Concise Report

A longitudinal study of anti-RNA polymerase III antibody levels in systemic sclerosis

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Objectives. Anti-RNA-polymerase antibodies (ARAs) are associated with the diffuse cutaneous subset of SSc (dcSSc) and particularly with scleroderma renal crisis (SRC). We analysed serial ARA levels and explored the relationship with clinical features and disease outcome.

Methods. A commercially available ELISA method with a recombinant peptide of RNA polymerase III was used and ARA levels were measured in a well-characterized cohort of SSc cases.

Results. ARA levels were measured in 64 SSc patients. Of them, 78% (n = 50) were females and 92% (n = 59) had dcSSc, 39% (n = 25) had SRC, 20% (n = 13) had pulmonary fibrosis (PF), 9% (n = 6) had pulmonary arterial hypertension and 3% (n = 2) had cardiac involvement. There was considerable inter- and intra-patient variability in ARA levels (11–210 U/ml). There was no correlation between absolute ARA levels and outcome. There was a moderate correlation between time to peak ARA level and development of significant PF (Pearson correlation = 0.689, P = 0.034), but no correlation between peak ARA levels and onset of SRC. ARA levels change correlated with change in skin score (correlation coefficient within subjects = 0.236, P = 0.011).

Conclusions. The pathogenic significance of ARA is unclear. Despite the very strong association of ARA with SRC, we could not show the clinically significant association between absolute levels of antibody and development of internal organ complications, which makes repeated measurements of ARA levels unnecessary. However, changes in ARA level over time occur and may reflect changes in skin score.

Key words: Systemic sclerosis, Scleroderma renal crisis, Anti-RNA polymerase antibody, Antibodies.

Introduction

ANAs in CTDs often target protein antigens of fundamental biological importance. Antibodies against RNA-polymerases (RNAP) I, II and III, for example, have been reported to occur in a variety of CTDs. Anti-RNAP sera from patients can be divided into two groups: those that precipitate RNAP III (usually in association with RNAP I or with RNAP I and II) and those that precipitate only the phosphorylated form of RNAP II. While the latter can be found in sera from patients with SLE as well as SSc, anti-RNA-polymerase antibodies (ARA) I and III are seen almost exclusively in SSc patients (reported specificity of 98–100%) [1–4]. This defines them as one of the three hallmark, generally mutually exclusive, SSc-specific auto-antibodies together with ACAs and anti-topoisomerase I antibodies (ATAs).

The prevalence of ARA III among SSc patients varies in different SSc cohorts. In the UK, two different SSc centres have reported a prevalence of ARA III of ~12% [5, 6], and similar frequency (10%) was observed among US patients by Chang et al. [1]. Much lower frequency has been reported in Korean (3.4%) and Italian (7.8%) patients [4, 7], whereas Okano et al. [3] found higher prevalence in a US centre (23%). The presence of ARA III has been strongly associated with the diffuse cutaneous subset of SSc (dcSSc) and with SSc-related renal disease (including scleroderma renal crisis, SRC). Among the ARA-positive SSc patients, dcSSc has been observed in 67–93%, whereas SSc-related renal involvement has been reported in 28–43% of the cases [1, 4, 5, 8, 9]. In addition, among a group of 95 patients from a single centre who developed SRC, 59% were ARA positive [10].

Over recent years, several reports have shown that ELISA can be used to identify ARA with very high sensitivity (91–96%) and specificity of 99% [2, 11]. High baseline ARA levels are associated with more severe skin disease but do not predict organ-based complications [2]. In a small group of patients, Kuwana et al. [2] measured ARA levels over time and noted that levels fluctuate and may reflect the changes in skin score.

We analysed serial samples from a large well-defined group of SSc patients to investigate change in ARA III levels over time and to look for any relationship between ARA III titres and clinical presentation or disease outcome.

Patients and methods

Patients

The study was approved by our institutional local research ethics committee (Royal Free and Medical School Local Research Ethics Committee), and the samples were provided after obtaining informed consent. From 1018 SSc patients’ sera, which had previously been tested for ANA by IIF on HEp-2 cell substrate, we identified 209 that had produced ANA patterns characteristic of ARA [11]. The presence of antibody was confirmed by the ELISA method in the sera from 150/157 patients in whom the presence of ARA was initially detected by immunoprecipitation and 52 patients identified only by IIF. In this study, we included the 33 patients who had serum samples taken on at least three occasions during the disease course; 21 of those had the first ARA level measured within 1 year of disease onset. An additional 31 subjects with ARA levels measured within 12 months of disease onset, but no subsequent samples available, were included for the cross-sectional analysis. All 64 subjects fulfilled the ACR preliminary criteria for the classification of SSc and were recruited from a single centre. Demographic and clinical data were collected...
through our clinical database and review of patient records. Clinically significant pulmonary fibrosis (PF) was defined as forced vital capacity (FVC) or carbon monoxide diffusing capacity of the lung (DLCO) <55% of predicted or a 15% decline from baseline in FVC or DLCO, with fibrosis confirmed on high-resolution CT. Pulmonary arterial hypertension (PAH) was considered when the pulmonary arterial pressure was >25 mmHg confirmed by right heart catheterization. Cardiac involvement was defined as haemodynamically significant cardiac arrhythmia, pericardial effusion or congestive heart failure, and renal involvement was defined as a documented SRC as defined by new-onset systemic hypertension >150/85 mmHg and a documented decrease in estimated glomerular filtration rate ≥30%.

**ELISA**

A commercially available ELISA method (Quantalite RNAP III, INOVA Diagnostics, San Diego, CA, USA) with a recombinant immunodominant fragment of RNAP III antigen was used, according to the manufacturer’s instructions [12]. We have previously verified that this ELISA robustly predicts positive results for ARA III by immunoprecipitation [11]. Sera to be tested were stored frozen at -20°C until assayed and sera from individual patients were all tested in the same assay.

**Statistical analysis**

Linear and logistic regression models were used as appropriate to assess the predictive value of baseline antibody levels and their change over the first 3 years of disease. Pearson’s correlation was used to look for relationship between time to peak antibody levels and time to onset of organ complications. Analysis of covariance as described by Bland and Altman [13] was used to assess association between changes in ARA levels and other clinical parameters. MINITAB 14 and STATATA 10 statistical packages were used for the statistical calculations.

**Results**

ARA levels were measured in 64 SSc patients. Fifty-two of these had ARA levels measured within 12 months of disease onset. Thirty-three of the patients had serial ARA levels measured between 3 and 19 times (mean ± S.D. = 7.4 ± 4.0) over a follow-up period of 21–142 months (mean ± S.D. = 63 ± 39); 21 of these had first ARA level measured within 12 months of disease onset. Overall, 78% (n = 50) were females and 92% (n = 59) had dcSSc. Six patients (9%) had overlap syndromes—five with PM/DM and one with SLE. SRC was the most frequent organ complication, seen in 39% (n = 25) of the subjects. Other internal organ problems included PF in 20% (n = 13), PAH in 9% (n = 6) and cardiac involvement in 3% (n = 2) of the patients.

There was considerable inter- and intra-patient variability in ARA levels (11–210 U/ml) (Fig. 1). For the 52 subjects for whom the first antibody level was measured within 1 year of disease onset, the levels varied between 39 and 210 U/ml (mean 113 U/ml). There was no association between baseline ARA levels and internal organ complications or severity of skin disease (peak skin score). For 21 of these subjects, serial ARA levels were available and change in antibody levels over 36 months from first assessment were calculated. There was an increase of between 10 and 59 U/ml (8–107% increase from baseline levels) in 10 patients, while the remaining 11 showed a decrease of between 9 and 102 U/ml (9–86% decrease from baseline levels). We found no association between change in ARA levels over the first 36 months of disease and organ complications or overall severity of skin disease. In addition, cumulative antibody levels over the first 3 years of follow-up were calculated and similarly these showed no correlation with clinical presentation.

ARA levels were measured within 2 months of development of clinically significant internal organ involvement in 13/25 patients with SRC (mean 106, range 55–169 U/ml) and 7/13 patients with PF (mean 86, range 15–113 U/ml). There was no association between absolute ARA levels and onset of severe internal organ problems. Time to peak antibody level was calculated and this varied between 3 and 188 months (median 25, inter-quartile range 13.5–45.5 months). There was a moderate correlation between time to peak ARA level and development of clinically significant PF (Pearson correlation = 0.669, P = 0.034), but no correlation between peak ARA levels and onset of renal crisis.

Change in serial ARA levels was compared with change in other clinical parameters. Serial skin scores were available for all the 33 patients with serial ARA levels. We found weak correlation between change in modified Rodnan skin score (mRSS) and change in ARA levels (correlation coefficient within subjects = 0.236, P = 0.011) (Fig. 2). Similarly, serial FVC and TLCO measurements were available for 29/33 subjects. There was also a weak negative correlation between lung function change over time and ARA levels (correlation coefficient within subjects = -0.221). There was no correlation between change in inflammatory markers and ARA levels.

Twenty-six patients had received immunosuppressive treatment during the assessment period and for 20 of them had antibody levels available when on and off treatment. Comparison between ARA levels in these patients while on immunosuppression (mean ± S.D. = 85 ± 41 U/ml) showed no significant difference from ARA levels when on no immunosuppressive treatment (mean ± S.D. = 93 ± 38 U/ml, P = 0.170). For 19 of the 22 patients who were treated with mycophenolate mofetil (MMF), ARA levels were available when on MMF and when on other immunosuppressive treatments or on no medication. We found a trend towards lower ARA levels with MMF treatment (mean ± S.D. = 85 ± 36 U/ml) compared with no treatment or therapy with other immunosuppressants (mean ± S.D. = 95 ± 34 U/ml, P = 0.07).

There were seven deaths over the follow-up period: three due to PAH, two due to PF and two due to ovarian cancer. Survival at 3 and 5 years was 95 and 93%, respectively. There was no association between mortality and ARA levels.

**Discussion**

In this study, we analysed ARA levels in 64 scleroderma patients, 33 of those with serial antibody measurements. We did not show any association between absolute ARA levels and clinical presentation or disease outcome. Although not as strongly associated with PF as ATA in cross sectional studies, there was a moderate correlation between time to peak ARA level and

![Figure 1. Variation in anti-RNA polymerase antibody level during follow-up of early scleroderma. Serial anti-RNA polymerase antibody level in 21 patients who had first antibody level measured within 12 months of disease onset, defined by the first non-Raynaud's manifestation of SSCs.](image)
development of clinically significant PF. We also confirm the previous observation, from a smaller study, that changes in mRSS reflect changes in ARA levels.

It is still unclear what the role of ANA is in the pathogenesis of SSc, although a growing body of evidence points towards a more active role in the inflammatory process. Anti-fibroblast antibodies have been shown to induce a pro-inflammatory fibroblast phenotype [14] and their presence has been strongly associated with ATA positivity, PF and increased mortality [15]. Strong correlation has also been shown to exist between ATA levels and skin score in SSc patients [16], and disappearance of ATA during follow-up predicts milder disease and better survival [17].

Despite the strong association between ARA positivity and specific SSc phenotype, little is known about the prognostic value of ARA titres. In a group of 90 ARA-positive SSc patients, ARA levels were measured within 12 months of first SSc symptom using ELISA [2]. The group with high ARA levels had higher maximum skin score \( (P = 0.002) \), higher frequency of tendon friction rubs \( (P = 0.002) \) and larger proportion of dcSSc patients \( (P = 0.049) \) compared with the group with low ARA levels, although no difference was found in terms of organ-based complications. We confirm some of their findings, demonstrating no correlation between baseline ARA levels and internal organ involvement, but we did not find any association between absolute ARA levels at baseline and maximum skin score or disease subset.

Interestingly, we did find statistically significant correlation between change in antibody levels and change in mRSS in a large proportion of our patients with serial ARA measurements. These results are also similar to those of Kuwana et al., [2] who measured serial ARA levels in six patients and observed close correlation between changes in antibody levels and changes in skin score over time in four of the subjects. In those series, two of the patients developed SRC and one severe PF after persistent increase in ARA levels. Unfortunately, for the majority of the patients from our cohort who developed SRC, this was the presenting symptom; therefore, we were unable to assess ARA levels before the event. After the SRC, ARA levels did not follow any specific pattern, with approximately half the cases showing an increase in levels and a similar proportion decreasing over time.

We demonstrated a trend towards lower ARA levels in patients receiving MMF compared with those on other immunosuppressive treatment or on no treatment. This trend may be important when considering therapeutic approaches that target B lymphocytes. It is noteworthy that preliminary reports have not shown major benefit from such strategies in scleroderma, although robust data are not yet available [18].

This is the first study that specifically looks for patterns in ARA level change over time in a large well-described SSc cohort. Our primary goal was to look for correlation between antibody levels and clinical presentation rather than to establish the prevalence of organ disease among anti-RNAP-positive patients. As we included only those subjects who had serial antibody levels available or those who had their first antibody level measured within 12 months of disease onset, as well as all patients identified in a tertiary referral centre, it is likely that the cohort was biased towards more severe disease. Nevertheless, it was comparable with other ARA-positive SSc cohorts in terms of disease subset (92% with dcSSc), and SRC frequency (39%). However, a weakness is the retrospective design, as a result of which ARA levels at the time of organ complication development were not available for the whole group of patients, which may have led to our failure to show significant association between absolute ARA levels and clinical presentation.

The apparent absence of clinically significant association between absolute level of antibody and development of internal organ complications in scleroderma suggests that serial measurement of ARA levels is of limited clinical usefulness. Nevertheless, changes in ARA level may reflect changes in skin score and, based on our results, we believe that larger prospective studies are needed to elucidate whether ARA can be used to monitor treatment response or disease progression.
Rheumatology key messages

- Change in ARA levels within patients correlates with change in skin score.
- No clear association exists between absolute ARA levels and clinical features or outcome in SSc.

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