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An updated review of anti-Ro52 (TRIM21) antibodies impact in connective tissue diseases clinical management

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Abstract

Anti-Ro52 (or anti-TRIM21) antibodies are part of the family of anti-Ro/SSA antibodies, historically markers of Sjögren syndrome and systemic lupus erythematosus. Anti-Ro52 antibodies represent one the most frequently encountered autoantibodies in patients with connective tissue disease (primary Sjögren syndrome, systemic lupus erythematosus, systemic sclerosis and idiopathic inflammatory myopathies). Because of their lack of specificity and detection in patients with non-autoimmune disorders, the usefulness of anti-Ro52 testing in connective tissue diseases is still matter of debate among clinicians and immunologists.

Autoantibodies are mainly diagnostic markers for autoimmune diseases but some of them can also be directly involved in the generation of tissue damage. Over the past decade several authors reported associations of anti-Ro52 antibodies with some clinical features – especially interstitial lung disease – and survival in patients with connective tissue diseases. There is also a growing evidence of the role of anti-Ro52 antibodies in the pathogenesis of connective tissue diseases. In this review, we comprehensively discuss the clinical associations of anti-Ro52 antibodies in the different connective tissue diseases and the recent advances on their potential role in the inflammatory response.

Keywords: Ro52, TRIM21, anti-Ro52 antibodies, anti-TRIM21 antibodies, connective tissue disease.

1. Introduction

The connective tissue is the framework and support structure for body tissues and organs (1). It is essential for structural and metabolic purposes but also plays a key role in antimicrobial defenses through various immune cells (2). Connective tissue diseases (CTD) are a heterogeneous group of systemic immune-mediated disorders, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren syndrome (SS), systemic sclerosis (SSc), idiopathic inflammatory myopathies (IIM) and mixed connective tissue diseases (MCTD). Autoimmune diseases (AID) are defined as pathological manifestations related to immune effectors responses against self-antigens (or autoantigens). Autoimmunity is physiologically controlled by central and peripheral tolerance mechanisms (3–5). In AID, the association of decreased tolerance signals and increased immune responses towards self-antigens in genetically predisposed individuals probably leads to the tolerance breakdown against autoantigens and subsequent tissue damages (6,7). Various effector cells are involved in the pathogenesis of AID, including innate (monocytes and dendritic cells) and adaptive (autoreactive CD4⁺ T cells, CD8⁺ T cells and B cells) immune cells (4,8,9). Autoantibodies can also be directly implicated in tissue damages through different mechanisms: complement-dependent cytotoxicity (CDC) (10,11), inflammation related to immune complexes deposits in tissues (12,13), stimulation or blockage of membrane receptors and cell structures (14,15), amplification of key inflammatory cytokines signaling pathways (type I interferons, TNF- α) (16,17). Autoantibodies are important tools in the diagnosis and management of CTD. If autoantibodies are mainly diagnostic markers for CTD, with variable sensitivity and specificity, some of them can also be involved in the generation of tissue damage and reflect the disease activity (such as anti-dsDNA antibodies in SLE and anti-HMGCR or anti-SRP antibodies in immune-mediated necrotizing myopathies) (18–21).

Anti-Ro52 (or anti-TRIM21) antibodies are one of the most frequently autoantibodies detected in sera of patients with CTD, in association with other specificities of autoantibodies in most of cases (22). Anti-Ro52 antibodies are not suitable diagnosis markers for CTD (lack of specificity) and their use as prognostic markers in CTD is debated. In this review we focus on the value of anti-Ro52 antibodies in CTD in clinical practice. We first provide a brief overview of anti-Ro/SSA antibodies history, Ro52 protein structure and functions, followed by a systematic literature review of epidemiologic data, clinical associations and recent preclinical advances regarding anti-Ro52 antibodies in CTD clinical setting.

2. Anti-Ro52 antibodies: generalities

2.1. History of anti-Ro/SSA antibodies

Antibodies directed against salivary and lacrimal gland extracts were first described in 1958 in sera of patients with SS, using an agar-gel diffusion precipitin test (23). A precipitin line was observed when the serum of patients with SS was tested with extracts of lacrimal and submandibular salivary glands. A similar reaction was observed with some kidney extracts. No reaction was observed with control sera and lacrimal gland, salivary gland and kidney extracts. Using another precipitin test (Ouchterlony technique), Anderson *et al.* identified two distinct antibodies in sera of patients with SS, directed against two distinct intracellular antigens detected in a wide range of cellular tissues (named SjD and SjT) (24). Intracellular localization of SjD and SjT antigens was demonstrated in tests conducted on lymphocytic leukemia extracts. Anti-SjD and anti-SjT antibodies were also detected in sera of patients with SLE, RA and SSc. The detection of SjD and SjT antigens in spleen extracts of a dead patient with RA and whose serum contained both corresponding antibodies indicated the autoimmune nature of these antibodies. A few years later, these two antigens were identified as Ro/SSA and La/SSB proteins, associated with small cytoplasmic ribonucleoproteins (or scRNP) (25,26). The antigenicity of Ro/SSA protein was independent of ribonucleic acids (RNA), since the precipitin activity was not affected by RNase and autoantibodies bound the protein after separation from RNA. In 1983, Wolin *et al.* demonstrated that a 60-kDa polypeptide (Ro60) was the major antigenic target of anti-Ro/SSA antibodies – isolated from sera of patients with SLE or other systemic autoimmune disorders – using immunoprecipitation and immunoblot techniques on human HeLa cells (27). In 1988, Ben-Chetrit *et al.* identified a 52-kDa protein (Ro52) as a second antigenic target of anti-Ro/SSA antibodies in sera of patients

with SS, using immunoblot tests (28). Purified anti-Ro60 and anti-Ro52 antibodies reacted exclusively with their corresponding antigens. Anti-Ro60 and anti-Ro52 antibodies had similar distributions on cellular structures in indirect immunofluorescence on HeLa cells, suggesting the colocalization of Ro60 and Ro52 proteins on the same ribonucleoproteic complex. In 1991, Itoh *et al.* demonstrated that Ro60 and Ro52 proteins were encoded by distinct genes with no sequence homology (29).

2.2. Structure and functions of Ro52 protein

Ro52 is a 52-kDa protein composed of 475 amino acids. Ro52 protein contains a RING finger domain, B-box motifs, a coiled-coil domain at the N-terminal region and a B30.2 (or PRYSPRY) region at the C-terminal region (30). The RING domain, B-box motifs and coiled-coil domain place Ro52 protein within the tripartite motif proteins (TRIM) family (31). Ro52 is also designated TRIM21 protein. TRIM proteins are mostly involved in innate immunity and antiviral responses but also play a role in the regulation of cell proliferation and survival (31). Ro52 (TRIM21) protein is predominantly expressed in lymphoid tissues (spleen, lymph nodes and thymus) and is located in the nucleus as well as the cytoplasm of cells (32). Ro52 protein has an E3 ubiquitin ligase activity and is essential in the ubiquitination of cellular proteins (33). Type 1 interferons are key mediators of antimicrobial defenses, especially antiviral responses. Interferon regulatory factors (IRF) are the main substrates of Ro52-mediated ubiquitination. Higgs *et al.* demonstrated that Ro52 protein negatively regulates IFN- β production by targeting IRF3 after TLR3 or TLR4 activation, with subsequent polyubiquitination and proteasomal degradation of IRF3 (34). IRF7 is another substrate of Ro52-mediated ubiquitination, resulting in a decrease in IFN- α production after TLR7 and TLR9 stimulation (35). More recently, Li *et al.* showed that Ro52 protein was also

involved in STING-mediated interferon- γ -inducible protein 16 (IFI-16) degradation through his E3 ligase activity, with subsequent decrease in type I interferon production (36). Ro52 can also act as a cytosolic Fc receptor for immunoglobulins coated with viral antigens, resulting in degradation of immune complexes and viral neutralization (37). Ro52 protein is induced by type 1 interferons and ensure a negative feedback regulation restricting type 1 interferon overproduction and prolonged immune system activation during antiviral immunity to avoid the development of autoimmune diseases (38). In a transgenic murine model, Ro52-knockout (Ro52^{-/-}) mice experienced a systemic autoimmune disease with lupus-like phenotype (including dermatitis, glomerulonephritis, hypergammaglobulinemia, anti-dsDNA antibodies) after ear injury, mediated by overproduction of cytokines involved in Th17 pathway (IL-17, IL-23) (32). Sabile *et al.* showed that Ro52 protein had a key role in cellular cycle in mammalian cells through p27 – a major cell cycle regulatory protein – ubiquitination and degradation (39). Jauharoh *et al.* demonstrated that Ro52 mediated cell apoptosis through negative regulation of the anti-apoptotic Bcl-2 protein (40). In another Ro52-knockout murine model, Brauner *et al.* showed higher specific antibody titers, higher proliferation of B cells after specific stimulation and higher expansion of splenic follicular B cells than controls, suggesting a potential role for Ro52 protein in the regulation of autoimmune antibodies production (41).

3. Anti-Ro52 antibodies and connective tissue diseases

3.1. Material and methods

We performed a systematic literature search of PubMed, Medline and EMBASE for all article types (abstracts or full papers) using the following keywords: “Ro52”, “TRIM21”. No restrictions concerning the date of publication were applied. In addition, we handsearched reference lists of papers initially detected to identify additional relevant reports. Only studies in English were included. We selected articles focusing on anti-Ro52 antibodies and CTD.

3.2. Sjögren syndrome

As described above, anti-Ro/SSA antibodies are historical markers of SS. In patients with primary SS, anti-Ro52 antibodies are detected in 60 % of cases (data pooled from recent studies; table 1) (22,42–45). Anti-Ro52 antibodies are most of the time associated with anti-Ro60 antibodies but isolated reactivity against Ro52 antigen is found in 20-30 % of patients (45,46). In SS secondary to other systemic autoimmune diseases, anti-Ro52 antibodies are detected in 40 % of patients (46,47). It is now well established that patients with SS and anti-Ro/anti-La antibodies had more “active” disease compared with patients without these autoantibodies. Anti-Ro/anti-La antibodies are associated with glandular dysfunction, parotid enlargement, clinical systemic features (Raynaud’s phenomenon, arthralgia/arthritis, vasculitis, renal involvement and peripheral neuropathy), cytopenia (leukopenia, thrombocytopenia) and immunological features (hypergammaglobulinemia, rheumatoid factor positivity, cryoglobulin) in patients with SS (48). Anti-Ro/anti-La antibodies are also associated with lung involvement, especially interstitial lung disease (ILD) (49). In the past

decade, several authors reported that anti-Ro52 antibodies were associated with clinical and biological hallmarks of SS (table 2). Retamozo *et al.* showed that high titers of anti-Ro52 antibodies were associated with severe salivary dysfunction, positive salivary gland biopsy, parotid enlargement, anemia, leukopenia and rheumatoid factor positivity (45). In this cohort, anti-Ro52 antibodies titers were correlated with gammaglobulin levels. In a more recently published Chinese cohort of 527 patients with primary SS (pSS), anti-Ro52 antibodies were associated with the presence of ILD (detection of anti-Ro52 antibodies in 36 % of patients with ILD vs. 24 % of patients without ILD, $p = 0.003$) (50). After logistic regression analysis, anti-Ro52 antibodies were independently associated with ILD (OR 7.9, 95 % CI 1.2- 13.4, $p = 0.005$). In a Korean cohort of primary SS patients, Song *et al.* found that patients with isolated anti-Ro52 antibodies (no associated anti-Ro60 reactivity) had more liver and muscle involvement (43). Preclinical studies showed a high expression of Ro52 antigen in the ductal epithelium in salivary glands of patients with primary SS, correlated with local mononuclear cells infiltration (51). Aqrabi *et al.* showed that Ro/SSA-specific B cells in salivary glands of patients with primary SS were exclusively plasma cells (52). Taken together, these data suggest that overexpression of Ro52 antigen is involved in the generation of specific antibodies in the salivary glands of patients with primary SS. Recently, Hillen *et al.* demonstrated the high expression of Ro52 protein on the surface of circulating plasmacytoid dendritic cells –antigen-presenting cells particularly implicated in anti-viral responses – associated with B cell hyperactivity and type 1 interferon activity in patients with primary SS (53). Anti-Ro52 antibodies appear to have a direct pathogenic role in SS as demonstrated in experimental models. In murine models of SS, mice immunized with Ro52 and aluminum adjuvant develop anti-Ro52 antibodies, salivary and lacrimal glandular inflammation with local anti-Ro52 antibodies deposits (54,55). Passive transfer of anti-Ro52 antibodies from immunized mice to naïve mice induced salivary and lacrimal glandular dysfunction. Sroka *et*

al. demonstrated that only anti-Ro52 antibodies directed against the coiled-coil domain of the protein – involved in the E3 ubiquitin ligase activity – induced salivary glandular dysfunction (56). In in-vitro manipulations, Espinosa *et al.* showed that anti-Ro52 antibodies from patients with primary SS inhibited the E3 ligase activity of Ro52 protein (57).

3.3. Systemic lupus erythematosus

In SLE, anti-Ro52 antibodies are detected in 40-50 % of patients, mostly in association with anti-Ro60 and anti-La antibodies (table 1) (42,58). The presence of isolated anti-Ro52 antibodies in SLE is rare, concerning less than 5 % of patients (44,46). Anti-Ro/SSA antibodies are associated with subacute lesions and photosensitivity in cutaneous lupus erythematosus (CLE) (59). Popovic *et al.* reported that levels of anti-Ro52 antibodies were higher than anti-Ro60 and anti-La antibodies levels in patients with CLE and that CLE patients with systemic disease showed higher levels of anti-Ro52 antibodies compared to patients with limited cutaneous disease (60). Oke *et al.* showed that Ro52 expression was upregulated in epidermal cells (keratinocytes) in inflammatory skin conditions – including CLE patients – and after in-vitro exposure to UV (61). This over-expression of Ro52 might offer more intense exposure of Ro52 epitopes and contribute to the breakage of tolerance and development of autoimmunity. In a Swedish cohort of 232 SLE patients, anti-Ro52 antibodies were associated with high immunoglobulin levels and decreased count of leukocytes and lymphocytes as in SS (table 2) (62). More interestingly, levels of anti-Ro52 antibodies were positively correlated with disease activity. Wang *et al.* also described a higher activity score in SLE patients with anti-Ro52 antibodies (63). Recently, Kunishita *et al.* demonstrated in a murine model of SLE (MRL/lpr mice) that TRIM21 dysfunction was associated with aberrant B cell differentiation, higher levels of anti-dsDNA antibodies and proteinuria (64).

Proportions of circulating B cells which differentiate into plasmablasts after in-vitro stimulation were also higher in SLE patients compared with healthy controls (64). Whether anti-Ro52 antibodies are directly implicated in TRIM21 dysfunction need to be further evaluated.

3.4. Systemic sclerosis

Anti-Ro52 antibodies are among the most frequently detected autoantibodies in SSc. Their prevalence is evaluated around 20 %, both in limited and diffuse forms of the disease (table 1) (47,65–68). Anti-Ro52 antibodies are associated with anti-Ro60 antibodies in more than 80 % of patients with SSc and are frequently detected in association with SSc-specific autoantibodies (anticentromere, anti-RNA polymerase III and anti-topoisomerase I antibodies) (68). Anti-Ro52 antibodies are the only detected autoantibodies in 5 % of SSc patients (65–67). In a multicentric cohort of 963 patients, Hudson *et al.* found that the presence of anti-Ro52 antibodies was associated with ILD (OR 1.53, 95 % CI 1.11- 2.12, $p = 0.009$) and overlap syndrome (OR 2.70, 95 % CI 1.01- 4.19, $p = 0.006$; table 2) (47). In this study, ILD severity – measured by the Medsger Disease Severity Scale – was higher in anti-Ro52 positive patients (OR 1.19, 95 % CI 1.04- 1.37, $p = 0.008$). Wodkowski *et al.* reported a higher prevalence of ILD (OR 2.70, 95 % CI 1.75- 4.14, $p < 0.0001$) and a significant risk of death (HR 1.87, 95 % CI 1.24- 2.82, $p = 0.003$) in SSc patients with isolated anti-Ro52 antibodies – i.e. no other SSc-specific or associated antibodies – compared with anti-Ro52-negative SSc patients (66). In the Australian SSc cohort, anti-Ro52 antibodies were independently associated with pulmonary arterial hypertension (PAH), Raynaud's phenomenon, telangiectasia and mortality but not with ILD (67, 69). Tangri *et al.* found a trend for a higher frequency of pulmonary arterial hypertension (PAH) in SSc patients with

anti-Ro52 antibodies (70). Discrepancies of results regarding ILD and PAH prevalences in SSc patients with or without anti-Ro52 antibodies could be explained by the heterogeneity of SSc subtypes and definitions among studies (use of chest high-resolution CT or not for ILD and estimated systolic pulmonary arterial pressure on echocardiography for PAH).

3.5. *Inflammatory myositis*

IIM are a heterogeneous group of muscle inflammatory diseases that historically included polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM). Recently, this classification has been revisited in line with the discovery of novel autoantigen specificities (71,72). PM are now subdivided into immune-mediated necrotizing myopathies and overlap myositis such as antisynthetase syndrome (ASS). Anti-Ro52 antibodies are the most commonly autoantibodies found in IIM, in almost 30 % of patients (table 1) (42,73). Anti-Ro52 antibodies are even more frequently detected in patients with ASS, in almost 40-60 % of cases (74–77). ASS is an overlap CTD syndrome associated with muscular, pulmonary, joint, cutaneous manifestations and the detection of specific autoantibodies (anti-aminoacyl-tRNA synthetase antibodies) in sera of patients (78). Unlike other systemic autoimmune disorders, anti-Ro52 antibodies frequently occur without anti-Ro60 antibodies in patients with ASS (74). The presence of anti-Ro52 antibodies in patients with ASS appears to be associated with disease severity, especially ILD prevalence and severity (table 2). In a French cohort of 89 ASS patients with anti-Jo1 antibodies, anti-Ro52 antibodies were associated with more symptomatic forms of ILD and more deterioration of myositis and joint involvement during follow-up (75). The authors also showed a trend to decreased survival in ASS patients with anti-Ro52 antibodies. Bauhammer *et al.* reported that anti-Ro52 antibodies were associated with more acute-onset forms of ILD with O2 dependency in ASS patients and that patients

with high titers were at higher risk of severe ILD (76). In a more recently published cohort of patients with ASS, Shi *et al.* showed that anti-Ro52 antibodies were significantly associated with more frequent ILD and anti-PL7 positive patients with anti-Ro52 antibodies had more rapidly progressive ILD (77). In a cohort of patients including ASS and anti-MDA5 positive DM, Xing *et al.* reported a higher frequency of ILD in patients with anti-Ro52 antibodies (79). In this study, DM patients with anti-MDA5 and anti-Ro52 antibodies had more severe ILD and decreased survival compared to patients with isolated anti-MDA5 antibodies. In juvenile myositis, after controlling for the presence of myositis specific autoantibodies, anti-Ro52 antibodies were also associated with higher prevalence of ILD (36 % of anti-Ro52 positive patients vs. 4 % of anti-Ro52 negative patients, $p < 0.001$), more frequent chronic disease course, less common remission and increased number of medications received during follow-up (80). Anti-Ro52 antibodies association with cancer is still matter of debate, as only one study reported a positive association in ASS adult patients (75), while others reporting no association (76,77). Interestingly, Gomez-Martin *et al.* demonstrated a decreased expression of Ro52 protein associated with a decrease in ubiquitination profile in peripheral CD4⁺ T cells and monocytes of patients with IIM compared with healthy controls (81). After nonspecific stimulation, they observed a higher synthesis of proinflammatory cytokines by T cells (IL-17A, TNF- α) and monocytes (IL-6, IFN- α) in IIM patients. In preclinical studies, Hassan *et al.* also found significantly higher TNF/IL-10 ratios in sera of myositis patients with anti-Ro52 antibodies (82). In muscle biopsies of patients with myositis, Krystufova *et al.* showed an increased expression of B-cell-activating factor receptor (BAFF-R), B-cell maturation antigen (BCMA) and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) – receptors of BAFF, a major cytokine involved in B cells maturation and survival – in patients with anti-Ro52 antibodies compared with anti-Ro52 negative patients (83). In muscle tissues of anti-Ro52 positive myositis patients, plasma cells were positively

correlated with plasmacytoid dendritic cells, BAFF-R, BCMA and TACI. These preclinical data support a potential role of anti-Ro52 antibodies in the pathogenesis of myositis.

3.6. *Mixed connective tissue disease*

MCTD is a debated CTD entity, first described by Sharp *et al.*, sharing SSc, SLE and myositis clinical features and characterized by the detection of anti-RNP antibodies in sera of patients (84). In a Norwegian MCTD cohort, anti-Ro52 antibodies were found in 30 % of patients (table 1) (85). Anti-Ro52 antibodies were detected with anti-Ro60 antibodies in approximately one third of patients. Anti-Ro52 antibodies were associated with more frequent ILD in MCTD patients (58 % of anti-Ro52 positive vs. 24 % of anti-Ro52 negative patients, $p = 0.001$) (85). Anti-Ro52 antibodies were also associated with ILD progression during follow-up in MCTD patients (OR 3.5, 95 % CI 1.2- 10.2, $p = 0.02$) (86).

4. Discussion

If it is well established that anti-Ro52 antibodies are not suitable markers for CTD diagnosis, they could represent valuable biologic prognostic markers in clinical practice. Data from preclinical studies suggest that anti-Ro52 antibodies could have a direct role in the pathogenesis of CTD. Anti-Ro52 antibodies are one of the most frequently detected autoantibodies in the serum of patients with CTD, especially in SS, SLE and myositis. Anti-Ro52 antibodies are also frequently found in others autoimmune disorders, as autoimmune hepatitis and primary biliary cirrhosis (in almost 40 % of patients) (87–89). Some patients with non-autoimmune disorders such as malignancies and infections have been tested positive for anti-Ro52 antibodies (44,90,91). In light with the low specificity of anti-Ro52 antibodies, some authors have questioned the usefulness of anti-Ro52 testing.

Even if we report discrepancies between studies, our systematic literature review tends to support an association between anti-Ro52 antibodies and CTD severity. One of the most interesting finding is probably the association of anti-Ro52 antibodies with ILD. ILD represents a frequent complication of CTD and remains a significant cause of morbidity and mortality in CTD patients (92). The association of anti-Ro52 antibodies and ILD appears to concern only CTD for which ILD is a usual manifestation (ASS, SSc, primary SS, MCTD). Anti-Ro52 antibodies appear to be associated with both the prevalence and the severity of ILD in these CTD. In a large cohort of different CTD, Wu *et al.* reported a higher prevalence of ILD in anti-Ro52⁺ antiRo60⁻ patients compared with anti-Ro52⁺ anti-Ro60⁺ patients (93). Recently, we confirmed that anti-Ro52 antibodies were independently associated with ILD prevalence at the time of CTD diagnosis, even after adjusting for the coexistence of anti-Ro60 antibodies (94). In our cohort, anti-Ro52 antibodies were also associated with less asymptomatic ILD forms at diagnosis. As reported in juvenile myositis, we found that anti-

Ro52 antibodies were associated with more relapses and immunosuppressive drugs used during the follow-up in CTD patients.

The role of anti-Ro52 antibodies in the pathophysiology of CTD is still debated. The fact that Ro52 (TRIM21) protein is highly expressed in lungs compared with other tissues of the body might reflect the need for tightly controlling type I interferon response in this site of frequent viral infections, and could explain in part the association between ILD and anti-Ro52 antibodies (95). Ro52 (TRIM21) is one of the most highly antigenic protein in humans (96). Anti-Ro52 antibodies could represent an epiphenomenon in tissues targeted by the immune response, especially in lungs. In-vitro studies showed a high Ro52 protein expression at cell surface in apoptotic cells or in response to cellular stress (61,97). Anti-Ro52 antibodies production could be secondary to the inflammatory reaction in tissues targeted by the initial anti-infectious or autoimmune responses, through a process of extension of the immune response to other proteins called “epitope spreading”. Reactivity against Ro52 protein could be related to the high level of the immune response and subsequent tissue damage reflecting the clinical severity of CTD patients. As described above, there is some evidence to suggest that anti-Ro52 antibodies might play a more direct role in the pathogenesis of CTD associated with anti-Ro52 antibodies. In several murine models, anti-Ro52 antibodies mediate salivary gland dysfunction and congenital heart block (54,55,98,99). As reported by Espinosa *et al.* in patients with primary SS, anti-Ro52 antibodies could inhibit the function of Ro52 protein (57). One hypothesis is that anti-Ro52 antibodies might directly amplify proinflammatory signals, especially type 1 interferons, through blocking the regulatory activity of Ro52 protein or through toll-like receptors stimulation (figure 1).

This systematic literature review needs precautions regarding data interpretation. As we previously discussed, findings regarding the clinical utility of anti-Ro52 antibodies in CTD are conflicting between the different cohorts. Use of different techniques with variable

sensitivity and specificity for autoantibodies detection and different definitions – notably for ILD diagnosis – could explain these discrepancies. In some CTD cohorts, information regarding the time point of anti-Ro52 antibodies testing or ILD evaluation (at diagnosis or during the follow-up) were missing. As the disease natural course and immunosuppressive drugs received can modify autoantibodies detection and clinical manifestations in CTD patients, extrapolation of results is difficult. Additional studies are needed to better precise clinical associations of anti-Ro52 antibodies and their role in CTD pathophysiology.

To conclude, anti-Ro52 antibodies are frequently detected in sera of patients with CTD. The usefulness of anti-Ro52 antibodies testing is still debated among immunologists and clinicians. If anti-Ro52 antibodies are not specific biologic markers of CTD, they could represent predictors of CTD-associated ILD and CTD severity. The detection of anti-Ro52 antibodies probably should lead clinicians to a careful screening of ILD in CTD patients at time of diagnosis, especially in ASS, primary SS and SSc. A better understanding of the role of anti-Ro52 antibodies in the pathogenesis of CTD could lead clinicians to integrate anti-Ro52 testing in evaluation, monitoring and management of patients with CTD in the future.

Take-home messages

- Anti-Ro52 (TRIM21) antibodies are one of the most frequently detected autoantibodies in patients with connective tissue diseases
- Anti-Ro52 antibodies appear to be associated with interstitial lung disease prevalence and severity in patients with connective tissue diseases
- Anti-Ro52 antibodies could represent an epiphenomenon of the strong immune response in severe patients or directly participate in tissue damages
- If anti-Ro52 antibodies are not suitable biologic markers for connective tissue diseases diagnosis, they could represent valuable prognostic markers in clinical practice

Competing interests

The authors declare no conflict of interests

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References

1. Kamrani P, Marston G, Jan A. Anatomy, Connective Tissue. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 [cited 2020 Dec 5]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK538534/>
2. Nezwek TA, Varacallo M. Physiology, Connective Tissue. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 [cited 2020 Dec 5]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK542226/>
3. Miller JF, Flavell RA. T-cell tolerance and autoimmunity in transgenic models of central and peripheral tolerance. *Curr Opin Immunol*. 1994;6(6):892–9.
4. Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature*. 2005;435(7042):590–7.
5. Sakaguchi S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol*. 2004;22:531–62.
6. Bach J-F. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med*. 2002;347(12):911–20.
7. Rai E, Wakeland EK. Genetic predisposition to autoimmunity--what have we learned? *Semin Immunol*. 2011;23(2):67–83.
8. Haskins K. Pathogenic T-cell clones in autoimmune diabetes: more lessons from the NOD mouse. *Adv Immunol*. 2005;87:123–62.

9. Horai R, Silver PB, Chen J, Agarwal RK, Chong WP, Jittayasothorn Y, et al. Breakdown of immune privilege and spontaneous autoimmunity in mice expressing a transgenic T cell receptor specific for a retinal autoantigen. *J Autoimmun.* 2013;44:21–33.
10. Kurlander RJ, Rosse WF. Monocyte-mediated destruction in the presence of serum of red cells coated with antibody. *Blood.* 1979 Nov;54(5):1131–9.
11. Berentsen S, Sundic T. Red Blood Cell Destruction in Autoimmune Hemolytic Anemia: Role of Complement and Potential New Targets for Therapy [Internet]. Vol. 2015, BioMed Research International. Hindawi; 2015 [cited 2020 Apr 2]. p. e363278. Available from: <https://www.hindawi.com/journals/bmri/2015/363278/>
12. Kramers C, Hylkema MN, van Bruggen MC, van de Lagemaat R, Dijkman HB, Assmann KJ, et al. Anti-nucleosome antibodies complexed to nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane in vivo. *J Clin Invest.* 1994;94(2):568–77.
13. van der Vlag J, Berden JHM. Lupus nephritis: role of antinucleosome autoantibodies. *Semin Nephrol.* 2011;31(4):376–89.
14. Morshed SA, Latif R, Davies TF. Characterization of thyrotropin receptor antibody-induced signaling cascades. *Endocrinology.* 2009;150(1):519–29.
15. Verschuuren JJGM, Huijbers MG, Plomp JJ, Niks EH, Molenaar PC, Martinez-Martinez P, et al. Pathophysiology of myasthenia gravis with antibodies to the acetylcholine receptor, muscle-specific kinase and low-density lipoprotein receptor-related protein 4. *Autoimmun Rev.* 2013;12(9):918–23.

16. Batteux F, Palmer P, Daëron M, Weill B, Lebon P. FCgammaRII (CD32)-dependent induction of interferon-alpha by serum from patients with lupus erythematosus. *Eur Cytokine Netw.* 1999;10(4):509–14.
17. Anquetil F, Clavel C, Offer G, Serre G, Sebbag M. IgM and IgA rheumatoid factors purified from rheumatoid arthritis sera boost the Fc receptor- and complement-dependent effector functions of the disease-specific anti-citrullinated protein autoantibodies. *J Immunol Baltim Md 1950.* 2015;194(8):3664–74.
18. ter Borg EJ, Horst G, Hummel EJ, Limburg PC, Kallenberg CG. Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long-term, prospective study. *Arthritis Rheum.* 1990;33(5):634–43.
19. Ehrenstein MR, Katz DR, Griffiths MH, Papadaki L, Winkler TH, Kalden JR, et al. Human IgG anti-DNA antibodies deposit in kidneys and induce proteinuria in SCID mice. *Kidney Int.* 1995;48(3):705–11.
20. Allenbach Y, Arouche-Delaperche L, Preusse C, Radbruch H, Butler-Browne G, Champtiaux N, et al. Necrosis in anti-SRP+ and anti-HMGCR+myopathies: Role of autoantibodies and complement. *Neurology.* 2018 06;90(6):e507–17.
21. Bergua C, Chiavelli H, Allenbach Y, Arouche-Delaperche L, Arnoult C, Bourdenet G, et al. In vivo pathogenicity of IgG from patients with anti-SRP or anti-HMGCR autoantibodies in immune-mediated necrotising myopathy. *Ann Rheum Dis.* 2019;78(1):131–9.
22. Lee SA, Kahng J, Kim Y, Park Y-J, Han K, Kwok S-K, et al. Comparative study of immunofluorescent antinuclear antibody test and line immunoassay detecting 15 specific

- autoantibodies in patients with systemic rheumatic disease. *J Clin Lab Anal.* 2012;26(4):307–14.
23. Jones BR. Lacrimal and salivary precipitating antibodies in Sjögren's syndrome. *Lancet Lond Engl.* 1958;2(7050):773–6.
 24. Anderson JR, Gray KG, Beck JS, Kinnear WF. Precipitating autoantibodies in Sjogren's disease. *Lancet Lond Engl.* 1961;2(7200):456–60.
 25. Clark G, Reichlin M, Tomasi TB. Characterization of a soluble cytoplasmic antigen reactive with sera from patients with systemic lupus erythmatosus. *J Immunol Baltim Md 1950.* 1969;102(1):117–22.
 26. Mattioli M, Reichlin M. Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus. Description of a cytoplasmic nonribosomal antigen. *Arthritis Rheum.* 1974;17(4):421–9.
 27. Wolin SL, Steitz JA. The Ro small cytoplasmic ribonucleoproteins: identification of the antigenic protein and its binding site on the Ro RNAs. *Proc Natl Acad Sci U S A.* 1984;81(7):1996–2000.
 28. Ben-Chetrit E, Chan EK, Sullivan KF, Tan EM. A 52-kD protein is a novel component of the SS-A/Ro antigenic particle. *J Exp Med.* 1988;167(5):1560–71.
 29. Itoh K, Itoh Y, Frank MB. Protein heterogeneity in the human Ro/SSA ribonucleoproteins. The 52- and 60-kD Ro/SSA autoantigens are encoded by separate genes. *J Clin Invest.* 1991;87(1):177–86.

30. Ottosson L, Hennig J, Espinosa A, Brauner S, Wahren-Herlenius M, Sunnerhagen M. Structural, functional and immunologic characterization of folded subdomains in the Ro52 protein targeted in Sjögren's syndrome. *Mol Immunol.* 2006;43(6):588–98.
31. Reymond A, Meroni G, Fantozzi A, Merla G, Cairo S, Luzi L, et al. The tripartite motif family identifies cell compartments. *EMBO J.* 2001;20(9):2140–51.
32. Espinosa A, Dardalhon V, Brauner S, Ambrosi A, Higgs R, Quintana FJ, et al. Loss of the lupus autoantigen Ro52/Trim21 induces tissue inflammation and systemic autoimmunity by disregulating the IL-23-Th17 pathway. *J Exp Med.* 2009;206(8):1661–71.
33. Wada K, Kamitani T. Autoantigen Ro52 is an E3 ubiquitin ligase. *Biochem Biophys Res Commun.* 2006;339(1):415–21.
34. Higgs R, Ní Gabhann J, Ben Larbi N, Breen EP, Fitzgerald KA, Jefferies CA. The E3 ubiquitin ligase Ro52 negatively regulates IFN-beta production post-pathogen recognition by polyubiquitin-mediated degradation of IRF3. *J Immunol Baltim Md* 1950. 2008;181(3):1780–6.
35. Higgs R, Lazzari E, Wynne C, Ní Gabhann J, Espinosa A, Wahren-Herlenius M, et al. Self protection from anti-viral responses--Ro52 promotes degradation of the transcription factor IRF7 downstream of the viral Toll-Like receptors. *PloS One.* 2010;5(7):e11776.
36. Li D, Wu R, Guo W, Xie L, Qiao Z, Chen S, et al. STING-Mediated IFI16 Degradation Negatively Controls Type I Interferon Production. *Cell Rep.* 2019;29(5):1249-1260.e4.

37. Foss S, Watkinson R, Sandlie I, James LC, Andersen JT. TRIM21: a cytosolic Fc receptor with broad antibody isotype specificity. *Immunol Rev.* 2015;268(1):328–39.
38. Strandberg L, Ambrosi A, Espinosa A, Ottosson L, Eloranta M-L, Zhou W, et al. Interferon-alpha induces up-regulation and nuclear translocation of the Ro52 autoantigen as detected by a panel of novel Ro52-specific monoclonal antibodies. *J Clin Immunol.* 2008;28(3):220–31.
39. Sabile A, Meyer AM, Wirbelauer C, Hess D, Kogel U, Scheffner M, et al. Regulation of p27 degradation and S-phase progression by Ro52 RING finger protein. *Mol Cell Biol.* 2006;26(16):5994–6004.
40. Jauharoh SNA, Saegusa J, Sugimoto T, Ardianto B, Kasagi S, Sugiyama D, et al. SS-A/Ro52 promotes apoptosis by regulating Bcl-2 production. *Biochem Biophys Res Commun.* 2012;417(1):582–7.
41. Brauner S, Ivanchenko M, Thorlacius GE, Ambrosi A, Wahren-Herlenius M. The Sjögren's syndrome-associated autoantigen Ro52/TRIM21 modulates follicular B cell homeostasis and immunoglobulin production. *Clin Exp Immunol.* 2018;194(3):315–26.
42. Schulte-Pelkum J, Fritzler M, Mahler M. Latest update on the Ro/SS-A autoantibody system. *Autoimmun Rev.* 2009;8(7):632–7.
43. Song J-S, Do JH, Lee S-W. The prevalence and the clinical relevance of anti-Ro52 in Korean patients with primary Sjögren's syndrome. *Rheumatol Int.* 2012;32(2):491–5.
44. Menéndez A, Gómez J, Escanlar E, Caminal-Montero L, Mozo L. Clinical associations of anti-SSA/Ro60 and anti-Ro52/TRIM21 antibodies: Diagnostic utility of their separate detection. *Autoimmunity.* 2013;46(1):32–9.

45. Retamozo S, Akasbi M, Brito-Zerón P, Bosch X, Bove A, Perez-de-Lis M, et al. Anti-Ro52 antibody testing influences the classification and clinical characterisation of primary Sjögren's syndrome. *Clin Exp Rheumatol*. 2012;30(5):686–92.
46. Menéndez A, Gómez J, Caminal-Montero L, Díaz-López JB, Cabezas-Rodríguez I, Mozo L. Common and specific associations of anti-SSA/Ro60 and anti-Ro52/TRIM21 antibodies in systemic lupus erythematosus. *ScientificWorldJournal*. 2013;2013:832789.
47. Hudson M, Pope J, Mahler M, Tatibouet S, Steele R, Baron M, et al. Clinical significance of antibodies to Ro52/TRIM21 in systemic sclerosis. *Arthritis Res Ther*. 2012;14(2):R50.
48. Ramos-Casals M, Solans R, Rosas J, Camps MT, Gil A, Del Pino-Montes J, et al. Primary Sjögren syndrome in Spain: clinical and immunologic expression in 1010 patients. *Medicine (Baltimore)*. 2008;87(4):210–9.
49. Palm O, Garen T, Berge Enger T, Jensen JL, Lund M-B, Aaløkken TM, et al. Clinical pulmonary involvement in primary Sjögren's syndrome: prevalence, quality of life and mortality--a retrospective study based on registry data. *Rheumatol Oxf Engl*. 2013;52(1):173–9.
50. Dong X, Zhou J, Guo X, Li Y, Xu Y, Fu Q, et al. A retrospective analysis of distinguishing features of chest HRCT and clinical manifestation in primary Sjögren's syndrome-related interstitial lung disease in a Chinese population. *Clin Rheumatol*. 2018;37(11):2981–8.
51. Aqrabi LA, Kvarnström M, Brokstad KA, Jonsson R, Skarstein K, Wahren-Herlenius M. Ductal epithelial expression of Ro52 correlates with inflammation in salivary glands of patients with primary Sjögren's syndrome. *Clin Exp Immunol*. 2014;177(1):244–52.

52. Aqrawi LA, Skarstein K, Øijordsbakken G, Brokstad KA. Ro52- and Ro60-specific B cell pattern in the salivary glands of patients with primary Sjögren's syndrome. *Clin Exp Immunol.* 2013;172(2):228–37.
53. Hillen MR, Urso K, Koppe E, Lopes AP, Blokland SLM, Pandit A, et al. Autoantigen TRIM21/Ro52 is expressed on the surface of antigen-presenting cells and its enhanced expression in Sjögren's syndrome is associated with B cell hyperactivity and type I interferon activity. *RMD Open.* 2020;6(2).
54. Szczerba BM, Kaplonek P, Wolska N, Podsiadlowska A, Rybakowska PD, Dey P, et al. Interaction between innate immunity and Ro52-induced antibody causes Sjögren's syndrome-like disorder in mice. *Ann Rheum Dis.* 2016;75(3):617–22.
55. Trzeciak M, Bagavant H, Papinska J, Deshmukh US. Immune Response Targeting Sjögren's Syndrome Antigen Ro52 Suppresses Tear Production in Female Mice. *Int J Mol Sci.* 2018;19(10).
56. Sroka M, Bagavant H, Biswas I, Ballard A, Deshmukh US. Immune response against the coiled coil domain of Sjögren's syndrome associated autoantigen Ro52 induces salivary gland dysfunction. *Clin Exp Rheumatol.* 2018;36 Suppl 112(3):41–6.
57. Espinosa A, Hennig J, Ambrosi A, Anandapadmanaban M, Abelius MS, Sheng Y, et al. Anti-Ro52 autoantibodies from patients with Sjögren's syndrome inhibit the Ro52 E3 ligase activity by blocking the E3/E2 interface. *J Biol Chem.* 2011;286(42):36478–91.
58. Ching KH, Burbelo PD, Tipton C, Wei C, Petri M, Sanz I, et al. Two major autoantibody clusters in systemic lupus erythematosus. *PloS One.* 2012;7(2):e32001.

59. Vera-Recabarren MA, García-Carrasco M, Ramos-Casals M, Herrero C. Comparative analysis of subacute cutaneous lupus erythematosus and chronic cutaneous lupus erythematosus: clinical and immunological study of 270 patients. *Br J Dermatol.* 2010;162(1):91–101.
60. Popovic K, Brauner S, Ek M, Wahren-Herlenius M, Nyberg F. Fine specificity of the Ro/SSA autoantibody response in relation to serological and clinical findings in 96 patients with self-reported cutaneous symptoms induced by the sun. *Lupus.* 2007;16(1):10–7.
61. Oke V, Vassilaki I, Espinosa A, Strandberg L, Kuchroo VK, Nyberg F, et al. High Ro52 expression in spontaneous and UV-induced cutaneous inflammation. *J Invest Dermatol.* 2009;129(8):2000–10.
62. Kvarnström M, Dzikaite-Ottosson V, Ottosson L, Gustafsson JT, Gunnarsson I, Svenungsson E, et al. Autoantibodies to the functionally active RING-domain of Ro52/SSA are associated with disease activity in patients with lupus. *Lupus.* 2013;22(5):477–85.
63. Wang Y, Xie J, Liu Z, Fu H, Huo Q, Gu Y, et al. Association of calreticulin expression with disease activity and organ damage in systemic lupus erythematosus patients. *Exp Ther Med.* 2017;13(5):2577–83.
64. Kunishita Y, Yoshimi R, Kamiyama R, Kishimoto D, Yoshida K, Hashimoto E, et al. TRIM21 Dysfunction Enhances Aberrant B-Cell Differentiation in Autoimmune Pathogenesis. *Front Immunol.* 2020;11:98.

65. Fujimoto M, Shimozuma M, Yazawa N, Kubo M, Ihn H, Sato S, et al. Prevalence and clinical relevance of 52-kDa and 60-kDa Ro/SS-A autoantibodies in Japanese patients with systemic sclerosis. *Ann Rheum Dis*. 1997;56(11):667–70.
66. Wodkowski M, Hudson M, Proudman S, Walker J, Stevens W, Nikpour M, et al. Monospecific anti-Ro52/TRIM21 antibodies in a tri-nation cohort of 1574 systemic sclerosis subjects: evidence of an association with interstitial lung disease and worse survival. *Clin Exp Rheumatol*. 2015;33(4 Suppl 91):S131-135.
67. Patterson KA, Roberts-Thomson PJ, Lester S, Tan JA, Hakendorf P, Rischmueller M, et al. Interpretation of an Extended Autoantibody Profile in a Well-Characterized Australian Systemic Sclerosis (Scleroderma) Cohort Using Principal Components Analysis. *Arthritis Rheumatol Hoboken NJ*. 2015;67(12):3234–44.
68. Parker JC, Burlingame RW, Bunn CC. Prevalence of antibodies to Ro-52 in a serologically defined population of patients with systemic sclerosis. *J Autoimmune Dis*. 2009;6:2.
69. Lee A, Patterson KA, Tan DJ, Wilson ME, Proudman SM, Stevens W, et al. Anti-Ro52/TRIM21 is independently associated with pulmonary arterial hypertension and mortality in a cohort of systemic sclerosis patients. *Scand J Rheumatol*. 2021;1–6.
70. Tangri V, Hewson C, Baron M, Bonner A, Fritzler M, Pope JE. Associations with Organ Involvement and Autoantibodies in Systemic Sclerosis: Results from the Canadian Scleroderma Research Group (CSRG). *Open J Rheumatol Autoimmune Dis*. 2013 16;3(2):113–8.
71. McHugh NJ, Tansley SL. Autoantibodies in myositis. *Nat Rev Rheumatol*. 2018 20;14(5):290–302.

72. Mariampillai K, Granger B, Amelin D, Guiguet M, Hachulla E, Maurier F, et al. Development of a New Classification System for Idiopathic Inflammatory Myopathies Based on Clinical Manifestations and Myositis-Specific Autoantibodies. *JAMA Neurol.* 2018 01;75(12):1528–37.
73. Koenig M, Fritzler MJ, Targoff IN, Troyanov Y, Senécal J-L. Heterogeneity of autoantibodies in 100 patients with autoimmune myositis: insights into clinical features and outcomes. *Arthritis Res Ther.* 2007;9(4):R78.
74. Rutjes SA, Vree Egberts WT, Jongen P, Van Den Hoogen F, Pruijn GJ, Van Venrooij WJ. Anti-Ro52 antibodies frequently co-occur with anti-Jo-1 antibodies in sera from patients with idiopathic inflammatory myopathy. *Clin Exp Immunol.* 1997;109(1):32–40.
75. Marie I, Hatron PY, Dominique S, Cherin P, Mouthon L, Menard J-F, et al. Short-term and long-term outcome of anti-Jo1-positive patients with anti-Ro52 antibody. *Semin Arthritis Rheum.* 2012;41(6):890–9.
76. Bauhammer J, Blank N, Max R, Lorenz H-M, Wagner U, Krause D, et al. Rituximab in the Treatment of Jo1 Antibody-associated Antisynthetase Syndrome: Anti-Ro52 Positivity as a Marker for Severity and Treatment Response. *J Rheumatol.* 2016;43(8):1566–74.
77. Shi J, Li S, Yang H, Zhang Y, Peng Q, Lu X, et al. Clinical Profiles and Prognosis of Patients with Distinct Antisynthetase Autoantibodies. *J Rheumatol.* 2017;44(7):1051–7.
78. Selva-O'Callaghan A, Pinal-Fernandez I, Trallero-Araguás E, Milisenda JC, Grau-Junyent JM, Mammen AL. Classification and management of adult inflammatory myopathies. *Lancet Neurol.* 2018;17(9):816–28.

79. Xing X, Li A, Li C. Anti-Ro52 antibody is an independent risk factor for interstitial lung disease in dermatomyositis. *Respir Med.* 2020;172:106134.
80. Sabbagh S, Pinal-Fernandez I, Kishi T, Targoff IN, Miller FW, Rider LG, et al. Anti-Ro52 autoantibodies are associated with interstitial lung disease and more severe disease in patients with juvenile myositis. *Ann Rheum Dis.* 2019;78(7):988–95.
81. Gómez-Martín D, Galindo-Feria AS, Barrera-Vargas A, Merayo-Chalico J, Juárez-Vega G, Torres-Ruiz J, et al. Ro52/TRIM21-deficient expression and function in different subsets of peripheral blood mononuclear cells is associated with a proinflammatory cytokine response in patients with idiopathic inflammatory myopathies. *Clin Exp Immunol.* 2017;188(1):154–62.
82. Hassan AB, Fathi M, Dastmalchi M, Lundberg IE, Padyukov L. Genetically determined imbalance between serum levels of tumour necrosis factor (TNF) and interleukin (IL)-10 is associated with anti-Jo-1 and anti-Ro52 autoantibodies in patients with poly- and dermatomyositis. *J Autoimmun.* 2006;27(1):62–8.
83. Kryštůfková O, Barbasso Helmers S, Venalis P, Malmström V, Lindroos E, Vencovský J, et al. Expression of BAFF receptors in muscle tissue of myositis patients with anti-Jo-1 or anti-Ro52/anti-Ro60 autoantibodies. *Arthritis Res Ther.* 2014;16(5):454.
84. Sharp G. Diagnostic criteria for classification of MCTD. In: *Mixed connective tissue diseases and antinuclear antibodies.* Elsevier. Amsterdam; 1987. p. 23–32.
85. Gunnarsson R, El-Hage F, Aaløkken TM, Reiser S, Lund MB, Garen T, et al. Associations between anti-Ro52 antibodies and lung fibrosis in mixed connective tissue disease. *Rheumatol Oxf Engl.* 2016;55(1):103–8.

86. Reiseter S, Gunnarsson R, Mogens Aaløkken T, Lund MB, Mynarek G, Corander J, et al. Progression and mortality of interstitial lung disease in mixed connective tissue disease: a long-term observational nationwide cohort study. *Rheumatol Oxf Engl*. 2018 01;57(2):255–62.
87. Montano-Loza AJ, Shums Z, Norman GL, Czaja AJ. Prognostic implications of antibodies to Ro/SSA and soluble liver antigen in type 1 autoimmune hepatitis. *Liver Int*. 2012;32(1):85–92.
88. Chou MJ, Lee SL, Chen TY, Tsay GJ. Specificity of antinuclear antibodies in primary biliary cirrhosis. *Ann Rheum Dis*. 1995;54(2):148–51.
89. Dörner T, Feist E, Held C, Conrad K, Burmester GR, Hiepe F. Differential recognition of the 52-kd Ro(SS-A) antigen by sera from patients with primary biliary cirrhosis and primary Sjögren's syndrome. *Hepatol Baltim Md*. 1996;24(6):1404–7.
90. Hervier B, Rimbert M, Colonna F, Hamidou MA, Audrain M. Clinical significance of anti-Ro/SSA-52 kDa antibodies: a retrospective monocentric study. *Rheumatol Oxf Engl*. 2009;48(8):964–7.
91. Ghillani P, André C, Toly C, Rouquette AM, Bengoufa D, Nicaise P, et al. Clinical significance of anti-Ro52 (TRIM21) antibodies non-associated with anti-SSA 60kDa antibodies: results of a multicentric study. *Autoimmun Rev*. 2011;10(9):509–13.
92. Fischer A, du Bois R. Interstitial lung disease in connective tissue disorders. *Lancet Lond Engl*. 2012;380(9842):689–98.
93. Wu S, Tang X, Wu L, Lu L-J, Feng X. Association of anti-Ro52 autoantibodies with interstitial lung disease in connective tissue diseases. *Ann Rheum Dis*. 2019 Oct 10;

94. Decker P, Moulinet T, Lopez B, Dubucquoi S, Bonnotte B, Lakomy D, et al. Clinical significance of anti-Ro52 (TRIM21) antibodies in adult patients with connective tissue diseases. *Eur J Int Med.* 2021;91:45–52.
95. Tissue expression of TRIM21 - Summary - The Human Protein Atlas [Internet]. [cited 2020 Dec 5]. Available from: <https://www.proteinatlas.org/ENSG00000132109-TRIM21/tissue>.
96. Burbelo PD, Ching KH, Han BL, Bush ER, Reeves WH, Iadarola MJ. Extraordinary antigenicity of the human Ro52 autoantigen. *Am J Transl Res.* 2010;2(2):145–55.
97. Igarashi T, Itoh Y, Fukunaga Y, Yamamoto M. Stress-induced cell surface expression and antigenic alteration of the Ro/SSA autoantigen. *Autoimmunity.* 1995;22(1):33–42.
98. Miranda-Carús ME, Askanase AD, Clancy RM, Di Donato F, Chou TM, Libera MR, et al. Anti-SSA/Ro and anti-SSB/La autoantibodies bind the surface of apoptotic fetal cardiocytes and promote secretion of TNF-alpha by macrophages. *J Immunol Baltim Md* 1950. 2000;165(9):5345–51.
99. Salomonsson S, Sonesson S-E, Ottosson L, Muhallab S, Olsson T, Sunnerhagen M, et al. Ro/SSA autoantibodies directly bind cardiomyocytes, disturb calcium homeostasis, and mediate congenital heart block. *J Exp Med.* 2005;201(1):11–7.

Table 1 Frequencies of anti-Ro52 antibodies in connective tissue diseases.

Disease	Anti-Ro52 frequency, %
Primary Sjögren syndrome	37 % ⁽²²⁾ , 67 % ⁽⁴³⁾ , 75 % ⁽⁴²⁾
Systemic lupus erythematosus	42 % ⁽⁴²⁾ , 50 % ⁽⁵⁸⁾
Systemic sclerosis	8 % ⁽⁶⁵⁾ , 20 % ^(47,66) , 35 % ⁽⁶⁷⁾ , 38 % ⁽⁶⁸⁾
Myositis	30 % ⁽⁷³⁾ , 35 % ⁽⁴²⁾
Dermatomyositis	17 % ⁽⁴²⁾ , 21 % ⁽⁷³⁾
Antisynthetase syndrome	29 % ⁽⁷⁷⁾ , 40 % ⁽⁷⁵⁾ , 43 % ⁽⁷⁶⁾ , 58 % ⁽⁷⁴⁾
Mixed connective tissue disease	29 % ⁽⁸⁵⁾

Table 2 Main studies results regarding clinical associations between anti-Ro52 antibodies and connective tissue diseases.

Reference	Year	Study design	CTD	Patients' number	Anti-Ro52 Abs detection	Main results	Limitations
Retamozo S ⁽⁴⁵⁾	2012	Prospective	pSS	187	ELISA	High titers anti-Ro52 Abs association with: - parotid enlargement, severe scintigraphic involvement - positive salivary gland biopsy - anaemia, leukopenia - positive rheumatoid factor Anti-Ro52 Abs titers correlation with serum gammaglobulin levels and rheumatoid factor titers No association between anti-Ro52 Abs and pSS-related ILD	-
Song J-S ⁽⁴³⁾	2012	Retrospective	pSS	96	LIA	Anti-Ro52 Abs independent association with: - liver involvement: OR = 6 [1.1-32.3], p = 0.04 - muscle involvement: OR = 9.5 [1.1-85.8], p = 0.04 No association between anti-Ro52 Abs and pSS-related ILD	Low number of pSS-related ILD patients (n = 5) Lack of statistical power
Dong X ⁽⁵⁰⁾	2018	Retrospective	pSS	527	-	Anti-Ro52 Abs independent association with pSS-related ILD: OR = 7.9 [1.2-33.6], p = 0.005	-
Menendez A ⁽⁴⁶⁾	2013	Prospective	SLE	141	ELISA	Anti-Ro52 Abs independent association with cytopenia: OR = 2.9 [1.1-8.5], p = 0.04 Anti-Ro52 Abs association with Raynaud's phenomenon, xerophthalmia, xerostomia, leukopenia, lymphopenia	-
Kvarnström M ⁽⁶²⁾	2013	Prospective	SLE	232	ELISA	Anti-Ro52-RING Abs levels correlation with disease activity (SLAM score) Anti-Ro52 Abs levels correlation with: - secondary SS - decreased salivary and tear production - gammaglobulin levels - leukopenia, lymphopenia	No study of organ-specific clinical features
Hudson M ⁽⁴⁷⁾	2012	Prospective	SSc	963	ALBIA	Anti-Ro52 Abs association with: - SSc-related ILD: OR = 1.5 [1.1-2.1], p = 0.009 - higher lung severity score (Medsger's scale): OR = 1.2 [1.1-1.4], p = 0.008 - overlap syndrome: OR = 1.8 [1.2-2.6], p = 0.006	-

Wodkowski M ⁽⁶⁶⁾	2015	Prospective	SSc	1574	LIA	Anti-Ro52 Abs association with: - SSc-related ILD: OR = 2.7 [1.8-4.1], p < 0.0001 - increased mortality: HR = 1.9 [1.2-2.8], p = 0.003	-
Marie I ⁽⁷⁵⁾	2012	Retrospective	ASS	89	ELISA	Anti-Ro52 Abs association with: - malignancy: OR = 4.5 [1.1-19.5], p = 0.04 - symptomatic form of ASS-related ILD - myositis and joint involvement deterioration No association between anti-Ro52 Abs and ASS-related ILD frequency	Small number of patients No study of anti-Jo1 negative ASS
Bauhammer J ⁽⁷⁶⁾	2016	Retrospective	ASS	61	Immunoblot	Anti-Ro52 Abs association with acute-onset ASS-related ILD	Small number of patients No study of anti-Jo1 negative ASS No data regarding ILD frequency and PFT
Shi J ⁽⁷⁷⁾	2017	Retrospective	ASS	124	?	Anti-Ro52 Abs association with rapidly progressive ASS-related ILD No association between anti-Ro52 Abs and malignancy	No data regarding ILD frequency and PFT
Xing X ⁽⁷⁹⁾	2020	Retrospective	ASS DM	153	Immunoblot	Anti-Ro52 Abs association with: - ILD: OR = 3.1 [1.3-7.6], p = 0.01 - higher HRCT scores of ILD and decreased survival rate (especially in anti-MDA5 positive patients)	No multivariate analysis adjusted for other autoantibodies No data regarding PFT
Sabbagh S ⁽⁸⁰⁾	2019	Prospective Retrospective	Juvenile myositis	371	ELISA	Anti-Ro52 Abs independent association with: - juvenile myositis-related ILD frequency and severity - Raynaud's phenomenon	No data regarding PFT
Gunnarsson R ⁽⁸⁵⁾	2016	Prospective	MCTD	113	Immunoblot	Anti-Ro52 Abs association with: - MCTD-related ILD frequency: OR = 4.4 [1.8-10.3], p < 0.002 - lower FVC and DLCO values	-
Reiseter S ⁽⁸⁶⁾	2018	Prospective	MCTD	135	Immunoblot	Anti-Ro52 Abs association with MCTD-related ILD progression: OR = 3 [1.2-10.2], p = 0.02	ILD progression defined by CT lung changes

Abs, antibodies; ALBIA, addressable laser bead immunoassay; ASS, antisynthetase syndrome; CTD, connective tissue disease; DM, dermatomyositis; ELISA, enzyme linked immunosorbent assay; HR, hazard ratio; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; LIA, line immunoassay; MCTD, mixed connective tissue disease; OR, odds ratio; PFT, pulmonary function tests; pSS, primary Sjögren syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

Figure 1 Schematic representation of the proposed role of anti-Ro52 antibodies in the pathogenesis of CTD (IFI-16, interferon factor inducible protein 16; IFN, interferon; IRF, interferon regulatory factor; RNA, ribonucleic acid; TLR, toll-like receptor; TNF, tumor necrosis factor).

