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ORIGINAL RESEARCH ARTICLE

Anti-CENP-A/B reactivity in samples exhibiting the centromere HEp-2 pattern is associated with a lower frequency of interstitial lung disease in limited cutaneous systemic sclerosis patients

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ABSTRACT

Introduction: Anti-centromere antibodies are associated with limited cutaneous systemic sclerosis (lcSSc) and a more favorable prognosis. The centromere HEp-2 pattern (AC-3) suggests the presence of antibodies against CENP antigens, mainly CENP-B/A. This study analyzed clinical and demographic associations of anti-centromere antibodies in a cohort of patients exclusively with the lcSSc form of SSc. The frequency of CENP-B and CENP-A reactivity in samples with the AC-3 pattern was also evaluated.

Method: Samples from 38 lcSSc patients with AC-3 were evaluated for reactivity to CENP-B/A using line-blot and ELISA. Clinical data from 68 lcSSc patients (20 AC-3 and 48 Non-AC-3) were analyzed.

Results: Of the AC-3 samples, 84% and 82% were reactive against CENP-B and CENP-A, respectively, by line-blot, and 92% were positive for CENP-B by ELISA. Concordance for CENP-B reactivity between ELISA and line-blot was 79%. Reactivity to both CENP-B and CENP-A was found in 68% of AC-3 samples, while one sample was positive only for CENP-A. Overall, 97% of AC-3 samples were reactive to CENP-B, and all were reactive to either CENP-B or CENP-A. Clinically, interstitial lung disease (ILD) was less frequent in AC-3 patients compared to Non-AC-3 (10.5% vs. 54.2%; p = 0.001). Other organ involvement frequencies were similar.

Conclusion: ILD was less frequent in lcSSc patients with a positive AC-3 pattern as compared to those with a non-AC-3 pattern, which could suggest a less severe prognosis. In addition, anti-CENP-B was the predominant autoantibody in samples yielding the AC-3 pattern, but anti-CENP-A reactivity was also prevalent, and exclusive anti-CENP-A reactivity was also observed.

Keywords: Autoantibodies, Centromere, Fluorescent antibody technique, HEp-2 cells, Immunoassay, Systemic scleroderma

Introduction

Systemic sclerosis (SSc) is a chronic, heterogeneous autoimmune rheumatic disease characterized by high mortality and morbidity. This condition involves immune dysregulation, vasculopathy in small arteries and capillaries, and excessive collagen production, resulting in fibrosis of the skin and internal organs (1-5). According to the extent of skin involvement, SSc can be classified into: a) Limited cutaneous SSc (lcSSc) that involves the face and the skin distal to the elbows and knees; b) Diffuse cutaneous SSc (dcSSc) that involves the face, chest, trunk, and the skin both distal and proximal to the elbows and knees; and c) Absent skin involvement (SSc *sine scleroderma*) (6). It is also possible to classify SSC according to the presence of autoantibodies. Some autoantibodies are more associated with lcSSc, such as anti-centromere, anti-Th/To, and anti-PM-Scl, while others are more associated with dcSSc and multi-organ involvement, such as anti-topoisomerase I, anti-RNA polymerase III, and anti-fibrillarin [a comprehensive review can be found elsewhere (3)]. Each

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of these autoantibodies is related to specific disease manifestations, which makes them valuable tools for estimating prognosis in a given patient (3). Furthermore, the 2013 American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) improved the classification criteria for SSc by introducing a scoring system that includes clinical and laboratory elements (7).

Anti-centromere antibody is one of the most frequent in SSc (8). However, these can also be found at lower frequencies in other autoimmune diseases, including Sjögren disease, primary biliary cholangitis, isolated Raynaud's phenomenon, and overlap syndromes (9). Nevertheless, they are considered highly specific for SSc (>90%) and have been reported to precede the onset of clinical disease by months or years (10). In fact, the guidelines of ACR/EULAR indicate that the overall diagnostic sensitivity and specificity of anti-centromere antibody detected by indirect immunofluorescence assay on HEp-2 cells (HEp-2 IFA) were 31% and 97.4%, respectively, compared with patients with other systemic autoimmune rheumatic diseases (SARD) (6, 11-13).

When tested by HEp-2 IFA, anti-centromere antibodies reveal a characteristic, discrete speckled nuclear pattern scattered throughout interphase cells and aligned at the chromatin mass on mitotic cells, compatible with the topography of the centromeres (Figure 1A). This pattern is classified as the AC-3 pattern according to the International Consensus on Antinuclear Antibody (ANA) Patterns (ICAP; Online) (14,15). Structurally, the centromere is the region where condensed chromatin assembles to the inner and outer kinetochore to attach to the microtubules, which are responsible for chromosome segregation during cell division. Although there are many CENP proteins (CENP-A, -B, -C, -D, -E, -F, -G, H) in the kinetochore (9,16), CENP-B and CENP-A are the main autoantigens, as they are most consistently correlated with the AC-3 positive pattern on HEp-2 IFA observed in autoimmune patients (12,17). CENP-C is also the target of autoantibodies and likely yields the AC-3 pattern, but is usually found in association with antibodies to CENP-B or CENP-A (9,18,19).

The 17kDa CENP-A and the 80kDa CENP-B share a cryptic linear epitope motif named G/A-PR/S-R-R mapped towards the C-terminal portion of CENP-B and the N-terminal charged region of CENP-A, which is the main epitope target of anti-centromere autoantibodies (16, 20-22). This may explain the nearly identical prevalence of reactivity to CENP-A and CENP-B in antigen-specific solid-phase assays among samples with the AC-3 centromere pattern in the HEp-2 IFA, leading some authors to suggest that ELISA could replace HEp-2 IFA, considering the level of expertise required for the HEp-2 IFA pattern analysis (12). However, it is important to remember that HEp-2-IFA is a screening assay and does not provide the exact specificity for the nuclear antigen. Although the correlation of the AC-3 pattern with CENP-B/A autoantibodies is high, it is not flawless, especially if the sample produces multiple HEp-2 IFA patterns that may override the AC-3 pattern (3).

The HEp-2 IFA test, previously known as antinuclear antibodies (ANA), is a highly sensitive method for the screening of anti-cellular antibodies (AC) (23). The HEp-2 IFA provides information on the antibody serum concentration (titer) and possible autoantigen target (pattern). Various techniques, including ELISA, CLIA (chemiluminescent immunoassay), immunodiffusion, and immunoblotting, can be applied to detect specific antigen reactivity (3,24). Multiplex bead-based assays and ELISAs, as well as dot/line-blots, allow for the simultaneous testing of several autoantibodies. However, these immunoassays usually use recombinant CENP-B or CENP-A proteins (12,25), which could affect sensitivity, as demonstrated for other autoantibody systems (26). Second-generation assays, like CytoBeads, combine IFA on HEp-2 cells and antigen-coated beads, creating a "2-in-1" solution for a one-step, two-level ANA test (27). This approach may be useful for diagnosing patients who might not be detected with a negative HEp-2 IFA test but are positive for CENP-B by other methods. In general, CENP-B/A-specific immunoassays tend to show good agreement rates (28).

Most studies addressing the clinical associations of anti-centromere antibodies comprise general cohorts of SSc patients. Because anti-centromere antibodies are strongly associated with the lcSSc form of the disease, the clinical traits traditionally associated with anti-centromere antibodies are those that characterize lcSSc. Therefore, it is not well established how the anti-centromere antibodies correlate with the clinical spectrum of lcSSc. In this study, the clinical associations of anti-centromere antibodies were analyzed in a pure cohort of lcSSc patients. In addition, the anti-centromere reactivity in HEp-2 IFA (AC-3 pattern) was compared with the results of specific immunoassays for anti-CENP-B and anti-CENP-A antibodies.

Objective

We analyzed the possible clinical and demographic associations of anti-centromere antibodies in a cohort of patients exclusively with the limited cutaneous form of SSc (lcSSc). In addition, we evaluated the frequency of reactivity to CENP-B and CENP-A in samples with the AC-3 pattern on the HEp-2 IFA test.

Methods

Patient samples

The patients were consecutively recruited from the Systemic Sclerosis Outpatient Clinic at Escola Paulista de Medicina, Federal University of São Paulo (UNIFESP), Brazil. Patients should meet the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2013 classification criteria for the limited cutaneous form of Systemic Sclerosis (lcSSc) (7). In accordance with the Declaration of Helsinki, the patients signed an informed consent form to participate in the study and the research was approved by the Ethics Committee at UNIFESP (Plataforma Brasil CAAE: 59126320.1.0000.5505).

Demographic and clinical features were cross-sectionally obtained from electronic medical records and reviewed by rheumatologists with expertise in SSc (C.K. and P.M.) as previously described (26,29,30). In brief, clinical data included age, sex, disease subtype, and disease duration (defined as the time between the first non-Raynaud symptom and the enrollment visit). Interstitial lung disease (SSc-ILD) was defined as the presence of interstitial abnormalities in chest

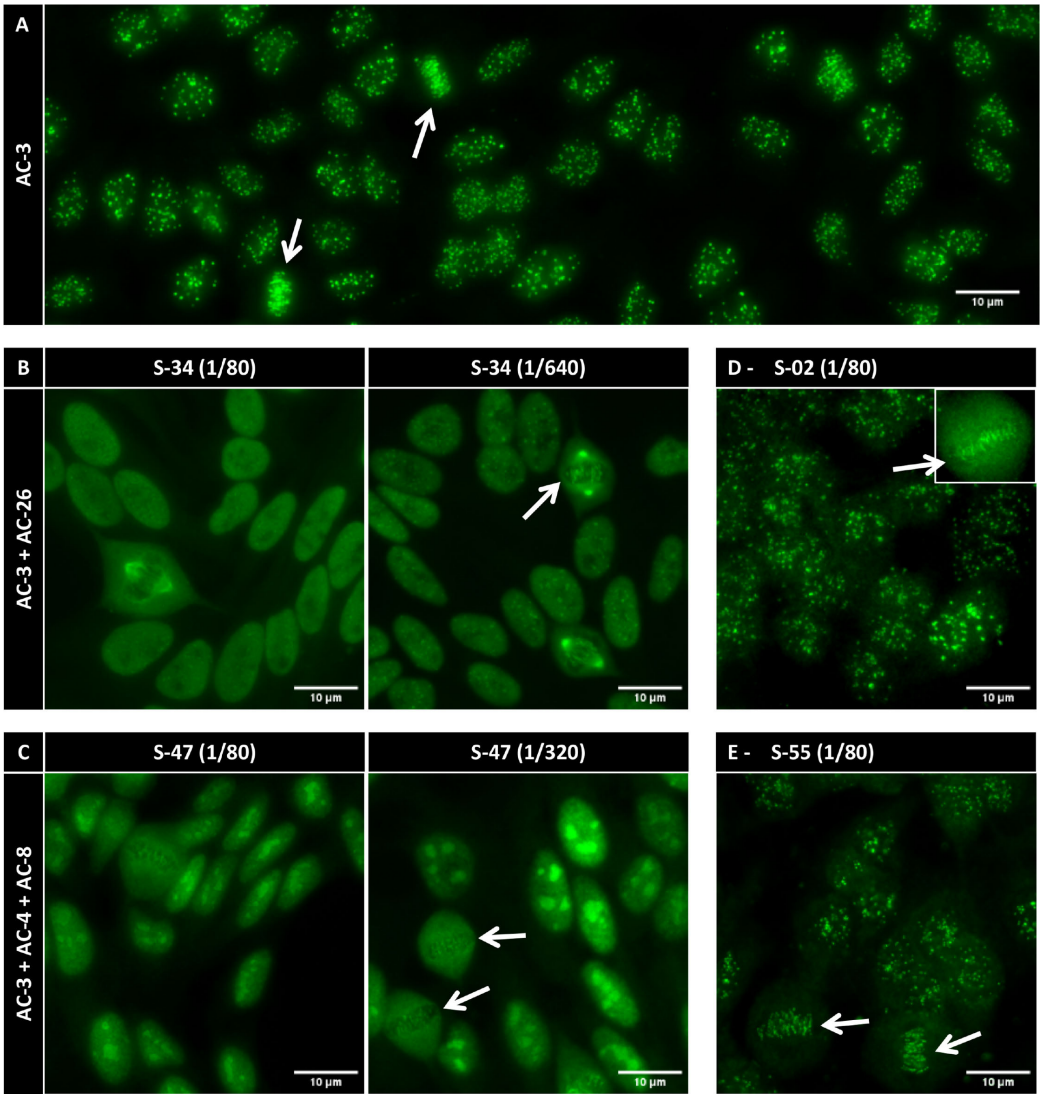

FIGURE 1 - Representative images of the HEp-2 IFA for samples with the AC-3 centromere pattern. (A) The typical AC-3 pattern. (B) Sample S34 with multiple patterns combining the centromere AC-3 and the NuMA-like AC-26 patterns; the AC-3 became evident at higher dilution. (C) Sample S47 with multiple patterns combining the nuclear fine speckled AC-4, the nucleolar homogeneous AC-8, and the centromere AC-3 patterns; the AC-3 became evident at higher dilution. (D-E) Sample S02 had reactivity to CENP-B in the line-blot but not in the ELISA. Sample S55 was negative for CENP-B in both methods, but was positive for anti-CENP-A in the line-blot (Figure 3D). Arrows in all panels indicate the characteristic metaphase plate of the AC-3 pattern. Scale bar = 10 µm.

high-resolution computerized tomography (HRCT) and a forced vital capacity (FVC) on pulmonary function test lower than 80%. Pulmonary arterial hypertension (PAH) was considered in patients with group I PAH confirmed by right heart catheterization, according to previously established criteria (31). Esophageal dysmotility was considered when confirmed in an esophagogram or esophageal manometry.

The lcSSc patients were tested in the HEp-2 IFA test and subdivided into two groups according to the presence of the AC-3 pattern in the HEp-2 IFA test respectively, the AC-3 group and the Non-AC-3 group.

Assays

The pattern and titer of the HEp-2-IFA were determined using commercial HEp-2 cell slides (#FA 1520-2010, Euroimmun; #51.100, AESKU), following the manufacturer's protocol, with a 1/80 starting dilution and serial dilutions up to 1/2560. The slides were analyzed and images captured at 400x magnification using a fluorescent microscope (Axio Imager.M2, Carl Zeiss).

Anti-CENP-B reactivity was assessed using an indirect ELISA kit (#ORG 633, Orgentec), following the manufacturer's protocol. A four-parameter logistic curve with four known concentration standards was applied (Figure 2B), and the interpolation of the samples' optical density allowed the determination of anti-CENP-B reactivity in each sample in arbitrary units (U/mL). Samples with >10 U/mL were considered positive for anti-CENP-B, as recommended by the manufacturer. In addition, reactivity to CENP-A and CENP-B was determined by immunoblot (Euroline Systemic Sclerosis Nucleoli profile kit; Cat# DL 1532-6401 G, Euroimmun) following the manufacturer's protocol (Figure 3). Although this kit can determine reactivity to other antigens, for this study, we only considered reactivity to the CENP antigens. The manufacturer recommends interpretation of the line-blot result as: (-) negative; (+) one plus as *borderline*; (≥++) two "pluses" or more, as positive. Because one plus (*borderline*) may not represent true positives, as we have shown for other autoantigens (26), we considered positive samples only those with the immunostaining intensity (≥++) two or more "pluses" (Fig. 3).

Data analysis

Immunofluorescence images were processed and panels assembled using ImageJ v1.53r software. Statistical analyses were performed using the software GraphPad Prism v7.0 or JASP v0.19.1. When comparing proportions, the two-tailed Fisher's exact test was applied. Quantitative and semi-quantitative parameters were assessed for normality distribution with the Shapiro–Wilk test, followed by comparison with Mann–Whitney or Student t-test according to the distribution pattern. Correlations were evaluated with the Spearman r-test. P values were considered significant when below 0.05. A Venn diagram was built with the Venny 2.1 online tool.

Results

There were 76 lcSSc patients, 38 classified into the AC-3 group and 48 classified into the Non-AC-3 group according to the presence of circulating anti-centromere antibodies. Concerning the AC-3 group, 29 (76%) patients showed a pure AC-3 pattern (Figure 1A) and nine (24%) patients showed a combination of the AC-3 pattern and other HEp-2 IFA patterns (Table 1). In this multiple pattern configuration, the centromere component tended to become more evident as the samples were further diluted (Figure 1B-C).

All samples were evaluated for anti-CENP-B reactivity in an indirect ELISA (Figure 2). Surprisingly, two samples that were originally not classified in the AC-3 group also tested positive, with reactivity above the cutoff of 10 U/mL (blue data-points in Fig. 2A). These two samples (S34 and S47) were re-evaluated by serial dilution HEp-2 IFA and showed the discrete speckles at the metaphase plate typical of the centromere pattern at 1/640 and 1/320, respectively (arrows in Figure 1B and 1C). Consequently, we reclassified these two samples as containing more than one pattern, including the AC-3, and thus part of the AC-3 group (n = 38). The AC-3 titer ranged from 1/80 to the highest dilution of 1/2560, with a median of 1/640 and a mean of 1/987 (Table 1 and Supplementary Fig. 1B).

As for the HEp-2 IFA pattern in the Non-AC-3 group, there were five negative samples (AC-0) and 43 with various patterns, such as nuclear fine speckled (AC-4; n = 12), nuclear coarse speckled (AC-5; n = 9), nucleolar (AC-8/9/10; n = 15), DNA topoisomerase I (topo I)-like (AC-29; n = 7), and miscellaneous patterns (AC-11, AC-18, AC-19, AC-21, AC-25; n = 5), including six samples (14%) with more than one pattern.

Regarding the anti-CENP-B reactivity measured by ELISA, three (8%) of the 38 samples with AC-3 had results below the cutoff (Fig. 2A), although all three samples had the AC-3 pattern at moderate intensity (titer 1/320; examples in Figures 1D and 1E). Therefore, 35 (92%) of the AC-3 samples were positive for anti-CENP-B by ELISA (Table 1).

Reactivity to CENP-B and CENP-A was also evaluated using a line-blot assay (Fig. 3). Most samples reacted with CENP-A and CENP-B (Fig. 4), and one sample reacted only with CENP-A (Fig. 3D). Seven samples reacted only with CENP-B (Figures 3E and 4). Altogether, the line-blot assay with the 38 AC-3 samples showed that 32 (84%) were reactive against CENP-B and 31 (82%) were reactive against CENP-A (Table 1 and Fig. 4). From the three samples negative for anti-CENP-B antibodies in ELISA (Fig. 2A), two were positive for anti-CENP-B

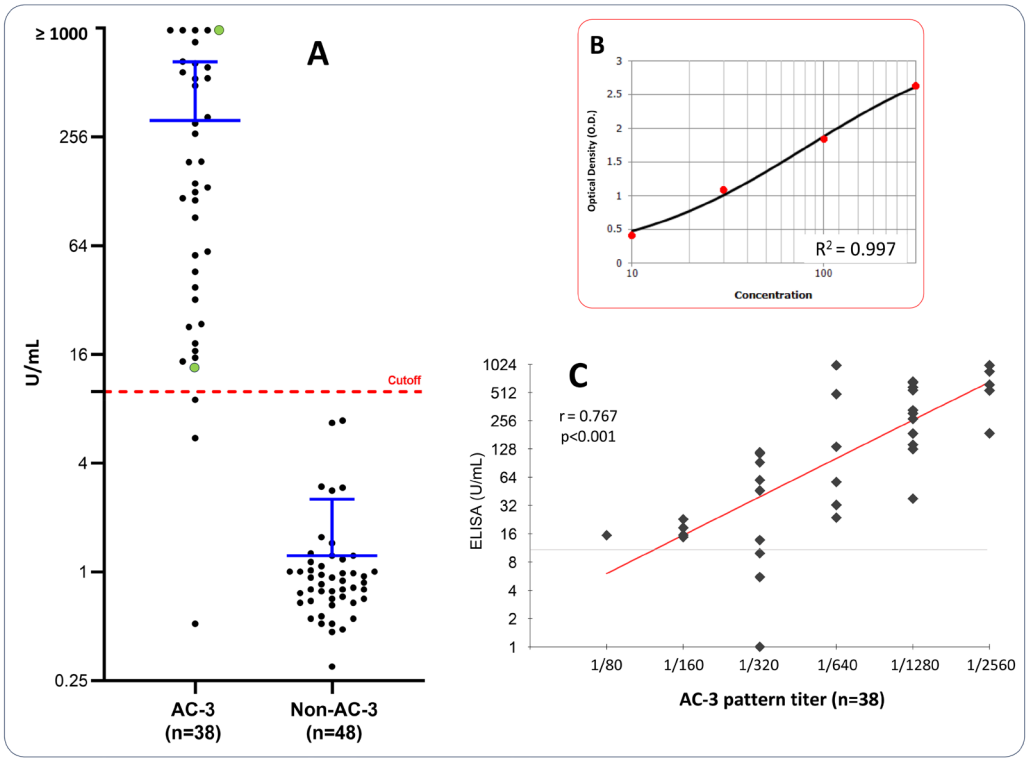

FIGURE 2 - Reactivity to CENP-B in ELISA. (A) Anti-CENP-B reactivity tested by indirect ELISA. Distribution of anti-CENP-B reactivity in U/mL. The cutoff (red dotted line) was set at 10 U/mL as recommended by the manufacturer. The two data points in green indicate the two samples in which AC-3 was not initially reported, but it was observed in the HEp-2-IFA upon re-evaluation with serial dilution, as detailed in Figures 1B and C. The blue line indicates the mean ±SD. (B) A representative standard four-parameter logistic curve for the ELISA with anti-CENP-B standards. (C) Correlation between Anti-CENP-B reactivity by ELISA and the AC-3 pattern titer.

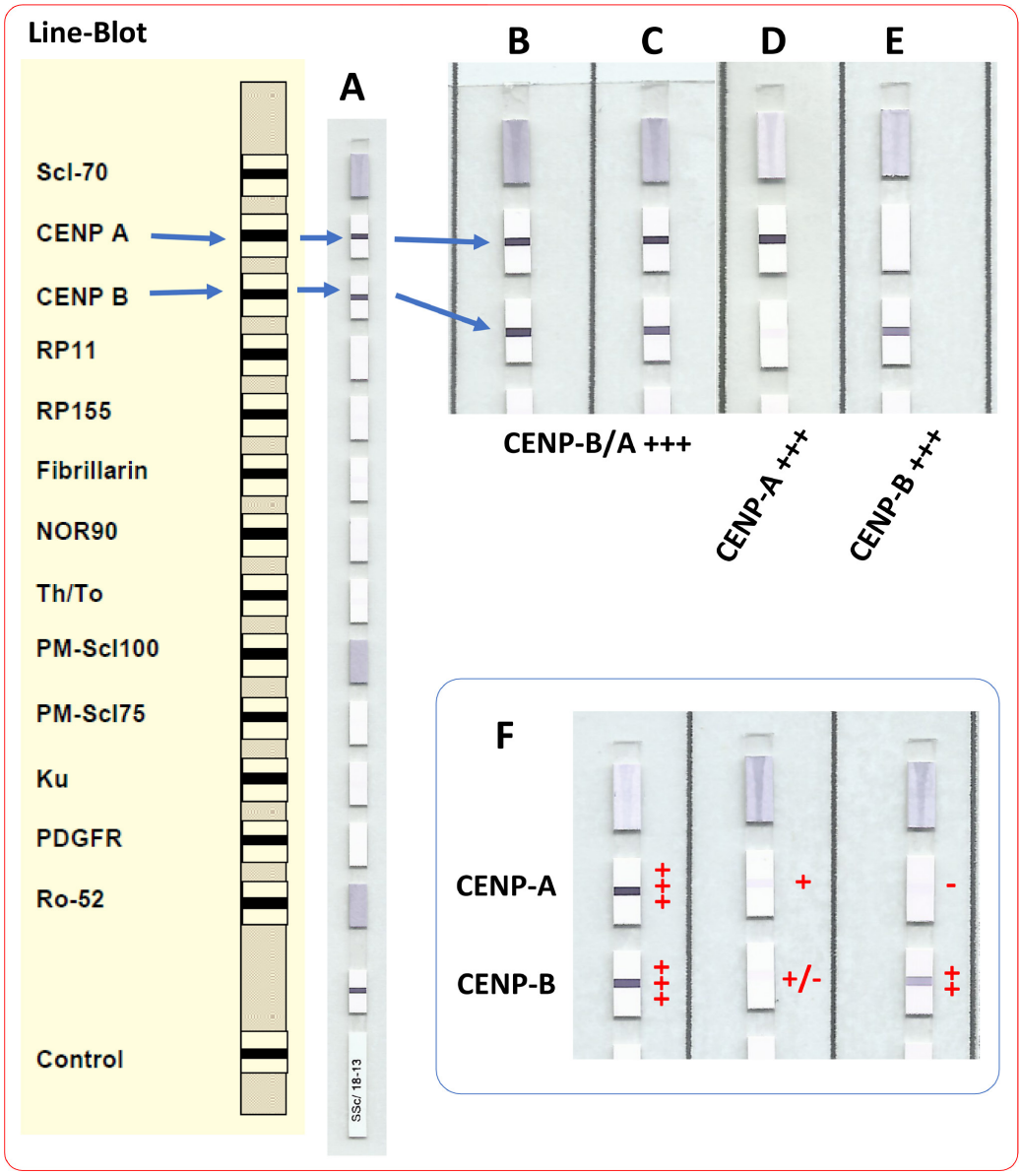

FIGURE 3 - Anti-CENP reactivity in line-blot. (A-E) Euroline Systemic Sclerosis profile, (A-C) representative samples with 3 pluses (+++) reactivity to anti-CENP-A and B, (D) representative sample with anti-CENP-A reactivity (+++) and CENP-B (+/-) considered negative, and (E) representative sample with anti-CENP-B reactivity (+++) only. (F) Examples for interpretation of line-blot results, only reactivity (≥++) was considered a true positive, as detailed in the methods.

in the line-blot assay, and one (S55, Fig. 1E) was positive only for anti-CENP-A (Figs 3D and 4).

When comparing the reactivity to CENP-B in ELISA and line-blot assay, 30 (79%) samples were reactive against CENP-B in ELISA and line-blot methods (Figure 4). One sample was reactive against CENP-B only in ELISA, and two samples were reactive against CENP-B only in line-blot. Altogether, all 38 AC-3 samples (100%) showed reactivity against either CENP-B or CENP-A in at least one of the antibody-specific immunoassays. Only one sample was reactive exclusively against CENP-A, meaning that 37 (97%) were reactive against CENP-B in at least one method (Figure 4).

The correlation between the AC-3 titer in the HEp-2-IFA and the CENP-B reactivity in U/mL levels obtained in ELISA in the 38 AC-3 samples was high, r = 0.767 (95% Confidence Interval 0.587-0.875; $p < 0.001$). The correlation between the intensity of CENP-B reactivity in ELISA and the line-blot assay was satisfactory, r = 0.594 (95% CI 0.330-0.772; $p < 0.001$) (Table 1, Fig. 2C and Supplementary Figs 1C-F).

Clinical information was available for 20 patients from the AC-3 group (of whom 19 showed positive anti-CENP-B reactivity in ELISA and/or line-blot, and one showed reactivity only to CENP-A) and for 48 patients from the Non-AC-3 group. The demographic data and clinical characteristics of these 68 lcSSc patients are depicted in Table 2. Patients in the AC-3 group were significantly older than those in the Non-AC-3 group, but the duration of the disease was similar in the two groups (Table 2 and Supplementary Fig. 1A). Regarding organ involvement, interstitial lung disease (ILD) was less frequently observed in patients in the AC-3 group (n = 2, 10.5%) compared to those in the Non-AC-3 group (n = 26, 54.2%; p = 0.001), but the other parameters of organ involvement had similar frequency in the two groups (Table 2).

TABLE 1 - Anti-CENP-B/A reactivity in 38 samples with the AC-3 pattern

HEp-2 IFA	Single pattern	Multiple patterns (AC-3 + others[§])	
AC-3 pattern	29 (76.3%)	9 (23.7%)	
Titer range	1/80 (n = 1) to 1/2560 (n = 6)		
Median titer	1/640		
Mean AC-3 titer (±SD)	1/987 (±1/809)		
CENP-B ELISA	Positives ≥10 U/mL	Negatives <10 U/mL	
Proportions	35 (92.1%)	3 (7.9%)	
Median reactivity	141.5	5.5	
Mean reactivity (±SD)	323.3 (±340.8)	5.3 (±4.7)	
Line-Blot	Positive (≥++)	Negative (–) and ***Borderline*** (+)	
CENP-B	32 (84.2%)	6 (15.8%)	
CENP-A	31 (81.6%)	7 (18.4%)	
Correlation [#]	CENP-B ELISA	Line-Blot CENP-B	Line-Blot CENP-A
AC-3 pattern titer	0.767***	0.432**	0.419**
CENP-B ELISA	–	0.594***	0.578***
Line-Blot CENP-B	–	–	0.676***

§ AC-4, AC-7, AC-8, AC-11, AC-21, AC-26. # Spearman r; **p < 0.01; ***p < 0.001

Discussion

In this study, we analyzed the clinical and demographic characteristics of lcSSc patients according to the presence of anti-centromere autoantibodies and investigated the anti-CENP-B/A reactivity in samples displaying the AC-3 pattern in the HEp-2-IFA test. We showed that even among patients with the lcSSc subtype, the presence of antibodies against centromere was associated with a lower frequency of lung involvement, specifically ILD, which could suggest a better prognosis and less severe disease. As expected, we confirmed the strong association between the AC-3 pattern and anti-CENP-B/A, as 100% of the AC-3 samples were reactive against CENP-B and/or CENP-A in at least one of the used immunoassays. Interestingly, however, the concordance rate between the solid phase assays themselves was weaker, as the agreement in anti-CENP-B reactivity between the ELISA and line-blot methods was only 79% as opposed to 100% concordance between HEp-2 AC-3 pattern and anti-CENP-B/A reactivity in solid-phase immunoassays. We also confirmed previous findings indicating that CENP-B is the dominant centromere autoantigen, as 37 (97%) of the AC-3 samples recognized CENP-B in at least one solid phase immunoassay, whereas 31 (82%) of the AC-3 samples recognized CENP-A. In addition, among the 38 samples tested for antibodies to CENP-B and CENP-A, seven (~18%) reacted exclusively with CENP-B, and one (~3%) was reactive solely against CENP-A. This result aligns with the concept that the AC-3 pattern in the HEp-2 IFA test is strongly associated with autoantibodies to CENP-B and/or CENP-A, CENP-B being the dominant autoantigen (12,19,32).

The first publications describing the targets of autoantibodies that recognize centromeric antigens, namely the CENP proteins, as well as their association with lcSSc, date back almost half a century, resulting from studies conducted in Dr Eng Tan's laboratory in the early 1980s. At that time, lcSSc was classified by the presence of calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia, collectively known as the CREST syndrome (33-35). In SSc, as mentioned, the main autoantigens in samples with the AC-3 pattern are CENP-A and CENP-B (21). Interestingly, the primary epitope on CENP-A, the G/A-PR/S-R-R motif, is also present on CENP-B and CENP-C. In fact, anti-CENP-A/B/C are frequently found in association in the same patient (18,19). It is important to note that the G/A-PR/S-R-R motif is not the only target, as these autoantibodies likely recognize other non-shared antigenic regions, providing strong evidence of intra- and intermolecular epitope spreading (22). This is supported by our findings, where one patient showed reactivity only to CENP-A and seven showed reactivity only to CENP-B (and not to CENP-A), although we cannot rule out the presence of autoantibodies against other CENP antigens in these samples. Autoantibodies against CENP-A/B/C, as well as the less common CENP-D and CENP-E, and the very rare CENP-O (36), are all associated with lcSSc or the CREST syndrome (21,22,37). CENP-D is primarily of the IgM type and tends to disappear over time (38). Anti-CENP-E has been found in approximately 40% of patients with anti-CENP (39). Autoantibodies against CENP-H are associated with Sjögren disease, particularly in patients without anti-Ro/La antibodies (40). Finally, perhaps the most distinctive among the non-CENP-A/B antigens is

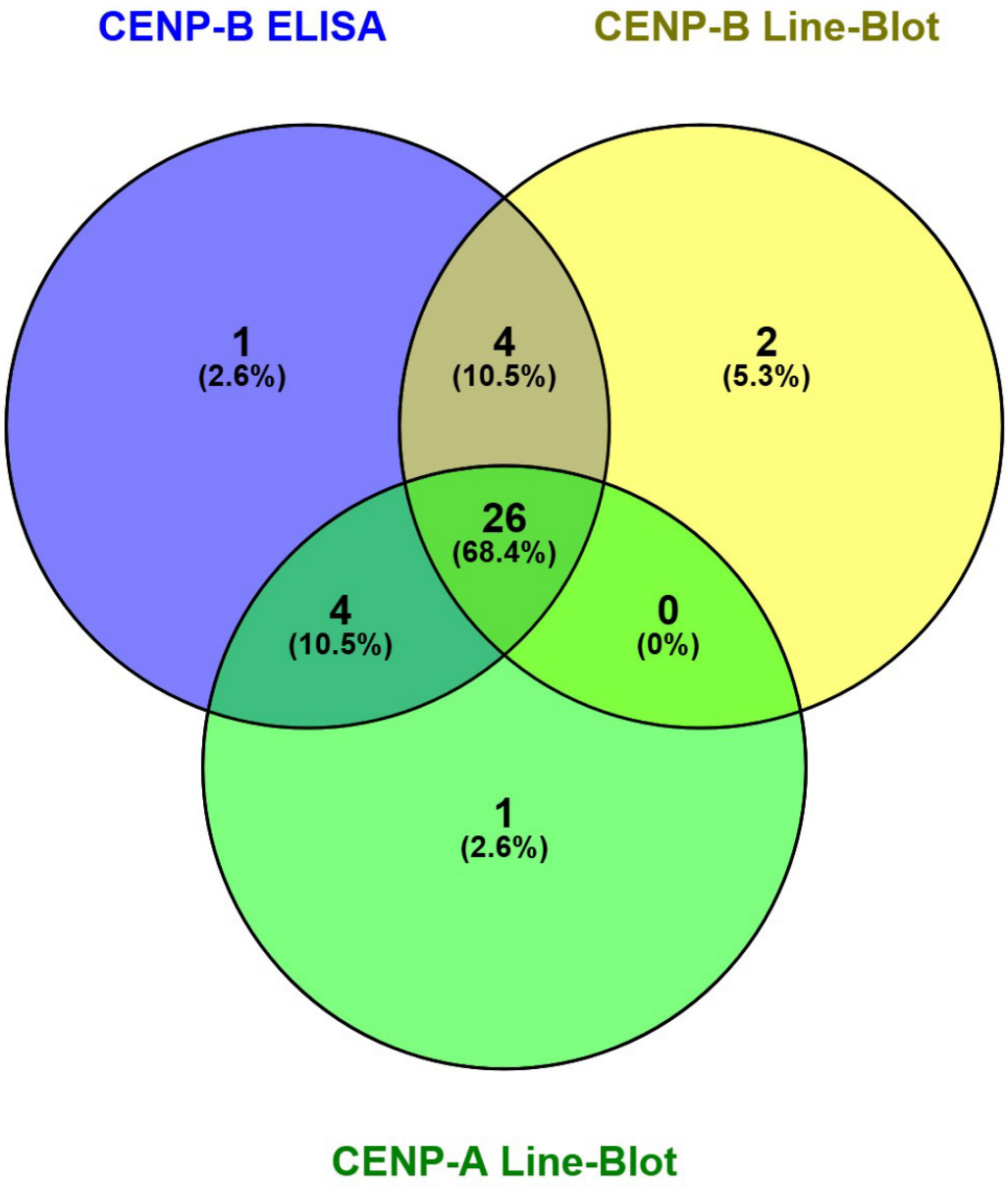

FIGURE 4 - Venn diagram for anti-CENP-B/A reactivity in ELISA and line-blot assays in samples from the AC-3 group.

CENP-F, a 330 kDa protein essential for cell cycle progression (41). Anti-CENP-F antibodies are associated with various types of malignancies rather than SSc, primary biliary cholangitis or Sjögren disease (42,43). These autoantibodies produce a different HEp-2 IFA pattern from the AC-3, referred to as the CENP-F-like pattern (AC-14) (41). The presence of anti-CENP-F antibodies may serve as a marker for cancer (44).

Choosing the most appropriate method to determine anti-centromere antibodies in patient samples is essential to ensure reliable results. In this study, no sample exhibited reactivity against CENP-B or CENP-A in the absence of the AC-3 pattern on the HEp-2 IFA. Conversely, all samples with the AC-3 pattern demonstrated reactivity against CENP-B/A in at least one assay. However, in some cases, the AC-3 pattern was visible only at higher dilutions and required a keen eye to identify the characteristic metaphase plate. Furthermore, we observed that some samples with the AC-3 pattern were negative in at least one of the solid-phase immunoassays. The concordance for CENP-B reactivity between ELISA and line-blot was less than 80%. Since clinical laboratories often use only one type of kit, they may fail to report samples with anti-centromere antibodies when relying solely on a solid-phase immunoassay. Thus, the data presented here provide additional evidence supporting the ACR/EULAR recommendation to use the indirect immunofluorescence assay on HEp-2 cells as the screening method for autoantibodies in rheumatic diseases, as commented elsewhere (45,46), and to consider the reported pattern when interpreting solid-phase immunoassay results.

TABLE 2 - Demographic and clinical features of the lcSSc patients according to the presence of anti-centromere pattern (AC-3) in HEp-2 IFA

Variable	AC-3 pattern (n = 20)	Non-AC-3 (n = 48)	*P*
Age, mean ± SD (years)	59.4 ± 12.5	50.5 ± 12.9	0.011
Female, n (%)	20 (100.0)	41 (85.4)	0.096
Disease duration, mean ± SD (years)	8.7 ± 6.3	6.6 ± 5.9	0.197
Organ involvement			
Digital ulcers, n (%)	5 (26.3)*	17 (35.4)	0.571
Esophageal dysmotility, n (%)	14 (73.7)*	38 (79.2)	0.747
Arthritis, n (%)	6 (31.6)*	19 (39.6)	0.588
FVC% of predicted, mean ± SD	84.5 ± 13.5	84.7 ± 19.9	0.967
ILD, n (%)	2 (10.5)*	26 (54.2)	0.001
PAH, n (%)	2 (10.5)*	7 (14.6)	1.000
Cardiac involvement, n (%)	0 (0.0)	2 (4.2)	1.000
Scleroderma renal crisis, n (%)	0 (0.0)	2 (4.2)	1.000

FVC: forced vital capacity; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension
*Data available for 19 patients

Previous studies have demonstrated that anti-centromere autoantibodies display a less severe SSc disease and better prognosis (11,32). In fact, anti-centromere autoantibodies correlate with less frequent elevations in serum creatine kinase, digital ulcers, joint contractures, interstitial lung disease (ILD), scleroderma renal crisis, arthritis, and myositis, among others (12,32). However, these studies have inferred these associations in cohorts of patients with both forms of SSc, raising the possibility that the obtained associations are secondary to the primary association of anti-centromere antibodies to lcSSc, the more benign form of the disease. In our cohort constituted exclusively by lcSSc patients, we could confirm a lower frequency of ILD among lcSSc patients with anti-centromere antibodies compared with those without these autoantibodies. This finding suggests that the presence of anti-centromere antibodies further discriminates a subgroup of lcSS patients with a more favorable prognosis. In a cohort comprising exclusively lcSSc patients, anti-centromere antibodies were associated with better prognosis and less severe disease. As proposed by a recent publication, individual autoantibodies associate with specific SSc characteristics (32). Since ILD is the leading cause of death in SSc patients (47,48), our results suggest a less severe disease, indicated by the less frequent ILD in SSc patients with anti-centromere autoantibodies.

This study has some limitations that should be acknowledged. First, it was a cross-sectional analysis, which does not allow for assessment of the longitudinal evolution of patients, including potential reclassification of the lcSSc over time. This, as well as the relatively short disease duration in many patients, may partially explain the presence of some autoantibodies typically associated with dcSSc in the Non-AC-3 group. Second, clinical data were not available for all of the patients with a positive AC-3 pattern; however, the clinical findings were consistent with previous cohorts from our region (29, 47). Third, while we compared HEp-2 IFA with two solid-phase immunoassays, there are other platforms, such as the bead-based assays. Notably, we did not evaluate reactivity to CENP-A by ELISA. Finally, the relatively modest sample size may limit the generalizability of the findings, particularly regarding the frequency of less common lcSSc manifestations such as pulmonary hypertension, suggesting longitudinal studies with larger cohorts may be appropriate.

Conclusion

In conclusion, ILD was less frequent in lcSSc patients with positive AC-3 pattern as compared to those with no anti-centromere reactivity, which could suggest a less severe prognosis within the lcSSc spectrum for those patients with anti-CENP reactivity. All samples with the AC-3 centromere pattern in HEp-2 IFA displayed reactivity to CENP-B or CENP-A in at least one of the applied tests, meaning the HEp-2 IFA method was 100% sensitive in detecting antibodies to CENP-A and CENP-B. One sample showed reactivity only to CENP-A, and of the 38 samples with AC-3, ~82% were positive for CENP-A. Regarding CENP-B reactivity, ~84% were positive by line-blot and ~92% by ELISA, but only 30 samples were positive for CENP-B in both the ELISA and line-blot methods, with a concordance of <80%. This means that anti-CENP-B is the predominant autoantibody in samples yielding the AC-3 pattern, but exclusive anti-CENP-A reactivity can also occur less frequently, as observed in only one sample in our cohort.

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language of the authors, and translated to English with the help of online tools such as ChatGPT with GPT-4-turbo (by OpenAI). The final text underwent proofreading for the English language; therefore, after using the online tools, the authors reviewed and edited the content and took full responsibility for the content of the publication.

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Author contributions

G.D.K.: Conceptualization; Methodology; Investigation; Writing – Original Draft.; D.L.: Writing – Original Draft; C.K. and P.M.: Data Curation; Formal Analysis; Validation.; L.D., J.K., and S.H.R.: Investigation; Resources; Data Curation; Formal Analysis.; L.E.C.A.: Conceptualization; Supervision; Writing – Review & Editing. All authors read and approved the final manuscript.

Disclosures

Conflict of interest: The authors declare that they have no competing interests.

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Data Availability Statement: The data presented in this study are available upon reasonable request from the corresponding author.

References

1. Denton CP, Khanna D. Systemic sclerosis. Lancet. 2017; 390(10103):1685-1699. CrossRef PubMed
2. Roofeh D, Khanna D. Management of systemic sclerosis: the first five years. Curr Opin Rheumatol. 2020;32(3):228-237. CrossRef PubMed
3. Stochmal A, Czuwara J, Trojanowska M, et al. Antinuclear antibodies in systemic sclerosis: an update. Clin Rev Allergy Immunol. 2020;58(1):40-51. CrossRef PubMed
4. van Leeuwen NM, Boonstra M, Bakker JA, et al. Anticentromere antibody levels and isotypes and the development of systemic sclerosis. Arthritis Rheumatol. 2021;73(12):2338-2347. CrossRef PubMed
5. Kowalska-Kępczyńska A. Systemic scleroderma-definition, clinical picture and laboratory diagnostics. J Clin Med. 2022; 11(9):2299. CrossRef PubMed
6. Johnson SR. New ACR EULAR guidelines for systemic sclerosis classification. Curr Rheumatol Rep. 2015;17(5):32. CrossRef PubMed
7. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis. 2013;72(11):1747-1755. CrossRef PubMed
8. Chepy A, Collet A, Launay D, et al. Autoantibodies in systemic sclerosis: from disease bystanders to pathogenic players. J Transl Autoimmun. 2025;10:100272. CrossRef PubMed
9. Kajio N, Takeshita M, Suzuki K, et al. Anti-centromere antibodies target centromere-kinetochore macrocomplex: a comprehensive autoantigen profiling. Ann Rheum Dis. 2021;80(5):651-659. CrossRef PubMed
10. Koenig M, Joyal F, Fritzler MJ, et al. Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis. Arthritis Rheum. 2008;58(12):3902-3912. CrossRef PubMed
11. Reveille JD, Solomon DH.; American College of Rheumatology Ad Hoc Committee of Immunologic Testing Guidelines. Evidence-based guidelines for the use of immunologic tests: anticentromere, Scl-70, and nucleolar antibodies. Arthritis Rheum. 2003;49(3):399-412. CrossRef PubMed
12. Hudson M, Mahler M, Pope J, et al.; Investigators of the Canadian Scleroderma Research Group. Clinical correlates of CENP-A and CENP-B antibodies in a large cohort of patients with systemic sclerosis. J Rheumatol. 2012;39(4):787-794. CrossRef PubMed
13. Matos P, Keppeke GD, Kayser C. Autoanticorpos específicos e associados na esclerose sistêmica: investigação e diagnóstico. Revista Paulista de Reumatologia. 2024;23(3):41-50. CrossRef
14. Chan EKL, Damoiseaux J, Carballo OG, et al. Report of the First International Consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns 2014-2015. Front Immunol. 2015;6:412. CrossRef PubMed
15. Andrade LEC, Klotz W, Herold M, et al. Reflecting on a decade of the international consensus on ANA patterns (ICAP): accomplishments and challenges from the perspective of the 7th ICAP workshop. Autoimmun Rev. 2024;23(9):103608. CrossRef PubMed
16. Akbarali Y, Matousek-Ronck J, Hunt L, et al. Fine specificity mapping of autoantigens targeted by anti-centromere autoantibodies. J Autoimmun. 2006;27(4):272-280. CrossRef PubMed
17. Prasad RM, Bellacosa A, Yen TJ. Clinical and Molecular Features of Anti-CENP-B Autoantibodies. J Mol Pathol. 2021;2(4): 281-295. CrossRef
18. Earnshaw WC, Rothfield N. Identification of a family of human centromere proteins using autoimmune sera from patients with scleroderma. Chromosoma. 1985;91(3-4):313-321. CrossRef PubMed
19. Earnshaw W, Bordwell B, Marino C, et al. Three human chromosomal autoantigens are recognized by sera from patients with anti-centromere antibodies. J Clin Invest. 1986;77(2):426-430. CrossRef PubMed
20. Muro Y, Azuma N, Onouchi H, et al. Autoepitopes on autoantigen centromere protein-a (CENP-A) are restricted to the N-terminal region, which has no homology with histone H3. Clin Exp Immunol. 2000;120(1):218-223. CrossRef PubMed
21. Mahler M, Fritzler MJ. Epitope specificity and significance in systemic autoimmune diseases. Ann N Y Acad Sci. 2010; 1183(1):267-287. CrossRef PubMed

22. Fritzler MJ, Rattner JB, Luft LM, et al. Historical perspectives on the discovery and elucidation of autoantibodies to centromere proteins (CENP) and the emerging importance of antibodies to CENP-F. Autoimmun Rev. 2011;10(4):194-200. CrossRef PubMed
23. Damoiseaux J, Andrade LEC, Carballo OG, et al. Clinical relevance of HEp-2 indirect immunofluorescent patterns: the International Consensus on ANA patterns (ICAP) perspective. Ann Rheum Dis. 2019;78(7):879-889. CrossRef PubMed
24. Keppeke GD, Agustinelli RA, Landoni DM, et al. Chapter 88 – Future Direction of Laboratory Testing for Autoimmune Diseases. In: Schmitz JL, Detrick B, O'Gorman MRG, editors. Manual of Molecular and Clinical Laboratory Immunology, 9th Edition. 2. 9 ed. Washington, DC: Wiley & ASM Press; 2024. p. 968-82. CrossRef
25. Mahler M, You D, Baron M, et al.; Canadian Scleroderma Research Group (CSRG). Anti-centromere antibodies in a large cohort of systemic sclerosis patients: comparison between immunofluorescence, CENP-A and CENP-B ELISA. Clin Chim Acta. 2011;412(21-22):1937-1943. CrossRef PubMed
26. Keppeke GD, Satoh M, Kayser C, et al. A cell-based assay for detection of anti-fibrillarin autoantibodies with performance equivalent to immunoprecipitation. Front Immunol. 2022;13:1011110. CrossRef PubMed
27. Sowa M, Hiemann R, Schierack P, et al. Next-generation autoantibody testing by combination of screening and confirmation—the CytoBead® Technology. Clin Rev Allergy Immunol. 2017;53(1):87-104. CrossRef PubMed
28. Jang J, Kim S, Kim HS, et al. Comparison of antinuclear antibody profiles obtained using line immunoassay and fluorescence enzyme immunoassay. J Int Med Res. 2021;49(6):3000605211014390. CrossRef PubMed
29. de Oliveira SM, Martins LVO, Lupino-Assad AP, et al. Severity and mortality of COVID-19 in patients with systemic sclerosis: a Brazilian multicenter study. Semin Arthritis Rheum. 2022;55:151987. CrossRef PubMed
30. Kayser C, de Oliveira Delgado SM, Zimmermann AF, et al. 2023 Brazilian Society of Rheumatology guidelines for the treatment of systemic sclerosis. Adv Rheumatol. 2024;64(1):52. CrossRef PubMed
31. Humbert M, Kovacs G, Hoeper MM, et al.; ESC/ERS Scientific Document Group. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Respir J. 2023;61(1):2200879. CrossRef PubMed
32. Żebryk P, Przymuszała P, Nowak JK, et al. Autoantibodies and clinical correlations in Polish systemic sclerosis patients: a cross-sectional study. J Clin Med. 2023;12(2):657. CrossRef PubMed
33. Fritzler MJ, Kinsella TD, Garbutt E. The CREST syndrome: a distinct serologic entity with anticentromere antibodies. Am J Med. 1980;69(4):520-526. CrossRef PubMed
34. Moroi Y, Peebles C, Fritzler MJ, et al. Autoantibody to centromere (kinetochore) in scleroderma sera. Proc Natl Acad Sci USA. 1980;77(3):1627-1631. CrossRef PubMed
35. Tan EM, Rodnan GP, Garcia I, et al. Diversity of antinuclear antibodies in progressive systemic sclerosis. Anti-centromere antibody and its relationship to CREST syndrome. Arthritis Rheum. 1980;23(6):617-625. CrossRef PubMed
36. Saito A, Muro Y, Sugiura K, et al. CENP-O, a protein localized at the centromere throughout the cell cycle, is a novel target antigen in systemic sclerosis. J Rheumatol. 2009;36(4):781-786. CrossRef PubMed
37. Earnshaw WC, Machlin PS, Bordwell BJ, et al. Analysis of anticentromere autoantibodies using cloned autoantigen CENP-B. Proc Natl Acad Sci USA. 1987;84(14):4979-4983. CrossRef PubMed
38. Ford AL, Kurien BT, Harley JB, et al. Anti-centromere autoantibody in a patient evolving from a lupus/Sjögren's overlap to the CREST variant of scleroderma. J Rheumatol. 1998;25(7):1419-1424. PubMed
39. Rattner JB, Rees J, Arnett FC, et al. The centromere kinesin-like protein, CENP-E. An autoantigen in systemic sclerosis. Arthritis Rheum. 1996;39(8):1355-1361. CrossRef PubMed
40. Hsu TC, Chang CH, Lin MC, et al. Anti-CENP-H antibodies in patients with Sjögren's syndrome. Rheumatol Int. 2006;26(4):298-303. CrossRef PubMed
41. Casiano CA, Landberg G, Ochs RL, et al. Autoantibodies to a novel cell cycle-regulated protein that accumulates in the nuclear matrix during S phase and is localized in the kinetochores and spindle midzone during mitosis. J Cell Sci. 1993;106(Pt 4):1045-1056. CrossRef PubMed
42. Landberg G, Erlanson M, Roos G, et al. Nuclear autoantigen p330d/CENP-F: a marker for cell proliferation in human malignancies. Cytometry. 1996;25(1):90-98. CrossRef PubMed
43. Rattner JB, Rees J, Whitehead CM, et al. High frequency of neoplasia in patients with autoantibodies to centromere protein CENP-F. Clin Invest Med. 1997;20(5):308-319. PubMed
44. Welner S, Trier NH, Frisch M, et al. Correlation between centromere protein-F autoantibodies and cancer analyzed by enzyme-linked immunosorbent assay. Mol Cancer. 2013;12(1):95. CrossRef PubMed
45. Bossuyt X, Fieuws S. Detection of antinuclear antibodies: added value of solid phase assay? Ann Rheum Dis. 2014;73(3):e10. CrossRef PubMed
46. Pisetsky DS, Lipsky PE. Role of antinuclear antibody determinations in classification criteria for systemic lupus erythematosus: comment on the article by Leuchten et al. Arthritis Care Res (Hoboken). 2019;71(5):696. CrossRef PubMed
47. de Oliveira Martins LV, Oliveira SM, Silvatti J, et al. Mortality in systemic sclerosis-associated interstitial lung disease in Brazil: a real-life, long-term follow-up observational study. J Clin Rheumatol. 2022;28(2):e532-e538. CrossRef PubMed
48. Hoffmann-Vold AM, Petelytska L, Fretheim H, et al. Predicting the risk of subsequent progression in patients with systemic sclerosis-associated interstitial lung disease with progression: a multicentre observational cohort study. Lancet Rheumatol. 2025;7(7):e463-e471. CrossRef PubMed