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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 of 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 a 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 (Outcherlony 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 ribonucleoprotein 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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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).

