Published in final edited form as:

Arthritis Care Res (Hoboken). 2017 June ; 69(6): 922–926. doi:10.1002/acr.22978.

Increased risk of digital vascular events in scleroderma patients who have both anti-centromere and anti-interferon-inducible protein 16 antibodies

Zsuzsanna H. McMahan, M.D., M.H.S, Fredrick M. Wigley, M.D., and Livia Casciola-Rosen, Ph.D.

Division of Rheumatology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Abstract

Objective—We sought to evaluate whether scleroderma patients who are double-positive for anti-interferon inducible protein 16 (IFI-16) antibodies and anti-centromere (CENP) antibodies are at increased risk for significant digital vascular events relative to patients positive for anti-CENP antibodies alone.

Methods—Sera from 165 scleroderma patients who tested positive for anti-CENP antibodies upon clinical evaluation were re-assayed for both anti-CENP and anti-IFI-16 antibodies by ELISA. Patients who were positive for anti-CENP antibodies alone were then compared to patients who were double-positive for both anti-IFI-16 and anti-CENP antibodies. The association between a history of significant digital vascular events (digital pits, ischemic digital ulcers, and/or gangrene) and double-positive antibody status was examined using Chi squared tests. After completion of univariate analysis, multivariable analyses were done to adjust for clinically relevant covariates.

Results—Of the 165 anti-CENP antibody positive patients, 21 (12.7%) also had anti-IFI-16 antibodies. Patients who were double-positive for anti-CENP and anti-IFI-16 antibodies were more likely to have had digital pits, ischemic digital ulcers, and/or gangrene ($p = 0.03$). After adjusting for clinically relevant covariates, (age, cutaneous subtype, disease duration, and smoking), double-positive patients remained at significantly higher odds of having severe Raynaud's (OR = 3.5; CI 1.1, 11.1; $p = 0.03$).

Conclusion—Scleroderma patients who are double-positive for antibodies recognizing CENP and IFI-16 are significantly more likely to have significant digital vascular events during the course of their disease. This study provides further evidence that anti-CENP and anti-IFI-16 antibodies are disease biomarkers that may be used for risk stratification of vascular events in scleroderma.

Corresponding author: Zsuzsanna McMahan, MD, MHS, 5200 Eastern Ave, Suite 5200, Mason F Lord building Center Tower, Baltimore, MD 21224, Tel: 410-550-2003; fax 410-550-2072, zmcma1@jhmi.edu.

Conflicts of interest: None of the authors has received any financial support or other benefits from commercial sources for the work reported in this manuscript, nor do any of the other authors have any financial interests which could create a potential conflict of interest, or the appearance thereof.

INTRODUCTION

Scleroderma is a systemic autoimmune disease characterized by vasculopathy, fibrosis, and internal organ dysfunction. Raynaud's phenomenon, which is present in more than 90% of patients, is often the earliest sign of vascular dysfunction and may be associated with ischemic digital ulcers or digital loss in a subset of patients. Structural vascular disease is found in digital cutaneous vessels, as well as in other organs, such as the lung where it may manifest as pulmonary arterial hypertension (PAH) (1). Identifying the patients who are at the highest risk of serious vascular complications is important but clinically challenging. While we know that patients with limited skin disease and anti-centromere (CENP) antibodies are at increased risk of digital loss and pulmonary arterial hypertension, the variability of developing these complications within this group can be quite significant (2, 3). For example, among anti-CENP antibody positive patients, only ~20% develop PAH, ~60% develop digital ischemic events, and ~18% develop digital gangrene (4). This suggests that other factors may be playing a role in the subset of patients with severe vascular events. Early identification of high risk patients would allow for early aggressive management (5).

We recently reported that antibodies targeting IFI-16 in the serum of scleroderma patients convey an associated risk of digital gangrene (6). In this study we sought to determine whether the presence of anti-IFI-16 autoantibodies among anti-CENP positive patients (or "double positive status") is a useful marker for identifying patients who are at the highest risk for significant digital vascular events associated with tissue damage and loss.

PATIENTS AND METHODS

The cohort included 165 patients randomly selected from the Johns Hopkins Scleroderma Center cohort database who met either 1980 American College of Rheumatology (ACR) criteria or at least three of five features of the CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias) syndrome for scleroderma. Patients were classified as having diffuse or limited cutaneous scleroderma based on the extent of skin involvement (7, 8). All patients had an available serum sample defined clinically as anti-CENP (CENP) antibody positive, and if multiple samples were available, the serum sample closest to the maximum Raynaud's severity score (see definition below) was selected (9). All serum samples were obtained during routine clinical visits at the Johns Hopkins Scleroderma Center and were stored at -80 C. As a control group, 30 healthy subjects without a history of scleroderma or other autoimmune disease were selected. Written informed consent was obtained from all patients. The present study was approved by the Johns Hopkins Institutional Review Board.

Clinical Phenotyping

Demographic and clinical data, including age, race, sex, smoking status, disease duration, scleroderma subtype, specific organ involvement, and autoantibody status, were previously obtained on each patient at the time of the clinical visit. Raynaud's severity was defined by the validated Medsger severity score for Raynaud's phenomenon (RP) (0= no RP, 1= RP with or without vasodilator required, 2 = digital pitting scars, 3 = digital tip ulceration, 4 digital gangrene) (9). Significant digital vascular events were defined by the presence of

digital pits, digital ischemic ulcers, and/or digital gangrene, to represent the spectrum of more severe Raynaud's. Digital pitting scars are defined as small, hard, keratinized depressions at the tips of the fingers. Digital tip ulcerations are defined as ulceration at the tip of the finger. Digital gangrene is defined as demarcated ischemic territory on a digit (usually at the tip) which is dark, cool and often intensely painful. This gangrenous area is clearly demarcated from viable painless flesh at the proximal border. Gangrene does not include non-digital vascular lesions. Digital loss is scored when loss of the distal (or part of) the finger was due to ischemic scleroderma vascular disease, and other causes including traumatic, amputation, and infection were excluded by the treating physician. Internal organ involvement was assessed using previously published criteria (9). Specific measures of pulmonary function were determined by findings on pulmonary function tests (PFTs) (forced vital capacity [FVC] and single breath diffusing capacity for carbon monoxide [DLCO], measured as the absolute value as well as the percent predicted value for sex and age) (10). Estimated right ventricular systolic pressure was measured by echocardiogram (Echo). For this study, patients were considered to have evidence of pulmonary arterial hypertension (PAH) measured by right heart catheterization (RHC) was done with a mean arterial pressure (mPAP) ≥ 25 mmHg and a pulmonary capillary wedge pressure (pcwp) ≥ 15 mmHg. An isolated low DLCO, which is associated with PAH, was determined by an FVC/DLCO ratio ≥ 1.6 (11, 12). Pulmonary, gastrointestinal, or renal involvement was considered present when the associated Medsger severity score was ≥ 1 (9).

IFI-16 ELISA

Serum levels of anti-IFI-16 antibodies were quantified using an ELISA assay as follows. Recombinant full-length human IFI-16, expressed and purified as described (6), was used to coat 96-well ELISA plates (100 ng/well, overnight at 4°C). Washes were included between this and all subsequent steps, and all further incubations were carried out at room temperature for 1 hour. Wells were blocked with 5% BSA in PBS/0.05% Tween 20 (PBST), followed by incubation with human sera diluted 1:400 in 1% BSA/PBST. Horseradish peroxidase-labeled goat anti-human antibody (1:10,000 dilution, Jackson ImmunoResearch) was added to each well, and color development was subsequently performed with SureBlue peroxidase reagent (KPL) before reading absorbances at 450 nm. A positive reference serum (1:400 dilution with an OD in the linear range) was included in every IFI-16 ELISA assay; all absorbances were calibrated relative to this reference absorbance. Sera were considered to be positive for anti-IFI-16 antibodies if their log-transformed calibrated OD value was >2 SD above the mean log-transformed OD of the 30 healthy controls.

CENP ELISA

This confirmatory testing was performed using a commercially available kit, per the manufacturer's protocol (Inova Diagnostics).

Statistical analysis

This cohort was comprised of 165 anti-CENP positive patients with scleroderma. Anti-IFI-16 antibody positivity and anti-IFI-16 antibody levels were compared across two groups: patients "double-positive" for both anti-CENP and anti-IFI-16, and those positive for anti-CENP antibodies alone. In this cross-sectional analysis, the evaluation for associations

between dichotomous variables was done using Chi-square or Fischer's exact tests. T-tests were used for parametric data to evaluate for significant differences between the means of continuous variables between two groups. Non-parametric continuous variables compared across two groups were evaluated with the Wilcoxon-Mann-Whitney test. The dependence of vascular outcomes, including presence of significant digital vascular events (digital pits, ulcers or gangrene), and PAH on double-positive status were evaluated using univariate logistic regression. Significance was tested using regression coefficients, and the association between the presence or absence of dichotomous vascular outcomes and anti-IFI-16 antibody status was expressed as the odds ratio (OR) and the corresponding 95% confidence interval (95% CI), or as a P-value (considered significant when 0.05). Multi-variable logistic regression models were then constructed to further evaluate the association between double-positive status and the vascular outcome (e.g. digital loss, PAH), when adjusting for clinically relevant covariates that were fixed in the model.

We subsequently did sensitivity analyses to evaluate the association between each of the aforementioned vascular outcomes (dependent variables) and double-positive antibody status (independent variable). These associations were then further evaluated in the multivariable model mentioned previously. We also performed sensitivity analyses to assess the association between double positive status (independent variable) and non-gangrenous digital vascular events (dependent variable).

RESULTS

Disease characteristics of anti-IFI-16 and anti-CENP antibody double positive patients

Sera from 165 scleroderma patients who were classified as anti-CENP antibody positive by clinical assays were evaluated for anti-IFI-16 antibodies. Patients had serum samples drawn between July 18, 2007 and May 26, 2015 and 157 patients (95%) met ACR/EULAR 2013 criteria and 8 patients (5%) met CREST criteria. Since the anti-CENP antibody status of the patient sera had been assayed by multiple different clinical labs over a period 8 years, we first confirmed the presence of anti-CENP antibodies in each serum using a commercially available ELISA assay. We subsequently assayed the sera for anti-IFI-16 antibodies, and found that 21/165 (12.7%) were also positive for anti-IFI-16 antibodies. In this study, patients' mean age was 61 years (19 to 90 years), and the median disease duration defined by the time of first symptom to the time of the serum sample was 14 years (1–56 years). Neither age nor disease duration were significantly different between the double-positive and the anti-CENP alone antibody groups (Table 1). Forty-two patients in the cohort had digital pits, 39 had digital ulcers, and 15 had digital gangrene. Of the 15 patients with gangrene, all had a prior history of digital ulcers and/or digital pits.

The phenotypic characteristics of the double-positive patient group and the CENP alone group are summarized in Table 1. Of the 21 double-positive patients, 18/21 (85.7%) were female, 18/20 (90%) were Caucasian, 2/20 (10%) were African American, and 19/21 (90.5%) had the limited cutaneous subtype. Seven out of twenty-one (33.3%) were former smokers, 4/21 (19.1%) were current smokers, and 10/21 (47.6%) had never smoked ($p=0.10$). The two groups were similar in most clinical features of their disease, with no significant differences in gastrointestinal, lung, and renal involvement (Table 1). The

prevalence of calcinosis, which has been associated with vascular hypoxia, was higher in the double positive group, though the difference did not reach statistical significance (71% vs. 56%, $p = 0.17$). Cardiopulmonary measures were compared between the two groups and there were no significant differences in forced vital capacity, DLCO, or estimated right ventricular systolic pressure. Mean pulmonary arterial pressure obtained by RHC was also similar between the double-positive and CENP only subsets (32 mmHg vs. 33 mmHg; $p = 0.88$).

Double positive antibody status is significantly associated with significant digital vascular events

Compared to patients who were positive for anti-CENP antibodies alone, double-positive antibody status was associated with significant digital vascular events as characterized by digital pits, ulcers, or gangrene (81% vs. 56%; $p = 0.03$). Anti-IFI-16 antibody levels were highest in patients with vascular ulcers or gangrene, though the number of patients with gangrene was small (Figure 1). A history of digital loss was more prevalent in the double-positive group (29% vs. 17%; $p = 0.19$). There was also a positive association between anti-IFI-16 antibody levels and a history of digital loss (OR 2.67; CI 1.15, 6.22; $p = 0.01$). After adjusting for age, disease duration, cutaneous subtype, and smoking status, double-positive patients remained at significantly higher odds of developing clinically significant digital vascular events than their counterparts who were positive for anti-CENP antibodies alone (OR = 3.55; CI 1.1, 11.3; $p = 0.03$) (Table 2).

Sensitivity Analysis

Sensitivity analyses were then performed to determine whether double-positive status associated positively with subsets of patients with significant digital ischemia. In the univariate analysis, we evaluated the effect of double-positive status on each individual outcome (pits, ulcers, gangrene). While the groups were likely underpowered, the effect of double-positive status on the development of digital pits (OR 3.25, $p = 0.075$; CI 0.89, 11.9), digital ulcers (OR 3.55, $p = 0.056$; CI 0.97, 13.03), and digital gangrene (OR 4.06, $p = 0.09$; CI 0.81, 20.50) each trended toward statistical significance. We then entered each of the three outcomes separately into the multivariable model. The association between double-positive status and digital pits (OR 3.41, $p = 0.069$; CI 0.91, 12.84), digital ulcers (OR 3.83, $p = 0.051$; CI 1.0, 14.77), and digital gangrene (OR 5.57, $p = 0.065$; CI 0.90, 34.50) remained, even after adjusting for potential confounders.

We performed further sensitivity analyses to determine whether double-positive status identified patients at high risk of non-gangrenous digital ischemic complications. In this analysis, the outcome was defined by digital pits and digital ulcers alone. The univariate analysis demonstrated that double-positive patients had a 3.4 increased odds of developing digital pits or ulcers compared to patients without double-positive status ($p = 0.04$; CI 1.06, 10.86). After adjusting for potential confounders in the multivariable model, this association remained statistically significant (OR 3.57, $p = 0.034$; CI 1.10, 11.55).

DISCUSSION

In this study, we evaluated 165 anti-CENP antibody positive patients with scleroderma, a group known to be at increased risk of vascular complications, to determine whether the additional presence of anti-IFI-16 antibodies (“double-positive”) informed risk stratification for severe vascular complications within this group. Here we show that double-positive patients have 3.5 increased odds of having clinically significant digital vascular events compared to patients who are positive for anti-CENP antibodies alone. This finding provides rationale for using these combined antibody assays to identify patients at higher risk for significant digital vascular events.

The value of extended autoantibody profiles in the diagnosis and management of patients with autoimmune rheumatic diseases is increasingly recognized (13). While clinical phenotypes are tightly associated with clinically available scleroderma-specific autoantibodies, (e.g. CENP, RNA pol3), significant heterogeneity in clinical outcomes and associated end-organ complications still exist within each of these autoantibody-specific groups. Identifying and characterizing, new and/or co-existing specificities that serve as biomarkers of clinical subsets will provide a more comprehensive clinical picture for the treating physician. Autoantibodies targeting IFI-16 add further to our ability to risk stratify patients with severe peripheral vascular disease. We previously reported in a case-control study that anti-IFI-16 antibodies associate with digital gangrene in scleroderma. In our current study we demonstrate that anti-IFI-16 antibodies associate not only with digital gangrene, but also associate with other forms of digital tissue injury including digital pits and ulcers. Further validation studies will be needed to determine whether these autoantibodies can be utilized to identify patients who would benefit from more aggressive management.

A growing body of evidence suggests that early intervention may improve outcomes in the management of pulmonary vascular disease. In PAH, a rationale for earlier diagnosis and treatment is supported by the fact that patients with World Health Organization (WHO) functional class I or II have significantly better outcomes than patients in higher functional classes (5, 15). Utilizing a similar paradigm, the aggressive treatment of progressive vascular disease, before the onset of tissue ischemia, in patients at particularly high risk for severe peripheral vascular complications (e.g. gangrene) could be warranted if appropriate tools for risk stratification and biomarkers of disease activity were available.

Our findings are limited by the retrospective study design, which inevitably led to some missing data. We did not fully evaluate other coexisting antibodies. Our study was cross-sectional and enriched in serum samples drawn close to the vascular event, because event proximity was found to be relevant in our prior work (6). This element of the study design may have improved our ability to see the association between anti-IFI-16 antibodies and severe Raynaud's. As this was a cohort study, the number of patients with digital vascular events was small and not equally matched with the number of controls, thus conclusions should be drawn cautiously. Our results show anti-CENP antibody positive patients who also have antibodies to IFI-16 are at particularly high risk for significant digital vascular events

and indicate that large, prospective studies evaluating the use of anti-IFI-16 antibodies as predictors of peripheral vascular disease are warranted.

Acknowledgments

We thank Adrienne Woods and Margaret Sampedro for their involvement in the dataset generation and sample acquisition.

Sources of Financial support: Dr. McMahan's funding for this project was provided by the Rheumatology Research Foundation, Scientist Development Award, by the Jerome L. Greene Foundation, and by the Scleroderma Research Foundation. Dr. Casciola-Rosen's work was supported by the NIH (R56-AR-062615, RO1-DE-12354 and P30-AR-053503). Dr. Wigley receives support from the Scleroderma Research Foundation and the Martha McCrory Professorship.

REFERENCES

- Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. *N Engl J Med*. 2009 May 7; 360(19):1989–2003. [PubMed: 19420368]
- Wigley FM, Wise RA, Miller R, Needleman BW, Spence RJ. Anticentromere antibody as a predictor of digital ischemic loss in patients with systemic sclerosis. *Arthritis Rheum*. 1992 Jun; 35(6):688–693. [PubMed: 1599523]
- Kampolis C, Plastiras S, Vlachoyiannopoulos P, Moyssakis I, Tzelepis G. The presence of anti-centromere antibodies may predict progression of estimated pulmonary arterial systolic pressure in systemic sclerosis. *Scand J Rheumatol*. 2008 Jul-Aug;37(4):278–283. [PubMed: 18612928]
- Steen VD. The many faces of scleroderma. *Rheum Dis Clin North Am*. 2008 Feb;34(1) 1,15;v.
- Humbert M, Gerry Coghlan J, Khanna D. Early detection and management of pulmonary arterial hypertension. *Eur Respir Rev*. 2012 Dec 1; 21(126):306–312. [PubMed: 23204118]
- McMahan Z, Shah A, Vaidya D, Wigley F, Rosen A, Casciola-Rosen L. Anti-IFI-16 antibodies in scleroderma are associated with digital gangrene. 2015
- LeRoy EC, Medsger TA Jr. Criteria for the classification of early systemic sclerosis. *J Rheumatol*. 2001 Jul; 28(7):1573–1576. [PubMed: 11469464]
- Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum*. 1980 May; 23(5):581–590. [PubMed: 7378088]
- Medsger TA Jr, Silman AJ, Steen VD, Black CM, Akesson A, Bacon PA, et al. A disease severity scale for systemic sclerosis: development and testing. *J Rheumatol*. 1999 Oct; 26(10):2159–2167. [PubMed: 10529133]
- Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med*. 1999 Jan; 159(1):179–187. [PubMed: 9872837]
- Chang B, Schachna L, White B, Wigley FM, Wise RA. Natural history of mild-moderate pulmonary hypertension and the risk factors for severe pulmonary hypertension in scleroderma. *J Rheumatol*. 2006 Feb; 33(2):269–274. [PubMed: 16465657]
- Steen V, Medsger TA Jr. Predictors of isolated pulmonary hypertension in patients with systemic sclerosis and limited cutaneous involvement. *Arthritis Rheum*. 2003 Feb; 48(2):516–522. [PubMed: 12571862]
- 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 Rheumatol*. 2015 Dec; 67(12):3234–3244. [PubMed: 26246178]
- Kawaguchi Y, Nakamura Y, Matsumoto I, Nishimagi E, Satoh T, Kuwana M, et al. Muscarinic-3 acetylcholine receptor autoantibody in patients with systemic sclerosis: contribution to severe gastrointestinal tract dysmotility. *Ann Rheum Dis*. 2009 May; 68(5):710–714. [PubMed: 18762475]

15. Visovatti SH, Distler O, Coghlan JG, Denton CP, Grunig E, Bonderman D, et al. Borderline pulmonary arterial pressure in systemic sclerosis patients: a post-hoc analysis of the DETECT study. *Arthritis Res Ther*. 2014 Dec 10.16(6) 493,014-0493-1.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

SIGNIFICANCE AND INNOVATIONS

- Scleroderma patients who are double-positive for anti-IFI-16 and anti-centromere autoantibodies are at increased risk of significant digital vascular events (digital pits, ulcers, or gangrene) relative to scleroderma patients who are only positive for anti-centromere antibodies.
- Among double positive patients, anti-IFI-16 levels were highest in patients with ischemic ulcers or gangrene.
- Anti-IFI-16 antibodies may highlight those anti-centromere antibody positive scleroderma patients who are at highest risk for significant digital vascular events.

Figure 1.

Anti-IFI-16 antibody levels are higher in patients with digital ulcers and gangrene than in comparator groups. Levels of anti-IFI-16 antibodies in anti-centromere antibody positive patients with scleroderma were assessed by ELISA and quantified by optical density (OD). Median anti-IFI-16 antibody levels were highest in patients with ischemic digital ulcers and digital gangrene.

Table 1

Demographic and disease characteristics of the 165 anti-CENP antibody positive scleroderma patients. Patients are grouped by the presence or absence of anti-IFI-16 antibodies.

Variable	Negative (n=144)	Positive (n=21)	P-value
Age at serum draw, year (range 19–90)	60.6±13.1	65.3±13.9	0.127
Age at Raynaud's onset (8–83)	40.2 ±15.1	42.9 ±17.4	0.449
Female, %	93.8	85.7	0.183
Race, %			
Caucasian	92.3	90.0	0.715
African American	6.3	10.0	
Other	1.4	0.0	
Ever smoker, %	42.3	52.4	0.382
Scleroderma type, limited, %	95.8	90.5	0.270
Disease duration, yrs (range 0–56) ⁺ °	13.6 (7.8–21.3)	17.9 (9.5–24.0)	0.153
Interval between MaxRP and serum (yrs) [^]	0.3 ±1.8	0.14 ±0.9	0.629
GI involvement (>1) [^] , %	63.9	71.4	0.500
Pulmonary involvement (>1) [^] , %	73.7	81.0	0.596
Renal involvement, %	19.6	20.0	1.000
Significant digital vascular events (>2), %[#]	55.6	81.0	0.033
Digital pits, % (n)	24 (35)	33 (7)	0.099
Digital ulcers, % (n)	22 (32)	33 (7)	0.093
Digital gangrene, % (n)	8 (12)	14 (3)	0.104
Calcinosis, %	55.6	71.4	0.169
Pulmonary function			
FVC, %predicted	88.2 ± 16.2	83.9 ± 13.3	0.253
DLCO, %predicted	69.8 ± 21.5	65.7 ± 21.9	0.429
RVSP, mmHg [°]	40.0 (32.5–49.0)	43.1 (28–56)	0.757
Mean pulmonary artery pressure (mPAP), [†]	31.8 ± 12.9 (n=31)	32.8 ± 13.8 (n=5)	0.875
Pulmonary artery hypertension, % ^{**}	63.3 (n=19)	80.0 (n=4)	0.640

⁺Disease duration from first symptom;

[°] median (IQR);

[^] MaxRP = maximum Medsger severity score;

[#] defined by pits, ulcers, or gangrene;

^{**} Pulmonary arterial hypertension based on right heart catheterization

Table 2

Multivariable logistic regression model evaluating the association between the presence of Raynaud's-associated significant digital vascular events (pits, ulcers, gangrene) and anti-IFI-16 antibody status among anti-CENP positive patients, adjusted for clinically relevant covariates *

Covariate	OR	95% CI	p-value
Anti-IFI-16 antibody positive *	3.55	1.1, 11.3	0.03
Age at serum	0.99	0.96, 1.01	0.31
Disease duration**	1.01	0.98, 1.05	0.58
Smoking (ever/never)	1.13	0.58, 2.18	0.72
Limited cutaneous subtype	1.00	0.21, 4.56	0.99

* Antibody levels are defined by the optical density dichotomized by positive/negative status; positive is defined as 2 standard deviations from the mean of normal controls; Disease duration defined from 1st symptom to serum sample