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Anti-RNA polymerase III antibodies: A marker of systemic sclerosis with rapid onset and skin thickening progression

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ABSTRACT

Anti-RNA polymerase III antibodies (ARA) are a specific marker for Systemic Sclerosis (SSc), associated to severe disease with major organ and diffuse cutaneous involvement.
In our series, ARA were found in 19 of 216 sera, in 15 cases as isolated antibodies' specificity, with a statistically negative association with other SSc-specific autoantibodies (p: 0.00003). The prevalence of ARA among 73 anticentromere and anti-topoisomerase I (topo I) negative sera, was 20.5%.
Patients with isolated ARA had more rapid disease onset, defined as the interval from the appearance of Raynaud's phenomenon to the first symptom other than Raynaud's, than patients with isolated anti-topo I antibodies (median: 2 months vs 13 months; p: 0.0013). A rapid onset of SSc (within 6 months from Raynaud's phenomenon onset) was found in all patients with isolated ARA and only in 34% of those with anti-topo I ($p<0.00001$). Moreover, the skin thickening in the first months after SSc onset was faster in the ARA group ($p<0.0001$). Nevertheless, the rates of internal organ involvement and of survival rates were similar between the two groups.
Our experience therefore suggests that ARA are a marker of very rapid onset of disease and skin thickening progression in SSc.

Contents

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1. Introduction

Systemic Sclerosis (SSc) is characterised by the occurrence of anti-nuclear antibodies (ANA) in virtually all the patients [1], mainly represented by four mutually exclusive specificities, namely anti-topoisomerase I (topo-I), anti-centromere, anti-nucleolar or anti-RNA polymerase III antibodies (ARA) [2–4], while anti-PM-Scl, U1-RNP and anti-Ku are detected in overlap syndromes [5–9]. ARA, considered highly specific for SSc, are found in 5–23% of patients [2,3,10–13], according to race differences or geographic factors [14–17]. They were originally proposed as markers of severe disease [3,10], and have been associated to predictors of poor prognosis, such as the diffuse cutaneous subset [3,5,10–12,18,19] and major organ involvement, including renal crisis [10–12,20] and right heart disease [21]. However, there are no information on the rapidity of disease onset and progression in SSc patients with ARA.

The gold-standard assay used for the detection of ARA is the immunoprecipitation, but an ELISA method using the recombinant major antigenic epitope has been recently validated [15,21–26], which allows the determination of ARA in routine laboratory, and more extensive clinical studies.

We have therefore used the ELISA test to evaluate ARA in 216 patients with SSc from one single Rheumatology Centre, with the main aim to assess the association of these antibodies with the rapidity of disease onset and evolution.

2. Patients and methods

2.1. Patients

Clinical and laboratory data were identified from medical records of 440 consecutive patients with SSc followed at our institution since 1983. More than 99% of patients were Caucasians of Italian ancestry. SSc was classified according to Le Roy criteria [27] to define the different clinical subsets. A rapid onset of SSc was defined when the time elapsed between Raynaud's phenomenon onset and the first SSc symptom other than Raynaud's was 6 months or less.

Patients with SSc were routinely evaluated according to standard procedures, analogous to those recommended by a consensus conference for the identification of core set of variables for SSc studies [28]. Skin thickness was measured using the modified Rodnan Skin Score (RSS) [29]. Lung function test with evaluation of Forced Vital Capacity (FVC) and diffusion lung capacity for carbon monoxide (DLCO), were performed at the time of diagnosis, and at least yearly in the first 5 years of follow-up. Results were expressed as percentages of predicted values based on age, sex, and height. Normal values were calculated by reference standard provided by the European Coal & Steel Community [30,31]. Patients with worsening of dyspnoea, or decrease of FVC and/or DLCO, or chest X-ray abnormalities were further evaluated with high resolution computed tomography (HRCT), and, in most cases, broncho-alveolar lavage (BAL) analysis. Active interstitial lung disease (ILD) was defined on the basis of ground glass opacities documented by HRCT and/or BAL findings. Pulmonary arterial hypertension (PAH) was defined using Doppler echocardiography, performed at least yearly, as screening test, and right heart catheterization as gold standard [28]. Scleroderma renal crisis was defined according to standard procedures [28]. PAH secondary to severe ILD was not considered as a separated outcome.

2.2. Methods

The serum of all 440 SSc patients was analysed by indirect immunofluorescence test (IIF) on HEp-2 cells (BioRad, Hercules, CA, USA) and considered positive at titre $\geq$1:80, and by counterimmunoelectrophoresis (CIE), using rabbit thymus and human or porcine spleen extracts as substrates [32–34].

Two hundred and sixteen available sera were also tested for ARA, using commercial ELISA with recombinant antigen (INOVA, San Diego, USA). The cut-off value, defined by the manufacturer as 20 U/ml, was recalculated using 78 sera of patients affected by different connective tissue diseases (CTDs), namely Systemic Lupus Erythematosus (30 sera), primary anti-phospholipid syndrome (15 sera), undifferentiated connective tissue disease (10 sera) and rheumatoid arthritis (23 sera). The new cut-off was calculated as the mean value plus 5SD and resulted to be 14.7 U/ml.

2.3. Statistical analysis

All the variables were expressed as mean values and SD or by median values and interquartile ranges (IQR). The parameters were compared by X^2 or Fisher tests, when indicated. Student's t or Mann–Whitney tests were used to perform comparison between groups. Survival rate was computed by Kaplan–Meier analysis and the difference between survival curves by Mantel–Cox (log-rank) test. Statistical significance was accepted at $p<0.05$.

3. Results

3.1. Laboratory data

In our cohort of 440 patients affected by SSc, anti-centromere and anti-topo I antibodies were positive in 207 (47%) and 97 sera (22%), respectively, while 136 sera (31%) resulted negative for both. ARA were searched in 216 available sera, that did not reflect the distribution of antibodies in the whole population: in fact, 62 of these were positive for anti-centromere (relatively under-represented), 81 for anti-topo I and 73 were negative both for anti-centromere and anti-topo I. ARA positivity was found in 1 of 62 anti-centromere positive sera, 3 of 81 anti-topo I positive sera, and as an isolated specificity in 15 out of 73 sera (20.5%) that were negative both for anti-centromere and anti-topo I. There was a significant negative association between the presence of ARA and that of other SSc-specific autoantibodies ($p=0.000032$).

In Fig. 1 the mean values of ARA titer in SSc patients and controls are reported. Comparing sera with isolated ARA and 4 sera in which ARA were associated with other ANA specificities, we found a significantly higher ARA titre in the first group (mean: 168.4 U/ml, SD: 70 U/ml vs mean: 35.7 U/ml, SD: 11.8 U/ml) ($p=0.0019$).

All 15 ARA positive sera without other specificities showed speckled IIF pattern with bright dots; in 4 of them, this was associated with punctuate nucleolar staining. No isolated nucleolar pattern was detected.