CLINICAL SIGNIFICANCE OF ANTI-RO52/TRIM21 ANTIBODIES IN SYSTEMIC AUTOIMMUNE RHEUMATIC DISORDERS: PRELIMINARY RESULTS

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Abstract

Although antibodies targeting Ro52 protein/TRIM21 are commonly detected in systemic autoimmune rheumatic disorders (SARDs), their clinical significance and pathobiologic role remains incompletely understood.

Objectives. We aimed to define clinical relevance and immunological associations of anti-Ro52/TRIM21 positivity in a cohort of patients with SARDs.

Methods. We retrospectively reviewed medical records of 97 consecutive SARDs who attended at least once our Rheumatology Department between March 2016 and June 2017. Clinical manifestations, rheumatoid factor (RF) (Latex and Waalter-Rose tests), antinuclear antibodies (ANA>1:100, immunofluorescence assays) as well as detailed ANA profile (nRNP/Sm, Sm, SS-A/Ro, Ro52/TRIM21, SS-B/La, Scl-70, PM/Scl, Jo-1, CENP-B, PCNA, dsDNA, nucleosome, histone, P-ribosomal protein, AMA-M2, DFS70) (line immunoblot assay) were systematically evaluated.

Statistical analysis was performed in SPSS19.0, assuming significance for $p<0.05$; the chi-squared test was applied to determine the association between anti-Ro52/TRIM21 status and clinical manifestations of SARDs.

Results. Only 27 patients (23.7%) had anti-Ro52/TRIM21 positive disease and were further analyzed. Isolated anti-Ro52/TRIM21 were detected in 7/27 (25.9 %) cases, while the majority had dual anti-Ro52/TRIM21 positivity and anti-Ro (n=7/20; 35%), anti-dsDNA (n=3/20, 15%), anti-Jo1 (n=3/20, 15%), anti-centromere B (n=2/20; 10%), anti-Scl70 (n=2/20; 10%), anti-RNP/Sm (n=2/20, 10%), anti-Pm/Scl (n=1/20, 5%), and RF (n=13; 65%).

Patients were classified as Sjögren's syndrome (n=7/27, 25.9%), myositis (n=3/27, 11.1%), systemic lupus erythematosus (n=3/27, 11.1%), systemic sclerosis (n=4/27,15.2%), mixed (n=3/27, 11.1%) or undifferentiated connective tissue disease (n=7/27, 25.9%).

More than half of anti-Ro52/TRIM21 positive patients presented with interstitial lung fibrosis (18/27, 66.6%), insolated (33.3%) or in association with anti-Jo1 (33.3%).

Conclusions. Anti-Ro52/TRIM21 antibodies should be systematically evaluated in patients with SARDs, as anti-Ro52 positivity (either isolated or in combination with different other ANA specificities) may be associated with definite clinical features.

Anti-Ro52/TRIM21 may be useful in identifying a subgroup of patients at risk for developing certain symptoms (particularly interstitial lung disease) of SARDs, with specific therapeutic outcomes.

Keywords: anti-Ro52/TRIM21 antibodies, systemic autoimmune rheumatic disorders

INTRODUCTION

Recently recognized as tripartite motif-containing 21 or TRIM21, Ro52 is a 52 kDa protein that acts as a shared target for autoantibodies in various systemic rheumatic and non-rheumatic autoimmune pathology including Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), idiopathic myositis – polymyositis/ dermatomyositis (PM/DM), mixed or undifferentiated connective tissue diseases (MCTD/ UCTD), rheumatoid arthritis, neonatal lupus erythematosus, primary biliary cirrhosis and autoimmune hepatitis (1,2).

Although typically assigned together with its matching Ro60 as the Ro/SS-A autoantigen, Ro52 may also function either independently, as a distinct entity associated with specific clinical settings (1,3), or combined with other nuclear specificities, such as double stranded (ds)DNA, topoisomerase 1 (Scl-70), centromere-B protein, Jo-1 and new aminoacyl-t-RNA synthetases (1,4).

Ro52 is usually involved in the complex processes of cell signalling and immunity, regulating cellular oxidative stress and mediating apoptosis (5,6). Why and how this protein becomes antigenic and

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may trigger autoimmunity is still controversial (1, 5,6).

Antibodies against Ro52 are frequently tested in clinical practice and are part of the standard extractable nuclear antigen (ENA) panel (2).

Although the clinical relevance of antibodies to Ro52/TRIM21 in a broad spectrum of systemic autoimmune conditions was recently updated, their pathobiologic, diagnostic and prognostic roles remain incompletely understood (1).

The main objective of our study was to define clinical significance and immunologic associations of anti-Ro52/TRIM21 antibodies in a cohort of patients with systemic autoimmune rheumatic disorders (SARDs).

PATIENTS AND METHODS

Patients

We retrospectively reviewed the medical records of 97 consecutive patients bearing a diagnosis of systemic autoimmune rheumatic disease, who attended at least once our Rheumatology Department between March 2016 and June 2017.

SARD was considered when a patient displayed one of the following disorders: systemic lupus erythematosus, idiopathic myositis (polymyositis/dermatomyositis), Sjögren's syndrome, systemic sclerosis, mixed connective tissue disease, undifferentiated connective tissue disease.

Only patients with anti-Ro52/TRIM21 positive SARD were qualified for enrolment in the current study, aiming to describe clinical and immunological associations of anti-Ro52/TRIM21 antibodies with various antinuclear antibodies (ANA) specificities.

The study received the approval of local Ethics Committee.

Clinical manifestations and laboratory tests

A wide spectrum of clinical manifestations was assessed including arthralgia/arthritis (erosive or non-erosive), muscle weakness and myalgia, Raynaud's phenomenon, sicca syndrome (dry mouth, dry eye), salivary gland enlargement, lung, kidney, liver and central nervous system involvement, peripheral neuropathy. Pleuritis and interstitial lung disease were evaluated through chest X-ray and/or CT scan as well as pulmonary function tests, while kidney involvement was defined as haematuria over 5 red blood cells on high power field or proteinuria over 500 mg per 24 h more than two times. Peripheral neuropathy and myopathy were confirmed by electrophysiological studies (nerve conduction velocity and electromyogram); inflammatory muscle involvement was defined as significantly decreased muscle force (impaired muscle testing) or severe myalgia with the evidence of myositis, such as typical findings on electromyogram or highly elevated levels of muscle enzymes.

Autoantibodies

ANA were tested by immunofluorescence assays, titres over 1:100 being classified as positive (Synevo Lab); a detailed ANA profile including nRNP/Sm, Sm, SS-A/Ro, Ro52/TRIM21, SS-B/La, Scl-70, PM/Scl, Jo-1, CENP-B, PCNA, dsDNA, nucleosome, histone, P- ribosomal protein, AMA-M2 and DFS70 was also done for each case (line immunoblot assay, Synevo Lab).

Statistical analysis

Statistical analysis was performed using the SPSS package for Windows version 19.0, $p<0.05$; the chi-squared test was applied to determine the association between the presence of anti-Ro52/TRIM21 and clinical manifestations of the autoimmune disease.

RESULTS

Demographics, clinical manifestations and immunological abnormalities in anti-Ro52 positive patients

Only 27 (23.7%) of the total of 97 consecutive patients with SARD were confirmed as ANA positivity with anti-Ro52 specificity and further analyzed.

Demographics, clinical as well as immunological tests of these patients are summarized in Table 1.

As expected, in our cohort the majority of cases were female in their fifth decade.

Clinical features

Bilateral and symmetrical small joints arthralgia and/or non-erosive arthritis (88.8%), interstitial lung disease (66.6%) and Raynaud's phenomenon (up to 60%) were commonly reported. About one third of patients presented with muscle involvement (33.3%) and dry mouth (29.6%), and one out of five patients with dry eyes (22.2%). Kidney involvement and peripheral neuropathy were only occasionally detected, exclusively in patients with severe disease.

TABLE 1. *Characteristics, clinical manifestations and laboratory features in 27 patients with SARD*

Demographics	
Age (years) (mean ± standard deviation)	47.9 ± 14.0
Gender (male/female) (%)	3/24 (11.1%/88.8%)
Clinical manifestations	
Dry mouth*	8 (29.6%)
Dry eye and/or foreign body sense*	6 (22.2%)
Arthralgia/Arthritis*	24 (88.8%)
Raynaud's phenomenon*	16 (59.2%)
Muscle involvement*	9 (33.3%)
Peripheral neuropathy*	1 (3.7%)
Lung involvement*	18 (66.6%)
Kidney involvement*	2 (7.4%)
Lab assays	
Antinuclear antibody*	27 (100%)
Anti-Ro52/TRIM 21 isolated*	7 (25.9%)
Anti-Ro52/TRIM21 associated*	20 (74%)
Anti-Ro/SS-A*	7 (35%)
Anti-RNP/Sm*	2 (10%)
Anti-ds DNA*	3 (15%)
Anti-Scl70*	2 (10%)
Anti-centromer B*	2 (10%)
Anti-Jo 1*	3 (15%)
Anti-Pm/Scl*	1 (5%)
Rheumatoid factor*	13 (65%)

*, n (%);

Immunological profile

7 out of 27 patients enrolled (26%) had confirmed *ANA positivity with isolated anti-Ro52/TRIM21,* while 20/27 cases (74%) had anti-Ro52/TRIM21 positivity associated with different other ANA specificities; anti-Ro/SS-A was present in 35% patients, anti-Jo-1 and anti-dsDNA in 15% each, anti-SCL-70, anti-centromere B and anti- Sm/RNP in other 10% each.

More than half of patients were seropositive for type IgM-RF (latex and Waaler-Rose tests).

SARD diagnosis

Different SARDs were diagnosed among anti-Ro52/TRIM 21 positive patients (Table 2). One out of four patients had SjS (25.9%) or were classified as having UCTD (25.9%), while four cases had CREST or SSc (15.2%); idiopathic myositis (PM/DM) (11.1%), SLE (11.1%) and MTCD (11.1%) were also identified.

Isolated anti-Ro52/TRIM21 positive SARD *versus* associated anti-Ro52/TRIM21 positive SARD

Subgroup analysis was further performed (patients with isolated anti-Ro52/TRIM21 positivity, patients with anti-Ro52/TRIM21 positivity associated with antibodies against other ANA specificities) (Table 3).

TABLE 3. *Clinical manifestations associated the presence of anti-Ro52 in SARD*

	Isolated anti-Ro52/TRIM21 positivity (n=7)	Associated anti-Ro52/TRIM21 positivity (n=20)	p
Sicca syndrome	0	14	<0.05
Arthralgia/arthritis	4	20	<0.05
Raynaud's phenomenon	4	12	<0.05
Muscle involvement	0	9	<0.05
Interstitial lung disease	5	13	<0.05

We found several clinical positive associations with either isolated or combined anti-Ro52 in our patients. Thus, sicca syndrome, non-erosive arthritis, Raynaud's, muscle and lung involvement were significantly correlated with the presence of both anti-Ro52 and other immune abnormalities) (chi-squared, $p<0.05$).

DISCUSSION

It is widely accepted that antibodies targeting Ro52/TRIM21 are documented in a variety of systemic autoimmune rheumatologic (e.g. Sjögren's syndrome, systemic lupus erythematosus, systemic scleroderma, inflammatory idiopathic myositis and overlap syndromes) and non-rheumatologic (e.g. au-

TABLE 2. *Diagnosis and immunology in anti-Ro52 positive patients*

Diagnosis	% patients with diagnosis*	Patients with another antibody positivity*	Patients with rheumatoid factor serositivity*
Sjögren syndrome	7 (25.9%)	7 (100%)	5 (71.4%)
Myositis (PM/DM)	3 (11.1%)	3 (100%)	2 (66.6%)
Systemic lupus	3 (11.1%)	3 (100%)	3 (100%)
Systemic sclerosis or CREST	4 (15.2%)	4 (100%)	1 (25%)
Mixed-CTD	3 (11.1%)	3 (100%)	2 (66.6%)
Undifferentiated - CTD	7 (25.9%)	0	0

*, **n (%); CTD, connective tissue disease; PM/DM, polymyositis/ dermatomyositis**

toimmune hepatitis, primary biliary cirrhosis and other autoimmune hepato-biliary syndromes) disorders (1,2).

Formerly grouped with anti-Ro60 as the anti-Ro/SS-A entity, anti-Ro52/TRIM21 have actually emerged as distinct antibodies and recognized as one of the most frequently detected ENAs (7-9). Although their role in the immunopathogenesis of different SARD remains debatable, anti-Ro52/TRIM21-positive antibodies appear to be associated with more marked pathology than anti-Ro52/TRIM21-negative patients, as indicated by distinct clinical settings and surrogate laboratory measures as well (1,2,9,10).

Anti-Ro52 and clinical associations

A variety of clinical and lab abnormalities have been positively linked with anti-Ro52 in systemic autoimmune diseases (1,11-18), while other associations are absent (1,19-22). Thus, interstitial lung disease and high pulmonary artery pressure (14,15), Raynaud's phenomenon (12), reflux disease, muscle involvement, immune anomalies including anti-Scl70 and anti-centromere B (14,16), anti-Jo-1 (21) and anti-La antibodies (21) were considered as positive associations with anti-Ro52; conversely, sicca symptoms (xerostomia, xerophtalmia), myositis, pulmonary (arterial) hypertension, psychiatric manifestations were classified as negative associations with anti-Ro52 antibodies (1, 14,16,19,20,22,23).

In our study, we demonstrated ANA positivity with anti-Ro52/TRIM21 specificities in about one fourth of consecutive SARD. The majority of cases had positive anti-Ro52/TRIM21 in association with other ANA specificities, commonly anti-Ro/SS-A, anti-Jo1 and anti-dsDNA antibodies. Evidently, anti-Ro52/TRIM21 antibodies were related to certain individual clinical features: the triad non-erosive arthritis, interstitial lung disease and Raynaud's phenomenon was frequently identified in our cohort, while sicca symptoms and muscle involvement in 25% of cases.

Interestingly, although clinical positive associations were reported in all our patients, a statistically significant difference was demonstrated among patients characterized by anti-Ro52/TRIM21 and other ANA specificities as compared with those with isolated anti-Ro52/TRIM21 antibodies.

A brief review of recently published papers about anti-Ro52 positivity and their clinical implications across systemic autoimmune diseases revealed interesting data (1).

Anti-Ro52/TRIM21 in Sjögren's syndrome

About one third of patients diagnosed with either primary or secondary Sjögren's syndrome have isolated anti-Ro52 antibodies (1,10-12) and over half of them have both anti-Ro52 and anti-Ro60 positivity (1,7,9-11). Although classically associated with certain specific clinical (parotid disease, autoimmune liver disease, muscle involvement) (1) and lab variables (erythrocyte sedimentation rate, anemia, leukopenia, serum imunoglobulins, ANA, rheumatoid factor) (1), anti-Ro52 antibodies are not significantly linked with dry mouth and/or dry eyes, as well as with Raynaud's phenomenon or interstitial lung disease (1,11). In addition, isolated anti-Ro52 negatively correlates with ocular and oral sicca syndromes (1, 23).

Anti-Ro52/TRIM21 in systemic sclerosis

Single anti-Ro52 as well as dual anti-Ro52 and ANA specificities (e.g. anti-centromere B and anti-Scl70) are reported among patients with diffuse cutaneous systemic sclerosis and CREST syndrome (24). Gastro-oesophageal reflux, interstitial lung disease or pulmonary hypertension are characteristic for patients with anti-Ro52 positive systemic sclerosis according to certain authors (1), while conflicting data are found with Raynaud's phenomenon (1,16,20). Moreover, other authors consider that myositis, cardiac disease, lung disease, arthritis, calcinosis and sicca symptoms in SSc are not associated with positive anti-Ro52 antibodies (1).

Anti-Ro52/TRIM21 in systemic lupus erythematosus

Both juvenile and adult lupus patients had not only anti-Ro52 positivity, but also a combination with serum biomarkers such as anti-Ro60 and anti-La antibodies (9,10,12). Raynaud's phenomenon and xerostomia are common clinical positive associations, while haematological cytopenia (mainly decreased white cells) and low anti-ds DNA are particularly described in such anti-Ro52 positive lupus patients (1). It seems that psychiatric manifestations are not listed among the clinical picture of a patient with SLE and positive anti-Ro52 (1).

Anti-Ro52/TRIM21 in inflammatory myositis

Finally, anti-Ro52 antibodies are usually identified in different types of myositis, especially in PM/

DM, the overall prevalence ranging between 26.3 and 37% (10,21,25). A remarkable correlation between anti-Ro52 positivity, aminoacyl-tRNA synthetase autoantibodies (particularly anti-Jo1), and pulmonary involvement (interstitial lung disease) (1,10,18,21,25) was described. One potential explanation for this specific association resides in the extensive expression of Ro52/TRIM in the lungs (1).

Besides, the severity of lung fibrosis, disease outcomes and response to conventional immunosuppressive drugs were evaluated in patients with myositis characterized by a dual, anti-Jo-1 and anti-Ro52, seropositivity; however, inconsistent data about the subtype of interstitial lung fibrosis and anti-Ro52 were reported (1,17,18). It seems that patients with anti-Ro52-positive anti-synthetase syndrome are resistant to remissive drugs (1).

We also found in our study that pulmonary manifestations were often related to the presence of anti-Ro52 antibodies (18 cases, 66.6%), either combined with anti-Jo1 or isolated (half of cases). Indeed, we haven't characterized the subtype of lung involvement as well as the response to some specific synthetic immunosuppressive drugs, but we intent to perform further research to detail this clinical and immunological link, and to follow-up disease outcomes under different remissive drugs.

Further studies in larger cohort of SARD are necessary in order to better describe clinical relevance, immunological associations as well as potential therapeutic implications of anti-Ro52 antibodies.

CONCLUSIONS

Anti-Ro52/TRIM21 antibodies should be systematically evaluated in patients with SARDs, as anti-Ro52 positivity (either isolated or combined with different ANA specificities) characterizes specific clinical features.

Anti-Ro52/TRIM21 may be useful in identifying a subgroup of patients at risk of developing certain symptoms (particularly interstitial lung disease) of SARDs, with particular therapeutic outcomes.

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