Additionally, anticentromere antibodies form a valuable prognostic marker for the development of ischaemic digital loss within the scleroderma spectrum.^{5,6}

More recently, anticentromere antibodies have been detected in sera from patients with autoimmune rheumatic disorders other than scleroderma, including primary Sjögren's syndrome (pSS), systemic lupus erythematosus, and rheumatoid arthritis.^{7–9} Relatively little is known, however, about the specific target of anticentromere antibodies in the various rheumatic disorders. In recent studies, we observed that centromere protein (CENP)-C is a target of autoantibodies in pSS, and identifies a subset of patients uniformly seropositive for Ro and La.¹⁰ Prompted by these findings, we next sought to determine whether the pattern of centromere protein autoreactivity found in pSS is different from that observed in scleroderma. If distinct recognition to centromere proteins is indeed linked to specific rheumatic disorders, the diagnostic value and specificity of anticentromere antibodies would be further enhanced, and additional insight into the unique immunising events underlying each disorder may be gained.

In these studies, we compared the recognition of CENPs B and C in sera from well defined cohorts of patients with pSS

and limited scleroderma. Our findings show that the two patient populations have clearly distinct patterns of CENP recognition. While most CENP positive patients with limited scleroderma recognise both CENP-B and CENP-C, patients with pSS predominantly recognise CENP-C alone.

METHODS

Patients

Forty five patients with pSS evaluated at the National Institutes of Health (NIH) were studied. This group constitutes all but two of the previously reported 47 patients with Sjögren's syndrome¹⁰ (two patients with secondary Sjögren's syndrome with underlying CREST syndrome were excluded from the present report), to examine CENP autoreactivity in pSS versus scleroderma. These 45 patients with pSS were compared with 33 patients with limited scleroderma assessed at the Johns Hopkins Scleroderma Center⁶ (table 1). Patients with pSS were selected on the basis of serum availability from consecutive patients in the large NIH pSS cohort. Those with scleroderma were selected from the Hopkins cohort on the basis of manifesting limited cutaneous disease¹¹; 18 of the 33 patients had suffered ischaemic digital loss. All 78 patients met classification criteria for pSS and scleroderma, respectively.^{12,13}

Abbreviations: ANA, antinuclear antibody; CENP, centromere protein; pSS, primary Sjögren's syndrome

Table 1 Demographic, clinical, and serological profile among 45 patients with primary Sjögren's syndrome and 33 patients with limited scleroderma

Variable	Sjögren's syndrome (n = 45)	Limited scleroderma (n = 33)	p Value
Age (years) (mean (SD))	58 (14)	57 (12)	0.72
Female	44 (98%)	31 (94%)	0.57
White	33 (73%)	28 (85%)	0.27
Xerophthalmia	36 (80%)	15 (45%)	0.002
Xerostomia	41 (91%)	14 (42%)	<0.0001
Raynaud's phenomenon	13 (29%)	32 (97%)	<0.0001
Satisfy diagnostic criteria	45 (100%)	33 (100%)	1.00
Anti-nuclear antibody	39 (87%)	33 (100%)	0.036
Centromere immunofluorescence staining pattern	3 (7%)	19 (58%)	<0.0001
Centromere protein B or C antibody	10 (22%)	18 (55%)	0.004

Data presented as frequency (%) unless otherwise indicated.

Methods

Demographic and clinical features were recorded on all patients. Furthermore, each patient with pSS underwent a uniform evaluation by protocol, including standardised queries about symptoms of xerophthalmia, xerostomia, and Raynaud's phenomenon.¹⁴ This protocol included a Schirmer's test, assessment of tear film break up time, eye lissamine green Van Bijsterveld score, and labial salivary gland biopsy. Those patients with scleroderma were similarly assessed for symptoms of xerophthalmia, xerostomia, and Raynaud's phenomenon, and for prevalent pulmonary hypertension, interstitial lung disease, and renal insufficiency, using accepted criteria.¹⁵

Antinuclear antibody (ANA) titre and immunofluorescence pattern were determined using Hep-2 cells as substrate. ANA titres of $\geq 1:160$ were considered positive. Anticentromere antibody was detected by its characteristic staining pattern on indirect immunofluorescence. Of note, this assay detects antibodies to any or all centromere proteins (alone or in combination) and does not distinguish between them. Among the patients with pSS, antibodies to SS-A (Ro) and SS-B (La) were assayed by enzyme linked immunosorbent assay (ELISA).

Immunoprecipitation of ³⁵S-methionine labelled CENP-B and CENP-C

³⁵S-Methionine labelled CENP-B and CENP-C were generated by coupled in vitro transcription/translation (IVTT) using the appropriate full length complementary DNAs (kind gifts from Dr A Pluta). Immunoprecipitations using these two radiolabelled substrates were carried out using patient sera as described.¹⁶ To determine whether antibodies to CENPs B or C, or both, were present in each serum, immunoprecipitated proteins were electrophoresed on 10% SDS-polyacrylamide gels and visualised by fluorography. In contrast to the immunofluorescence method used to assess ANA status and titre (above), the immunoprecipitation assay allows determination of precisely which CENP proteins are recognised by the anticentromere positive sera. We have focused on CENPs B and C in this study.

Analysis

The association of demographic and clinical variables with disease category (pSS v limited scleroderma) was compared using the Student *t* test for continuous variables and Fisher's exact test for categorical variables. The proportions of pSS and scleroderma sera recognising the CENP-B and CENP-C antigens were similarly assessed. Odds ratios were calculated, using logistic regression, to estimate the strength of association between distinct patterns of CENP recognition (CENP-B alone; CENP-C alone; or both CENP-B and CENP-C) with these two diseases and with their defining presenting

symptoms. In the circumstance where one of the four cell frequencies, in the related 2×2 table, was equal to zero, the odds ratio was calculated according to the modification suggested by Fleiss.¹⁷ We also examined the sensitivity, specificity, and predictive value of the distinct CENP autoreactivity patterns, to distinguish between the two rheumatic disorders among the studied patients.

RESULTS

The demographic, clinical, and serological profiles of the 78 patients with pSS and limited scleroderma are shown in table 1. Mean (SD) age of the patients with pSS was 58 (14) years, which was similar to that in the limited scleroderma group (57 (12) years). All but three of the 78 patients were women and most were white.

These patients showed the characteristic features of their respective rheumatic disorders (table 1). Thirty six of the 45 patients with pSS (80%) reported having daily, persistent, troublesome dry eyes for more than three months. Forty one (91%) experienced a recurrent sensation of sand or gravel in their eyes. In addition, 41 (91%) conveyed a daily sensation of dry mouth for more than three months, while 37 (82%) answered affirmatively to frequently drinking liquids to aid in swallowing dry foods. Nearly one third (n = 13; 29%) had digital cold sensitivity consistent with Raynaud's phenomenon. We reviewed the profiles of these 13 patients with pSS and Raynaud's phenomenon to be certain no others signs of limited scleroderma were present. Antibodies to Ro and La were detected by ELISA in 28 (62%) and 12 (27%) of these patients, respectively. Thirty seven patients (82%) with pSS had an abnormal Schirmer's test in at least one eye, while 42 of 44 (95%) so tested had an abnormal tear film break up time. In addition, the lissamine green Van Bijsterveld score was abnormal in 37 of 44 tested patients (84%). Labial salivary gland biopsy was positive (with clusters of 50 or more lymphocytes, histiocytes, and plasma cells per 4 mm²) in 40 of these patients (89%). Among the 33 patients with limited scleroderma, 15 (45%) reported dry eyes (p = 0.002), 14 (42%) dry mouth (p<0.0001), and all but one (97%) experienced Raynaud's phenomenon (p<0.0001).

Next, we examined the frequency of ANA seropositivity and anticentromere antibody status, by characteristic immunofluorescence staining pattern, in the two disorders. The large majority of those with pSS were seropositive (n = 39, 87%) for ANA, yet only three (7%) had a centromere staining pattern. In contrast, all 33 patients with limited scleroderma were uniformly seropositive for ANA (p<0.036) and more than half (n = 19, 58%) showed a centromere immunofluorescence pattern (p<0.0001). Subsequently, to determine whether antibodies directed against the same or different CENPs were present in the sera of patients with pSS and scleroderma, in vitro translated ³⁵S-methionine labelled

CENP-B and CENP-C were immunoprecipitated using these sera. Sera were categorised as being CENP negative, CENP-B positive only, CENP-C positive only, or both CENP-B and CENP-C positive. In fig 1, representative examples of sera from patients with either limited scleroderma (lane 1) or pSS (lanes 2–4), recognising both CENP-B and CENP-C (lane 1), CENP-B alone (lane 2), CENP-C alone (lane 3), or neither centromere protein (lane 4), are shown. Of note, CENP-B radiolabelled more strongly than CENP-C—hence the difference in signal intensity in the lanes. The frequency and patterns of CENP reactivity in the two patient groups were clearly distinct. Antibodies to CENP proteins were detected in 10 of 45 (22%) patients with pSS, compared with 18 of 33 (55%) patients with scleroderma ($p = 0.004$).

Interestingly, sera from patients with pSS and scleroderma had strikingly distinct patterns of CENP recognition. Seven of 10 CENP positive patients with pSS (70%) recognised CENP-C alone, compared with one of 18 CENP positive patients with scleroderma (6%) (OR = 40 (95% CI, 3.5 to 450)) ($p = 0.003$). Thus, within this subset of 28 patients with these two well defined rheumatic disorders who were seropositive for CENP-B or CENP-C, recognition of CENP-C alone showed 70% sensitivity, 94% specificity, and 87.5% positive predictive value in distinguishing pSS sera from scleroderma sera. In contrast, the majority (15 of 18 (83%)) of CENP positive scleroderma sera recognised both CENP-B and CENP-C, compared with none of 10 pSS sera (OR = 93 (95% CI, 4.4 to 1979)) ($p = 0.0001$). Dual CENP-B and CENP-C positivity therefore showed 83% sensitivity, 100% specificity, and 100% positive predictive value in differentiating CENP positive patients with scleroderma from those with pSS in this study. Lastly, three of 10 CENP positive patients with pSS (30%), compared with two of 18 (11%) CENP positive patients with scleroderma, had antibodies recognising CENP-B alone ($p = 0.32$).

Of note, among those with scleroderma, there were 19 patients who were anticentromere antibody positive by immunofluorescence assay. In this group, 13 had antibodies against both CENP-B and CENP-C, two had antibodies

Figure 1 Immunoprecipitation of in vitro translated ^{35}S -methionine labelled centromere proteins (CENP). Sera from four different patients with either limited scleroderma (lane 1) or primary Sjögren's syndrome (pSS) (lanes 2–4) were used to immunoprecipitate ^{35}S -methionine labelled CENP-B (upper panel) and CENP-C (lower panel). The profiles shown are representative of those sera that have antibodies to both CENP-B and CENP-C (lane 1), CENP-B only (lane 2), CENP-C only (lane 3), or neither CENP (lane 4). The immunoprecipitations were all carried out at the same time and electrophoresed on a single gel. To equalise the immunoprecipitation band intensities for making the figure, two different autorad exposures were used: a longer exposure was used for the CENP-C data and a shorter one for the CENP-B data. Intervening lanes have been spliced out because the gel was not loaded in the exact order in which the data are presented.

Table 2 The association of xerophthalmia, xerostomia, Raynaud's phenomenon, and ACR diagnosis with dual recognition of centromere proteins B and C*

Model	OR	95% CI	p Value
Raynaud's phenomenon	27	1.3 to 540	0.0046
Xerophthalmia	0.19	0.04 to 1.02	0.055
Xerostomia	0.14	0.02 to 0.96	0.038
ACR diagnosis of scleroderma v primary SS	93	4.4 to 1979	0.0001

*Univariate relations modelled using logistic regression analysis. ACR, American College of Rheumatology; CI, confidence interval; OR, odds ratio; SS, Sjögren's syndrome.

against CENP-B, and four did not have antibodies against either protein. Of the 14 patients with scleroderma who were anticentromere antibody negative, 11 were negative for both CENP-B and CENP-C, two were dual CENP-B and CENP-C positive, and one was CENP-C positive only. Among the 45 patients with pSS, only three had anticentromere antibodies as assessed by immunofluorescence assay, and these three all had antibodies to CENP-B. Of the remaining 42 anticentromere antibody negative sera from the patients with pSS, seven had antibodies to CENP-C alone.

Finally, as Raynaud's phenomenon, xerophthalmia, and xerostomia are common features of both pSS and scleroderma, we examined the association of these characteristic disease symptoms, and that of a well defined diagnosis, with autoantibody recognition to both CENP-B and CENP-C. The presence of Raynaud's phenomenon was strongly related to dual CENP-B and CENP-C recognition (OR = 27 (95% CI, 1.3 to 540)) (table 2). In contrast, xerophthalmia and xerostomia were each associated with an 80% reduction in the prevalence of dual CENP recognition. Thus the presence of dry eyes (OR = 0.19 (95% CI, 0.04 to 1.02)) and dry mouth (OR = 0.14 (95% CI, 0.02 to 0.96)) substantially diminished the likelihood that autoantibodies to both CENP-B and CENP-C were present. Of note, dual CENP recognition was most strongly related to a well defined diagnosis of scleroderma (OR = 93 (95% CI, 4.4 to 1979)).

DISCUSSION

In the present report, we examined the pattern of autoantibody recognition to centromere proteins in two autoimmune rheumatic disorders with several overlapping clinical features. Sera from patients with pSS and those with limited scleroderma were found to differ markedly in this regard. While CENP positive patients with pSS recognise predominantly CENP-C alone, this pattern was very uncommon in CENP positive patients with scleroderma. In contrast, dual recognition of CENP-B and CENP-C is a feature found exclusively in patients with limited scleroderma and was absent in those with pSS.

Establishing and distinguishing the diagnosis of one rheumatic disorder from another can be quite challenging, particularly early in the disease course. Overlapping symptoms common to several rheumatic disorders contribute to this challenge. In particular, xerostomia and xerophthalmia—symptoms characteristic of pSS—are often observed in scleroderma^{18, 19} and were present in almost half the patients with scleroderma described in this study. Similarly, Raynaud's phenomenon—a hallmark feature in scleroderma—was observed in approximately one third of the patients with pSS in this report, and has been described previously.²⁰ Given these considerations, the detection of characteristic autoantibody patterns in addition to the signs and symptoms elucidated by history and physical

examination represents a meaningful part of the diagnostic evaluation of patients presenting with rheumatological disorders.

One such valuable serological test, anticentromere antibody, has been a standard component of rheumatological evaluation for more than a decade, and has predominantly been associated with the limited form of scleroderma.^{2 4 21 22} Several reports have emphasised, however, that anticentromere antibody—as detected by immunofluorescence pattern on Hep-2 cells—is not specific to scleroderma alone. For example, anticentromere antibody was observed in 12 of 216 patients with systemic lupus erythematosus (5.6%),²³ uniformly in a series of 12 patients with pSS,²⁴ and during evolution from a lupus–Sjögren's syndrome overlap to the CREST syndrome.²⁵ Several studies have shown that anticentromere antibodies recognise specific centromeric proteins, identified as autoantigens in scleroderma.^{26–28} These antigens (including CENP-B and CENP-C) have also been detected in patients with Raynaud's phenomenon and sicca symptoms, and among individuals with the CREST syndrome and the sicca complex.²⁹ Whether the pattern of CENP recognition differs from one to another rheumatic disorder has not yet been defined.

CENP-B and CENP-C are components of the human centromere.³⁰ CENP-B functions in the recognition of α satellite DNA sequences found at centromeres,³¹ whereas CENP-C has been identified as a component of the inner kinetochore plate.²⁸ Recently, CENP-C was also shown to bind to centromeric DNA sequences in vivo.³⁰ Of note, while CENP-B is present at both functional and inactive centromeres, CENP-C is located exclusively at the kinetochore of functional centromeres.³² The anti-centromere pattern on immunofluorescence is well correlated with the presence of antibodies to CENP-B but not to CENP-C. Thus the distinct patterns of recognition of specific centromere proteins in pSS (CENP-C only) and scleroderma (CENP-B and CENP-C) may reflect differences in the structures targeted by the immune system at disease initiation in the two processes. While active centromeres are the likely target in scleroderma, CENP-C targeting in pSS probably occurs through other mechanisms.

A limitation of the present report is its cross sectional nature. Serological status for CENP-B and CENP-C was determined for each patient with pSS and scleroderma at a single point in time. We are therefore unable to evaluate whether antibodies directed against centromere proteins are necessary to initiate the development of these disorders, or are merely markers of disease. Second, the present report aggregates demographic, clinical, and serological information culled from two distinct cohorts that were collected at separate institutions, for different purposes, using distinct protocols. Third, we were unable to evaluate the prevalence and potential discriminatory value of CENP-A autoreactivity among the patients studied. Notwithstanding these limitations, however, serological analysis of the different cohorts for CENP-B and CENP-C was conducted in a uniform fashion from sera collected from the 78 patients in this study, and clearly identifies a distinct pattern of centromere protein recognition in pSS compared with limited scleroderma.

The identification of new and specific serological patterns may provide a valuable diagnostic tool for the better characterisation of patients presenting with xerophthalmia, xerostomia, and Raynaud's phenomenon. Thus, among such patients—particularly those presenting in an undifferentiated manner early in their course—the opportunity to assay for CENP-B and CENP-C reactivity pattern may offer a discriminating serological test to identify the underlying autoimmune disorder. The pattern of CENP reactivity may also be of predictive value during longitudinal care of such patients. Interestingly, among the 10 patients described here

with pSS and CENP recognition, seven who were CENP-C positive did not show a centromere pattern by traditional indirect immunofluorescence. These findings suggest that other assay systems (for example, ELISA or immunoprecipitation) may be necessary to capture the full pattern of CENP reactivity in pSS and scleroderma and they warrant confirmation by other centres.

In the present report, sera from patients with pSS and limited scleroderma were found to differ noticeably in pattern of CENP recognition. The patterns of CENP-C seropositivity in isolation, and of dual CENP-B and CENP-C recognition, correctly identified the phenotype in the vast majority of these anticentromere positive patients with pSS and limited scleroderma. These findings suggest that obtaining antibodies to specific centromere antigens will be useful diagnostically, and imply that distinct mechanisms underlie the unique patterns of centromere autoreactivity in pSS and scleroderma.

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REFERENCES

- Moroi Y, Peebles C, Fritzler MJ, Steigerwald J, Tan EM. Autoantibody to centromere (kinetochore) in scleroderma sera. *Proc Natl Acad Sci USA* 1980;**77**:1627–31.
- Fritzler MJ, Kinsella TD. The CREST syndrome: a distinct serologic entity with anticentromere antibodies. *Am J Med* 1980;**69**:520–6.
- 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**:382–7.
- Steen VD, Ziegler GL, Rodnan GP, Medsger TA. Clinical and laboratory associations of anticentromere antibody in patients with progressive systemic sclerosis. *Arthritis Rheum* 1984;**27**:125–31.
- Wigley FM, Wise RA, Miller R, Needleman BW, Spence RJ. Anticentromere antibody as a predictor of digital ischemic loss in patients with systemic sclerosis. *Arthritis Rheum* 1992;**35**:688–93.
- Schachna L, Wigley FM, Morris S, Gelber AC, Rosen A, Casciola-Rosen L. Recognition of Granzyme B-generated autoantigen fragments in scleroderma patients with ischemic digital loss. *Arthritis Rheum* 2002;**46**:1873–84.
- Tramposch HD, Smith CD, Senecal JL, Rothfield N. A long-term longitudinal study of anticentromere antibodies. *Arthritis Rheum* 1984;**27**:121–4.
- Chan HL, Lee YS, Hong HS, Kuo TT. Anticentromere antibodies (ACA): clinical distribution and disease specificity. *Clin Exp Dermatol* 1994;**19**:298–302.
- 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.
- Pillemer SR, Casciola-Rosen L, Baum BJ, Rosen A, Gelber AC. Centromere protein C is a target of autoantibodies in Sjögren's syndrome and is uniformly associated with antibodies to Ro and La. *J Rheumatol* 2004;**31**:1121–5.
- Leroy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;**15**:202–5.
- Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;**61**:554–8.
- Leroy EC, Medsger TA. Criteria for the classification of early systemic sclerosis. *J Rheumatol* 2001;**28**:1573–6.
- Atkinson JC, Travis WD, Pillemer SR, Bermudez D, Wolff A, Fox PC. Major salivary gland function in primary Sjögren's syndrome and its relationship to clinical features. *J Rheumatol* 1990;**17**:318–22.

- 15 **Medsger TA**, Silman AJ, Steen VD, Black CM, Akesson A, Bacon PA, et al. A disease severity scale for systemic sclerosis: development and testing. *J Rheumatol* 1999;**26**:2159–67.
- 16 **Casciola-Rosen LA**, Pluta AF, Plotz PH, Cox AE, Morris S, Wigley FM, et al. The DNA mismatch repair enzyme PMS1 is a myositis-specific autoantigen. *Arthritis Rheum* 2001;**44**:389–96.
- 17 **Fleiss JL**. *Statistical methods for rates and proportions*, 2nd edition. New York: John Wiley, 1981.
- 18 **Cipoletti JF**, Buckingham RB, Barnes EL, Peel RL, Mahmood K, Cignetti FE, et al. Sjogren's syndrome in progressive systemic sclerosis. *Ann Intern Med* 1977;**87**:535–41.
- 19 **Alarcon-Segovia D**, Ibanez G, Hernandez-Ortiz J, Velazquez-Forero F, Gonzalez-Jimenez Y. Sjogren's syndrome in progressive systemic sclerosis (scleroderma). *Am J Med* 1974;**57**:78–85.
- 20 **Garcia-Carrasco M**, Siso A, Ramos-Casals M, Rosas J, de la RG, Gil V, et al. Raynaud's phenomenon in primary Sjogren's syndrome. Prevalence and clinical characteristics in a series of 320 patients. *J Rheumatol* 2002;**29**:726–30.
- 21 **Silver RM**. Clinical aspects of systemic sclerosis (scleroderma). *Ann Rheum Dis* 1991;**50**(suppl 4):854–61.
- 22 **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 relationship to CREST syndrome. *Arthritis Rheum* 1980;**23**:617–25.
- 23 **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.
- 24 **Katano K**, Kawano M, Koni I, Sugai S, Muro Y. Clinical and laboratory features of anticentromere antibody positive primary Sjogren's syndrome. *J Rheumatol* 2001;**28**:2238–44.
- 25 **Ford AL**, Kurien BT, Harley JB, Scofield RH. Anti-centromere autoantibody in a patient evolving from a lupus/Sjogren's overlap to the CREST variant of scleroderma. *J Rheumatol* 1998;**25**:1419–24.
- 26 **Earnshaw WC**, Machlin PS, Bordwell BJ, Rothfield NF, Cleveland DW. Analysis of anticentromere autoantibodies using cloned autoantigen CENP-B. *Proc Natl Acad Sci USA* 1987;**84**:4979–83.
- 27 **Vazquez-Abad D**, Tian L, Zanetti M, Rothfield NF. A cross-reactive idiotype in scleroderma. *Clin Exp Immunol* 1997;**108**:420–7.
- 28 **Saitoh H**, Tomkiel J, Cooke CA, Ratrie H, Maurer M, Rothfield NF, et al. CENP-C, an autoantigen in scleroderma, is a component of the human inner kinetochore plate. *Cell* 1992;**70**:115–25.
- 29 **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.
- 30 **Politi V**, Perini G, Trazzi S, Pliss A, Raska I, Earnshaw WC, et al. CENP-C binds the alpha-satellite DNA in vivo at specific centromere domains. *J Cell Sci* 2002;**115**:2317–27.
- 31 **Pluta AF**, Saitoh N, Goldberg I, Earnshaw WC. Identification of a subdomain of CENP-B that is necessary and sufficient for localization to the human centromere. *J Cell Biol* 1992;**116**:1081–93.
- 32 **Jabs EW**, Tuck-Muller CM, Anhalt GJ, Earnshaw W, Wise RA, Wigley F. Cytogenetic survey in systemic sclerosis: correlation of aneuploidy with the presence of anticentromere antibodies. *Cytogenet Cell Genet* 1993;**63**:169–75.