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Antinuclear Antibody Negative Systemic Sclerosis

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Abstract

Objective—To examine the demographic and clinical characteristics of systemic sclerosis (SSc) patients without antinuclear antibodies (ANA) compared to ANA positive patients.

Methods—SSc patients enrolled in the Scleroderma Family Registry and DNA Repository were included. Relevant demographic and clinical data were entered by participating sites or obtained by chart review. ANA and SSc related antibodies were determined in all investigated patients using commercially available kits at our laboratories.

Results—This study included 3249 patients, of whom 208 (6.4%) were ANA negative. The proportion of male patients was higher in the ANA negative group (OR 1.65; p=0.008). ANA negative patients experienced less vasculopathic manifestations of SSc. The percent predicted diffusing capacity of carbon monoxide (DLco) was higher in ANA negative patients (p=0.03). Pulmonary arterial hypertension (PAH) per right heart catheterization was less common in the ANA negative group (OR= 0.28; p=0.03). Furthermore, patients with negative ANA had a lower prevalence of telangiectasias and digital ulcers/pits (OR= 0.59; p=0.03 and OR=0.38; p=0.01, respectively). Although diffuse cutaneous involvement was more common, the modified Rodnan Skin Score (mRSS) was lower in the ANA negative group (2.4 points lower, p=0.05). Furthermore, they experienced more malabsorption (p=0.05). There was no difference in the frequency of pulmonary fibrosis or scleroderma renal crisis. All-cause mortality was not different between the two groups (p=0.28).

Conclusions—In conclusion, the results of this study suggest that SSc patients who are ANA negative constitute a distinct subset of SSc with less vasculopathy (less PAH, digital ulcers and fewer telangiectasias), a greater proportion of males and possibly, more frequent lower gastrointestinal involvement.

1. Introduction

Systemic Sclerosis (SSc) is an autoimmune disease characterized by fibrosis of skin and internal organs, as well as vasculopathy and immune dysregulation. SSc is a clinically heterogeneous disease that can range from limited skin involvement and minimal internal organ disease to rapidly progressive organ involvement and skin fibrosis resulting in premature death.

Autoantibody formation is one of the hallmarks of SSc. Several studies have shown that the autoantibodies found in patients with SSc carry considerable value in diagnosis and in predicting various clinical outcomes [1–4]. Although SSc related autoantibodies are associated with specific genotypes as well as characteristic clinical manifestations, the role of ANA antibodies and its subsets in the pathogenesis of SSc is unclear. While the great majority of patients with SSc have circulating antinuclear antibodies (ANA) (90–95%), a small percentage of patients are ANA negative (5–10%) [1, 2]. Although the typical clinical presentations of the different subsets of ANA positive patients have been extensively examined, the detailed demographic and clinical characteristics of patients without detectable ANA have not been clearly explored.

The purpose of this study was exploratory and to describe the clinical manifestations of this SSc subgroup by determining their clinical and demographic differences compared to ANA positive patients. Our hypothesis was that ANA negative patients are a subgroup of SSc with a distinct clinical presentation.

2. Patients and Methods

2.1 Study population

Patient information was obtained from the Scleroderma Family Registry and DNA Repository[5] database. Patients were recruited at the University of Texas – Houston and from the following participating sites including: the participating Canadian Scleroderma Research Group (CSRG) sites, University of California Los Angeles, University of Michigan, Georgetown University, Boston University, Medical University of South Carolina, Johns Hopkins University, University of Utah, Northwestern University, University of Alabama Birmingham and University of Minnesota. All patients that agreed to be enrolled in the National Scleroderma Family Registry and DNA Repository at the participating sites were included in the current study. Of note, 390 of the Canadian patients included in our study were also investigated in a recently published study that investigated the frequency of autoantibody negative SSc patients constituting a small overlap of 12% in the study population between these two studies [6].

All study patients fulfilled the 1983 American College of Rheumatology preliminary criteria for SSc [7] or had ≥3 of the 5 clinical features of the CREST syndrome (Calcinosis, Raynaud’s phenomenon, Esophageal dysfunction, Sclerodactyly or Telangiectasias) with sclerodactyly being mandatory [8].

2.2 Autoantibodies

Presence of antinuclear antibodies (ANA) was investigated in all patients at the time of enrollment using indirect immunofluorescence on HEp-2 cells as the antigen substrate in the Rheumatology laboratory of the University of Texas Health Science Center at Houston. A titer of >1:80 was considered positive. All ANA titers and patterns were determined by the same investigator (FCA). Anticentromere antibodies (ACA) were determined by the pattern of immunofluorescence staining on HEp-2 cells. Antitopoisomerase antibodies (anti-topo), anti-U1-RNP (RNP), anti-SSA (anti-Ro60) and anti-SSB (anti-La) were determined by passive immunodiffusion against calf thymus extract with commercial kits (Inova Diagnostics, San Diego, CA, USA). Anti-RNA polymerase III (RNA Pol-III) antibodies were determined by enzyme-linked immunosorbent assay (MBL, Co. Ltd, Nagoya, Japan). To be considered ANA negative both the ANA and all other autoantibodies listed above had to be negative. The comparison group was defined as patients with a positive ANA.

A repeat ANA testing was performed in a subgroup of ANA negative patients from whom a follow-up serum sample was available (n=19). Only one patient became positive after repeat testing, this patient was excluded from the analysis.

2.3 Clinical manifestations

Cross-sectional demographic and clinical data were entered directly or captured from medical records utilizing a standard abstract form. Clinical manifestations were entered based on the findings of latest clinic visit.

Age, sex, disease type, disease duration (calculated from the onset of both Raynaud's and the first non-Raynaud's phenomenon symptom attributable to SSc), and modified Rodnan skin score (mRSS) at the time of study entry, were recorded. Disease type (limited cutaneous versus diffuse cutaneous SSc) [8] was defined based on the extent of skin involvement as assessed by the physician upon enrollment.

Malabsorption was defined as diarrhea associated with >10% loss of body weight. Scleroderma renal crisis was defined as accelerated hypertension, rapidly progressive renal insufficiency and/or microangiopathic hemolysis [9]. Electrocardiography and 2-dimensional echocardiography findings were recorded and were used to determine the presence of pericarditis or clinically significant pericardial effusion.

Interstitial lung disease (ILD) was defined as imaging changes consistent with scleroderma-related fibrosis including honeycombing, increased interstitial markings or ground glass opacity on chest radiograph, chest computed tomography (CT) or high resolution CT (HRCT) of the chest and restrictive lung disease indicative of ILD was determined when the forced vital capacity (FVC) predicted value was < 70%. Only 35 patients or 3% of the 1098 that were classified as having ILD by these means had an FEV1 (forced expiratory volume) to FVC ratio below 0.7 indicative of a possible obstructive process. These patients were excluded from the ILD analyses to prevent misclassification.

Pulmonary arterial hypertension (PAH) was defined by mean pulmonary artery pressure ≥ 25 mm Hg on right heart catheterization (RHC) with pulmonary wedge pressure <15.

Pulmonary Hypertension (PH) was defined as a right ventricular systolic pressure (RVSP) ≥ 50 mm Hg on 2-dimensional echocardiography when RHC was not available; this cut-off value was chosen because previous studies have shown RVSP >50 corresponds most reliably to pulmonary hypertension on RHC [10, 11].

Not all enrolled patients underwent all diagnostic studies reflecting the practice pattern of participating sites and individual physicians. Specifically, pulmonary function test results were available for analysis in 2624 cases, whereas echocardiogram was available for 1356 patients. When more than one echocardiogram was available the highest measurement was recorded. Chest imaging (chest X-ray, chest CT, or HRCT) was available in 756, 400 and 754 patients respectively. Right heart catheterization (RHC) was performed when clinically indicated in 415 patients, 381 were ANA positive and 31 were ANA negative.

2.4 Vital Status

A vital status search was performed only in patients that were enrolled at the University of Texas - Houston for whom personal identifiers were available. The vital status of these 1475 patients was determined by the National Death Index (NDI), at Centers for Disease Control and Prevention (CDCP) and the online Social Security Death Index (SSDI). The ascertainment of vital status was complete based on the available information in the above mentioned data bases. The censoring date was January 30th 2013.

All study subjects provided written informed consent and the study was approved by all participating sites.

3. Data Analysis

Analysis was carried out using STATA 12 (Statacorp LP, College Station, TX, USA) statistical package. Clinical manifestations were considered the outcome (dependent) variables and ANA status was the independent variable. Initial comparisons were conducted by chi-square or t-test depending on whether the outcome being analyzed was categorical or continuous, respectively.

Multivariable analysis adjusting for potential confounders, specifically age at enrollment, disease duration, disease type (limited or diffuse cutaneous disease) and gender was performed. The same confounding variables were used for all multivariable analysis. Linear regression was used for continuous variables while logistic regression was used for the categorical. The results were adjusted for multiple comparisons using the False Discovery Rate (FDR) or Benjamini-Hochberg <5% adjustment method. The FDR method was used because several independent variables such as markers of pulmonary hypertension were not independent from each other.

All-cause mortality analysis with disease onset as the starting point was performed by Cox proportional-hazards regression and was graphically depicted by Kaplan-Meier survival curve.

4. Results

4.1 Demographic characteristics

A total of 3249 patients were included in this study, of whom 208 (6.4%) were ANA negative. Table 1 shows the demographic and basic clinical characteristics of patients with and without ANA antibodies. There was no difference for mean age at disease onset which was 44 years for both groups. When considering the onset of Raynaud's phenomenon as the starting point for disease onset there was still no significant difference for mean age at disease onset. There were more males in the ANA negative group. No differences were observed in the ethnic composition between the two groups.

4.2 Clinical Characteristics

The mean disease duration from non-Raynaud's phenomenon onset to diagnosis was 1.9 years for ANA negative and 2.2 years for ANA positives patients. The mean disease duration from Raynaud's phenomenon onset to diagnosis and the average disease duration from SSc onset (non-Raynaud's phenomenon) to study entry were not different between the two groups (Table 1). Similar findings for this analysis were observed if the disease duration was measured from Raynaud's phenomenon onset (12 years for ANA positive and 13 for ANA negative, p=0.20). ANA negative patients had a higher proportion of diffuse skin involvement than their ANA positive counterparts (Table 1). The severity of skin involvement as evaluated by the modified Rodnan skin score (mRSS) was not different in the initial comparison (Table 2). Although ANA negatives had a higher percentage of patients with diffuse skin involvement, the multivariable analysis adjusting for potential confounders including disease type (plus age at enrollment, disease duration, and gender) showed that the fibrosis severity as measured by mRSS was lower in the in the ANA negative group (Table 3).

The FVC % predicted was lower in ANA negative patients; however, the multivariable analysis adjusting for the above mentioned confounders failed to show a significant difference (Table 3). The DLco % predicted was higher in the ANA negative patients (Table 2). Pulmonary arterial hypertension (PAH) verified by right heart catheterization (RHC) was significantly less common in the ANA negative group (Table 2). Only 7 ANA negative patients had PAH compared to 213 ANA positive patients.

When patients who had PAH diagnosed per RHC and patients that did not have a RHC but had an RVSP>50 mmHg per echocardiogram were included in the analysis, a total of 2040 patients were analyzed (1907 were ANA positive and 133 were ANA negative). In this analysis, we again observed a statistically significant difference between the two groups for pulmonary hypertension (PH) (Table 2).

After adjusting for potential confounders (disease type, age at enrollment, disease duration, and gender), the difference in prevalence of PAH by RHC between the two groups remained statistically significant (Table 3). When centromere antibody status was added to the model, the relationship was still significant (p= 0.009). PH (per RHC or RVSP>50) was also statistically significant in the multivariable model but the level of significance was not sustained after adjusting for multiple comparisons (Table 3). ANA negative patients were

found to have a lower prevalence of telangiectasias and digital ulcers/pits (Table 2). As shown in Table 3, these differences remained significant in the multivariable model.

ANA negative patients experienced more malabsorption (Table 2). This finding remained significant in the multivariable analysis (Table 3).

There was no difference in creatine kinase (CK) levels, fibrotic changes per chest imaging or ejection fraction by echocardiogram (LVEF<50%) between the two groups. Also no difference was observed for the presence of Raynaud's phenomenon, GERD or calcinosis. Of note, there were no cases of primary biliary cirrhosis (PBC) in the ANA negative group and 12 in the ANA positive group but this finding did not reach statistical significance.

Ten patients had scleroderma renal crisis (SRC) in the ANA negative group. While the ANA status (negative versus positive) was not associated with higher occurrence of SRC in the overall cohort, ANA negative patients were more likely to develop SRC than patients with anti-topo or ACA (OR= 3.83, CI 1.49–9.84, p=0.005 and OR= 11.86, CI 3.68–38.22, p= <0.001, respectively). Only RNA-polymerase III, which is a well-known risk for SRC, was associated with a higher risk compared to the ANA negative group (OR 4.87; CI=3.19–7.44; p<0.001) after adjusting for confounders).

4.3 Survival Analysis

Among 1475 patients in whom a death search could be performed, 300 had died. The ANA status was not associated with overall mortality either in the univariable or the multivariable analysis. The univariable analysis showed a hazard ratio (HR) 0.96 (CI 0.62–1.49, p=0.87). Multivariable analysis correcting for potential confounders resulted in a HR=0.83 (CI 0.53–1.30, p=0.42). Kaplan-Meier survivor function is shown in figure 1.

4.4 ANA follow up

Nineteen ANA negative patients in our database had a follow-up serum sample. The repeat ANA was performed at a mean of 4.5 years after the initial determination (SD 4.3 years). Out of these, only one patient who was initially ANA negative seroconverted to ANA positive upon follow-up testing. The pattern was atypical (few bright speckles).

5. Discussion

This study is the first to specifically focus on the demographic and clinical features of ANA negative SSc in a large and diverse sample of patients. Our study was of sufficient size for statistical analysis and our survival cohort was also larger than published cohorts to date. We observed that ANA negative patients represent a distinct subset of patients who have the fibrotic features of SSc but are less likely to have the vasculopathic features of the disease.

We observed that PAH and clinical markers of vasculopathy such as digital ulcerations and telangiectasias were less common in ANA negative patients.

Our findings show that, although SSc is relatively uncommon in males, ANA negative patients in our study were more commonly male. The ANA negative patients might also have a higher frequency of malabsorption.

Only a few studies have previously described some of the clinical characteristics of ANA negative patients. Hamaguchi et al delineated the clinical manifestations of SSc patients based on antibody subsets and included 203 Japanese SSc patients, 10 of whom were ANA negative (5% of their sample) [12]. They reported lower severity of skin involvement, rare incidence of PAH, ILD and pitting scars (one patient each) in the ANA negative group.

The German network for SSc published a study focusing on the correlation of clinical features and the different antibody subsets [13]. Within their sample of 863 patients, 50 (5.8%) were ANA negative. In their study, ANA negative cases had significantly less frequent PAH, digital ulcers and Raynaud's phenomenon.

More recently Hudson et al published their observations on autoantibody negative SSc in the CSRG cohort [6]. Their sample included 874 patients. They divided their patients into groups based on their ANA and/or ENA (Extractable Nuclear Antigens) status as positive (+) versus negative (−). ENA antibodies also included cytoplasmic antibodies. This resulted in 3 different study groups (group 1: ANA+/ENA+, group 2: ANA+/ENA− and group 3: ANA−/ENA−). Their sample included 15 patients (1.7%) who were autoantibody negative (group 3). Three hundred ninety of the patients examined in our study were also studied by Hudson et al, indicating a small overlap of 12% in the study population between our studies. Only univariable comparisons without correction for potential confounders were performed due to sample size limitations and were done between the ANA+/ENA− group and the remaining two groups separately. They reported lower prevalence of Raynaud's phenomenon and telangiectasias in the autoantibody negative group. They did not observe any other clinical differences, possibly due to sample size limitations or differences in the study design.

Despite the small overlap with this last study (390 patients), thanks to our multi-institutional international effort our cohort was able to support a more in depth analysis and represents the largest sample of ANA negative SSc patients described in the literature to date. The data collected from this endeavor enabled us to conduct for the first time, robust clinical correlations given the significantly larger sample size, wider geographic catchment area and multi-ethnic population. Encouragingly the results of the above mentioned studies support several of our findings. Similar to our results, these studies indicate that ANA negative patients have less often vasculopathic features of disease.

The association of SSc vasculopathic features with ANA positivity might have pathophysiological implications. This relationship was still present after correcting for ACA which has been associated with susceptibility to PAH. Therefore any circulating ANA is potentially a risk for PAH compared to the absence of ANA. Our analysis showed a significant difference for overall markers of vasculopathy in ANA positive compared to ANA negative patients including PAH, telangiectasias and digital ulcers/pits. A correlation between PAH and telangiectasias was previously described by Shah et al which supports the notion that vasculopathic features tend to occur together and that there is a subgroup of patients who have more vasculopathy [14].

Several studies have described the relationship of specific antinuclear antibodies including ACA, RNP and Th/To to the presence of PAH. More recently, Nihtyanova et al also reported an association between PAH and RNA-polymerase III antibody positivity.[15] The relationship of ACA with digital ulcers, telangiectasias and microvascular damage per capillaroscopy has also been observed [1–3, 16–18]. However, the pathophysiologic role of these antibodies has not been determined (direct cause of vascular damage, or an epiphenomenon). It is also possible that ANA negative patients have other antibodies that are not currently detected by our traditional assays causing the observed clinical differences or that the ANAs are associated with other antibodies that are causing the vascular damage. Studies designed to understand the pathophysiology of this important manifestation of SSc are needed.

Several studies have linked autoantibody production with genetics in SSc. Multiple polymorphisms in the human leukocyte (HLA) regions have been linked to SSc and the association of some of the class II haplotypes with the different ANA subsets has been described [19–21]. Also familial SSc studies have shown a concordance of autoantibodies between family members [22, 23]. Although the relationship between ANA and the genetic background has been studied, the genetics of the ANA negative subgroup are not known. The discovery that these patients are more commonly male underlines the importance of exploring the genetics of this subset. If genetics do not play a role than other causes for the observed clinical differences such as hormonal changes or differentially expressed epigenetic mechanisms by gender should be considered.

ANA negative patients have a lower frequency of PAH which is a common cause of mortality for SSc patients; however their all-cause mortality was not different. This could be due to severe GI involvement or ILD. Dedicated longitudinal prospective studies are needed to analyze this finding in more detail. We considered the possibility of detection bias in our study given that ANA negative SSc is rare and can be easily missed unless clinically evident, but this would have biased toward female sex, longer disease duration before diagnosis and diffuse disease. We observed more diffuse disease in the ANA negative group. However, the severity of skin fibrosis as measured by mRSS was lower in the multivariable analysis that included adjustment for disease type. Interestingly, the ANA negative group did not have longer time to diagnosis as we expected, and had a higher proportion of males. It is also important to mention that although RHC was obtained for only a subgroup of patients which could arguably bias the results of our analysis, we observed that the proportion of ANA negative and positive patients was not statistically different between the patients that had a RHC versus the patients that did not have one (7% vs 6% in ANA negatives and 92% vs 93% in ANA positives, p=0.4).

Taken together, these data support the concept that ANA negative SSc patients are a subgroup of patients with distinct demographic and clinical manifestations. Further prospective clinical studies as well as genetic studies examining this subpopulation of patients are warranted. Also studies examining the pathophysiologic link between ANAs and vasculopathy in SSc should be considered.

The ANA negativity appears stable over time, as almost all patients in a follow-up subset of the original cohort that had an initial negative determination remained negative for up to 14 years when retested. Only one patient that was initially negative became positive upon follow up. The repeated ANA had an IIF pattern of few bright speckles also known as NSp-I or “multiple nuclear dots” (MND).[24, 25] Among a variety of potential MND targets, this pattern was seen in patients with sp100 antibodies, which are very specific for primary biliary cirrhosis (PBC) [26, 27]. However this particular patient has not developed PBC up until now. We acknowledge that longitudinal data were only available in a small subgroup of ANA negative patients. Nevertheless, we believe this limited longitudinal study provides important pilot data on stability of ANA negativity over time, which needs to be verified in larger follow-up studies.

The present study has some limitations. Reflecting the practice patterns of treating physicians, the data on clinical manifestations and diagnostic studies were not available in all patients. Furthermore, the diagnostic tests and basic laboratory studies were done at different centers. Although the data collection and patient inclusion for this study was performed before the 2013 ACR/EULAR classification criteria were published, we set out to determine how many of our patients fulfilled these criteria. Based on the available data, we were able to classify 97.5 percent of our sample. However our data does not include capillaroscopy information for all the participating sites therefore we cannot make assumptions on the 80 remaining patients which were not classified.

It is possible that some of the observed associations are false-positive findings; however, almost all the statistically significant differences observed remained significant after adjusting our analysis for multiple comparisons. Furthermore, we observed negative associations with several independent vasculopathic features supporting the notion that these associations are true biological findings rather than random spurious results.

As this is a cross-sectional study, the information on highest ever mRSS was not available. We could not investigate cause-specific mortality and prevalence of malignancy as this information was not captured in our data base. The paucity of data on chemical studies to clearly define malabsorption is also a limitation. Although we realize that our definition of malabsorption was relatively non-specific and did not include measures of bacterial overgrowth, stool fat measurements or laboratory tests for malabsorption such as carotene, we feel that clinically significant weight loss along with diarrhea is a reasonable surrogate. Moreover although the survival analysis was performed only on patients who were enrolled at UT Houston (due to lack of personal identifiers in our data base for those enrolled at other centers), we do not think this introduces a systemic bias as the percentage of ANA negative patients was not different between the patients who had mortality data available and the patients who did not (6.8% vs 5.9%, p=0.3). Finally, we cannot report on percentage of patients who were ANA positive at a lower titer (i.e. 1:40) because this was not examined in our laboratory.

The strengths of the current study are that the ANA testing was centrally performed and read by the same investigator and our large sample which is multi-ethnic and representative of

the SSc population across a wide geographic area in the United States and Canada. Finally, this sample includes the largest ANA negative SSc sample reported to date.

6. Conclusion

In conclusion, the results of this study suggest that SSc patients who are ANA negative constitute a distinct subset of SSc with less vasculopathy (less PAH, digital ulcers and fewer telangiectasias), a greater proportion of males and possibly, more frequent lower gastrointestinal involvement.

It is important to understand the clinical characteristics and genetics of ANA negative patients with SSc because this will allow further understanding the role of ANA in the pathophysiology of SSc.

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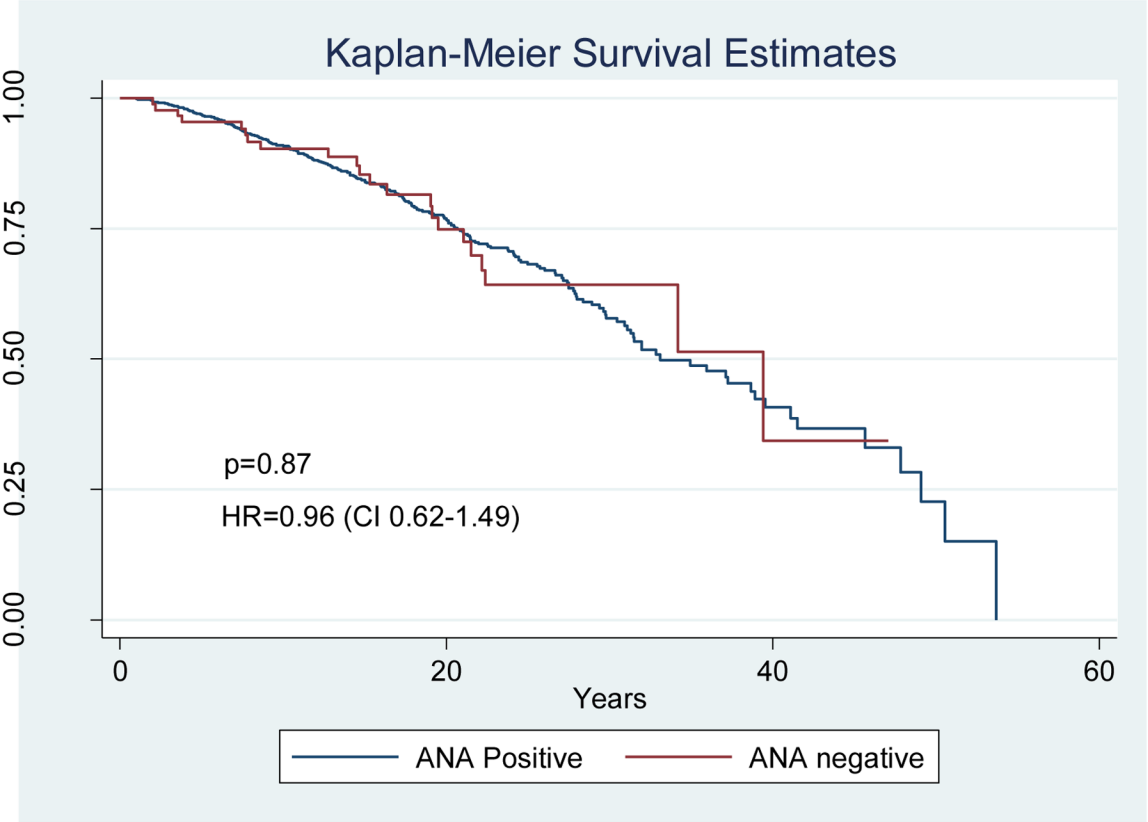

Figure 1.
Kaplan-Meier survivor function of ANA negative SSc patients compared to ANA positive patients

Table 1

Analysis of demographic and basic clinical parameters in systemic sclerosis (SSc) patients who are ANA negative compared with ANA positive patients.

	ANA negative (n=208)	ANA positive (n=3041)	*p*
Age at disease onset, mean +SD years	44.7+13.7	44.6+13.5	0.97
Disease duration to diagnosis, mean +SD	1.9+3.6	2.2+4.0	0.43
Disease duration to enrollment, median (IQR)	7.8(11.64)	6.9(10.99)	0.10
Gender (male),%	41(19.7)	402(13.2)	0.008
Ethnicity			
Caucasian,%	182(88.3)	2516(83.7)	0.07
African American,%	12(5.8)	221(7.3)	0.41
Hispanic,%	10(4.8)	204(6.7)	0.28
Asian,%	1(0.4)	42(1.4)	0.27
American Indian,%	1(0.4)	23(0.7)	0.65
Unknown or mixed,%	2(0.09)	35 (1.2)	0.78
Disease type (diffuse),%	105(49.8)	1240(40.1)	0.007

Table 2

Comparisons of clinical parameters in systemic sclerosis (SSc) patients who are ANA negative versus ANA positive.

	ANA negative (n=208)	ANA positive (n=3041)	OR [a]	b coef [a]	95% CI	*p*	*Adjusted P* [*]
mRSS, mean +SD	12 (10)	11 (10)	N/A	0.25	−2.25, 2.77	0.84	0.85
Telangiectasias,%	71/114(62)	1505/2061(73)	0.60	N/A	0.41, 0.91	0.01	0.03
Digital ulcers and pits,%	41/105(39)	1088/1925(56)	0.49	N/A	0.32, 0.73	0.001	0.008
Calcinosis,%	26/95(27)	518/1791(28)	0.92	N/A	0.58, 1.47	0.74	0.85
FVC%, mean +SD	80.6+21.7	84.1+19.4	N/A	−3.5	−6.69, −0.44	0.02	0.04
DLCO%, mean +SD	72+25	68+22	N/A	4.02	0.25, 7.8	0.03	0.05
Fibrosis per imaging,%	50/67(74)	716/1102(64)	1.66	N/A	0.93, 2.96	0.08	0.13
MPAP per RHC, mean +SD	23.4+18	29.4+13	N/A	−6.20	−11.30, −1.10	0.01	0.03
PAH per RHC,%	7/31(22.5)	221/405(55.4)	0.23	N/A	0.09, 0.55	<0.001	0.008
PH (per RHC and/or RVSP>50),%	13/133(9.7)	332/1907(17.4)	0.51	N/A	0.28, 0.92	0.02	0.04
GERD,%	116/130(89)	1834/2144(85)	1.40	N/A	0.79, 2.47	0.24	0.36
Malabsorption,%	48/94(51)	576/1597(36)	1.84	N/A	1.21, 2.80	0.004	0.02
CK, mean +SD	249 (309)	209 (704)	N/A	39.34	−380, 429	0.85	0.85
Renal crisis,%	10/208(4.8)	103/3041(3.3)	1.44	N/A	0.74, 2.8	0.28	0.38
Low LVEF(<50), %	4/106(3.7)	39/1614(2.4)	1.58	N/A	0.55, 4.51	0.39	0.48

Abbreviations: ANA= antinuclear antibodies, mRSS= modified Rodnan skin score, FVC= Forced vital capacity, DLCO= diffusing capacity of carbon monoxide, PAH= Pulmonary arterial hypertension, PH= Pulmonary Hypertension, MPAP= Mean pulmonary artery pressure, RHC= Right heart catheterization, RVSP= Right ventricular systolic pressure, GERD= Gastroesophageal reflux disease, CK= creatine kinase, LVEF= Left ventricular ejection fraction. OR =odds ratio; b coef= b coefficient; 95% CI=95% confidence interval; SD= standard deviation.

[a] Linear regression was used for continuous data; logistic regression was used for categorical data

[*] After False Discovery Rate (FDR) or Benjamini-Hochberg adjustment method for multiple comparisons.

Table 3

Multivariable analysis of clinical parameters in systemic sclerosis (SSc) patients who are ANA negative compared with ANA positive patients.

	OR [a]	b coef [a]	95% CI	*P*	*Adjusted P*
mRSS	N/A	−2.48	−4.54, −0.42	0.018	0.05
Telangiectasias	0.59	N/A	0.38, 0.91	0.01	0.03
Digital ulcers and pits	0.38	N/A	0.24, 0.59	<0.001	0.01
Calcinosis	0.63	N/A	1.08, 0.37	0.09	0.15
FVC%	N/A	−2.18	−5.33, 0.95	0.17	0.25
DLCO%	N/A	5.16	1.33, 8.99	0.008	0.03
Fibrosis per imaging	1.46	N/A	0.81, 2.64	0.20	0.27
MPAP per RHC	N/A	−5.58	−10.85, −0.32	0.04	0.07
PAH per RHC	0.28	N/A	0.11, 0.70	0.006	0.03
PH (per RHC and/or RVSP>50)	0.52	N/A	0.28, 0.94	0.03	0.06
GERD	1.32	N/A	0.75, 2.35	0.33	0.41
Malabsorption	1.66	N/A	1.08, 2.56	0.02	0.05
CK	N/A	26.64	−394, 447	0.90	0.90
Renal crisis	1.23	N/A	0.62, 2.43	0.53	0.61
Low LVEF (<50)	1.31	N/A	0.45, 3.83	0.61	0.65

Abbreviations: ANA= antinuclear antibodies, mRSS= modified Rodnan skin score, FVC= Forced vital capacity, DLCO= diffusing capacity of carbon monoxide, PAH= Pulmonary arterial hypertension, PH= Pulmonary Hypertension, MPAP= Mean pulmonary artery pressure, RHC= Right heart catheterization, RVSP= Right ventricular systolic pressure, GERD= Gastroesophageal reflux disease, CK= creatine kinase, LVEF= Left ventricular ejection fraction, OR=odd ratio; b coef= b coefficient; 95% CI=95% confidence interval; SD= standard deviation.

[a] Linear regression was used for continuous data; logistic regression was used for categorical data. The multivariable analysis was adjusted for age, disease duration, gender and disease type.

[*] After False Discovery Rate (FDR) or Benjamini-Hochberg adjustment method for multiple comparisons.