

Publication of the Month

October 10/11: Impact of separate anti-Ro52 determination

Key messages:

- *Anti-Ro52 and anti-Ro60 should be tested separately due to their distinct autoantibodies systems.*
 - *Information on isolated or associated anti-Ro52 reactivity is crucial in clinical diagnosis.*
 - *Pulmonary manifestations are often associated with the presence of anti-Ro52 antibodies.*
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Clinical significance of anti-Ro52 (TRIM21) antibodies non-associated with anti-SSA 60 kDa antibodies: Results of a multicentric study
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Background: Recent publications have shown that anti-Ro52 antibodies are directed against a macromolecular complex (called TRIM21) different from the antigen of anti-Ro60 antibodies. Ro52-antibodies are most common in myositis, systemic sclerosis and autoimmune liver diseases. The identification of the two distinct autoantibody systems and the ability to differentiate them through serological diagnosis raises the question on the significance of Ro52 antibodies non-associated to Ro60 antibodies.

Summary: 155 sera confirmed as Ro52 positive but Ro60 negative were checked for association with other autoantibodies. While 57% of the samples showed isolated anti-Ro52 antibodies the remaining samples showed antibodies associations with mainly Sm/SmRNP or Chromatin (57%), Jo-1 (26%) and CENP-B (14%).

Out of the 155 patients, 73% had autoimmune diseases including myositis, SLE, Sjögren's syndrome and systemic sclerosis/CREST.

Anti-Ro52 positive patients mainly show pulmonary manifestations (34/155) caused by autoimmune diseases (n=25) but also with non-immune origin (n=9).

Conclusions: Separate detection of anti-Ro52 antibodies might be useful in related antisynthetase syndrome diagnosis. The presence of anti-Ro52 antibodies should probably precede development of autoimmune disease and must induce sequential follow-up of positive patients, particularly in interstitial lung disease progression.

Comment: Most Anti-SS-A/Ro tests do not distinguish between anti-Ro52 and anti-Ro60 but measure both simultaneously. However, the antibodies are independent markers and particularly when one is present without the other, it may be associated with different diseases. Therefore, the diagnosis is more precise and specific, when these two antibody populations are measured independently.





Review

Clinical significance of anti-Ro52 (TRIM21) antibodies non-associated with anti-SSA 60 kDa antibodies: Results of a multicentric study

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ABSTRACT

Ro52 antigen has recently been identified as TRIM21 protein, but the clinical significance of anti-Ro52/TRIM21 antibodies remains controversial. The aim of this multicentric study was to investigate the significance of anti-Ro52 antibodies without anti-SSA/Ro60 antibodies in various connective diseases. Sera were selected by each laboratory using its own method (ELISA, immunodot or Luminex technology), and then performed with ANA Screen BioPlex™ reagent (BIO-RAD). Among the 247 screened sera, 155/247 (63%) were confirmed as anti-Ro52 positive and anti-SSA/Ro60 negative. These sera were analyzed for the detection of other antibodies in relation with clinical settings.

Isolated anti-Ro52 antibodies were detected in 89/155 (57%) sera. For the remaining sera (66/155), the main antibodies associations were Sm/SmRNP or Chromatin (n = 38; 57%), Jo1 (n = 17; 26%) and CenpB (n = 9; 14%). Clinical data from the 155 patients showed high prevalence in autoimmune diseases (73%) including myositis or dermatomyositis (n = 30), lupus (n = 23); Sjögren and/or sicca syndrome (n = 27); CREST or Systemic sclerosis (n = 11) and autoimmune hepatitis (n = 11). We found that pulmonary manifestations were often associated with the presence of anti-Ro52 antibodies (n = 34, 22%), in addition with anti-tRNA synthetases, anti-SRP or anti-Ku antibodies (18/34) or isolated in half of cases (16/34).

Separate detection of anti-Ro52 antibodies might be useful in related antisynthetase syndrome diagnosis. The presence of anti-Ro52 antibodies should probably precede development of autoimmune disease and must induce sequential follow-up of positive patients, particularly in interstitial lung disease progression.

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Contents

| | |
|--|-----|
| 1. Introduction | 509 |
| 2. Patients and methods | 510 |
| 2.1. Inclusion criteria | 510 |
| 2.2. Antibodies detection | 510 |
| 2.3. Definition of isolated anti-Ro52 antibodies | 510 |
| 3. Results | 510 |
| 4. Discussion | 511 |
| 5. Conclusion | 512 |
| Take-home messages | 513 |
| Acknowledgements | 513 |
| References | 513 |

1. Introduction

Antibodies to SSA antigen (Ro52/Ro60), historically described as a marker for Sjögren syndrome and systemic lupus erythematosus are now known not to be directed to the same macromolecular complex [1]. It is well admitted that Ro52 and Ro60 (SSA) antigens consisted of

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two different proteins coded by distinct cDNAs [2,3]. The Ro52 gene has been mapped to the end of the short arm of human chromosome 11 [4] and Ro52 antigen has recently been identified as TRIM21 protein, belonging to the tripartite motif (TRIM) protein family. Indeed, the 52 kDa Ro antigen is identified as a family member of the RING/Bbox/coiled-coil (RBCC) tripartite motif protein and as an ubiquitin ligase that is over expressed in peripheral blood of mononuclear cells in Sjögren syndrome and SLE patients [5,6]. So, anti-Ro52 antibodies are named anti-TRIM21 antibodies and the clinical significance of anti-Ro52/TRIM21 antibodies remains controversial, because some non-immunological interactions may exist between TRIM21 protein and human IgG [7]. Anti-Ro60 (SSA) and anti-Ro52 (TRIM21) represent two distinct autoantibodies systems. Whereas Ro52 has an extraordinary immunogenicity [8] and may be the most antigenic protein recognized in humans, separate detection is desirable in a clinical diagnostic setting [1,9]. Clinically, the presence of anti-Ro52 antibodies has been reported in a wide variety of diseases. In autoimmune diseases, the frequency of anti-Ro52 antibodies is high in myositis, Systemic sclerosis [10] but also in autoimmune liver diseases [11,12]. These antibodies also tend to be associated with non-autoimmune diseases such as viral infections or neoplastic diseases [13].

The aim of this multicentric study was to investigate the clinical significance of anti-Ro52 (TRIM21) antibodies in patients recruited from various departments of medicine and displaying anti-Ro52 but not anti-SSA/Ro60 antibodies.

2. Patients and methods

2.1. Inclusion criteria

In this retrospective multicentric study, patients were selected on the basis of two criteria: (i) the presence of antibodies to TRIM21/Ro52 and the absence of anti-SSA/Ro60 antibodies in serum and (ii) knowledge of their medical status determined from their medical records.

2.2. Antibodies detection

During 18 months, consecutive sera were selected by each of the 10 laboratories through auto-immunity exploration using its own method, ELISA, immunodot or Luminex technology, reported in Table 1. The sera were checked for a second time with another methodology to standardize anti-Ro52 determination. Then, anti-Ro52 reactivity was confirmed using ANA Screen BioPlex® reagent (BIO-RAD). This fully-automated multiplexed reagent permitted simultaneous and separate detection of anti-dsDNA; -chromatin;

-ribosomal protein; -SSA60; -SSA52; -SSB; -Sm; -SmRNP; -RNPA; -RNP68; -Scl70; -Jo1 and -Cenp B. In all used methods, Ro52 antigen was a recombinant protein.

Immunodots, provided by D-tek, were also performed to check other anti-tRNA synthetase reactivities such as anti-PL7, anti-PL12 or to reveal the presence of antibodies against SRP, PMScl or Ku antigens. Inhibition ELISA, described elsewhere [12], was also performed to detect anti-SLA (Soluble Liver Antigen) in autoimmune hepatitis.

2.3. Definition of isolated anti-Ro52 antibodies

Patients with isolated anti-Ro52 antibodies were defined as patients with anti-Ro52 antibodies non-associated with antibodies directed to antigens neither detected by BioPlex® reagent nor by immunodot or ELISA.

3. Results

Among the 247 tested sera, only sera confirmed as anti-Ro52 positive and anti-SSA/Ro60 negative were later analyzed. Non-selected sera were divided into two groups in relation with the primary test:

- not enough sensitivity for Ro60: sera were finally Ro52 and Ro60 positive.
- too sensitive for Ro52: sera were finally Ro52 and Ro60 negative.

After confirmation, data from 155/247 (63%) patients were studied: 131 women (85%) and 24 men (15%), mean age: 51.7 ± 17 years (range 13–88). These sera were principally collected from internal medicine (38%), rheumatology (14%) and pneumology (12%) departments. They were analyzed for the detection of other antibodies by BioPlex® reagent in relation with clinical settings, as shown in Fig. 1.

Among the 155 selected sera, 89/155 (57%) showed isolated anti-Ro52 antibodies and 66/155 (43%) showed anti-Ro52 antibodies associated with at least one other autoantibody detected by ANA Screen BioPlex® reagent. The sex-gender and mean age of the “isolated anti-Ro52” group were not different from the initial group (82 vs 85% of women; 54.7 ± 17 years vs 51.7 ± 17 years as mean age and same medicine department recruitment).

For the remaining sera (66/155) with non-isolated anti-Ro52 antibodies, the main antibodies associations were Sm/SmRNP or Chromatin ($n = 38$; 57%), Jo1 ($n = 17$; 26%) and CenpB ($n = 9$; 14%). If we considered the results of immunodots and ELISA, we had in a second time 78 versus 89 patients with true isolated anti-Ro52 antibodies. So, we detected the presence of two anti-PL12, one anti-PM-Scl, one anti-Ku and seven anti-SLA antibodies in 11 patients with anti-Ro52 antibodies.

Clinical data analysis from the 155 patients showed that 73% of them had an autoimmune disease. The most common associated

Table 1
Multicentric selection of sera with isolated anti-Ro52 antibodies.

| | Tested (n) | Anti-Ro52 (+)/ anti-Ro60 (–) (n) | Kept/tested % | Isolated Anti-Ro52 (n) | Isolated/total anti-Ro52 (%) | Method |
|----------------------------|------------|----------------------------------|---------------|------------------------|------------------------------|---|
| Béclère Hospital | 7 | 6 | 86 | 5 | 83 | Dot Euroimmun (ANA Profil 3 euroline) |
| Bichat Hospital | 9 | 8 | 89 | 5 | 63 | Dot Euroimmun (ANA Profil 3 euroline) |
| Mondor Hospital | 24 | 15 | 63 | 6 | 40 | Luminex (AtheNa) |
| Pitié-Salpêtrière Hospital | 94 | 65 | 69 | 34 | 52 | Luminex (Fidis, AtheNA, QuantaPlex) |
| St Antoine Hospital | 30 | 19 | 63 | 11 | 58 | Dot Innogenetics (INNOLIA-ANA) |
| St Louis Hospital | 19 | 14 | 74 | 10 | 71 | (Varelisa, Phadia Ro52 + 60) Dot Innogenetics (INNOLIA-ANA) and Dot Euroimmun (ANA Profil 3 euroline) |
| Tenon Hospital | 48 | 14 | 29 | 9 | 64 | Dot Innogenetics (INNOLIA-ANA) (research kit Ro52, Phadia) |
| HEGP Hospital | 6 | 6 | 100 | 6 | 100 | Dot Innogenetics (INNOLIA-ANA) |
| Necker Hospital | 1 | 1 | 100 | 1 | 100 | Dot Euroimmun (ANA Profil 3 euroline) |
| Cochin Hospital | 9 | 7 | 78 | 2 | 29 | Dot Euroimmun (ANA Profil 3 euroline) |
| Total | 247 | 155 | 63 | 89 | 57 | |

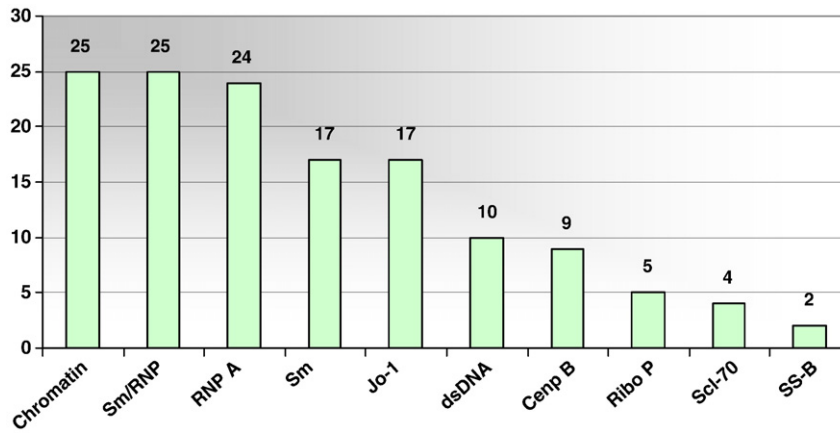


Fig. 1. Target of anti-Ro52 associated auto-antibodies (detected by BioPlex).

autoimmune diseases were myositis or dermatomyositis (n = 30, including 17 with anti-Jo1, four with anti-PL12, one with anti-PM-Scl and one with anti-SRP antibodies); lupus (n = 23); Sjögren and/or sicca syndrome (n = 27); CREST syndrome or Systemic Sclerosis (n = 11) and auto-immune hepatitis (n = 11, including 7 with anti-SLA antibodies).

The prevalence of pulmonary manifestations associated with the presence of anti-Ro52 antibodies (34/155, 22%) was high, including 9 myositis, 5 Sjögren syndrome and 7 CREST syndrome or Systemic Sclerosis.

As shown in Fig. 2, for each disease, some patients have isolated anti-Ro52 antibodies and some patients have associated anti-Ro52 antibodies.

Then, we focused particularly on the pulmonary tropism of anti-Ro52 antibodies positive patients. Among these 34 affected patients, 25 have an identified autoimmune disease as myositis (n = 7), Sjögren syndrome (n = 5), CREST syndrome or systemic sclerosis (n = 7), rheumatoid arthritis (n = 2), dermatomyositis (n = 2) or MCTD (Mixed Connective Tissue Disease) (n = 2). The nine remaining patients have pulmonary diseases of apparently non-immune origin: 4 with interstitial lung disease, 2 with fibrosis, 2 with pulmonary arterial hypertension and one with BOOP (bronchiolitis obliterans with organizing pneumonia). In this 34 patients group, 16 patients have isolated anti-Ro52 antibodies with neither antibodies against antigens

detected by ANA BioPlex reagent nor anti-Ku, anti-PMscl, anti-PL12 or anti-PL7 antibody. Then, we compared the frequency of pulmonary infections in isolated anti-Ro52 patients group versus the others: interstitial lung disease seemed to be more frequent in the isolated anti-Ro52 group (37.5% versus 27.7%), whereas pulmonary fibrosis and pulmonary arterial hypertension were similar, respectively 31 vs 28% and 13 vs 16% as related in Table 2.

4. Discussion

In this study, we looked at the clinical relevance of anti-Ro52/TRIM21 antibodies in autoimmune and non-autoimmune diseases. This multicentric retrospective study was performed with the results of autoimmunity tests in different laboratories in various hospitals. Then, in our recruitment, we had a high prevalence of autoimmune diseases (73%) and our results differ from published series [13,14] in which Anti-Ro52 was not consistently associated with autoimmune disease and weakly predictive with autoimmunity. However, in the absence of any neoplastic or viral diseases, or any treatment inducing ANAs (such as IFN alpha), it could be useful to confirm the anti-Ro52 status during follow-up. As previously reported [15,16], anti-Ro52 is the most common autoantibody detected in polymyositis with antisynthetase syndrome. We also confirmed the variability of detection of anti-Ro60 and anti-Ro52/TRIM21 antibodies using different commercial tests, because only

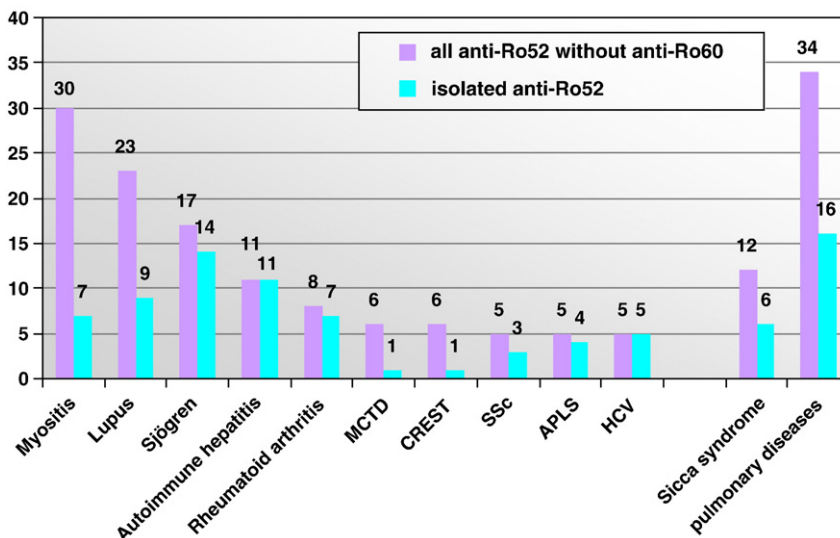


Fig. 2. Main diseases or clinical manifestations associated with anti-Ro52 antibodies.

Table 2
Pulmonary manifestations in 34 patients.

| | Isolated anti-Ro52 Ab (n = 16) | Associated anti-Ro52 Ab (n = 18) |
|---------------------------|-----------------------------------|-------------------------------------|
| Interstitial lung disease | 6 (37.5%) | 5 (27.7%) |
| Pulmonary fibrosis | 5 (31%) | 5 (27.7%) |
| Pulmonary arterial | 2 (12.5%) | 3 (16%) |
| Hypertension | | |
| Lung cancer | 1 (6%) | 0 |
| BOOP ^a | 0 | 1 (5%) |

^a Bronchiolitis obliterans with organizing pneumonia.

63% of selected sera were confirmed in a second time. Anti-Ro52/TRIM21 antibodies were very frequent possibly because of the antigen's immunogenicity and ubiquitous nature [1] and often associated with SSA/Ro60 antibodies in Sjögren syndrome and SLE. Several methods with own specificity and sensitivity can be used to detect separately anti-Ro60 and anti-Ro52 antibodies. Indeed, hidden reactivities could exist when using blended Ro52 and Ro60 antigens in one assay. They should be detected separately because they can mask each other's reactivity. Then, detecting anti-Ro52 and anti-SSA/Ro60 simultaneously adds sensitivity in Lupus and Sjögren syndrome.

As anti-SSA antibodies temporally precede other SLE associated antibodies such as anti-dsDNA, anti ribonucleoprotein and anti-Sm, and can be present on average 3.4 years before the diagnosis of SLE [17], detection of anti-Ro52 antibodies may be of great interest for the early diagnosis of this disease or Sjögren syndrome. Since autoantibodies could be present before the occurrence of clinical symptom, clinicians should be aware of this possibility and should regularly monitor patients at follow-up. In a recent Korean study, Song et al. [18] show that anti-Ro52 was the most frequently detected antibody in primary Sjögren's syndrome. Patients with anti-Ro52 had higher frequency of liver and muscle involvements than those without, while anti-Ro60 exhibited negative association with liver involvement. They confirm that anti-Ro52 has been reported to be found in mothers of children with neonatal lupus and congenital heart block as well as in patients with various autoimmune diseases such as systemic lupus erythematosus, primary Sjögren's syndrome, systemic sclerosis, inflammatory myopathy and autoimmune hepatitis. In liver diseases, patients who had anti-Ro52 were in a more advanced histological stage and had higher serum bilirubin and IgM levels at the time of diagnosis. Anti-Ro52 were highly specific for PBC primary biliary cirrhosis in autoimmune liver diseases and may be diagnostically relevant in patients who are negative for anti-mitochondrial antibodies [11]. Furthermore, Liaskos found that patients with type 1 autoimmune hepatitis who had anti-SLA antibodies, had simultaneously anti-Ro antibodies but not due to cross-reactivity [19]. It was controversial whether anti-Ro52 is a cause or a result of liver involvement. The role of anti-Ro52 in liver involvement was not clear, anti-Ro52 might be related to the structural or functional deterioration of liver and it might be necessary to evaluate liver functions in patients having anti-Ro52 antibodies at the time of diagnosis. Other studies demonstrate that Anti-La, but not anti-Ro52 or anti-Ro60, is a strong predictor of internal organ infection in primary Sjögren's syndrome [20].

It was also shown that high levels of anti-Ro52 antibodies are associated with the highest relative risks of congenital atrioventricular block (CABV) [21]. All cardiac complications were associated with moderate or high maternal anti-Ro levels and the risk of fetal tissue injury and cardiac complication correlate with anti-Ro52 levels. Eftekhari et al. [22] show that anti-Ro52 antibodies blocking the cardiac 5-HT4 serotonergic receptor could explain neonatal lupus congenital heart block. By homology scanning, a cross-reactivity was found between the peptide derived from the 5-HT4 receptor, and a peptide corresponding to residues 365–382 of the Ro52 protein. Anti-

Ro52 antibodies have been implicated in the occurrence of congenital heart block, particularly with the homology between Ro52 antigen and the receptor of 5-HT4 [23]. Recently, spontaneous rupture of atrioventricular valve tensor was shown to be a late manifestation of Anti-Ro/SSA antibody-mediated cardiac disease [24].

Furthermore, Ro52 that ubiquitinates various members of the Interferon Regulatory Factors family is an inducible protein of the TRIM family that translocates to the nucleus upon interferon alpha stimulation [5,36]. Ro52 antigen can interact with different molecules, is an ubiquitin ligase involved in the proteosomal destruction [6,25] and UV-induced cutaneous inflammation [26]. Indeed, Ro52 autoantibodies are specifically associated with cutaneous lupus erythematosus and photosensitivity. It has been reported [7] that Ro52 antigen binds the Fc portion of circulating IgG, which may further block opsonisation and blebs clearance. Increased and inefficient apoptosis may also lead to the inflammatory process and then to secondary necrosis [27,28]. Ro52 is thought to modify the role or stability of its substrates through ubiquitination and this modification might result in the Ro52-mediated biological events [6,28]. Recently, TRIM21 was described as a cytosolic IgG receptor [29] that played an important role in intracellular immunity, mediating antibody neutralization.

Significant association between isolated anti-Ro52 reactivity and myositis and to a lesser extent with systemic sclerosis has been described [30]. Monospecific anti-Ro52 is the most common serological marker in about 24% of patients with idiopathic inflammatory myopathy (IMM) and co-occurred with anti-Jo1 in 56% of the cases [31]. The high frequency of anti-Ro52 in antisynthetase positive patients is not due to cross-reactivity between anti-Jo1 and Ro52 [10]. Anti-Ro52 is indeed an independent autoantibody in myositis [16]. Anti-Ro52 is not associated with any particular clinical symptom to the contrary of what had been suggested in antisynthetase syndrome [32] and primary biliary cirrhosis [11].

Anti-tRNA synthetases syndrome is an original model of autoimmunization with a link between an initial specific muscular lesion and the appearance of autoantibodies which maintain and aggravate the phenomenon. A breaking of tolerance against tRNA synthetases and neoantigens located in the alveolar lung membrane might exist that could induce an immune response and pulmonary aggression [33]. The presence of anti-Ro52 antibody could be associated with a particular phenotype of the disease, with a poor prognosis or perhaps it may precede the development of pulmonary infection and follow-up of those patients must be recommended [32,34].

Although isolated anti-Ro52 antibodies were detected in various autoimmune diseases, the main clinical reported data were interstitial lung disease. Association between the presence of anti-Ro52 antibodies and pulmonary infections may be related to the gene location of Ro52 antigen on human chromosome 11. Indeed, the chromosome 11p15.5 segment may harbour genes involved in the development and progression of lung cancer and the Ro52 gene is a candidate tumor suppression because of its function as a transcriptional regulator [35,36]. Ro52 targets many transcription factors to deregulate pro-inflammatory cytokine production belonging to the IL23-TH17 pathway and links to the development of tissue specific inflammation and systemic autoimmunity.

5. Conclusion

Beside tRNA-antisynthetases syndrome, it will be helpful to test anti-Ro52 antibodies in pulmonary infections for the diagnosis of idiopathic pneumopathies. Further investigations should be useful to determine the clinical interest of anti-Ro52 determination in the diagnosis of related antisynthetase syndrome, without classical antibodies or to classify as autoimmune idiopathic interstitial lung disease.

Take-home messages

- Ro52 (TRIM21) is distinct from Ro60 (SSA) antigen.
- Anti-Ro52 and anti-Ro60 (SSA) should be tested separately.
- Anti-Ro52 antibodies are detected preferentially in autoimmune diseases.
- Anti-Ro52 antibodies are often present when patients develop lung infections, even in the absence of tRNA antisynthetase antibodies.
- Presence of anti-Ro52 antibodies should probably precede development of autoimmune disease and must induce sequential follow-up of positive patients.

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