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# Current state of technologies and recognition of anti-SSA/Ro antibodies in China: A multi-center study

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## Abstract

**Background:** Previous studies have demonstrated that Ro60 and Ro52 have different clinical implications, and anti-Ro52 antibodies are an independent serum marker of systemic autoimmune diseases, including Sjögren's syndrome. Many different assays have been adopted to detect anti-Sjögren's syndrome antigen A (SSA)/Ro antibodies, while to date no specific approach has been recommended as optimal for anti-SSA/Ro antibody testing. Herein, we performed a multi-center study to explore the current clinical utility of different strategies for anti-SSA/Ro antibody testing in China.

**Methods:** Twenty-one tertiary care centers were included in this questionnaire-based study. The self-administered questionnaire mainly includes testing methods for anti-SSA/Ro antibodies, reporting system of results, and interpretation of results by clinicians.

**Results:** Six different methods were applied to detect anti-SSA/Ro antibodies in the 21 centers. Line immunoassay (eight different commercial kits) was the most frequently adopted method (21/21, 100%), with different cutoff values and strategies for intensity stratification. There were two reporting systems: One was reported as "anti-SSA antibodies" and "anti-Ro52 antibodies" (12/21, 57%), while the other was "anti-SSA/Ro60 antibodies" and "anti-SSA/Ro52 antibodies" (9/21, 43%). Notably, six centers (29%) considered either positive anti-Ro60 or anti-Ro52 antibodies as positive anti-SSA antibodies, all of which adopted the latter reporting system.

**Conclusion:** Significant variabilities existed among anti-SSA/Ro assays. Nearly 30% of centers misinterpreted the definition of positive anti-SSA antibodies, which may be attributed to the confusing reporting systems of line immunoassay. Therefore, we

Chen and Hu contributed equally to this paper.

advocate standardization of the nomenclature of anti-SSA/Ro antibodies, changing the "anti-SSA/Ro52" label in favor of the "anti-Ro52" antibodies for a clear designation.

**KEYWORDS**
anti-Ro52 antibodies, anti-Ro60 antibodies, anti-SSA/Ro antibodies, detection assay, reporting system, Sjögren's syndrome

## 1 | INTRODUCTION

Sjögren's syndrome (SS) is a common systemic autoimmune disease characterized by exocrinopathy and a triad of symptoms: dryness of the mouth and eyes, fatigue, and joint pain. Autoantibodies directed against Sjögren's syndrome antigen A (SSA)/Ro autoantigens are important serological biomarkers in SS, found in nearly two-thirds of patients.[1] Anti-SSA/Ro antibodies are also associated with other autoimmune diseases, such as systemic lupus erythematosus, systemic sclerosis, and idiopathic inflammatory myopathies. Previous studies initially demonstrated that anti-SSA/Ro antibodies may recognize two cellular proteins with molecular weights of approximately 52 and 60 KDa,[2,3] also referred to as Ro52 and Ro60, respectively. These two autoantigens were originally considered to constitute a stable macromolecular ribonucleoprotein complex and interact closely with each other.[4] However, subsequent studies have provided evidence that anti-Ro52 antibodies are an independent serum marker and the two proteins have different clinical implications.[5]

Ro60, also known as TROVE2, predominantly localizes to the nucleus and nucleolus and may recognize the misfolded precursor-5S ribosomal RNA to target the defective RNA for degradation, which is thought to serve a role in noncoding RNA quality control.[6,7] The presence of anti-Ro60 antibodies has been reported to be strongly associated with some key features of SS, such as sensory peripheral neuropathy.[8] Ro52 is encoded by the tripartite motif (TRIM) 21 gene and belongs to the TRIM family. The protein is situated mostly in cytoplasm and can translocate into the nucleus in a pro-inflammatory situation.[9] Ro52 functions as an E3 ubiquitin ligase, and anti-Ro52 antibodies were found to bind the RING domain of Ro52 and inhibit the E3 ligase activity of Ro52 by sterically blocking the E2/E3 interface.[10] Mouse models showed that Ro52-induced antibodies were capable of causing SS-like disorders.[11,12] Additionally, higher mean titers of anti-Ro52 antibodies are closely associated with severe scintigraphic involvement, positive salivary gland biopsy, parotid enlargement, leukopenia, and rheumatoid factor positivity, indicating more aggressive disease in patients with SS.[13] Furthermore, anti-Ro52 antibodies are the most common myositis-associated antibodies. Recent studies have suggested that anti-Ro52 antibodies are strongly associated with interstitial lung disease, more severe disease activity, unresponsiveness to immunosuppressants, and poorer prognosis in myositis.[14,15]

Anti-SSA/Ro antibodies are the most frequently identified markers in the standard extractable nuclear antigen panel. Anti-SSA/Ro antibodies can be detected by assays including RNA precipitation, double-immunodiffusion (DID), immunoblotting (IB), fluoroimmunoenzymatic assay, line immunoassay (LIA), enzyme-linked immunosorbent assay (ELISA), chemiluminescence assay (CLIA), and multi-bead immunoassay (MBA). Depending on the assay platforms and kits used, the sensitivity and specificity may vary significantly. No specific approach to date has been recommended as optimal for the detection of anti-SSA/Ro antibodies, due to the lack of well-designed studies comparing the sensitivity and specificity of these methods. Although a number of classification criteria for SS have been proposed since 1965,[16–18] the differences between anti-Ro60 and anti-Ro52 antibodies have not yet been clearly stated in the classification criteria. Here, we performed the first multi-center study based on the Chinese Sjögren's Syndrome Collaborative Research Group to explore the current clinical utility of different strategies for anti-SSA/Ro antibody testing and recognition of anti-SSA/Ro antibodies in China.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

All centers in the Chinese Sjögren's Syndrome Collaborative Research Group ($n$ = 28) were invited to participate in the questionnaire-based study. A total of 21 centers that had given their informed consents to participate in the study were finally included, which encompass the major regions of China. This study was approved by the Medical Ethics Committee of the leading centers (Shenzhen People's Hospital, identifier: YKLS2019-15–01; Peking Union Medical College Hospital, identifier: JS-2038).

### 2.2 | Questionnaire

All the centers have independent departments of Rheumatology and clinical immunology laboratories for detecting autoantibodies. The self-administered questionnaire mainly covered the following topics: testing methods for anti-SSA/Ro antibodies, reporting system of results, and interpretation of results by clinicians. The results of the

questionnaires were reconfirmed by the individual centers before analysis.

## 3 | RESULTS

### 3.1 | Testing methods of anti-SSA/Ro antibodies

A total of six different methods were applied in the 21 centers, including DID, IB, LIA, ELISA, CLIA, and MBA. As shown in Figure 1, LIA kits from eight different companies were used to detect anti-SSA/Ro antibodies in all of the centers (21/21, 100%), of which three were from foreign companies. ELISA was applied in four centers (4/21, 19%); CLIA in two centers (2/21, 10%); and DID, IB, and MBA in only one center, respectively (1/21, 5%). Seven (7/21, 33%) centers adopted two or more than two methods to detect the anti-SSA/Ro antibodies.

### 3.2 | Reporting system of anti-SSA/Ro antibody results

The characteristics of the methods used in the centers are recapitulated in Table 1. The results of anti-Ro60 and anti-Ro52 antibody testing were reported separately in all the centers. IB, LIA, and MBA included both the detection of anti-Ro60 and anti-Ro52 antibodies, whereas ELISA and CLIA were used only for the detection of anti-Ro60 antibodies, without anti-Ro52 antibodies.

There were two reporting systems in the eight different commercial LIA kits applied in this study (Table 2). One was reported as "anti-SSA antibodies" ("anti-Ro60 antibodies" were shown as "anti-SSA antibodies") and "anti-Ro52 antibodies" (12/21, 57%), while the other was shown as "anti-SSA/Ro60 antibodies" and "anti-SSA/Ro52 antibodies" (9/21, 43%). In addition, all the LIA reporting systems performed the intensity measurement, with different cutoff values for the determination of positive and negative results. Of the eight different LIA kits, three kits estimated the samples with a qualitative scale of values from negative (–) to strongly positive (+++), while five kits solely categorized the samples as negative (–) or positive (+), without intensity stratification.

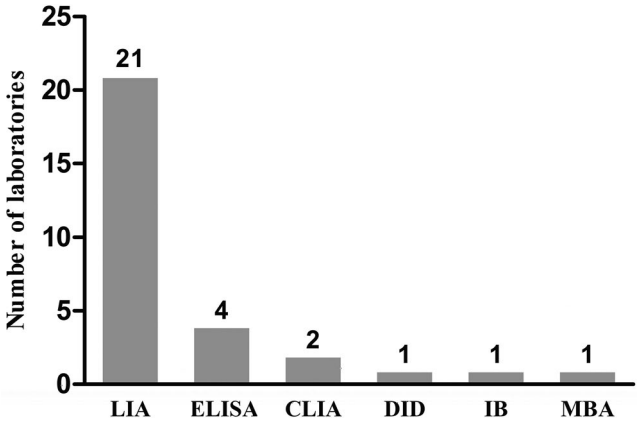


**FIGURE 1** Number of laboratories adopting different assays to detect anti-SSA/Ro antibodies. CLIA, chemiluminescence assay; DID, double-immunodiffusion; ELISA, enzyme-linked immunosorbent assay; IB, immunoblotting; LIA, line immunoassay; MBA, multi-bead immunoassay

### 3.3 | Definition of positive anti-SSA antibodies

According to the reporting systems in the different laboratories, the clinicians made determinations regarding anti-SSA antibody positivity. Notably, six centers (29%) considered either positive anti-Ro60 or anti-Ro52 antibodies as a positive anti-SSA antibody result (Table 2). That is, positivity for anti-Ro52 antibodies would be determined as a positive anti-SSA antibody result in nearly one-third of centers. Importantly, all the six centers adopted the reporting system indicated as "anti-SSA/Ro60 antibodies" and "anti-SSA/Ro52 antibodies."

## 4 | DISCUSSION

The results of one cohort study including 181 Chinese patients with SS revealed that positive labial salivary gland biopsy is strongly associated with the presence of anti-SSA antibodies.[19] Accordingly, anti-SSA/Ro antibodies have represented a mandatory criterion for the classification of patients with SS, particularly in individuals with a negative labial salivary gland biopsy.[16–18] In addition, key phenotypic features of SS were more prevalent and correlated with disease severity in SS patients with anti-SSA antibodies.[20] Therefore, anti-SSA/Ro antibody testing is critical in the diagnosis and management of SS. Nevertheless, to date, no classification or diagnostic criteria for SS have specified the "gold standard" method for anti-SSA/Ro antibody testing. Therefore, we conducted the first multi-center study based on the Chinese Sjögren's Syndrome Collaborative Research Group to explore the clinical utility of anti-SSA/Ro antibody testing and reporting practices in the clinical setting. Our findings reveal significant variabilities among anti-SSA/Ro assays in the detection of anti-SSA/Ro reactivity in different centers, with LIA as the most frequently adopted method. Furthermore, we are surprised to find that nearly one-third of the centers misinterpreted the definition of positive anti-SSA antibodies and considered either positive anti-Ro60 or anti-Ro52 antibodies as a positive anti-SSA antibody test result. This finding indicates that the diagnosis and management of SS could be misleading according to the current serological determination of anti-SSA/Ro antibodies, a problem that remains underestimated despite being previously reported in the literature.[21–25] Importantly, this study suggests that clinicians should differentiate between anti-Ro60 and anti-Ro52 antibody test results and advocates optimization of the reporting systems for these two antibody tests.

Although many methods have been proposed and evaluated for anti-SSA/Ro antibody detection, there is still no single, ideal assay

**TABLE 1** Characteristics of the methods used in the 21 centers

| | | | | | LIA | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Center | Method types | DID | IB | LIA | Kit[a] | Intensity stratification | Ro60 antigen source | ELISA | CLIA | MBA |
| 1 | 3 | Y | Y | Y | 1 | Y | Native | - | - | - |
| 2 | 1 | - | - | Y | 1 | Y | Native | - | - | - |
| 3 | 2 | - | - | Y | 1 | Y | Native | - | - | Y |
| 4 | 1 | - | - | Y | 1 | Y | Native | - | - | - |
| 5 | 2 | - | - | Y | 1 | Y | Native | Y | - | - |
| 6 | 1 | - | - | Y | 1 | Y | Native | - | - | - |
| 7 | 1 | - | - | Y | 1 | Y | Native | - | - | - |
| 8 | 2 | - | - | Y | 1 | Y | Native | Y | - | - |
| 9 | 1 | - | - | Y | 1 | Y | Native | - | - | - |
| 10 | 1 | - | - | Y | 1 | Y | Native | - | - | - |
| 11 | 2 | - | - | Y | 1 | Y | Native | Y | - | - |
| 12 | 3 | - | - | Y | 2 | Y | NS | Y | Y | - |
| 13 | 1 | - | - | Y | 2 | Y | NS | - | - | - |
| 14 | 1 | - | - | Y | 2 | Y | NS | - | - | - |
| 15 | 1 | - | - | Y | 3 | Y | NS | - | - | - |
| 16 | 1 | - | - | Y | 4 | - | NS | - | - | - |
| 17 | 2 | - | - | Y | 5 | - | NS | - | Y | - |
| 18 | 1 | - | - | Y | 6 | - | NS | - | - | - |
| 19 | 1 | - | - | Y | 6 | - | NS | - | - | - |
| 20 | 1 | - | - | Y | 7 | - | NS | - | - | - |
| 21 | 1 | - | - | Y | 8 | - | NS | - | - | - |

Abbreviations: anti-SSA, anti-Sjögren's syndrome antigen A antibodies; CLIA, chemiluminescence assay; DID, double-immunodiffusion; ELISA, enzyme-linked immunosorbent assay; IB, immune blotting; LIA, line immunoassay; MBA, multi-bead immunoassay; NS, not clearly stated; Y, applied; -, not applied.

[a]A total of eight different commercial kits of line immunoassay; the numbers indicate the different kits.

providing both high sensitivity and specificity. Several assays are predominantly used for the detection of anti-SSA/Ro antibodies, including RNA precipitation assay, DID, IB, LIA, ELISA, CLIA, and MBA. Despite the highest sensitivity and specificity of RNA precipitation assay for the detection of anti-SSA/Ro antibodies and being therefore used as the reference method in the previous study by Manoussakis et al.,[26] the complexity of RNA precipitation assay has limited its application in clinical practice. DID and IB have also been employed with relatively satisfactory specificity. However, besides a lower sensitivity, testing large numbers of samples using DID can be expensive and time consuming. LIA is similar to IB in that it includes a strip that has a broad spectrum of specific antigens in different areas and allows for multiplexed testing. It is the most frequently used method for anti-SSA/Ro antibody detection in China,[27] which is consistent with the result in this study that all of the centers adopted LIA. However, previous studies have highlighted a higher sensitivity but a lower specificity in anti-SSA/Ro antibody test by LIA.[28] During the last decades, semi-quantification method ELISA has been developed and commonly used to detect anti-SSA/Ro antibodies in clinical practice.[26] Nevertheless, comparative studies have reported similar sensitivities and specificities between ELISA and LIA,[29] as well as inconsistent results between various ELISA kits,[30] which may be explained by the diverse composition of the antigen preparations and different antigen-binding epitopes. With rapid advances in immunological detection technologies, the detection of anti-SSA/Ro antibodies has gradually entered the automatic and quantitative era. In the automated CLIA test, the enzymes linked to the detection antibodies produce a luminescence via a chemical reaction, which provides a quantitative determination of anti-SSA/Ro antibodies. A recent systematic meta-analysis demonstrated that CLIA had good specificity compared with indirect immunofluorescence in ANA testing.[31] In addition, MBA has shown certain advantages over conventional techniques, including the feasibility of high-throughput analyses for multiple antigens, minimal labor for automation, and reduced cost of samples.[32] However, the antigen composition of the bead-based assays also varies significantly, and issues with false-positive results are the main concern for MBAs.[33] Similarly, in this multi-center study, significant variabilities among anti-SSA/Ro assay kits were also observed in the detection of anti-SSA/Ro antibodies, with LIA as the most frequently used assay, which may be attributed

**TABLE 2** Reporting system and definition of positive anti-SSA antibodies in the 21 centers

| Center | Kit[a] | Reporting system of LIA | Positive anti-SSA[b] |
|---|---|---|---|
| 1 | 1 | Anti-SSA and anti-Ro52[c] | Anti-SSA (+) |
| 2 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 3 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 4 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 5 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 6 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 7 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 8 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 9 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 10 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 11 | 1 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 12 | 2 | Anti-SSA/Ro60 and anti-SSA/Ro52[d] | Anti-SSA/Ro60 (+) |
| 13 | 2 | Anti-SSA/Ro60 and anti-SSA/Ro52 | Anti-SSA/Ro60 (+) or anti-SSA/Ro52 (+) |
| 14 | 2 | Anti-SSA/Ro60 and anti-SSA/Ro52 | Anti-SSA/Ro60 (+) or anti-SSA/Ro52 (+) |
| 15 | 3 | Anti-SSA and anti-Ro52 | Anti-SSA (+) |
| 16 | 4 | Anti-SSA/Ro60 and anti-SSA/Ro52 | Anti-SSA/Ro60 (+) or anti-SSA/Ro52 (+) |
| 17 | 5 | Anti-SSA/Ro60 and anti-SSA/Ro52 | Anti-SSA/Ro60 (+) |
| 18 | 6 | Anti-SSA/Ro60 and anti-SSA/Ro52 | Anti-SSA/Ro60 (+) or anti-SSA/Ro52 (+) |
| 19 | 6 | Anti-SSA/Ro60 and anti-SSA/Ro52 | Anti-SSA/Ro60 (+) or anti-SSA/Ro52 (+) |
| 20 | 7 | Anti-SSA/Ro60 and anti-SSA/Ro52 | Anti-SSA/Ro60 (+) |
| 21 | 8 | Anti-SSA/Ro60 and anti-SSA/Ro52 | Anti-SSA/Ro60 (+) or anti-SSA/Ro52 (+) |

Abbreviations: anti-SSA, anti-Sjögren's syndrome antigen A antibodies; LIA, line immunoassay; (+), positive.

[a]A total of eight different commercial kits of line immunoassay.

[b]Definition of positive anti-SSA antibodies.

[c]The result is reported as "anti-SSA antibodies" and "anti-Ro52 antibodies," that is, "anti-Ro60 antibodies" are shown as "anti-SSA antibodies" in this reporting system.

[d]The result is reported as "anti-SSA/Ro60 antibodies" and "anti-SSA/Ro52 antibodies."

to multiplexed testing and simultaneously reduced cost. Therefore, it is important for clinicians and researchers to understand each testing method and its value, as well as its limitations.

In this study, three centers adopted CLIA and MBA to detect the anti-SSA/Ro antibodies, indicating a trend toward the application of automatic and quantitative methods in the autoantibody detection.[34] Studies have suggested that combining different detection methods could increase the diagnostic information.[28,35] Nevertheless, two-thirds of the centers in our study adopted only one method to detect anti-SSA/Ro antibodies, which may result in mis-determination of the antibodies. In fact, even within a given assay, differences between antigen source, methods of purification, and cutoff values may yield different results. In this study, although all the included centers used LIA to detect anti-SSA/Ro antibodies, the LIA kits came from up to eight different companies, suggesting that significant variation may exist. Moreover, the cutoff values and the positive stratification strategies also vary markedly within different commercial LIA kits. Five commercial LIA kits only reported positive or negative results, without intensity stratification, which would increase the difficulties in quantification and comparability when using different assays.

Although anti-Ro52 antibodies are frequently found in combination with anti-Ro60 antibodies, they have different clinical significance. Ro60 and Ro52 antigen are encoded by different genes and located in different intracellular regions. Furthermore, although most of the commercial Ro60 antigens on the market are claimed to be native antigens, some recombinant Ro60 antigens are still adopted due to their reduced cost. Most of the Ro52 antigens on the market are recombinant antigens or even synthetic peptide fragments, containing a limited number of epitopes of the native Ro52 antigen. Anti-Ro52 antibodies are not specific in diagnosing autoimmune diseases, including SS. Anti-Ro52 antibodies without anti-Ro60 antibodies are not reported routinely in some immunological laboratories. Consequently, ELISA and CLIA used in this study solely focused on the Ro60 reactivity but did not screen specifically for Ro52. However, recent evidence has indicated a potential significance of anti-Ro52 antibodies in SS[11–13] and other autoimmune diseases,[36] especially myositis.[14,15] Therefore, separate detection of anti-Ro60 and anti-Ro52 antibodies is advisable due to the representation of two distinct autoantibody systems. Single reactivity to either Ro60 or Ro52 can be missed when measured with an ELISA based on a mixture of both antigens.[37] In the

current study, anti-Ro60 and anti-Ro52 antibodies were separately detected in all of the centers. Furthermore, previous studies have suggested that serum anti-SSA/Ro levels were significantly higher in SS patients with severe keratoconjunctivitis sicca.[38] Higher mean titers of anti-Ro52 antibodies were closely associated with more aggressive disease in patients with SS.[13] Therefore, in addition to separate detection of anti-Ro60 and anti-Ro52 reactivity, it may be necessary to adopt methods that are based on quantitative detection of the two antibodies rather than anti-Ro60 antibodies alone.

Moreover, there were two reporting systems of results in different commercial LIA kits. One is reported as "anti-SSA antibodies" and "anti-Ro52 antibodies"; the other is "anti-SSA/Ro60 antibodies" and "anti-SSA/Ro52 antibodies." It is important to point out that nearly one-third of centers in this study considered the sole presence of anti-Ro52 antibodies as positive anti-SSA antibodies. The high frequency of anti-SSA antibody mis-determination is surprising, which would lead to mismanagement of individuals suspected with SS and heterogeneously diagnosed patient populations, especially in those without anti-Ro60 antibodies. Notably, all these six centers adopted the commercial kits reported as "anti-SSA/Ro60 antibodies and anti-SSA/Ro52 antibodies." The findings indicated that the misinterpretation of anti-SSA/Ro antibodies may not be only related to the mis-understanding of anti-Ro60 and anti-Ro52 antibodies by clinicians, but may also be partially explained by confusing reporting systems of the LIA kits. Therefore, in order to avoid confusion, it may be reasonable to standardize the nomenclature of anti-SSA antibodies, changing the "anti-SSA/Ro52" label in favor of the "anti-Ro52 antibodies" for an unambiguous designation. Regardless of the testing strategies used, clear and adequate communication between clinicians and laboratory staff regarding the significance of a positive result is imperative.

There are several limitations in this study. First, although this is a multi-center study including 21 centers encompassing the major regions of China, a larger number of centers would be able to obtain a more robust conclusion. Second, a self-administered questionnaire was used in this study, which has not been validated before. However, all of the participating centers are members of the Chinese Sjögren's Syndrome Collaborative Research Group, and the results of the questionnaires have been reconfirmed by the individual centers before the final analysis. Moreover, it would be necessary to carry out further studies to compare the sensitivity and specificity of the different methods, as well as the consistency among the different commercial kits in China.

## 5 | CONCLUSIONS

Our results demonstrate significant variabilities among anti-SSA/Ro assays in the detection of anti-SSA/Ro reactivity, with LIA as the most frequently used assay in China. Nearly one-third of the centers misinterpreted the definition of positive anti-SSA antibodies and considered positive anti-Ro52 antibodies alone as a positive anti-SSA antibody result, which may be attributed to the confusing reporting systems of LIA and may result in overdiagnosis of SS. Therefore, we suggest clinicians and researchers should recognize the differences between anti-Ro60 and anti-Ro52 antibodies, and we advocate standardization of the nomenclature of anti-SSA/Ro antibodies, changing the "anti-SSA/Ro52" label in favor of "anti-Ro52" antibodies for a clear designation. Moreover, in addition to separate detection of anti-Ro60 and anti-Ro52 reactivity, we also propose a quantitative detection of the two antibodies rather than anti-Ro60 antibodies alone.

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## CONFLICT OF INTERESTS

All the authors have no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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