Clinical significance of coexisting antitopoisomerase I and anticientromere antibodies in patients with systemic sclerosis: a EUSTAR group-based study

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ABSTRACT

Objective. To determine the clinical characteristics of simultaneous occurrence of antitopoisomerase (ATA) and anticientromere (ACA) autoantibodies in systemic sclerosis (SSc).

Methods. Data of patients (n=4,687) fulfilling the ACR criteria for SSc and followed in the EULAR Scleroderma Trials and Research (EUSTAR) cohort were analysed. Sera from patients with simultaneous ATA and ACA were reanalysed centrally by indirect immunofluorescence, enzyme immunoassay, and immunoblot to confirm antibody status.

Results. A total of 29 patients (0.6%) had been documented double-positive for both ATA and ACA in the EUSTAR database. Sera of 14 cases were available for central analysis, of which 8 were confirmed to unequivocally contain both antibodies. The double-positive patients were on average 52.4 years of age, 87.5% were female, and 62.5% had diffuse cutaneous (dc) SSc. Compared with matched ACA single-positive disease, cutaneous and visceral complications were more prevalent in double-positive cases, but this prevalence did not differ significantly in comparison to ATA single-positives.

Conclusions. Coexistence of ATA and ACA can be found at low prevalence in SSc. The clinical features of double-positive patients are not clearly dissimilar to those of patients harbouring only ATA. The data do not support a direct involvement of these antibodies in the pathogenesis of established SSc, but may lack statistical power.

Introduction

Systemic sclerosis (SSc) is an uncommon multisystem connective tissue disease characterised by microvascular damage and extensive fibrosis of the skin as well as internal organs (1). In addition, immunological abnormalities are typically observed in patients with SSc, the most prominent being the presence of circulating serum autoantibodies directed against specific nuclear components. In more than 90% of the patients with SSc, antinuclear antibodies (ANA) predominantly reacting with topoisomerase I, centromere antibodies, RNA polymerases, or Th/To are detectable (2, 3). These antibodies are considered highly specific for SSc and their presence is associated with distinct clinical manifestations, organ involvement, and risk of mortality (3, 4). Furthermore, SSc-specific autoantibodies represent risk markers that predict progression from isolated Raynaud’s phenomenon to definite SSc (5).

The first and hence best documented antibody specificities in SSc are anticientromere (ACA) and antitopoisomerase I (ATA) antibodies. ACA and ATA have been routinely measured in clinical laboratories for many years, and numerous detection systems are nowadays commercially available (6, 7). In general, ACA and ATA occur in ~60% of SSc patients worldwide, but the reported range (40 to 90%) is very wide (1-3). Genetic variations, classification criteria for SSc, and choice of antibody detection methods may all have contributed to the differences in the reported antibody frequencies (8-10). Interestingly, ACA and ATA are virtually always mutually exclusive (2, 3, 8-10).

The prevalence of clinical disease features and laboratory abnormalities in SSc was recently assessed in a large, multinational SSc cohort, organised by the EULAR Scleroderma Trials and Research (EUSTAR) group (11). Patients evaluated in this database fulfilled...
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the American College of Rheumatology (ACR) classification criteria of SSc (12). About one-third of EUSTAR patients were positive for ATA and another third was positive for ACA. The majority of patients with ATA suffered from the diffuse form of cutaneous scleroderma (dcSSc), whereas ACA occurred mostly in patients with limited cutaneous SSc (lcSSc). There was a significantly shorter lag period between the onset of Raynaud’s phenomenon and the first non-Raynaud’s clinical feature of disease in EUSTAR patients with ATA, as compared to ACA positive patients. On multivariate analysis, autoantibody status was found to be independently associated with the prevalence of organ manifestations. For example, the presence of ATA was the strongest predictor of complications by pulmonary fibrosis and digital ulcers, whereas the absence of ACA strongly predicted the presence of synovitis, joint contractures, and pulmonary fibrosis (11). The predictive value of ATA and ACA, thus, raises the possibility that these autoantibodies are directly or indirectly involved in the pathogenesis of SSc. Likewise, it may also be suggested that distinct and independent pathogenic mechanisms associated with these well-defined antibodies are involved in SSc.

Although individuals are generally positive for either ATA or ACA, the concomitant existence of ATA and ACA in a single patient with SSc has occasionally been reported in the literature (13-16). The clinical and laboratory characteristics of these cases may contribute to our understanding of the pathogenic role of ATA and ACA in SSc. However, the exceptionally rare prevalence of cases expressing both autoantibodies has hampered such evaluation. The objective of this study was to take advantage of the large EUSTAR database and to identify multiple SSc patients with coincident ATA and ACA, allowing a meaningful comparison of the clinical features of patients harbouring both antibody specificities (‘double-positives’) with those of patients having only one specificity (‘single-positives’).

In a first step, the EUSTAR database was assessed for patients who were registered as being positive for both ATA and ACA. Second, in order to circumvent the problem of the variety in antibody detection methods used at the different participating centres, serum samples of these patients were re-analysed centrally. Finally, clinical characteristics of patients with SSc who were unequivocally positive for both ATA and ACA were compared to those of patients having only ATA or ACA.

**Materials and methods**

**The EUSTAR database and autoantibody status**

The structure and organisation of the EUSTAR database have been described previously (11). For each individual SSc patient entered in the EUSTAR database after informed written consent, a minimal essential data set (MEDS) is recorded that covers demographic data, disease duration, organ involvement, and basic laboratory data. The presence or absence of ANA, ACA, and ATA in a patient’s serum is documented in the MEDS via simple yes/no answers with positivity being defined as “above the cut-off value of the test employed by the examining laboratory”.

**Patient samples**

This study focused on patients who were registered in the EUSTAR database up to November 2007, fulfilled the ACR-criteria for SSc (12), and were documented by the respective centre as being positive for both ACA and ATA on at least one of their annual visits. The unique patient numbers of these cases were identified and the corresponding EUSTAR centres were requested to confirm the autoantibody status and medical data documented in the MEDS. Centres were also asked for more detailed information, i.e. the antibody detection method used, the assay manufacturer, the cut-offs employed, and the original results obtained. In addition, centres were asked for availability of stored serum samples and requested to send an aliquot of the sera to the reference laboratory of this study (Medical Immunology, University Hospital Basel, Switzerland) for central re-analysis. All serum samples were stored at -80°C until testing.

**Autoantibody measurement**

ANA were determined by indirect immunofluorescence (IIF) using HEp-2 cells (Inova Diagnostics, San Diego, CA) as substrate, and applying serial serum dilutions of 1:40 to 1:5120. Titres of 1:80 or more were regarded as positive. For each sample, the end-point titre and IIF staining pattern were independently recorded by three experienced observers. A fine speckled nucleolar and nucleolar staining pattern of interphase cells accompanied by a dense staining of the metaphase plate in mitotic cells was regarded indicative for ATA, whereas a pattern of discrete dots in interphase and metaphase nuclei was regarded as characteristic for ACA (17, 18). This implies theoretically that the IIF pattern triggered by ATA may mask the ACA pattern when occurring in one sample. Therefore, in cases where the ATA-like pattern appeared dominant, the presence of concomitant ACA was evaluated by competitive inhibition assays. Briefly, serum samples were pre-incubated with soluble topoisomerase I (10 ug/ml) for 1 hour at room temperature using a 1:1 mixture of both native antigen purified from bovine thymus (Inova Diagnostics) and recombinant human topoisomerase I protein (Phadia, Freiburg, Germany) before being tested on HEp-2 slides. In addition to the HEp-2 cell IIF analyses, ATA and ACA were specifically detected and quantified by enzyme immunoassays (EIAs) and by line immunoblots assays (LIAs). These two methods currently represent widely used techniques for the detection of ATA and ACA in European clinical laboratory practice. For the EIAs, commercial ELISA kits (Inova Diagnostics) and an automated fluorescence EIA (FEIA) system (EliA™; Phadia) were used. The centromere antigen used in both systems is recombinant CENP-B protein, but the ELISA additionally contains recombinant CENP-A. The topoisomerase I antigen is purified from bovine thymus in the ELISA for ATA detection, but is of recombinant origin in the FEIA. Assay procedures and cut-off values (20 U for ELISA, and 10 U/ml for FEIA) were followed exactly according to the instructions.
of the respective manufacturer. For the LIAs, membrane strips coated with recombinant CENP-B and topoisomerase I (Euroimmun, Lübeck, Germany), or with recombinant CENP-B and native topoisomerase I (Innogenetics, Brussels, Belgium) were used. LIA were performed, evaluated and compared with controls according to the manufacturer’s instructions. All serum samples were additionally tested for the presence of anti-RNA Polymerase III antibodies (ARA) by an ELISA assay (Inova Diagnostics), and for antibodies against SS-A (SS-A60 and Ro52), SS-B, Sm (SmD), U1-RNP, and Jo-1 by FEIA (Phadia).

Data analyses
SSc presentations were analysed cross-sectionally for differences in demographic and clinical features. For each patient the available data of the last visit was used. Group means and medians were compared by t-tests and Wilcoxon Rank sum test, respectively. Group percentages were compared by chi-square or Fisher’s exact test. The dataset was analysed using Stata version 11.0 (StataCorp Inc., College Station, TX, US). Each double-positive case was compared with five gender- and centre-matched controls harbouring either ACA or ATA alone.

Results
As of 31st of October 2007, a total of 4,687 SSc patients from 168 centres in 25 European and 19 non-European countries met the study criteria. Almost all of these subjects were Caucasian. A total of 1,681 (35.9%) patients were documented in the database as being positive for ATA, whereas 1,546 (32.9%) patients were positive for ACA. A total of 1,500 (32.0%) patients were documented as being negative for both ATA and ACA. Finally, there were 29 (0.6%) patients who had been scored positive for both ATA and ACA by the respective investigator. All of the patients who were claimed to show positivity for both ATA and ACA were also scored positive for ANA. Serum samples from 14 of these patients were available for central analysis. The 15 patients of whom no serum was available were excluded from the data analysis.

Coexistence of ATA and ACA in patients with SSc
Of the 14 patients in whom both ACA and ATA had been detected by the documenting centre, sera from 8 patients gave an unequivocal positive result by both IIF and ELISAs upon central reassay. These 8 sera clearly showed a staining pattern by IIF on HEp-2 substrate cells consistent with the presence of ATA (titers ranging from 1:160 to 1:1280). In the remaining 3 sera, the typical ACA pattern became only visible after preabsorption of the ATA.

Fig. 1. ANA patterns revealed by IIF using HEp-2 substrate cells. (A) Serum from a SSc patient positive for ACA revealing a typical pattern of discrete dots in interphase and metaphase. (B) A patient with ATA demonstrating the characteristic fine speckled nucleoplasmic and nucleolar staining pattern of interphase cells accompanied by a dense staining of the metaphase plate in mitotic cells. (C) A combined ACA and ATA pattern observed with the serum of a patient double-positive for ACA and ATA. (D) A dominant ATA pattern masking the ACA pattern in the serum of a patient double-positive for high levels of ATA and relatively low levels of ACA. Note that the typical ACA pattern is not readily visible with this patient’s serum. (E) In the latter case, the ACA stain became only visible after preabsorption of the ATA.
reactivity by preincubation of serum samples with the topoisomerase I antigen (Fig. 1D-E). Interestingly, these sera had relatively low titers of ACA (range 1:80–1:320) and relatively high ATA titers (range 1:640 to 1:1280), indicating that ATA, when present in relatively high concentrations, masks the ACA staining pattern in IIF testing. In serum samples of two patients, only ACA could be detected, but these samples were taken at different time points than the ones at which both ACA and ATA were observed by the respective centres. As ATA titers may change in time (19, 20), ATA could have theoretically been disappeared in these cases. However, because the coexistence of ACA and ATA could no longer be unequivocally confirmed, the two patients were excluded from subsequent data analysis.

The 4 remaining patients that were registered positive for both ACA and ATA in the EUSTAR database were classified as false positive for one of the two antibodies after central re-analysis. In 2 of these 4 cases, ACA were unequivocally detectable, but the sera did not have the typical ATA staining pattern by IIF, and did not show reactivity to topoisomerase I by the EIAs that employ recombinant protein (i.e. FEIA (Phadia) and LIA (Euroimmun)). However, the sera demonstrated positive reactivity for topoisomerase I when EIAs were used that incorporate native anti-SS-A/Ro antibodies after central re-analysis. In 2 of these cases, the sera reacted with the SS-A/Ro antigen purified from natural sources (i.e. ELISA (Inova) and LIA (Innogenetics)). Interestingly, the clinical centres that had registered the serum samples of these two patients as ACA and ATA double-positive, reported having used such native topoisomerase I EIA for ATA detection. Based on these results the two cases were considered ACA, but not ATA positive and were excluded from this analysis. Likewise, one additional case was excluded, because its ATA positivity appeared to have been based on a borderline positive EIA result (i.e. 11 U/ml by EliA™ (Phadia); cut-off 10 U/ml) by the clinical centre. As expected, the presence of ATA could not be unequivocally confirmed upon re-analysis of the serum by any of the detection methods. Finally, one case was unequivocally ATA positive, but ACA could not be detected by any of the used methods. It is of interest to note that the serum of this patient did not have the classical ACA staining pattern by IIF, but produced a discrete speckled pattern on interphase HEp-2 cells without concomitant staining of mitotic cells. We concluded that this staining pattern, also known as multiple nuclear dots pattern (21), had mistakenly been interpreted by the centre as ACA pattern, and we excluded this case from further analysis.

Prevalence of other nuclear autoantibodies in SSc patients with coexisting ATA and ACA

The presence of autoantibodies against SS-A/Ro, SS-B/La, SmD, U1-RNP, Jo-1, and RNA polymerase III in the serum of all 8 SSc patients with coexisting ACA and ATA is detailed for each individual in Table I. Regarding the three major SSc-specific autoantibodies (1-3), none of the patients who were positive for both ATA and ACA had detectable levels of ARA. Four patients positive for ACA and ATA were also positive for anti-SS-A/Ro antibodies. Sera of two of these patients reacted with the Ro60 antigen only, whereas serum of one patient showed antibodies against Ro60 only, and one patient had antibodies to both SS-A/Ro antigens. No significant levels of antibodies against SS-B, Sm (SmD), U1-RNP, and Jo-1 could be detected in any of the ATA and ACA double-positive patients.

Clinical associations of coexistingATA and ACA in patients with SSc

Clinical and serological data from the last available EUSTAR visit of the eight SSc patients harbouring both serum antibodies are shown in Table I. The eight double-positive patients were on average 53.2 years of age at their last visit, similar to the ATA single-positive and ACA single-positive control subjects (Table II). Most of them (87.5%) were female, as was the case -as a consequence of gender matching- in the ACA and ATA single-positive control groups. The frequency of dcSSc in the ACA and ATA double-positive group (62.5%) was not significantly different from the ATA single-positive controls, but significantly higher than in the ACA single-positive control group. In the double-positive group, the Raynaud’s phenomenon had begun at a slightly but statistically not significant younger age than in the other groups, but the age at the first non-Raynaud’s feature of SSc and the time interval

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Table I. Clinical features of the eight SSc patients with coexisting ATA and ACA. The age at last follow-up is given in years. Lung fibrosis refers to any interstitial lung involvement seen on conventional chest x-ray.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Gender</th>
<th>Age</th>
<th>Age at RO</th>
<th>Age at Non-RO</th>
<th>SSc subset</th>
<th>Max mRSS</th>
<th>Last mRSS</th>
<th>Renal crisis</th>
<th>PH</th>
<th>Lung fibrosis</th>
<th>DLCO</th>
<th>Alive</th>
<th>Other ANA</th>
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<tr>
<td>1</td>
<td>F</td>
<td>67</td>
<td>47</td>
<td>51</td>
<td>Limited</td>
<td>4</td>
<td>4</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>74</td>
<td>Yes</td>
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</tr>
<tr>
<td>2</td>
<td>F</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>Limited</td>
<td>10</td>
<td>10</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>64</td>
<td>Yes</td>
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</tr>
<tr>
<td>3</td>
<td>F</td>
<td>54</td>
<td>36</td>
<td>37</td>
<td>Diffuse</td>
<td>7</td>
<td>5</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>61</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>66</td>
<td>57</td>
<td>59</td>
<td>Limited</td>
<td>5</td>
<td>5</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>90</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>33</td>
<td>7</td>
<td>13</td>
<td>Diffuse</td>
<td>20</td>
<td>20</td>
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<td>No</td>
<td>Yes</td>
<td>40</td>
<td>Yes</td>
<td>Ro60, Ro52</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>73</td>
<td>15</td>
<td>69</td>
<td>Diffuse</td>
<td>22</td>
<td>22</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>37</td>
<td>Yes</td>
<td>Ro52</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
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<td>25</td>
<td>26</td>
<td>Diffuse</td>
<td>18</td>
<td>18</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>94</td>
<td>Yes</td>
<td>Ro60</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>43</td>
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<td>35</td>
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<td>30</td>
<td>14</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>115</td>
<td>Yes</td>
<td>–</td>
</tr>
</tbody>
</table>

RO: Raynaud’s onset; max mRSS: maximal modified Rodnan skin score; last mRSS: modified Rodnan skin score at last follow-up; DU: digital ulcer; PH: pulmonary hypertension; DLCO: diffusion capacity of the lung for carbon monoxide (% of normal).
between the onset of Raynaud’s phenomenon and the first non-Raynaud’s feature of SSc was similar between all groups. None of the double-positive patients had left-ventricular heart failure, pulmonary hypertension (PH), proteinuria and none had experienced a renal crisis.

We also compared other clinical data of the double-positive SSc patients with those of patients that were documented as having either ACA or ATA alone (Table II). As known from the clinical comparison between ACA and ATA single-positives reported previously (11), the current study confirmed that the prevalence of a number of disease features differs between ATA single-positive and ACA single-positive SSc patients upon statistical testing. Most notably, musculoskeletal, cardiopulmonary and cutaneous manifestations were more frequent in the ATA single-positives compared with the ACA-single positives. Considering the ATA and ACA double-positive cases, the prevalence of all organ manifestations was statistically similar to those in the ATA single-positives although there was a trend for more severe or prevalent involvement of the musculoskeletal system and of the pulmonary parenchyma in terms of lung fibrosis.

Taken together, the data confirm that positivity for either ATA or ACA is associated with significant differences in the prevalence of life-threatening organ complications of the pulmonary vasculature and parenchyma. Although patients with coexisting ATA and ACA tend to have more severe disease in some organ systems, the data do not demonstrate that double-positive patients are truly clinically different from ATA single-positive patients.

**Discussion**

We have evaluated the prevalence of co-existing ATA and ACA in patients with SSc and found the simultaneous occurrence of ATA and ACA, two antibodies which are commonly regarded as mutually exclusive. In our rigorous analysis, the combined prevalence is low and perhaps even lower than that reported in smaller samples (14-16, 22). This study illustrates that the detection of ACA and ATA is not without problems. Due to the lack of a diagnostic gold standard, it is difficult to define when a specific test truly detects the antibody and classifies a sample as positive. First, tests may be positive due to unspecific reactivity with non-target epitopes contaminating the antigen extract, as is documented in this study. Second, although ANA immunofluorescence may be considered the standard method for ANA detection, it is however not specific because IIF patterns may be wrongly interpreted. Even the recognition of the ACA-associated pattern which is considered to be the most specific staining, is not without difficulty. As was shown in this study, one pattern may mask another pattern in the presence of two or more autoantibodies, especially when different titres are present. Third, the use of a cut-off value to discriminate between positive versus negative clearly represents an oversimplification. This is shown by the borderline positive result renders an improved post-test probability (23). Another aspect of our work was that double-positive patients did have a high prevalence of Ro antibodies (anti-Ro52 and anti-Ro60) as described previously (24, 25), but did not harbour ARA as an SSc-specific antibody.
indicates that there is no promiscuity of the antibody response. Importantly, our study is the first large multicentre prospective cohort study to systematically compare the clinical features of patients with both antibodies to those harbouring either antibody alone, a method which is likely to reduce centre-related selection bias as a potential confounder (26). The main finding is that all clinical features of patients harbouring both serum autoantibodies are not statistically dissimilar from those harbouring solely ATA. Interestingly, none of the double-positive patients had PH. These observations however must be interpreted with caution due to the lack of statistical power. Furthermore, the differences between the single-positive groups, although confirmed in larger cohorts (11), lack adjustment for multiple statistical testing and therefore should be regarded as descriptive.

The absence of a clear additive or synergistic effect of double antibody positivity on visceral involvement and disease evolution may be used as an argument against a direct involvement of ATA and ACA in the clinical phenotype of SSC. This notion is also supported by the lack of an association between the serum levels of SSC-specific antibodies and clinical activity, although limited data have suggested some association of ATA levels with the extent of organ involvement and the difficult to measure disease activity (19, 27, 28). Our data therefore do not support serial testing for these antibodies in accord with clinical practise (3). Other arguments against a direct pathogenic relevance of ACA and ATA in the SSC disease process are given by the fact that these antibodies may be detectable long term in healthy individuals without subsequent disease development (3, 5, 22), and the observation that they can remain detectable after autologous stem cell transplantation despite complete disease remission (29).

Finally, the finding of a low prevalence of double-positives which is lower than the expected probability of coincidence (13) does not support the postulate that environmental factors contribute to the ANAs in scleroderma. This notion is also suggested by the low prevalence of SSC-specific ANA in first degree relatives and spouses of affected patients (30). In contrast to environmental factors, strong correlations with SSC-specific autoantibodies have recently been observed in multiple studies for certain HLA-class II alleles (31-33). Highly associated allelic combinations in the HLA-DQB1 locus have been demonstrated with ACA and in the HLA-DPA1/B1 loci with ATA (33), suggesting that the alleles may drive different immunological processes involved in the production of these different autoantibodies. For this reason it would have been interesting to evaluate the HLA genotype of the ATA-ACA double positive patients in our study, but genetic data were not collected in this EUSTAR project.

It should be noted that we have restricted our study to SSC patients diagnosed according to the ACR criteria and thus have excluded patients who have limited forms of SSC (34). This bias however is not likely to diminish the power by which clinical differences between double-positive and ATA single-positive patients may have been detected. Nevertheless, our analysis is limited by the observational nature of the cohort, the large confidence intervals of the clinical characteristics in the double-positive group due to the small number of patients.

In summary, our prospective analysis suggests a low prevalence of coexisting ATA and ACA in SSC after exclusion of false positives. The absence of a clearly discernible synergistic or additive effect of antibody status with respect to disease evolution, as well as cutaneous or visceral involvement does not support a direct pathogenic involvement of these antibodies in the established disease process, although such effect cannot be completely excluded due to limited statistical power.

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References

13. KIKUCHI M, INAGAKI T: Bibliographical study of the concurrent existence of antici-


