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Leiden
The Netherlands

Systemic sclerosis: are anti-nuclear antibodies our guiding stars?

Boonstra, M.

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Association of anti-topoisomerase I antibodies of the IgM Isotype with disease progression in anti-topoisomerase I-positive Systemic Sclerosis

M. Boonstra, J.A. Bakker, A. Grummels, M.K. Ninaber, N. Ajmone Marsan, C.M. Wortel,
T.W.J. Huizinga, S. Jordan, A.M. Hoffman-Vold, O. Distler, R.E.M. Toes, H.U. Scherer,
J.K. de Vries-Bouwstra

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Abstract

Background

Anti-topoisomerase I auto-antibodies (ATA) in systemic sclerosis (SSc) are associated with diffuse skin involvement and interstitial lung fibrosis. Thus far, however, the relations between the ATA response and disease course have not yet been fully evaluated.

Objectives

To gain insight into the relation between characteristics of the ATA immune response and clinical disease course in ATA+ SSc.

Methods

ATA-IgG, -IgM and -IgA levels were assessed in consecutive serum samples of baseline ATA-IgG+ patients from the Leiden Combined Care In Systemic Sclerosis cohort (CCISS). One-year disease progression was defined by a relevant increase in modified Rodnan Skin Score (mRSS), decline in pulmonary function tests, development of digital ulcers, renal crisis, pulmonary hypertension and/or mortality. Validation was performed in ATA+ SSc patients from the Oslo University Hospital and University Hospital Zurich.

Results

Of 103 ATA-IgG+ patients available in the CCISS cohort, 81 patients had clinical data available to assess one-year disease progression. Of these 81 patients, 23 patients (28%) showed disease progression. At baseline, disease-progressors were significantly more often ATA-IgM+ compared to non-progressors (21/23 [91%] vs 33/58 [57%], $p < 0.01$). This finding was confirmed in the independent validation samples.

Conclusion

In ATA-IgG+ SSc patients, presence of ATA-IgM, which might be taken as a surrogate for an ongoing auto-reactive B cell immune response, is associated with disease progression.

Introduction

Anti-topoisomerase I antibodies (ATA) are highly specific for Systemic Sclerosis (SSc) (1). Patients with isolated Raynaud's phenomenon have an increased risk of progression to SSc when ATA positive (2), indicating presence of ATA in a preclinical phase. In established SSc, ATA are associated with diffuse cutaneous SSc (dcSSc), severe interstitial lung disease (ILD) and their presence indicates an unfavorable prognosis (3-7). This association with a typical clinical phenotype suggests that the immune response involved in ATA production may play a role in disease pathophysiology. The exact pathogenicity of ATA, however, has not yet been elucidated.

In daily clinical practice, ATA+ SSc is heterogeneous. Not all ATA+ patients demonstrate a severe disease course, some patients experience only moderate skin and lung fibrosis (6, 8). Based on the hypothesis that topoisomerase I represents a candidate autoantigen in the pathogenesis of SSc, different groups have studied immunization with topoisomerase I in mouse models. These models demonstrated that a specific antibody response can be induced, resulting in varying extents of fibrosis in skin and lungs of immunized mice (9, 10).

Previous small studies in SSc have shown that IgG and IgA levels of ATA correlate with skin scores (11-13). Loss of the ATA response, on the other hand, has been associated with a favorable disease course in a small patient group (14). However, the relations between ATA isotype profile and isotype levels and disease course have not yet been fully evaluated in larger SSc cohorts. By taking advantage of our well described SSc cohort with annual, prospective and comprehensive clinical data available, we investigated the association between presence and levels of ATA-IgG, -IgA and -IgM and disease course in ATA-IgG+ SSc.

Patients and Methods

Patient population

The Combined Care in Systemic Sclerosis (CCISS) cohort Leiden is a prospective cohort that started in April 2009, including all consecutive SSc patients evaluated at the Leiden University Medical Center (15). Ethical approval for data collection was obtained from the local ethics committee (CME number B16.037). All participants provided written informed consent. This research was done without patient involvement. As described previously (15), all patients undergo annual extensive screening during a 1 to 2 day health care program, including detailed physical examination, modified Rodnan skin score (mRSS) assessment (16), laboratory testing (with autoantibody screening at baseline), pulmonary function test and optionally:

echocardiography (mandatory at baseline), holter evaluation (mandatory at baseline), cardiopulmonary exercise tests (CPET) and high-resolution computed tomography (HRCT) (mandatory at baseline). Patients are requested to fill in the Scleroderma Health Assessment Questionnaire (HAQ) (17), Short Form-36 (SF-36) (18, 19), Mouth Handicap in Systemic Sclerosis scale (MHSS) (20, 21), EuroQol-5D (EQ-5D) (22, 23) and Scleroderma Clinical Trial Consortium Gastrointestinal Tract Instrument 2.0 (SCTC GIT 2.0) (24, 25) questionnaires at every visit. Additionally, every visit serum samples are collected and stored in the Leiden Scleroderma Biobank. All patients entering the cohort before September 24th, 2016, who were ATA-IgG+ were selected for the present study. Only patients who had a clinical SSc diagnosis at inclusion and fulfilled the ACR/EULAR 2013 SSc classification criteria (26) at any point during their disease course were evaluated.

Disease progression

Clinical data were collected, with censoring at January 1st, 2018. Skin progression was defined as a ≥ 5 point and $\geq 25\%$ increase in mRSS (27). Worsening of lung involvement was defined as $\geq 10\%$ relative decline in forced vital capacity (FVC) with follow-up FVC $< 80\%$ predicted or $\geq 5\%$ to $< 10\%$ relative decline in FVC and either a $\geq 15\%$ relative decline in DLCO with follow-up DLCO $< 80\%$ predicted or increase of lung involvement as determined by HRCT, towards $> 20\%$ lung involvement (28). Patients were considered disease progressors in case of skin- and/or lung progression, incident digital ulcers (DU), newly diagnosed myocardial involvement, scleroderma renal crisis, pulmonary hypertension (PH) or in case of death. Use of aggressive immunosuppression in both progressors and non-progressors was assessed, including hematopoietic stem cell transplantation (HSCT), cyclophosphamide and mycophenolate mofetil.

Anti-topoisomerase I assay and measurements

Total ATA-IgG, -IgA, and -IgM levels of consecutive samples collected before January 1st, 2017 were measured in baseline and follow-up sera by fluorescence enzyme-linked immune sorbent assay [FEIA], using Phadia250[®] system [Thermo Fisher Scientific, Nieuwegein, The Netherlands]. If necessary, sera were diluted to obtain a reliable ATA isotype-specific level. For ATA-IgG, the manufacturer specified a cut-off value of 7 aU/mL. For ATA-IgA and ATA-IgM, no manufacturer cut-off values were available. Therefore, sera of 51 non-rheumatic subjects were measured and the cut-off value was determined as the mean plus two standard deviations of the measurements. A cut-off for ATA-IgM was determined at 432 aU/mL and for ATA-IgA at 77 aU/mL. To evaluate specificity of the assay, ATA-isotype levels from 5 ANA+ SSc patients lacking SSc specific antibodies and from 5 ACA+ SSc patients were additionally assessed. None of these patients were positive for any of the isotypes in the ATA assay.

Data validation

For validation of the main findings, baseline serum samples of ATA-IgG+ patients from the Oslo University Hospital (29) and from the University Hospital Zurich (30) were tested for the presence and levels of ATA isotypes using the same methodology. Baseline and follow-up clinical data were additionally collected. At both centers longitudinal data of SSc patients is being collected according to the EUSTAR recommendations (31). Details of these cohorts can be found elsewhere (29, 30). Collection and analysis of biomaterial and their clinical associations have been approved by the Cantonal Ethics Committee in Switzerland (PB_2016-02014 and BASEC-Nr. 2018-01873) and by the Data Protection Authority in Norway (No.2006/119). All patients provided informed consent.

Statistical analysis

Descriptive statistics were used to characterize the study population clinically. Contingency tables were evaluated by Fisher's exact, χ^2 or Mann-Whitney test as appropriate. Correlations between isotype levels were assessed by Spearman's correlation coefficient. Disease progression over time was analysed by Kaplan-Meier survival analysis. P values < 0.05 were considered significant. To exclude relevant bias by expression of ATA-IgM and ATA-IgA in patients negative for ATA-IgG and by evaluating a higher cut-off for ATA-IgM to define positivity, a sensitivity analysis was performed (Supplementary Figure 5). In addition, we evaluated the robustness of the data by re-analyze using a different cut off for ATA IgM. Statistical analysis was performed using SPSS version 23.0 and GraphPad Prism 7.

Results

Baseline characteristics and ATA isotype expression of the population under study

In total, 103 ATA-IgG+ patients from the CCISS cohort were included. Of these patients, a total of 333 samples were available (range 1-8 per patient). Sixty-nine percent of patients were female with a mean age of 53 years, and 48% had diffuse cutaneous SSc (Table 1). At baseline, median duration since first non-Raynaud's symptom was 2.8 years. Clinical follow-up was available for 3.4 years (range 0.0-8.4). All but one patient evaluated were ATA-IgA+ at baseline. This patient was also low in his ATA-IgG level (24 aU/mL). At baseline, 65% ($n=67/103$) of patients were ATA-IgM+. Antibody isotype levels at baseline correlated weakly (ATA-IgG and ATA-IgM [$r_s=0.25$, $p=0.01$], ATA-IgG and ATA-IgA [$r_s=0.30$, $p<0.01$], ATA-IgA and ATA-IgM [$r_s=0.45$, $p<0.01$]) and were not correlated with disease duration (Supplementary Figure 1 + 2). Correlations between baseline ATA isotype levels and skin scores are presented in Figure 1, levels of ATA-IgG correlated with skin scores ($r_s=0.41$, $p<0.01$), other isotypes did not correlate with skin scores.

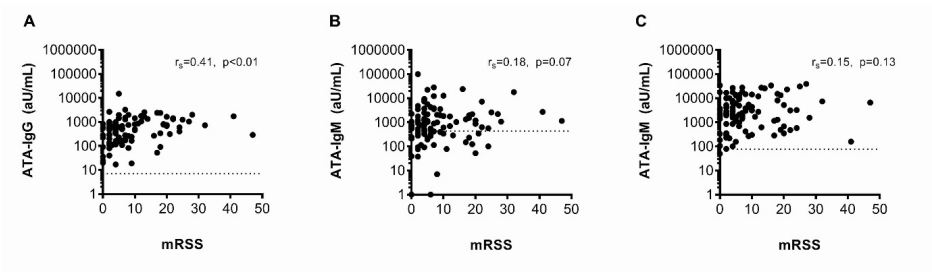


Figure 1. Correlations between baseline levels of ATA-IgG (panel A), ATA-IgM (panel B) and ATA-IgA (panel C) and modified Rodnan Skin Score (mRSS) of patients from the Leiden Combined Care in Systemic Sclerosis (CCISS) cohort (n=103). ATA-IgG levels correlate with skin scores ($r_s=0.41$, $p<0.01$). [ATA=anti-topoisomerase I].

During follow-up, 12 patients died (combined pulmonary and cardiac failure n=6, cardiac ischemia n=1, sepsis during hematopoietic stem cell transplant work-up n=1, gastro-intestinal ischemia n=1, influenza-related n=1, multi-organ failure during acute myeloid leukemia treatment n=1, unclear n=1).

Loss and gain of ATA-isotype response is only frequent for ATA-IgM

Change of isotype profile over time was assessed in 75 patients as 28 patients did not have follow-up samples available (Table 2, Supplementary Figure 3). Of these patients, four patients showed a loss of the ATA-IgG+ response (5%); all four were ATA-IgM- at baseline. Two of these patients were treated with IV cyclophosphamide before baseline sampling, one was treated with HSCT before baseline sampling and one was treated with HSCT 3 months after baseline sampling. Three of these four patients were ATA-IgA+ at baseline and two of them also showed loss of the ATA-IgA response. In total, there were 4 patients that lost ATA-IgA response over time (5%), of whom one also lost the ATA-IgM response, but remained ATA-IgG+. Loss and gain ATA-IgM response over time was more common compared to other isotypes. Thirty-one percent (n=14/45) of patients lost and 10% (n=3/29) of ATA-IgM- patients gained an ATA-IgM+ response when followed from baseline.

Table 1. Baseline characteristics of all anti-topoisomerase I-IgG+ Systemic Sclerosis patients in the study

all patients (n=103)	
Demographic	
female, n(%)	70 (68)
age, mean[<i>yrs.</i>] $\pm$ SD	53.0 $\pm$ 14.8
smoking (ever), n(%)	50 (49)
Disease duration	
since onset first Raynaud symptom, median [<i>yrs.</i>] (IQR)	5.8 (2.1-13.4)
since onset first non-Raynaud symptom, median [<i>yrs.</i>] (IQR)	2.8 (0.8-9.3)
Organ involvement	
dcSSc, n(%)	49 (48)
modified Rodnan Skin Score, median (IQR)	6 (2-12)
FVC, mean [% of predicted] $\pm$ SD	87 $\pm$ 27
DLCO, mean [% of predicted] $\pm$ SD	63 $\pm$ 17
history of renal crisis, n(%)	3 (3)
digital ulcers, n(%)	14 (14)
pulmonary hypertension, n(%)	5 (5)
Previous use of immunosuppression*	
HSCT, n(%)	7 (7)
CYC (ever), n(%)	24 (23)
MMF (ever), n(%)	1 (1)

ATA=anti-topoisomerase antibodies, CYC=cyclophosphamide, dcSSc=diffuse cutaneous Systemic Sclerosis, DLCO=diffusing capacity of the lung, FVC=forced vital capacity, HSCT=hematopoietic stem cell transplantation, IQR=interquartile range, MMF=mycophenolate mofetil, SD=standard deviation, *yrs.*=years

* Immunosuppression= use of either HSCT, CYC or MMF

Table 2. Changes in presence of anti-topoisomerase I isotypes in paired (first and last available serum) samples of 75 ATA-IgG SSc patients with follow-up samples available

	ATA isotype status at baseline/last follow-up			
	+/+ (n)	+/- (n)	-/- (n)	-/+ (n)
ATA-IgG	71	4	-	-
ATA-IgM	31	14	27	3
ATA-IgA	70	4	1	0

ATA=anti-topoisomerase I autoantibody
*Status of the first available serum sample/status of the last available serum sample

Disease progression is more frequent in ATA-IgG+ SSc patients positive for ATA-IgM
To assess the association between ATA isotype profile and disease progression, we used data of 81 patients with one-year clinical follow-up available. During the first year starting from sampling, none of these patients received HSCT, 16 patients were treated with cyclophosphamide and 7 received mycophenolate mofetil.

In total 23 patients showed disease progression according to predefined criteria. This consisted of death (n=4; including combined pulmonary and cardiac failure n=3 and , multi-organ failure during acute myeloid leukemia treatment n=1), skin progression (n=12), lung progression (n=4), digital ulcers (n=5). None of the patients developed clinically meaningful myocardial involvement or renal crisis. Correlations between ATA isotype levels at baseline and one-year change in mRSS, FVC and DLCO % predicted are shown in Supplementary Figure 4. Baseline levels of ATA-IgM and ATA-IgA correlated with a decrease in FVC % predicted, and ATA-IgM additionally correlated with a decrease in DLCO % predicted. Baseline levels of ATA-IgG, -IgM and -IgA were not correlated with one-year change in mRSS.

Table 3. Baseline characteristics of ATA-IgG+ patients stratified according to one-year disease progression

	progressors (n=23)	non- progressors (n=58)	p
Demographic			
female, n(%)	14 (61)	39 (67)	0.59
age, mean[yrs.]±SD	55.3±16.3	51.9±13.9	0.21
smoking (ever), n(%)	12 (52)	30 (52)	0.95

Table 3. Baseline characteristics of ATA-IgG+ patients stratified according to one-year disease progression (continued)

	progressors (n=23)	non- progressors (n=58)	p
Disease duration			
since onset first Raynaud symptom, median [yrs.] (IQR)	3.8 (1.3-8.4)	5.6 (2.1-12.9)	0.21
since onset first non-Raynaud symptom, median [yrs.] (IQR)	1.9 (0.6-4.5)	3.5 (0.7-11.4)	0.07
Organ involvement			
dcSSc, n(%)	12 (52)	28 (48)	1.00
modified Rodnan Skin Score, median (IQR)	6 (2-19)	6 (3-13)	0.86
FVC, mean [% of predicted] ±SD	89±26	89±28	0.92
DLCO, mean [% of predicted] ±SD	62±18	64±16	0.83
history of renal crisis, n(%)	0 (0)	2 (4)	1.00
digital ulcers, n(%)	0 (0)	5 (9)	0.31
pulmonary hypertension, n(%)	2 (9)	2 (4)	0.59
Previous use of immunosuppression*			
HSCT, n(%)	0 (0)	7 (12)	0.18
CYC (ever), n(%)	4 (17)	16 (28)	0.34
MMF (ever), n(%)	1 (4)	0 (0)	0.28
Use of aggressive immunosuppression* during one-year follow-up			
HSCT, n(%)	0 (0)	0 (0)	-
CYC, n(%)	11 (19)	5 (26)	0.52
MMF, n(%)	1 (5)	6 (10)	0.67
ATA characteristics			
IgG level [aU/mL], median(IQR)	813 (542-1263)	396 (115-832)	<0.01
IgA positivity, n(%)	23 (100)	57 (98)	1.00
IgA level [aU/mL], median(IQR)	9898 (2743-16656)	2045 (462-5314)	<0.01
IgM positivity, n(%)	21 (91)	33 (57)	0.04
IgM level [aU/mL], median(IQR)	1065 (869-3853)	588 (223-1610)	0.01

ATA=anti-topoisomerase antibodies, CYC=cyclophosphamide, dcSSc=diffuse cutaneous Systemic Sclerosis, DLCO=diffusing capacity of the lung, FVC=forced vital capacity, HSCT=hematopoietic stem cell transplantation, IQR=interquartile range, MMF=mycophenolate mofetil, SD=standard deviation, yrs.=years
* Aggressive immunosuppression= use of either HSCT, CYC or MMF.
In 22 patients, clinical follow-up data was not available; therefore, they could not be stratified into either progressors or non-progressors.

In total, 23 patients (28%) showed disease progression according to pre-specified criteria during the first year. Clinical characteristics and ATA isotype profiles at baseline stratified for disease progression are presented in Table 3. At baseline, there were no differences in clinical characteristics between patients with and those without disease progression. Treatment strategy was also comparable between patients with and without disease progression. Strikingly, while the clinical characteristics were similar, ATA isotype levels at baseline were significantly higher and ATA-IgM positivity was significantly more frequent in patients with disease progression (91% vs 57%, $p<0.01$). Kaplan-Meier analysis underlined the prognostic value of ATA-IgM positivity (Log-Rank - Mantel-Cox $p=0.02$, Figure 2). Sensitivity analysis did not influence these results (Supplementary Figure 5).

Validation in other cohorts

To confirm our results, we additionally performed ATA isotype level measurements in 90 ATA-IgG+ SSc patients (n=60 from University Hospital Zurich and n=30 from Oslo University Hospital). Baseline characteristics of these patients are presented in Supplementary Table 1. Cross-sectional analysis confirmed the correlation between ATA-IgG levels and skin scores at baseline ($r_s=0.37$ $p<0.01$). Additionally, in this sample set a correlation between ATA-IgG and respectively FVC ($r_s=-0.30$ $p<0.01$) and DLCO ($r_s=-0.24$ $p=0.03$) was found. Clinical follow-up at one year was available in 63 patients of the validation samples. During this year, 5 patients died, skin progression was observed in 6, lung progression in 7, incident renal crisis developed in 1 and digital ulcers developed in 5 patients. In total, 24 patients from the validation sample set experienced disease progression. Again, there were no clinical differences between disease progressors and non-progressors at baseline, but disease progressors more often expressed ATA-IgM (96%, vs. 71%, $p=0.04$) (Supplementary Table 1). Thus, these data confirm that ATA-IgG+ SSc patients that are also positive for ATA-IgM, have a higher risk for disease progression as compared to ATA-IgG+ patients not positive for ATA-IgM.

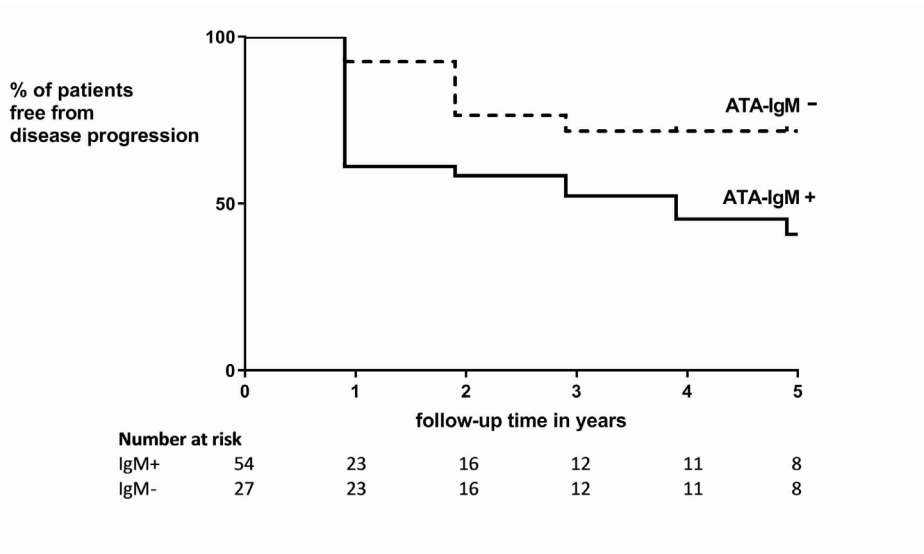


Figure 2. Disease progression over in time in ATA-IgM positive and negative SSc patients from the Leiden Combined Care in Systemic Sclerosis (CCISS) cohort with at least one year follow-up available (n=81). Disease progression occurs more often in ATA-IgM positive patients (Log-Rank - Mantel-Cox $p=0.02$). [ATA=anti-topoisomerase I].

Discussion

This study shows that ATA-IgG+ SSc patients, additionally positive for ATA-IgM, more often experience disease progression compared to ATA-IgG+, ATA-IgM- patients. Importantly, progressors could not be identified based on baseline clinical parameters. In addition, we show that ATA-IgG+ patients are almost always ATA-IgA+. Alteration from a positive to negative response (or vice versa) for ATA-IgG and ATA-IgA isotypes is relatively rare, while loss and gain of the ATA-IgM response occurs frequently. Over one-third of patients ATA-IgM+ at baseline, becomes ATA-IgM- during follow up.

Our observations of high levels of ATA-IgA, and only part of ATA-IgG+ patients harboring ATA-IgM+, are consistent with previous findings from the early 1990's (32, 33). The sustained ATA-IgG response found in SSc patients, with little or no fluctuations with disease activity and not sero-reverting (in some cases even after high dose cyclophosphamide treatment in the context of hematopoietic stem cell transplantation), points to the notion that this response is long-lived and that its generation depends on T cell help. Hence, it is conceivable that long-lived plasma cells secreting ATA-IgG without the need for antigenic triggering may be responsible for a large fraction of the ATA-IgG levels measured in serum. However, we consider

it possible that there is also a short-lived, more dynamic part of the ATA-response, triggered due to the continuous presence of autoantigens and potentially, additional/external (yet unknown) triggers such as TLR-ligands. Such triggers would be able to recruit naïve B cells from the repertoire and explain why IgM-secreting plasma cells arise that, due to their short life span (i.e., the lack of a long-lived memory compartment) and the short half-life of IgM, more closely reflect disease-relevant processes, with possible clinical consequences in the near future.

ATA-IgG levels have previously been described to be correlated with skin scores (11-13). A study of Kuwana et al. in 28 SSc patients reported that 21% of ATA-IgG+ patients lost their ATA-IgG response over time, which was associated with a favorable disease course (14). Notably, although non-significant, none of these patients were ATA-IgM+ at baseline, while one-third of patients that persisted to be ATA-IgG also were ATA-IgM+ at baseline. In our cohort, loss of ATA-IgG response over time was less common (5%). This discrepancy between the study of Kuwana et al. and ours might be explained by methodological differences. Kuwana et al. used a cut-off of 3 times the standard deviation of samples of healthy controls for their ELISA assays (34). We used a cut-off for ATA-IgG as pre-specified by the manufacturer and used in clinical routine, which corresponds to the mean plus 8 standard deviations (data not shown). Consequently, Kuwana et al. might have included patients with already lower ATA-IgG levels at baseline. In addition, in another study including 21 patients, decreasing levels were accompanied by skin showing atrophic changes, while increasing levels were associated with new onset or worsening of organ involvement. Thus, our work and that from others show that the ATA-response is related to disease course. Nonetheless, the frequency of ATA-IgM+ in patients not experiencing disease progression implicates that ATA-IgM status solely is not sufficient to function as a biomarker in every day clinical practice, but might be of additional help for clinical trial enrichment. As disease progression is highly unlikely in patients negative for ATA-IgM (<10%), ATA-IgM status might be of help to decide to refrain from aggressive treatment like HSCT.

Our hypothesis that ATA or its underlying immune response is (at least partly) responsible for clinical heterogeneity, might not seem to rhyme with the heterogeneity observed among patients who are all ATA-IgG+ and ATA-IgM+ from the first measurement onwards. A pathophysiologic explanation of the clinical heterogeneity within ATA-IgG+, ATA-IgM+ SSc might be found in the presence of additional triggers for ATA or its underlying immune response to become pathogenic. For example, it has been speculated that ATA triggers adhesion and activation of monocytes by binding to DNA-topoisomerase I expressed on fibroblasts. This potentially could lead to amplification of the fibrogenetic cascade (35-37). In line with this, it is tempting to speculate that the presence of ATA may only be pathogenic in case there is insufficient clearance of apoptotic bodies of endothelial cells containing DNA-topoisomerase I. Consequently, the production of ATA-IgG might be an ongoing process in all

ATA+ patients, however if not accompanied by the presence of extracellular DNA-topoisomerase I, the ability of ATA to trigger fibrosis is lost. Clinically, this might result in different ATA+ subsets of patients depending on the level of endothelial cell apoptosis. This could also fit with the observation that more severe capillary loss is associated with more severe organ involvement independent of auto-antibody subtype (38). Alternatively, other characteristics of ATA or its underlying immune response, such as epitope recognition patterns, the extent of T-cell and/or B-cell activation or interaction with cytokines could be important for pathogenicity.

This study has some limitations to be considered. As we only included patients positive for ATA-IgG at baseline, we cannot exclude that there might be patients positive for ATA-IgM and/or ATA-IgA solely. However, based on our sensitivity analysis, we conclude that in SSc patients continuous expression of ATA-IgM without switching to ATA-IgG does hardly occur. Also, as data were derived from a cohort study, treatment was uncontrolled. However, significant treatment differences between disease progressors and non-progressors were not observed. In addition, because of the exploratory character of the study, we deliberately did not correct for multiple testing as this would lead to increased chances of false negative findings (39), which cannot be easily justified in an explorative study. Instead, we validated our main findings in an independent cohort. Finally, we used a composite of several individually validated scores for different organs to define overall disease progression, including all-cause mortality. We acknowledge that a precise determination of cause of death is often difficult, leading to weak data quality. To address this, recorded causes of death are described in the results section. Using a composite end point for disease progression is common in SSc studies (40, 41), as the heterogeneous nature of the disease with multiple organs involved implicates the use of composite indices. Availability of a validated composite for disease progression could have substantiated our findings. However, although it lacks validation, our composite has face validity and most importantly, as our data have been validated in an independent second cohort, our analyses is robust.

In conclusion, our results indicate that the ATA immune response is relevant for the disease course of SSc. Further research of the ATA response by characterization of specific epitopes and other antibody characteristics such as Fc-glycosylation are relevant for understanding of the disease pathogenesis. Most important, our data indicate that expression of an ATA-IgM response associates with an unfavorable disease course, a finding that we validated in other cohorts. Whether IgM positivity of other SSc specific auto-antibodies is of equal importance in explaining disease course remains to be evaluated.

Supporting information

Supplementary data is available at the website of Arthritis & Rheumatology or can be obtained by contacting the first author

REFERENCES

1. Reveille JD, Solomon DH. Evidence-based guidelines for the use of immunologic tests: Anticentromere, Scl-70, and nucleolar antibodies. *Arthritis Care Res (Hoboken)*. 2003;49(3):399-412.
2. Koenig M, Joyal F, Fritzler MJ, Roussin A, Abrahamowicz M, Boire G, et al. Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis. *Arthritis Rheum*. 2008;58(12):3902-12.
3. Patterson KA, Roberts-Thomson PJ, Lester S, Tan JA, Hakendorf P, Rischmueller M, et al. Interpretation of an Extended Autoantibody Profile in a Well-Characterized Australian Systemic Sclerosis (Scleroderma) Cohort Using Principal Components Analysis. *Arthritis & rheumatology*. 2015;67(12):3234-44.
4. Mierau R, Moinszadeh P, Riemekasten G, Melchers I, Meurer M, Reichenberger F, et al. Frequency of disease-associated and other nuclear autoantibodies in patients of the German Network for Systemic Scleroderma: correlation with characteristic clinical features. *Arthritis Res Ther*. 2011;13(5):R172.
5. Vanthuyne M, Smith V, De Langhe E, Van Praet J, Arat S, Depresseux G, et al. The Belgian Systemic Sclerosis Cohort: correlations between disease severity scores, cutaneous subsets, and autoantibody profile. *J Rheumatol*. 2012;39(11):2127-33.
6. Steen VD. Autoantibodies in systemic sclerosis. *Semin Arthritis Rheum*. 2005;35(1):35-42.
7. Khanna D, Denton CP. Evidence-based management of rapidly progressing systemic sclerosis. *Best Pract Res Clin Rheumatol*. 2010;24(3):387-400.
8. Boonstra M, Ninaber MK, Ajmone Marsan N, Huizinga TWJ, Scherer HU, de Vries-Bouwstra JK. Prognostic properties of anti-topoisomerase antibodies in patients identified by the ACR/EULAR 2013 systemic sclerosis criteria. *Rheumatology (Oxford)*. 2019.
9. Yoshizaki A, Yanaba K, Ogawa A, Asano Y, Kadono T, Sato S. Immunization with DNA topoisomerase I and Freund's complete adjuvant induces skin and lung fibrosis and autoimmunity via interleukin-6 signaling. *Arthritis Rheum*. 2011;63(11):3575-85.
10. Mehta H, Goulet P-O, Nguyen V, Pérez G, Koenig M, Sénécal J-L, et al. Topoisomerase I peptide-loaded dendritic cells induce autoantibody response as well as skin and lung fibrosis. *Autoimmunity*. 2016;49(8):503-13.
11. Hu PQ, Fertig N, Medsger TA, Jr., Wright TM. Correlation of serum anti-DNA topoisomerase I antibody levels with disease severity and activity in systemic sclerosis. *Arthritis Rheum*. 2003;48(5):1363-73.
12. Perera A, Fertig N, Lucas M, Rodriguez-Reyna TS, Hu P, Steen VD, et al. Clinical subsets, skin thickness progression rate, and serum antibody levels in systemic sclerosis patients with anti-topoisomerase I antibody. *Arthritis Rheum*. 2007;56(8):2740-6.
13. Hasegawa M, Imura-Kumada S, Matsushita T, Hamaguchi Y, Fujimoto M, Takehara K. Anti-topoisomerase I antibody levels as serum markers of skin sclerosis in systemic sclerosis. *J Dermatol*. 2013;40(2):89-93.
14. Kuwana M, Kaburaki J, Mimori T, Kawakami Y, Tojo T. Longitudinal analysis of autoantibody response to topoisomerase I in systemic sclerosis. *Arthritis Rheum*. 2000;43(5):1074-84.
15. Meijs J, Schouffoer AA, Ajmone Marsan N, Kroft LJ, Stijnen T, Ninaber MK, et al. Therapeutic and diagnostic outcomes of a standardised, comprehensive care pathway for patients with systemic sclerosis. *RMD open*. 2016;2(1):e000159.
16. Clements P, Lachenbruch P, Siebold J, White B, Weiner S, Martin R, et al. Inter and intraobserver variability of total skin thickness score (modified Rodnan TSS) in systemic sclerosis. *J Rheumatol*. 1995;22(7):1281-5.
17. Clements PJ, Wong WK, Hurwitz EL, Furst DE, Mayes M, White B, et al. The Disability Index of the Health Assessment Questionnaire is a predictor and correlate of outcome in the high-dose versus low-dose penicillamine in systemic sclerosis trial. *Arthritis Rheum*. 2001;44(3):653-61.
18. Ware JE, Kosinski M, Keller S. SF-36 physical and mental health summary scales: a user's manual: Health Assessment Lab.; 1994.
19. Newnham EA, Harwood KE, Page AC. Evaluating the clinical significance of responses by psychiatric inpatients to the mental health subscales of the SF-36. *J Affect Disord*. 2007;98(1):91-7.
20. Mouthon L, Rannou F, Bérezné A, Pagnoux C, Arène J-P, Fois E, et al. Development and validation of a scale for mouth handicap in systemic sclerosis: the Mouth Handicap in Systemic Sclerosis scale. *Ann Rheum Dis*. 2007;66(12):1651-5.
21. Schouffoer A, Strijbos E, Schuerwegh M, Mouthon L, Vlieland MV. Translation, cross-cultural adaptation, and validation of the Mouth Handicap in Systemic Sclerosis questionnaire (MHISS) into the Dutch language. *Clin Rheumatol*. 2013;32(11):1649.
22. Dolan P. Modeling valuations for EuroQol health states. *Med Care*. 1997;35(11):1095-108.
23. Lamers L, Stalmeier P, McDonnell J, Krabbe P, Van Busschbach J. Kwaliteit van leven meten in economische evaluaties: het Nederlands EQ-5D-tarief. *Ned Tijdschr Geneesk*. 2005;149(28):1574-8.
24. Meijs J, Pors D, Vliet VT, Huizinga T, Schouffoer A. Translation, cross-cultural adaptation, and validation of the UCLA Scleroderma Clinical Trial Consortium Gastrointestinal Tract Instrument (SCTC GI) 2.0 into Dutch. *Clin Exp Rheumatol*. 2013;32(6 Suppl 86):S-41-8.
25. Khanna D, Hays RD, Park GS, Braun-Moscovici Y, Mayes MD, McNearney TA, et al. Development of a preliminary scleroderma gastrointestinal tract 1.0 quality of life instrument. *Arthritis Care Res (Hoboken)*. 2007;57(7):1280-6.
26. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/ European League against Rheumatism collaborative initiative. *Arthritis Rheum*. 2013;65(11):2737-47.
27. Khanna D, Furst DE, Clements PJ, Allanore Y, Baron M, Czirjak L, et al. Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. *Journal of Scleroderma and Related Disorders*. 2017;2(1):11-8.
28. Khanna D, Mittoo S, Aggarwal R, Proudman SM, Dalbeth N, Matteson EL, et al. Connective Tissue Disease-associated Interstitial Lung Diseases (CTD-ILD) - Report from OMERACT CTD-ILD Working Group. *J Rheumatol*. 2015;42(11):2168-71.
29. Hoffmann-Vold AM, Midtvedt O, Molberg O, Garen T, Gran JT. Prevalence of systemic sclerosis in south-east Norway. *Rheumatology (Oxford)*. 2012;51(9):1600-5.

30. Frauenfelder T, Winklehner A, Nguyen TD, Dobrota R, Baumuegger S, Maurer B, et al. Screening for interstitial lung disease in systemic sclerosis: performance of high-resolution CT with limited number of slices: a prospective study. *Ann Rheum Dis*. 2014;73(12):2069-73.
31. Jordan S, Maurer B, Toniolo M, Michel B, Distler O. Performance of the new ACR/EULAR classification criteria for systemic sclerosis in clinical practice. *Rheumatology (Oxford)*. 2015;54(8):1454-8.
32. Verheijen R, de Jong BA, van Venrooij WJ. A recombinant topoisomerase I ELISA: screening for IgG, IgM and IgA anti-topo I autoantibodies in human sera. *Clin Exp Immunol*. 1992;89(3):456-60.
33. Hildebrandt S, Jackh G, Weber S, Peter HH. A long-term longitudinal isotypic study of anti-topoisomerase I autoantibodies. *Rheumatol Int*. 1993;12(6):231-4.
34. Kuwana M, Medsger TA, Jr., Wright TM. Detection of anti-DNA topoisomerase I antibody by an enzyme-linked immunosorbent assay using overlapping recombinant polypeptides. *Clin Immunol Immunopathol*. 1995;76(3 Pt 1):266-78.
35. Henault J, Robitaille G, Senecal JL, Raymond Y. DNA topoisomerase I binding to fibroblasts induces monocyte adhesion and activation in the presence of anti-topoisomerase I autoantibodies from systemic sclerosis patients. *Arthritis Rheum*. 2006;54(3):963-73.
36. Senecal J, Hénault J, Raymond Y. The pathogenic role of autoantibodies to nuclear autoantigens in systemic sclerosis (scleroderma). *J Rheumatol*. 2005;32(9):1643.
37. Henault J, Tremblay M, Clement I, Raymond Y, Senecal JL. Direct binding of anti-DNA topoisomerase I autoantibodies to the cell surface of fibroblasts in patients with systemic sclerosis. *Arthritis Rheum*. 2004;50(10):3265-74.
38. Markusse IM, Meijs J, de Boer B, Bakker JA, Schippers HPC, Schouffoer AA, et al. Predicting cardiopulmonary involvement in patients with systemic sclerosis: complementary value of nailfold videocapillaroscopy patterns and disease-specific autoantibodies. *Rheumatology (Oxford)*. 2017;56(7):1081-8.
39. Feise RJ. Do multiple outcome measures require p-value adjustment? *BMC Med Res Methodol*. 2002;2:8.
40. Sullivan KM, Goldmuntz EA, Keyes-Elstein L, McSweeney PA, Pinckney A, Welch B, et al. Myeloablative Autologous Stem-Cell Transplantation for Severe Scleroderma. *N Engl J Med*. 2018;378(1):35-47.
41. Avouac J, Walker UA, Hachulla E, Riemekasten G, Cuomo G, Carreira PE, et al. Joint and tendon involvement predict disease progression in systemic sclerosis: a EUSTAR prospective study. *Ann Rheum Dis*. 2016;75(1):103-9.