Anti-Ro52 antibodies positivity in antisynthetase syndrome: a single centre cohort study

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ABSTRACT

Objective. Although antisynthetase antibodies (ARS) are the established markers of the so-called antisynthetase syndrome (ASSD), in these patients the concomitant positivity of anti-Ro52 antibodies, reported in up to the 50% of cases, is not rare. Several studies focused on the effect of different ARS specificities on the evolution of ASSD, the most recent showing no effects. On the contrary, the role of co-occurring anti-Ro52 antibodies in ASSD is still debated. We investigated the potential of anti-Ro52 antibodies in identifying a clinical phenotype of ASSD or influencing prognosis, irrespectively to the underlying ARS specificity.

Methods. Retrospective analysis of clinical, imaging and laboratory characteristics, therapeutic approaches and outcome at baseline and at last follow-up, of 60 ASSD patients progressively enrolled at our Hospital.

Results. We identified 34 anti-Ro+ and 26 anti-Ro- ASSD patients. Classic triad prevalence at baseline was similar between the two groups, whereas interstitial lung disease (ILD) (p-value=0.01) and myositis (p-value=0.03) were significantly more prevalent in anti-Ro52+ and in anti-Ro52- patients at last follow up, respectively. No differences in therapeutic approaches, oxygen need and ILD patterns were observed. Overall mortality was 25% (15 subjects). No differences in mortality, overall and disease related, between anti-Ro52+ and anti-Ro52- patients were observed (p-value=0.764), despite the more frequent ILD occurrence in anti-Ro52+ patients. Survival curves were not different at any time point (Log-rank test, p-value 0.98).

Conclusion. Anti-Ro52 antibodies affect time course and clinical characteristics of ASSD. Although ILD is significantly more associated to anti-Ro52 antibodies, no difference in mortality was observed compared to anti-Ro52 negative patients.

Introduction

Antisynthetase syndrome (ASSD) is a systemic autoimmune disease characterised by the positivity of anti-aminocyl-transfer-RNA synthetases antibodies (ARS) and the occurrence of the classic triad arthritis, myositis, and interstitial lung disease (ILD) (1). The occurrence of accompanying findings, such as the Raynaud’s phenomenon and mechanic’s hands (MHs) is not rare in ASSD (2). ILD is the most common manifestation of the classic triad leading to both acute and chronic lung damage (3) and it has the highest impact on prognosis (4). Non-specific interstitial pneumonia (NSIP) is the most common pattern of ILD, followed by usual interstitial pneumonia (UIP), organising pneumonia (OP), and NSIP superimposed with OP (5). The leading prognostic role of ILD in these patients prompted a focused search for clinical, instrumental, and laboratory markers able to predict lung involvement occurrence and progression (1, 2). ARS specificities have been associated with different incidence of arthritis, myositis, and ILD only at disease onset, whereas no significant differences in the clinical spectrum time course and prognosis of the disease have been observed among the groups (6, 7). Anti-Ro52 antibodies (anti-Ro52) are systemic autoantibodies addressed against the Tripartite motif-containing protein 21 (TRIM21) (8), which can be found in several connective tissue diseases (CTDs), and up to 50% of ASSD patients (6). Furthermore, anti-Ro52 have been associated with ILD in the spectrum of various rheumatologic diseases (9), and also seem to indicate a more aggressive ILD...
course (10). Although a possible role of anti-Ro52 in ASSD sub-phenotyping has been previously suggested (11), their impact on ILD’s responsiveness to treatment and prognosis has not been fully elucidated, with contrasting results (11-15). Therefore, with this study we aim to investigate whether anti-Ro52 antibodies are associated with some specific disease characteristics or influence the final prognosis in our cohort of ASSD patients.

Methods
We performed a retrospective chart analysis on our ASSD patient cohort. We identified 60 ASSD patients with at least one year of follow-up, diagnosed and evaluated at our Hospital between June 2010 and December 2019. Approval of the study was obtained from the local Institutional Review Board. Data on clinical, instrumental and laboratory characteristics, along with administered treatments were collected. The detection of myositis-specific and associated antibodies (MSA and MAA) was performed in our Hospital laboratory through a line blot analysis (Euroline Autoimmune Inflammatory Myopathies 16 Ag, EuroImmun, Lübeck, Germany).

Patient history was recorded by the primary care physician (PCP), whereas clinical manifestation, laboratory data, and treatments were recorded by a single biologist expert in autoimmunity (CA). The diagnosis of ASSD was made in the presence of one ARS plus at least one finding from the clinical triad (arthritis, myositis or ILD). Patients with more than one ARS positive were not included in the study, as well as those positive for myositis-associated autoantibodies other than the anti-Ro52. Positivity for anti-RNP, anti-Sm, and anti-Scl70 at the ENA screen was considered, and positive patients were included in the study. Statistical significance was defined as a p-value ≤0.05. Analyses were performed with STATA software package (2018, release 15.1; StataCorp, College Station, TX, USA).

Results
General characteristics
Our cohort included 60 patients, mainly females (39 patients, 65%) with a median (IQR) age of onset of 54 (46-68) years. The median (IQR) diagnostic delay was 7 months (3-36), whereas the median disease duration was 90 (51-142) months. Fifty patients (83%) were anti-Jo1, 4 (7%) anti-PL-7, 3 (5%) anti-PL12, 2 (3%) anti-EJ and 1 (2%) anti-OJ positive. At disease onset and last follow-up, arthritis was observed in 42 (70%) and 48 (80%) patients, ILD in 34 (57%) and 55 (92%) patients, myositis in 28 (47%), and 42 (70%) patients, respectively. A complete ASSD was observed in 12 patients (20%) at the onset and in 31 (52%) at the last follow-up (n = 31, 52%). De novo triad findings were observed in 31 out of 41 patients with incomplete ASSD (76%). Among the 48 patients with arthritis, 21 (44%) satisfied the 2010 ACR/EULAR criteria for RA (16). ILD onset was acute in 15

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ILD: occurrence of restrictive pulmonary function tests (PFTs) pattern and/or a reduction of diffusing capacity of the lungs for carbon monoxide (DLCO) >20%, and/or evidence of ground glass/reticular pattern on chest high-resolution computed tomography (HRCT). As previously described (1) ILD presentation was defined as acute when dyspnoea occurred acutely and progressed rapidly (within 4–6 weeks from symptom onset), chronic when dyspnoea occurred insidiously and progressed slowly, and asymptomatic when lung involvement was only detected through HRCT or PFTs.

Muscle involvement: presence of muscle enzymes’ elevation (creatine phosphokinase and/or aldolase increase >50%, as compared with upper normal values) along with typical electromyography and/or muscle biopsy and/or muscle magnetic resonance alterations. Muscle involvement was classified as classic (muscle-related strength deficit) or hypomyopathic (no muscle-related strength deficit).

Arthritis: evidence of joint swelling/tenderness detected by a Rheumatologist at the physical exam. The pattern of joint involvement was collected, and in all patients, we evaluated if the 2010 ACR/EULAR criteria for Rheumatoid arthritis (RA) (16) were satisfied.

Accompanying findings were defined as follows:
- Fever: body temperature of ≥38°C for more than 10 days, not otherwise explained, and considered disease-related after adequate differential diagnosis.
- Mechanic’s hands (MHs): thickened, hyperkeratotic, and fissured aspect of the radial sides of the fingers, without other explanations.
- Raynaud’s phenomenon (RP): transient fingers’ ischaemia after exposure to the cold, confirmed by a clinician.

All patients underwent HRCT at our Radiology Department. Two experienced radiologists (LP and AV) re-evaluated HRCT images and classified the ILD pattern separately. In case of inconsistencies, an agreement was reached after discussion with other skilled specialists (LC, FM, VV). PFTs with DLCO determination were all performed in the Lung Transplantation Centre Unit of our Hospital and supervised by two experienced clinicians (VV and FM). The occurrence of muscle strength deficit and arthritis, as well the occurrence of accompanying manifestation was evaluated by 3 experienced Rheumatologists (LC, GZ, and AB).

Clinical manifestation were considered concomitant if they occurred less than 3 months apart. ASSD was defined as complete or incomplete, according to the occurrence of all triad findings or not. The triad findings that appeared during the follow-up were defined as de-novo manifestations.

Outcomes were defined as O2 therapy need, overall mortality, disease-related mortality. Patients’ characteristics were reported using median and interquartile range (IQR) for the quantitative variables, and absolute/relative frequency values for qualitative variables. The comparison between the groups was performed by the parametric unpaired sample t-test or by the non-parametric Kruskal-Wallis test for quantitative variables, and by the Chi-square or by the Fisher exact test for categorical variables. The Kaplan-Meier method and Log-rank test were used to estimate survival and evaluate whether there were differences among anti-Ro52 positive and negative patients. Statistical significance was defined as a p-value ≤0.05. Analyses were performed with STATA software package (2018, release 15.1; StataCorp, College Station, TX, USA).
cases (27%), chronic in 26 (47%), and asymptomatic in 14 (26%). The main HRCT pattern observed was NSIP (40 patients, 67%), followed by NSIP + OP (10 cases, 17%), UIP (3 cases, 5%), and OP (2 cases, 3%). Of note, in all cases, the extent of ILD was greater than the 10% of lung parenchyma. At disease onset, the median FVC and DLCO were respectively the 87% and the 63% of the predicted value. The most pathologic FVC and DLCO had a median value of 84.5% and 60% of the predicted value, respectively. The 42 patients with muscle involvement had classic onset in 28 cases (67%), and hypomyopathic in 14 (33%). Overall, 15 patients (25%, 6 male and 9 female) died during the follow-up. The median (IQR) age of deceased patients was 78 (67-82) years with a median (IQR) disease duration of 100 (60-124) months. Causes of death were disease-related in 9 (60%) patients and non-disease-related in 6 (40%). In all cases, disease-related deaths were due to ILD. The median (IQR) age of patients that died for disease (ILD)-related causes was 76 (62-82) years with a median (IQR) disease duration of 74 (42–104) months. Of note, O2 therapy was started in 18 out of the 55 patients with ILD (33%), in particular in all patients that died for ILD.

Differences in clinical characteristics between anti-Ro52 positive and anti-Ro52 negative patients

Anti-Ro52 antibodies were positive in 34 patients (57%) and negative in 26 (43%). The results of collected data according to anti-Ro52 status (positive or negative) at both disease onset and last follow-up are reported in Table I (overall characteristics), Figure 1 (HRCT patterns of ILD involvement), and Table II (ongoing and withdrawn therapies). Anti-Ro52 positive patients were less commonly males ($p=0.001$, OR 0.157, 95% CI 0.048–0.509), had less commonly myositis at last follow-up ($p=0.04$, OR 0.260, 95% CI 0.073–0.921), and presented more frequently ILD ($p=0.01$, OR 17.7, 95% CI 0.929–335) than anti-Ro52 negative patients. No time-related differences were observed in particular for the age at disease onset. In Supplementary Tables S1 and S2 we reported the characteristics of anti-Ro52 positive and negative patients stratified according to the ARS specificities (anti-Jo1 and non-anti-Jo1 positive).

The detected overall mortality was 9 (26%) and 6 (23%) patients in anti-Ro52+ and anti-Ro52- groups, respectively ($p=0.764$). Of note, among the non-disease-related deaths, we observed only one case of neoplasm more than 5 years from ASSD onset in an anti-Ro52- female. Another patient died in car crash, one for ruptured cerebral aneurism, 3 for cardiovascular disease. Disease-related deaths accounted for 5 (55% of the group) cases in anti-Ro52+ and 4 (67% of the group) cases in anti-Ro52- patients ($p=1.00$). In all cases, disease-related death were due to ILD. The median (IQR) age of patients deceased for disease (ILD) related causes...
was 74 (68–78) years in anti-Ro52 positive patients, and 78 (62–82) in anti-Ro52 negative patients (p=0.512). The median (IQR) disease duration in patients deceased for ILD related causes was 82 (58–110) months in anti-Ro52 positive patients, and 74 (35–110) in anti-Ro52 negative patients (p=0.720). By adjusting mortality for age, sex, disease duration, and time from lung involvement detection the survival in anti-Ro52 positive and negative patients was still similar. Survival curves estimated by Kaplan-Meier function did not show differences at any time point for both overall (Log-rank test, p-value 0.786) and disease-related mortality (Log-rank test, p-value 0.991) as shown in Figure 2. O2 therapy was started in 12 patients (35%) and 6 patients (29%) in the anti-Ro52 positive and negative patients, respectively (p=0.606). No substantial treatment differences were observed in the 2 groups, with a similar rate of ongoing and withdrew drugs (Table II).

**Discussion**

In our cohort of ASSD, anti-Ro52 status was associated with some peculiar characteristics. In particular, anti-Ro52 positive ASSD were mainly females, had increased frequency of ILD and a reduced prevalence of muscle involvement. Conversely, anti-Ro52 negative ASSD patients were more commonly males and displayed ILD less frequently. In our cohort, anti-Ro52 status did not affect the radiographic pattern of ILD, the lung function or the prognosis, in terms of both survival and need of oxygen therapy. We think that the early diagnosis and treatment of ILD, together with the tight control of lung involvement with both pulmonary function tests and chest HRCT, may explain why the prognosis is not different between anti-Ro52 positive and negative patients. Furthermore, it is also interesting to observe that the prognosis between anti-Ro52 positive and negative patients was not affected by the different gender distribution we observed between groups.

To date, only a few studies evaluated the clinical relevance of anti-Ro52 antibodies in ASSD, with contrasting results (11–13, 15). Although 2 studies (12, 13) focuses only on anti-Ro status, due to the very low prevalence of anti-Ro60 in myositis in general and in ASSD in particular (11, 17), it seems reasonable that the evaluated patients were anti-Ro52 positive.

La Corte et al. (13) showed that anti-Ro positivity, although associated with a more extended ILD, did not affect the presenting PFTs and the final prognosis of ASSD. In addition, Bauhammer et al. (15) showed that anti-Ro52 positivity might identify patients with a better response to rituximab, thus acting as possible biomarker for targeted treatment. Conversely, Vancsa et al. (12) evidenced that anti-Ro positive ASSD had increased prevalence of ILD, especially with acute onset, as well as deterioration of PFTs, reduced treatment responsiveness, and impaired patients’ survival. These results, with the exception of PFTs deterioration, have been confirmed in another cohort including ASSD characterised for anti-Ro52 antibodies (11).

To considering previous reports, our results are in line with those of Vancsa et al. (12) for the increased prevalence of ILD in anti-Ro52 ASSD, and with those of La Corte et al. (13) and Marie

### Table II. Therapeutic approaches in patients affected by interstitial lung disease according to anti-Ro52 antibodies status.

<table>
<thead>
<tr>
<th>Anti-Ro52 positive</th>
<th>Anti-Ro52 negative</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>34 (100)</td>
<td>21 (81)</td>
</tr>
<tr>
<td>Prednisone ongoing</td>
<td>31 (91)</td>
<td>20 (95)</td>
</tr>
<tr>
<td>Cyclophosphine ongoing</td>
<td>22 (65)</td>
<td>10 (48)</td>
</tr>
<tr>
<td>Cyclophosphine withdrawal</td>
<td>7 (21)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>Mycophenolate ongoing</td>
<td>4 (12)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Mycophenolate withdrawal</td>
<td>3 (9)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Hydroxychloroquine ongoing</td>
<td>4 (12)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Hydroxychloroquine withdrawal</td>
<td>0 (0)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Methotrexate ongoing</td>
<td>4 (12)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>Methotrexate withdrawal</td>
<td>2 (6)</td>
<td>5 (24)</td>
</tr>
<tr>
<td>Azathioprine ongoing</td>
<td>2 (6)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Azathioprine withdrawal</td>
<td>6 (18)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Rituximab ongoing</td>
<td>5 (15)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Rituximab withdrawal</td>
<td>1 (3)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Cyclophosphamide ongoing</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cyclophosphamide withdrawal</td>
<td>1 (3)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Other* ongoing</td>
<td>1 (3)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Other withdrawal</td>
<td>2 (6)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Association ongoing</td>
<td>10 (29)</td>
<td>7 (33)</td>
</tr>
</tbody>
</table>

*Baricitinib, tocilizumab, abatacept, leflunomide, sulfasalazine, human immunoglobulins.

![Fig. 2. Kaplan-Meier survival curve for disease-related deaths.](image)
et al. (11) for the lack of differences in the two groups for baseline PFTs’ values and ILD pattern. Our study also retrieved some previously unreported findings, such as the increased prevalence of male gender in anti-Ro52 negative patients and the reduced prevalence of myositis in anti-Ro52 positive patients. These results may suggest that anti-Ro52 antibodies could identify a peculiar sub-phenotype of ASSD, represented by female patients with high prevalence of arthritis and ILD and low prevalence of myositis. However, it is interesting that in previous studies, no gender differences have been reported between the anti-Ro52 positive and negative ASSD (11-13). One possible explanation of the difference we observed could be related to the hormonal status of the patients, since TRIM21 expression is increased by oestrogens (18).

Of note, given the substantial homogeneity of treatment approach we used for ASSD (19), the therapies prescribed did not differ between the 2 groups. According to this result and the substantially equal prognosis of both anti-Ro52 positive and negative patients, we are not able to define if these antibodies may be used for the stratification of the therapeutic approach of ASSD, as suggested by Bauhammer et al. (15). Based on obtained results, we think that anti-Ro52 antibodies may be helpful in the setting of ASSD, not only because they may suggest the diagnostic suspect, but also because they seem to be associated with specific clinical and demographic features and with a more common occurrence of ILD.

This study has some limitations, mainly due to its retrospective and unicentric design. Furthermore, some patients were addressed to our Unit after the diagnosis, thus increasing the risk of some bias in data collection. However, antibody testing was repeated in our centre for every patient, while CT scans were evaluated by our radiologists even when performed in other centres. Other tests and assessment such as pulmonary function test or electromyography, if performed elsewhere, were repeated only if clinically necessary. Although this could be a limit, it represents surely a correct management decision, because addressed to reduce the costs for the National Health system.

Overall, the presence of anti-Ro52 antibodies seems to be related to a higher prevalence of ILD. This compels not only to evaluate lung disease at the onset but also to perform a strict follow-up with lung imaging and assure proper and timely initiation of appropriate therapy.

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References

1. CAVAGNA L, NUSO L, SCRÈ CA et al.: Clinical spectrum time course in Anti Jo-1 positive antisynthetase syndrome: results from an international retrospective multicenter study. Medicine (Baltimore) 2015; 94: e1144.