Rethinking the Routine Detection of Centromeric Autoantibodies

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Abstract

Systemic sclerosis is an autoimmune condition marked by vascular complications and fibrosis. Essential to its diagnosis are antinuclear antibodies with subsets such as anti-topoisomerase I, ant centromere antibodies, and anti-RNA polymerase III being particularly telling. This ten-year retrospective analysis delves into the diagnostic potential of combined centromere protein B antibodies and centromere protein A antibodies detection in anticentromere antibodies-positive serum samples. From a cohort of 94,164 antinuclear antibodies-positive participants, 756 displayed a distinctive centromeric indirect immunofluorescence staining. Of these, 95.8% were confirmed for centromere protein B antibodies. Interestingly, of those who were centromere protein B antibodies-negative initially, 53% tested positive for centromere protein A antibodies. Our study underscores the nuanced differences in diagnostic sensitivity and specificity between the two antibodies in the context of systemic sclerosis, with centromere protein A antibodies emerging as a potentially more sensitive marker. We propose a nuanced approach to anticentromere antibodies detection, giving prominence to indirect immunofluorescence on HEp-2 cells, and raising questions about the necessity of distinguishing between specific centromeric proteins.

Keywords: Antinuclear Antibodies; Centromere Protein A Antibodies; Centromere Protein B Antibodies; Diagnostic Sensitivity; Systemic Sclerosis

Introduction

Systemic sclerosis (SSc) is a severe autoimmune disorder marked by vascular damage and excessive fibrosis in both the skin and internal organs, culminating in premature mortality [1]. As per the Leroy classification [2], the disease manifests in two primary forms: diffuse and limited scleroderma. A pivotal immunological marker for diagnosing SSc is the presence of circulating antinuclear antibodies (ANA), detectable in over 90% of patients. Among these ANAs, specific antibodies for the diagnosis of systemic sclerosis include those against topoisomerase (anti-TOPO I), centromere (ACA), and the RNA polymerase enzyme (anti-RNAP III).

Anti-centromere antibodies (ACA) are instrumental in diagnosing SSc, with a reported prevalence ranging from 20–40%, predominantly associated with the limited cutaneous variant of the disease. While ACAs exhibit a specificity of over 95% for SSc, they have also been identified in a spectrum of autoimmune disorders, including systemic lupus erythematosus (SLE), primary biliary cirrhosis (PBC) [3], rheumatoid arthritis (RA), Sjögren Syndrome (SjS), and Raynaud’s phenomenon.
Historically, ACAs were pinpointed using indirect immunofluorescence (IIF) on HEp-2 cells, showcasing a distinctive discrete-speckled staining pattern indicative of centromere localization. This detection was subsequently corroborated by immunoassays employing the recombinant centromeric proteins (CENP) B. Among the myriad of centromeric proteins (CENP) encompassing CENP-A, -B, -C, -D, -E, -F, -G, -H, and -O, CENP B is perceived as the “primary” autoantigen in SSc sera [4-6]. Several factors underpin this assertion. The CENP B protein was cloned in 1987 by Earnshaw et al., and tailored ELISAs were swiftly introduced for autoantibody detection [7,8]. Moreover, it was previously ascertained that the archetypal IIF staining pattern was solely linked with autoantibodies targeting CENP-B[7]. Since then, a limited number of studies have juxtaposed the relevance of detecting autoantibodies against both CENP B and CENP A.

Aiming to refine the ACA detection procedure, we delved into the significance of concurrently searching for CENP B and/or CENP A from ACA-positive serum samples gathered in routine practice.

Material and Methods

We performed a retrospective study of anti-ACA positive results over a 10-year period from 01/12/2011 to 31/12/2022 in patients referred to the University Hospital of Marseilles for suspected autoimmune disease. This study utilized data from healthcare records, and all serum samples were part of the Marseilles Biobank (registered as DC 2012_1704). The study received approval and registration from the institution under GDPR number 20-390 and met local requirements for data collection and data protection. Diagnoses were routinely conducted in medical departments, primarily internal medicine, of Marseille hospitals, based on established criteria.

Systemic Sclerosis (SSc) was diagnosed based on the criteria established in 2018 by Asano et al. [9]. Systemic Lupus Erythematosus (SLE) was diagnosed based on standard criteria [3,10]. Sharp’s syndrome was identified using the Alarcon-Segovia criteria [11], and other conditions diagnosed included primary biliary cirrhosis (PBC), rheumatoid arthritis (RA), Sjögren Syndrome (SjS), and Raynaud’s phenomenon.

Statistical Analysis

Analysis was performed using R version 3.03 (R Development Core Team) and GraphPad Prism V6.05 (GraphPad Software, La Jolla, CA, USA). Data are described as Mean ± standard deviation in the tables. Shapiro-Wilk test was used to test for data normality and two-tailed student t-test was used to test variable differences between groups. Pearson’s Chi-squared test was used to test difference in frequencies between groups for categorical variables.

Correlations between markers were evaluated using Pearson correlation analysis. Significance levels are indicated on graphs (*: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001). The study was conducted in accordance to the STROBE statement.

Results

Over a ten-year period, a total of 94,164 patients tested positive for anti-nuclear antibodies (ANA) detected through indirect immunofluorescence (IIF) on HEp-2 cells. Among these patients, 756 exhibited a distinct centromeric IIF staining pattern. In accordance with routine clinical practice, the initial screening focused exclusively on anti-CENP B autoantibodies. Among this subset, 724 patients (95.8%) tested positive for anti-CENP B autoantibodies, while 32 were negative. Subsequently, the detection of anti-CENP A autoantibodies was introduced for the 32 patients who initially tested negative for anti-CENP B autoantibodies, and it was also extended to 48 available samples from patients who were positive for anti-CENP B autoantibodies (Figure 1).
Figure 1: Overview of 94,164 patients who tested positive for anti-nuclear antibodies (ANA) detected through indirect immunofluorescence (IIF) on HEp-2 cells.

Among the 32 patients initially negative for anti-CENP B autoantibodies, 17 individuals (53%) were found to be positive for anti-CENP A autoantibodies. Within this group, 6 patients (37%) were diagnosed with Systemic Sclerosis (SSc), 6 (37%) had other autoimmune diseases (including rheumatoid arthritis, Sharp syndrome, lupus), and 4 cases remained unknown. Furthermore, 15 patients tested negative for both anti-CENP B and anti-CENP A autoantibodies, and none of them presented with SSc.

Among all patients tested positive for anti-CENP B autoantibodies (n=48), each one was also positive for anti-CENP A autoantibodies. Within this subset, 27 patients (56%) were diagnosed with SSc (Figure 2). Additionally, other autoimmune diseases (including rheumatoid arthritis, Sharp syndrome, lupus, celiac disease, hepatitis, spondylarthritis), Raynaud’s phenomenon, other clinical contexts, and unknown conditions were also observed.
**Figure 2**: Comparison of patients with anti-CENP A B Positive and Patients with only anti-CENP A positive

A relative assessment of sensitivity and specificity of anti-CENP B and anti-CENP A autoantibodies was performed for patients with SSc in whom both autoantibodies were evaluated (n=80). The comparative analysis of their analytical characteristics revealed a higher sensitivity for anti-CENP A autoantibodies and a higher specificity for anti-CENP B autoantibodies (Table 1).

![Comparison of anti-CENP A and B autoantibodies](image)

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<th>Anti-CENP A+ autoantibodies</th>
<th>Anti-CENP B+ autoantibodies</th>
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<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>Value 1,000 0,8957 to 1,000</td>
<td>Value 0,8182 0,6561 to 0,9139</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>0,3191 0,2040 to 0,4617</td>
<td>0,5532 0,4125 to 0,6859</td>
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**Table 1**: Comparative Analysis of Anti-CENP A and B Autoantibodies in SSc Patients. This table presents the relative sensitivity and specificity of anti-CENP A and B autoantibodies in 80 SSc patients. The data illustrates a higher sensitivity for anti-CENP A and increased specificity for anti-CENP B in diagnosing SSc.

**Discussion**

In the presence of a characteristic centromeric IIF staining, it is customary to confirm the presence of anti-centromere autoantibodies (ACA) by specifically detecting anti-CENP-B autoantibodies [12]. In our retrospective study, we made a noteworthy observation: all patients who tested positive for anti-CENP-B autoantibodies also tested positive for anti-CENP-A autoantibodies, with a substantial 56% of them being diagnosed with Systemic Sclerosis (SSc). Additionally, among patients exclusively positive for anti-CENP-A autoantibodies, we identified 6 cases diagnosed with SSc. Consequently, in the cohort of patients examined in our study, anti-CENP-A autoantibodies exhibited superior sensitivity, whereas anti-CENP-B autoantibodies demonstrated superior specificity for the diagnosis of SSc.

As demonstrated in prior studies, our findings underscore the notion that ACA, whether directed against CENP-B or CENP-A, lack specificity for SSc, as they are also detected in other autoimmune diseases such as Primary Raynaud’s phenomenon, Lupus, Sharp syndrome, and even certain infectious diseases. Notably, CENP-A is a 17 kDa protein sharing substantial sequence homology with histone H3 [13]. Research by Mahler et al. has suggested that anti-CENP-A reactivity may be initiated by epitope spreading from histone H3, with autoantibodies against CENP-A peptides potentially preceding those targeting CENP-B [14]. Therefore, anti-CENP-A
autoantibodies could serve as an early marker for SSc, offering enhanced sensitivity. Moreover, some studies have proposed that anti-CENP-A antibodies could serve as a more specific biomarker for SSc compared to antibodies targeting CENP-B [14].

In our study, it’s important to note that we observed a higher specificity of anti-CENP-B autoantibodies, albeit in a limited number of patients with available sera. D. Villalta et al. reported sensitivity and specificity percentages for ACA (anti-CENP-B) and ACA (anti-CENP-A) [12]. Considering the lack of definitive results regarding the detection of “specific” anti-centromeric proteins, we propose a simplified approach—detecting these autoantibodies solely through indirect immunofluorescence (IIF) on HEp-2 cells, without the need for confirmation using recombinant centromeric proteins. It’s important to emphasize that, like many autoimmune diseases, the diagnosis of SSc relies on a combination of clinical and biological criteria. Among these criteria, anti-centromere autoantibodies are included, with no specificity regarding a particular centromere protein specified in their definition. Additionally, the latest recommendations for ANA evaluation do not mention the necessity for confirming the anti-centromeric pattern obtained via IIF [15].

References