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# Serological intermolecular epitope spreading in a patient with primary Sjögren's syndrome

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## SUMMARY

Sjögren's syndrome (SS) is one of the prototypic systemic autoimmune diseases characterised by autoreactive T and B cells, sicca symptoms and various extraglandular manifestations. SS is characterised by autoantibodies (anti-Ro52/tripartite motif containing-21 [TRIM21], anti-Ro60 and anti-La) that are important diagnostic biomarkers. Patients have typically stable serostatus; that is, patients who are positive for one or more of these autoantibodies tend to remain thus and vice versa. We describe a rare instance where a woman in her 50s was diagnosed with primary SS and developed new autoantibodies subsequently through serological epitope spreading. She demonstrated primarily glandular features only and clinical stability despite serological evolution. In this case report, we discuss the significance of this molecular feature and the clinical implications for our understanding of autoimmunity.

## BACKGROUND

Sjögren's syndrome (SS) is one of the prototypic systemic autoimmune diseases characterised by autoreactive T and B cells, sicca symptoms and in a good percentage of patients, various extraglandular manifestations.<sup>1</sup> While most patients experience stable disease, around 5%–10% of SS patients develop lymphoma and related complications.<sup>1</sup> Autoantibodies to the Ro/La ribonucleoprotein (RNP) complex (anti-Ro52, anti-Ro60 and anti-La) are highly characteristic and are the by-products of failed immunological tolerance. They are best known as being diagnostic biomarkers,<sup>2</sup> yet little is known about the origins and mechanisms of sustained autoantibody production. While these SS-associated autoantibodies are generally stable in patients, we discuss a rare instance of a patient who develops autoantibodies subsequent to diagnosis in an act of intermolecular epitope spreading.

## CASE PRESENTATION

A woman in her 50s was referred to the immunology clinics following the finding of a high-titred antinuclear antibody (ANA) test (speckled > 1:2560) in the context of recurrent thromboembolic events. Her background history included recurrent provoked and unprovoked deep vein thromboses on rivaroxaban, a left middle cerebral artery stroke on aspirin and rosuvastatin, and being a lifelong non-smoker. There is no family history of autoimmune disorders.

On further history, she described several years of a dry mouth complicated by severe dental caries and occasional dry eyes necessitating the use of

daily lubricating eye drops. She complained of some global alopecia but no fatigue, arthralgias, salivary gland swellings, cutaneous lesions, oral/nasal ulcers, neuropathic symptoms, Raynaud's, psychiatric or constitutional symptoms.

On examination, she was normotensive. There was no appreciable synovitis in her hands. Her cardiorespiratory examination was unremarkable. She had slightly prominent and non-tender parotid glands but no adenopathy. There was no hepatosplenomegaly. Schirmer's test in unanaesthetised eyes after 5 min was 10 mm and 5 mm in the right and left eyes, respectively.

## INVESTIGATIONS

Key investigations revealed anti-Ro52 autoantibody without anti-Ro60/La, normal C3/C4 complements, anti-double-stranded DNA not detected, no detectable rheumatoid factor, mild lymphopaenia ( $0.9 \times 10^6/L$ ), normal biochemistry and HIV was not detected. Prothrombotic screens including antiphospholipid antibodies were unremarkable. Targeted human leucocyte antigen (HLA) class II phenotyping was performed by polymerase chain reaction-sequence-specific oligonucleotide (PCR-SSO) typing (online supplemental table 1). Our patient did not receive a minor salivary gland biopsy.

## OUTCOME AND FOLLOW-UP

Our patient was formally diagnosed with primary SS as per American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria.<sup>2</sup> Hydroxychloroquine 200 mg daily was introduced as an immunomodulating agent; however, she self-ceased this after several weeks as found it not helpful to her situation. Three years after diagnosis, repeat serology revealed the development of new autoantibodies to Ro60 and La; yet, her clinical course remained relatively stable to that date (figure 1). This was assessed by the EULAR SS disease activity index (ESSDAI). At a further 3 years, despite the previous serological evolution, she still remained clinically stable (figure 1). She did not display any new features of serological evolution with a persistently high-titred ANA (> 1:2560 speckled across the 3 years), no other new antiextractable nuclear antigens or rheumatoid factors [RhF], and relatively stable total IgG level ranging from 11.5 to 11.9 g/L (< 15.6).

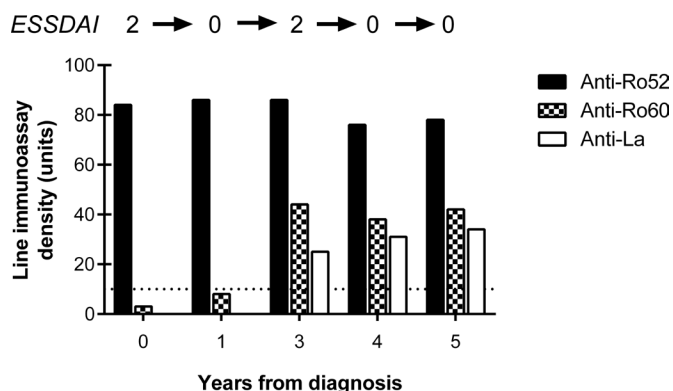
## DISCUSSION

Autoantibodies are the archetypal autoimmune biomarkers for systemic autoimmune disorders like SS. For the latter, they form part of the diagnostic criteria making them important laboratory



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**Figure 1** Longitudinal serological profiles and corresponding European Alliance of Associations for Rheumatology (EULAR) Sjögren's syndrome disease activity index (ESSDAI). The patient scores 2 points in year 0 for mild lymphopaenia ( $0.9 \times 10^9/L$ ) and 2 points in year 3 for transient parotidomegaly. The dotted horizontal line represents the autoantibody cut-off as stipulated by the manufacturer. Measurement of autoantibodies was performed by line immunoassay. Original figure created by author Lee.

parameters for the disease.<sup>2</sup> IgG antibodies towards the Ro/La RNP complex (anti-Ro52/anti-Ro60 and anti-La) can be found in up to 73% and 45% of cases, respectively, in large cohort studies.<sup>3</sup> The Ro/La RNP complex is a cytoplasmic conglomeration of non-coding RNAs (Y RNAs) and several proteins including Ro52, Ro60 and La, to which autoantibodies are commonly found towards in diseases like SS and systemic lupus erythematosus (SLE).<sup>4</sup>

In the diagnostic laboratory, these autoantibodies may be measured by line immunoassays and (ELISAs). Other key autoantibodies that are usually measured, but not part of the latest diagnostic criteria, include the ANA test and RhF. These are positive in 79% and 49% of patients, respectively; however, these are not specific for SS.<sup>3</sup> The ANA is commonly measured by indirect immunofluorescence microscopy on the HEp-2 substrate (or variations thereof). Positivity rate is dependent on the laboratory's dilution cut-off and the definition of 'nuclear' staining. In around 15% of SS patients, these autoantibodies are not detectable<sup>5</sup> and are termed seronegative SS, requiring other supportive evidence—such as minor salivary gland biopsies—to diagnose SS. Seronegative SS patients may also be positive for novel autoantibodies that are not routinely measured in the diagnostic laboratory, including antisalivary protein 1 and antiparotid secretory protein.<sup>6</sup> These autoantibodies are thought to be early autoantibodies and typically appear before the anti-Ro/La autoantibodies. Other novel, specific autoantibodies, such as antienolase, are associated with severe pathologies or end-organ manifestations and therefore may be helpful in identifying subsets of patients.<sup>6</sup>

Ro/La autoantibodies are thought to be persistent from long-lived memory B-cell responses.<sup>7,8</sup> Despite minor fluctuating titres of autoantibodies, the Ro52/Ro60 autoantibodies in SS tend to persist and once positive, remains so.<sup>9,10</sup> Likewise, it is unusual to develop new autoantibodies such as anti-Ro60 in patients tracked longitudinally.<sup>11</sup> This points to an immune response and autoantibodies that is likely 'hard-wired' and reliant on adaptive memory responses.

However, what occurs in human disease is in stark contrast to experimental mice models that show spontaneous and rapid epitope spreading of autoantibodies from Ro52 to Ro60 and La autoantigens after immunisation.<sup>12,13</sup> To our knowledge, no other case of SS in the literature has documented the epitope spreading of Ro52 to Ro60 and La. In spite of the immunological

maturation, this did not coincide with an appreciable evolution of her disease with relatively stable EULAR SS disease activity indices (ESSDAI) throughout the last few years of observation (figure 1).

It is also conceivable that other SS patients follow a similar trajectory yet be overlooked in the clinic as they may not have had autoantibodies serially tested. Serial autoantibody (antiextractable nuclear antigen) testing is currently dissuaded as there is little clinical benefit and it is costly.<sup>14</sup> Furthermore, Ro/La autoantibodies may appear many years before the onset of clinical symptoms and diagnosis in SS.<sup>15</sup> Thus, epitope spreading and development of autoantibodies at the start of failed immunological tolerance may be occurring in this predisease phase which may continue in the symptomatic phase. As support for this theory, SS patients who also have more Ro/La autoantibodies also tend to have autoantibodies of other specificities and higher ANA titres, indicating likely antigen epitope spreading.<sup>16</sup>

One factor that may influence the tendency to develop autoantibodies is the patient's HLA genotype. Interestingly, our patient did not harbour any recognised SS HLA class II risk alleles (online supplemental table 1), particularly HLA-DRB1\*0301.<sup>17</sup> These HLA risk alleles are also associated with SS patients that tend to have greater serological activity and epitope spread to Ro/La autoantigens, as opposed to remain singularly positive for anti-Ro52 or anti-Ro60.<sup>18,19</sup> At a pathophysiological level, these risk HLA alleles probably relate to the T cells' abilities to recognise and engage in Ro/La autoantigens.<sup>19</sup> This is supported in part by the recent observations of a higher diversity of T cell clonotypes associated with Ro/La positivity in SS patients.<sup>20</sup>

The natural molecular evolution and the pathogenesis of autoantibodies is still poorly understood. It is conceivable that dying or apoptotic cells cause the exposure of these RNP autoantigens.<sup>21</sup> The defective clearance of these cells and autoantigens coupled with other stimulatory factors such as Toll-like receptor signalling contributes to the autoantibody pathogenesis and clinical manifestations.<sup>22</sup> In addition, molecular mimicry between pathogens and these autoantigens may provide a mechanism for the development of autoantibodies and in SLE, it is hypothesised that key cross-reactive, 'driving' epitope sequences, to which autoantibodies appear early to, can help promote diversification of the immune response.<sup>23</sup>

Autoantibodies may hold prognostic information in SS. Seropositive SS patients with anti-Ro/La autoantibodies are more likely to be younger and develop extraglandular manifestations including haematological, neurological and immunological complications.<sup>24,25</sup> Although isolated anti-Ro52 SS patients have rarely been studied, a recent study found that patients with isolated anti-Ro52 (which was initially the case for our patient) may represent a severe subset of SS patients with higher disease activity over patients with additional autoantibodies.<sup>26</sup> Among seropositive SS patients, those with isolated anti-Ro52 are rarer (around 11%) compared with those with anti-Ro60 and/or anti-La. Anti-Ro52 autoantibodies are found in a range of autoimmune diseases (38% in a diagnostic laboratory cohort) and non-autoimmune diseases such as malignancies and interstitial lung disease (ILD).<sup>27</sup> By themselves, the autoantibodies are non-specific; yet hold distinct clinical utility from anti-Ro60, warranting separating detection in the diagnostic laboratory.<sup>28</sup> One recognised utility for anti-Ro52 is in the identification of ILD patients that have poorer prognoses over anti-Ro52-negative patients.<sup>29</sup>

These observations support the accurate serological phenotyping of patients with SS. Whether there is clinical benefit to periodic monitoring of autoantibodies to the Ro/La RNP remains to be decided in longitudinal studies. Although autoantibody titres may fluctuate longitudinally, it is rare for there to be the acquisition or loss of a particular autoantibody.<sup>7,11,30</sup> It may not be unreasonable,

however, to consider rechecking these Ro/La autoantibodies if there has been a clinical change; for example, if the patient has developed features of a secondary autoimmune disorder. In our department, rechecking of autoantibodies are variable, are clinician-dependent and may occur every 6–12 months at their regular follow-up, or only once at baseline.

In brief, we describe an interesting case of clinically stable SS despite molecular evolution. Given that serological manifestations including autoantibodies may precede the onset of clinical features and SS diagnosis by many years and appear in otherwise healthy individuals,<sup>15 31</sup> it is reasonable for us to continue periodically monitoring the patient to check for the development of severe extraglandular manifestations. However, SS is a disease that generally sees clinical stability in longitudinal studies,<sup>1</sup> providing some reassurance. Autoantibody profiles tend to also remain very stable and to our knowledge, we provide the first described instance of an SS patient longitudinally developing new autoantibodies to the Ro/La RNP complex. It is possible that we have captured the early stages of our patient's disease. Future studies are required to unearth the origins and mechanisms of autoantibodies and epitope spreading, as this will likely lead to insights into the immunopathogenesis of autoimmunity and possible targeted therapeutics.

### Learning points

- ▶ Autoantibodies are key by-products of failed immune tolerance in the diagnosis and prognosis of patients with primary Sjögren's syndrome (SS).
- ▶ The serostatus (detected/not detected) of autoantibodies to ribonucleoproteins tend to remain stable longitudinally and yield prognostic clinical information.
- ▶ Further research to unearth the origins and mechanisms of failed humoral tolerance and hence, the development of autoantibodies, will be instrumental in developing appropriate, targeted therapies in SS.

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Case reports provide a valuable learning resource for the scientific community and can indicate areas of interest for future research. They should not be used in isolation to guide treatment choices or public health policy.

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