Monospecific anti-Ro52/TRIM21 antibodies in a tri-nation cohort of 1574 systemic sclerosis subjects: evidence of an association with interstitial lung disease and worse survival

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ABSTRACT

Objective. Autoantibodies directed against Ro52/TRIM21 are common in systemic sclerosis (SSc) but their clinical significance remains uncertain. The aim of this study was to assess the clinical correlates and survival of subjects with monospecific anti-Ro52/TRIM21 antibodies, i.e., anti-Ro52/TRIM21 antibodies in the absence of other SSc-related antibodies.

Methods. A tri-nation (Canada, Australia, USA) cohort of 1574 SSc subjects was formed, demographic and clinical variables were harmonised and sera were tested using a common diagnostic platform. Statistical analyses were performed to determine associations between the presence of monospecific anti-Ro52/TRIM21 antibodies and outcomes of interest, including interstitial lung disease (ILD) and survival.

Results. 103 (6.5%) had monospecific anti-Ro52/TRIM21 antibodies, 324 (20.6%) had anti-Ro52/TRIM21 antibodies overlapping with other SSc-related antibodies and 1147 (72.9%) were negative for anti-Ro52/TRIM21 antibodies. Monospecific subjects were less likely to be White compared to negative subjects (68% vs. 82%, odds ratio (OR) 0.48, 95% confidence interval (CI) 0.30–0.75, p=0.0011). ILD was the only clinical variable significantly associated with monospecific anti-Ro52/TRIM21 antibodies compared to negative subjects (adjusted OR 2.70, 95% CI 1.75–4.14, p=0.0001). Subjects with monospecific anti-Ro52/TRIM21 antibodies were at significantly increased risk of death compared to subjects without anti-Ro52/TRIM21 antibodies (log rank p=0.0003; adjusted hazard ratio (HR) 1.87, 95% CI 1.24–2.82, p=0.0029).

Conclusion. The results obtained from this unique tri-nation cohort represent the strongest evidence to date that anti-Ro52/TRIM21 antibodies are independently associated with the presence of ILD and poor survival in SSc. These data provide strong support for the predictive and prognostic value of this serological biomarker in SSc.

Introduction

Two main types of SS-A/Ro autoantibodies have been described. One is directed at a 60 kDa protein known as SS-A/Ro60, which is a component of a small cytoplasmic ribonucleoprotein (scRNP) macromolecular complexes. Another, that often coexists with SS-A/ Ro60 autoantibodies, is directed against a 52 kDa (Ro52) protein that is not normally part of the scRNP complex but is an E3 ubiquitin ligase and member of the tripartite motif (TRIM) family of proteins known as TRIM21 (1, 2); hence, the preferred terminology of Ro52/TRIM21 will be used in this report.

Anti-Ro52/TRIM21 antibodies have been reported in a wide variety of autoimmune diseases, often overlapping with other autoantibodies (3-5). Hence, they have often been considered non-specific markers of autoimmune inflammation. The fact that these autoantibodies have also been detected in sera of patients with neoplasia (6), viral infections or even healthy individuals who later developed auto-immune diseases (7) has provided further support for this. In SSc, a recent report on a Spanish cohort of 132 consecutive SSc patients did not find any clinical associations with anti-Ro52/TRIM21 (8). On the other hand, anti-Ro52/TRIM21 antibodies have been reported to be as-

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Funding information and competing interests on page S-135.
associated with interstitial lung disease (ILD) in various autoimmune diseases (9, 10), in particular in association with anti-Jo1 antibodies (11), which are well known to be associated with ILD. We previously reported an association between anti-Ro52/TRIM21 antibodies and ILD in SSC where, if present, ILD was 1.5 times more likely (12). However, the relationship between anti-Ro52/TRIM21 and ILD in SSC (and other autoimmune diseases) may have been confounded by the presence of concomitant antibodies known to be associated with ILD, particularly anti-topoisomerase I in SSC (13). We have previously shown that overlap with SSC-specific autoantibodies can confound the associations with other autoantibodies (14). The aim of this study was therefore to assess the clinical correlates of monospecific anti-Ro52/TRIM21 antibodies, i.e. anti-Ro52/TRIM21 antibodies in the absence of other SSC-related antibodies.

Materials and Methods
The Tri-Nation cohort comprises SSC subjects included in the Canadian Scleroderma Research Group (CSRG), the Australian Scleroderma Cohort Study (ASCS) and the American Genetics versus Environment in Scleroderma Outcomes Study (GENISOS) cohorts. Ethics committee approval for this study was obtained at McGill University (Montreal, Canada) and at all participating CSRG, ASCS, and GENISOS study sites. All subjects provided informed written consent to participate in the study. Selection of study subjects in and harmonisation of clinical variables between the 3 study cohorts have been described (15). Briefly, over 98% of the CSRG (16) and ASCS subjects, and all GENISOS subjects meet the 2013 ACR/EULAR classification criteria for SSC (17). Demographic information regarding age, sex and ethnicity was collected by subject self-report. Disease duration was recorded by study physicians and defined as the interval between the onset of the first non-Raynaud disease manifestation and baseline study visit. Skin involvement was assessed using the modified Rodnan skin score. Limited cutaneous disease (lcSSc) was defined as skin involvement distal to the elbows and knees with or without facial involvement; diffuse cutaneous disease (dcSSc) was defined as skin involvement proximal to the elbows and knees and/or of the trunk. A history of inflammatory myositis, calcinosis, inflammatory arthritis and scleroderma renal crisis was recorded by a study physician. To assess gastrointestinal involvement, subjects answered yes/no to 6 questions concerning gastroesophageal reflux disease, dysphagia, antibiotics for bacterial overgrowth, episodes of pseudo-obstruction, fecal incontinence and hyperalimentation. The presence of interstitial lung disease (ILD) was determined using a clinical decision rule that was recently published (18). Using this algorithm, ILD was considered present if a high resolution computed tomography (HRCT) scan of the lung was interpreted by an experienced radiologist as showing ILD or, in the case where no HRCT was available, if either a chest x-ray was reported as showing either increased interstitial markings (not thought to be due to congestive heart failure) or fibrosis, and/or if a study physician reported the presence of typical “velcro-like crackles” on physical examination. Pulmonary hypertension was defined as an estimated systolic pulmonary artery pressure (sPAP) ≥45 mmHg measured using the Doppler flow measurement of the tricuspid regurgitant jet on cardiac echocardiography (an estimate that correlates strongly with right heart catheter studies) (19) for CSRG and GENISOS subjects, or mean pulmonary artery pressure (mPAP) ≥25 mmHg with a pulmonary capillary wedge pressure (PCWP) <15 mmHg on right heart catheterisation for ASCS subjects.

Serology
Autoantibody analyses of the CSRG and GENISOS cohorts were performed in a central laboratory, Mitogen Advanced Diagnostics Laboratory, University of Calgary and the ASCS analyses were performed in Australia using an identical immunoassay kit and protocols. Serum aliquots were stored at -80°C until needed for diagnostic assays. Antibodies against Ro52/TRIM21, centromere (CENP A and CENP B), topoisomerase I, RNA polymerase III (RP11 and RP155), fibrillarin, Nor90, Th/To, Ku, PDGFR, PM75 and PM100 were detected and digitally quantified by the Euroline systemic sclerosis profile line immunoassay (LIA) (Euroimmun, Luebeck, Germany) according to the manufacturer’s instructions.

Statistical analysis
Subjects were divided into those exclusively positive for anti-Ro52/TRIM21 antibodies (i.e. monospecific anti-Ro52/TRIM21 antibodies subjects), those with anti-Ro52/TRIM21 antibodies overlapping with other measured antibodies (i.e. overlapping anti-Ro52/TRIM21 antibodies subjects), and those altogether negative for anti-Ro52/TRIM21 antibodies. Descriptive statistics were used to compare 20 selected variables between 1) monospecific anti-Ro52/TRIM21 antibody positive versus negative subjects and 2) overlapping anti-Ro52/TRIM21 antibodies versus negative subjects. Adjusting for multiple comparisons, p<0.00125 was considered statistically significant. Multivariate logistic regression adjusting for baseline differences in age and ethnicity was used to determine the association between anti-Ro52/TRIM21 antibody groups and ILD. Kaplan Meier analysis and Cox proportional hazard models adjusting for baseline differences in age and ethnicity were used to compare survival between autoantibody subsets. p-values <0.05 were considered statistically significant for these 3 latter analyses. All statistical analyses were performed with SAS v.9.2 (SAS Institute, USA).

Results
A total of 1574 SSC subjects were included in this study, of whom 103 (6.5%) had monospecific anti-Ro52/TRIM21 antibodies, 324 (20.6%) had anti-Ro52/TRIM21 antibodies overlapping with other SSC-related antibodies and 1147 (72.9%) were negative for anti-Ro52/TRIM21 antibodies (Table 1). Monospecific subjects were less likely to be White compared to negative subjects (68% vs. 82%, odds ratio (OR) 0.48, 95% confidence interval (CI) 0.30–0.75, p=0.0011). Subjects with overlapping anti-Ro52/TRIM21 anti-
In unadjusted survival analysis (Fig. 1), multivariate analysis.

the prevalence of monospecific anti-Ro52/TRIM21 antibodies in this large SSc cohort was second most common autoantibody in whole group (12, 20), the prevalence of anti-Ro52/TRIM21 antibodies modifies the association between anti-Ro52/TRIM21 and ILD (22) and worsening forced vital capacity (23). Our data provide evidence for a novel predictive and prognostic biomarker in SSc. Of note, though, subjects with overlapping anti-Ro52/TRIM21 antibodies did not have a higher frequency of ILD either in univariate or multivariate analysis. In unadjusted survival analysis (Fig. 1), subjects with monospecific anti-Ro52/TRIM21 antibodies were at increased risk of death compared to negative subjects (log rank p=0.0003). Again, after adjusting for differences in baseline demographic characteristics, subjects with monospecific anti-Ro52/TRIM21 antibodies were still at significantly increased risk of death compared to subjects without anti-Ro52/TRIM21 antibodies (hazard ratio (HR) 1.87, 95% CI 1.24–2.82, p=0.0029; Table III).

Discussion

Although anti-Ro52/TRIM21 is the second most common autoantibody in SSc sera (12, 20), the prevalence of monospecific anti-Ro52/TRIM21 antibodies in this large SSc cohort was less than 10%. Nonetheless, leveraging this large unique tri-nation dataset using a common serological platform, we found strong evidence that monospecific anti-Ro52/TRIM21 antibodies are independently associated with ILD and increased mortality in SSc. Currently, there are few robust clinical biomarkers in SSc-ILD aside from C-reactive protein, which has been shown to be associated with worse pulmonary function (21) and anti-topoisomerase I (Scl-70) with ILD (22) and worsening forced vital capacity (23). Our data provide evidence for a novel predictive and prognostic biomarker in SSc. Of note, though, subjects with overlapping anti-Ro52/TRIM21 antibodies did not have a higher frequency of ILD. It is possible that the presence of other SSc-related antibodies modifies the association between anti-Ro52/TRIM21 and ILD. The role of anti-Ro52/TRIM21 in the pathophysiology of autoimmune diseases remains largely unknown.

Table I. Baseline characteristics of the study cohort, as a group and according to anti-Ro52/TRIM21 antibody status. The monospecific anti-Ro52/TRIM21 antibody positive group was exclusive of anti-CENP, topoisomerase I, RNA polymerase III, fibrillarin, NOR90, Th/To, Ku, PDGFR, PM75 and PM100 antibodies. Adjusting for multiple comparisons, p<0.00125 was considered statistically significant.

<table>
<thead>
<tr>
<th>Sociodemographics</th>
<th>Whole group (n=1574)</th>
<th>Monospecific anti-Ro52/TRIM21 positive (n=103)</th>
<th>Overlapping anti-Ro52/TRIM21 positive (n=324)</th>
<th>Anti-Ro52/TRIM21 negative (n=1147)</th>
<th>Monospecific vs. Negative</th>
<th>Overlapping vs. Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>% mean</td>
<td>SD</td>
<td>% mean</td>
<td>SD</td>
<td>% mean</td>
<td>SD</td>
<td>% mean</td>
</tr>
<tr>
<td>Sociodemographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>86%</td>
<td>1355</td>
<td>82%</td>
<td>84</td>
<td>90%</td>
<td>290</td>
</tr>
<tr>
<td>White</td>
<td>82%</td>
<td>1244</td>
<td>68%</td>
<td>66</td>
<td>86%</td>
<td>273</td>
</tr>
<tr>
<td>Age, years</td>
<td>55.1</td>
<td>12.8</td>
<td>53.2</td>
<td>13.1</td>
<td>58.7</td>
<td>12.6</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>9.5</td>
<td>9.2</td>
<td>7.9</td>
<td>7.7</td>
<td>10.3</td>
<td>9.5</td>
</tr>
<tr>
<td>Age at disease onset, years</td>
<td>45.5</td>
<td>13.7</td>
<td>45.2</td>
<td>13.2</td>
<td>48.4</td>
<td>13.9</td>
</tr>
<tr>
<td>Clinical variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Modified Rodman skin score (0-51)</td>
<td>11.4</td>
<td>10.2</td>
<td>11.3</td>
<td>9.4</td>
<td>10.9</td>
<td>9.8</td>
</tr>
<tr>
<td>Limited cutaneous disease</td>
<td>61%</td>
<td>963</td>
<td>52%</td>
<td>53</td>
<td>68%</td>
<td>221</td>
</tr>
<tr>
<td>Inflammatory myositis</td>
<td>9%</td>
<td>129</td>
<td>11%</td>
<td>10</td>
<td>8%</td>
<td>24</td>
</tr>
<tr>
<td>Calcinosis</td>
<td>26%</td>
<td>405</td>
<td>18%</td>
<td>18</td>
<td>30%</td>
<td>95</td>
</tr>
<tr>
<td>Inflammatory arthritis</td>
<td>28%</td>
<td>443</td>
<td>27%</td>
<td>28</td>
<td>30%</td>
<td>97</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>82%</td>
<td>1278</td>
<td>75%</td>
<td>76</td>
<td>87%</td>
<td>279</td>
</tr>
<tr>
<td>GERD/reflux</td>
<td>52%</td>
<td>808</td>
<td>51%</td>
<td>50</td>
<td>56%</td>
<td>177</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>7%</td>
<td>94</td>
<td>6%</td>
<td>5</td>
<td>8%</td>
<td>24</td>
</tr>
<tr>
<td>Antibiotics for bacterial overgrowth</td>
<td>3%</td>
<td>42</td>
<td>1%</td>
<td>1</td>
<td>4%</td>
<td>12</td>
</tr>
<tr>
<td>Episodes of pseudo-obstruction</td>
<td>18%</td>
<td>217</td>
<td>9%</td>
<td>6</td>
<td>21%</td>
<td>56</td>
</tr>
<tr>
<td>Fecal incontinence</td>
<td>3%</td>
<td>23</td>
<td>9%</td>
<td>5</td>
<td>2%</td>
<td>4</td>
</tr>
<tr>
<td>Hyperalimentation</td>
<td>1.6</td>
<td>1</td>
<td>1.4</td>
<td>0.9</td>
<td>1.7</td>
<td>1</td>
</tr>
<tr>
<td>Number of GI symptoms (0-6)</td>
<td>4%</td>
<td>60</td>
<td>6%</td>
<td>6</td>
<td>3%</td>
<td>11</td>
</tr>
<tr>
<td>Scleroderma renal crisis</td>
<td>14%</td>
<td>172</td>
<td>8%</td>
<td>6</td>
<td>20%</td>
<td>56</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>36%</td>
<td>548</td>
<td>57%</td>
<td>57</td>
<td>36%</td>
<td>116</td>
</tr>
<tr>
<td>Interstitial lung disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CENP: centromere proteins; CI: confidence interval; GERD: gastro-esophageal reflux disease; GI: gastrointestinal; NOR: nucleolar organiser; PDGFR: platelet derived growth factor; OR: odds ratio; SD: standard deviation; TRIM: tripartite motif.
Table II. Logistic regression model to estimate the association between the presence of anti-Ro52/TRIM21 antibodies and ILD, adjusting for baseline demographic differences.

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>-0.23</td>
<td>0.80</td>
<td>0.60</td>
<td>1.06</td>
</tr>
<tr>
<td>Age</td>
<td>0.01</td>
<td>1.01</td>
<td>1.01</td>
<td>1.02</td>
</tr>
<tr>
<td>Monospecific vs. negative anti-Ro52/TRIM21 subjects</td>
<td>0.99</td>
<td>2.70</td>
<td>1.75</td>
<td>4.14</td>
</tr>
<tr>
<td>Overlapping vs. negative anti-Ro52/TRIM21 subjects</td>
<td>0.05</td>
<td>1.05</td>
<td>0.80</td>
<td>1.37</td>
</tr>
</tbody>
</table>

Nevertheless, some reports suggest a pathogenic role. In general, the autoantibody binding target, Ro52/TRIM21, is a regulator of type I interferon (IFN) and proinflammatory cytokine production (2). In turn, IFNα upregulates Ro52/TRIM21 and promotes its nuclear translocation (24). This self-perpetuating process has the potential to contribute to the inflammatory cascade. In tissue, Ro52/TRIM21 expression is increased in cutaneous lupus erythematosus and ultraviolet light-induced skin lesions and translocation to apoptotic blebs has been hypothesised as a mechanism for its immunogenicity (25). Evidence also exists to support a similar mechanism occurring during cardiomyocyte apoptosis, as well as direct cross-reactivity with cardiac membrane proteins involved in the control of electric signal generation and/or conduction, as in congenital heart block (26, 27). Finally, in primary Sjögren syndrome, a single nucleotide polymorphism in the Ro52 gene has been shown to be associated with anti-Ro52/TRIM21 autoantibodies (28). Although anti-Ro52/TRIM21 autoantibodies have been shown to be associated with severe disease refractory to steroids in auto-immune hepatitis, the pathogenic mechanisms, if any, are not known (29). Similarly, the pathogenic role of anti-Ro52/TRIM21 and other autoantibodies associated with interstitial lung disease is not known (30).

This study is not without limitations. In particular, defining ILD in the context of longitudinal observational cohort studies is very complex, given issues of missing data and verification bias. We defined ILD using a clinical decision rule that was recently published (18). Still, measurement error may have contributed to some of the negative findings of the study. On the other hand, when dealing with relatively uncommon serological profiles (there were only 6.5% of subjects with monospecific anti-Ro52/TRIM21 antibodies), large well-phenotyped cohorts are required to obtain robust estimates. Thus, the limitations of our data are counter-balanced by its strengths, which include large sample size and detailed clinical phenotypic data. Finally, subjects identified as having “monospecific” anti-Ro52/TRIM21 antibodies may in fact have had other autoantibodies that are undetected by the immunoassays employed in this study. This might include some associated with connective tissue disease-related ILD such as anti-Jo1, which were not included among those tested for this study. However, we have previously reported a very low prevalence of anti-Jo1 antibodies in the CSRG SSc cohort (approximately 1%) (12). Thus, the presence of these autoantibodies is unlikely to have influenced the results of this study in a meaningful manner.

We found that monospecific anti-Ro52/TRIM21 antibodies were strongly associated with ILD and an independent predictor of mortality in this large SSc cohort. This provides the strongest evidence to date for the predictive and prognostic value of this serological biomarker in SSc and contributes important clinically meaningful data.

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