
Smoking and systemic sclerosis: influence on microangiopathy and expression of anti-topoisomerase I antibodies in a monocentric cohort

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ABSTRACT

Objective. To investigate whether systemic sclerosis (SSc) patients exposed to active tobacco smoke exhibit a different autoantibody profile or are at higher risk for severe microangiopathy compared to never-smokers, and to assess differences between men and women.

Methods. We performed an exploratory observational study in a cohort of SSc patients fulfilling the 2013 ACR/EULAR classification criteria. According to the smoking habit, patients were categorised as ever-smokers or never-smokers. Microvascular damage was assessed at baseline using nailfold videocapillaroscopy. The presence of SSc-specific autoantibodies was investigated.

Results. The studied population was composed of 361 patients (279 women, 82 men). Of these, 208 (58%) were ever-smokers and 153 (42%) were never-smokers. Anti-centromere, anti-topoisomerase I (ATA) and anti-RNA polymerase III antibodies were found, respectively, in 90 (43%), 41 (20%), and 11 (5%) ever-smokers, and in 66 (43%), 40 (26%) and 5 (3%) never-smokers (all $p > 0.05$). Scleroderma patterns early, active and late were present respectively in 12%, 44% and 21% of ever-smokers, and in 9%, 48%, and 29% of never-smokers (all $p > 0.05$). In multivariable logistic regression, being a never-smoker was significantly associated with ATA positivity (OR 1.77, 95% CI 1.04–2.99, $p = 0.034$). In the gender-based sub-cohorts, 36 (27%) female patients who never smoked were ATA positive, compared to 16 (11%) ever-smoking women ($p < 0.001$).

Conclusion. We observed a significant association between smoking history and positivity of ATA and we outlined the idea of a different effect of smoking on autoantibody expression between men and women.

Introduction

Systemic sclerosis (SSc) is a complex connective tissue disease with elusive pathogenesis, characterised by vasculopathy, autoimmunity, and progressive fibrosis (1). Microangiopathy is an early manifestation of SSc (1) and Raynaud's phenomenon (RP) is often the presenting symptom of the disease. Smoking has detrimental effects on the intensity of RP and on vascular outcomes in patients with SSc (2). Smoking habit is also associated with impaired hand function in patients with SSc (3) and smoking cessation is advised to reduce the symptoms of RP. Nailfold videocapillaroscopy (NVC) is the most widely used tool for the assessment of microvascular damage in patients with SSc and smoking is one of the factors potentially affecting microangiopathy (4). Smoking has distinct effects in different autoimmune diseases, ranging from protective to deleterious (5) but, in rheumatology, robust evidence highlights the role of smoking in autoantibody production and in disease pathogenesis. In rheumatoid arthritis (RA), one of the mechanisms hypothesised for disease onset, is the generation of anti-citrullinated protein antibodies (ACPA) in response to citrullination of peptides in the lungs under the noxious stimulus of smoking in genetically predisposed individuals (6). Moreover, recent findings suggest that, in RA patients, smoking is associated with the presence of multiple autoantibodies rather than with the single positivity of ACPA (7). In systemic lupus erythematosus (SLE), two studies (8, 9) demonstrated higher risk of anti-double stranded DNA (anti-dsDNA) antibody positivity in current smokers compared to former and never-smokers. Conversely, in two SSc cohorts, ever-smokers have been found to be at lower risk of being anti-topoisomerase

Competing interests: none declared.

I antibodies (ATA) positive compared to never-smokers (10, 11), but little is known about eventual differences in the effects of tobacco smoke on autoantibody status between male and female patients. The aim of our study was to evaluate the influence of smoking on nailfold NVC patterns and on the expression of SSc-specific autoantibodies in a cohort of SSc patients. Secondly, we compared findings between men and women.

Materials and methods

Patients

We conducted an explorative, observational, monocentric study on adult patients enrolled in the Leiden Combined Care in SSc (CCISS) cohort between 2nd April 2009 and 31st December 2018. All patients had to fulfil the 2013 ACR/EULAR classification criteria for SSc (12). For cases enrolled before their availability, the above-mentioned criteria were retrospectively applied. Being interested in NVC findings, we selected all patients in which NVC was performed at baseline. Research on the CCISS cohort is approved by the Ethics Committee of Leiden University Medical Center (approval no. P09.003) and all patients gave written informed consent. The study was conducted in conformity with the principles of the Declaration of Helsinki.

Variables

All data were collected in a dedicated electronic database. Medical records were checked in case of missing variables. Baseline characteristics were considered at time of inclusion in the cohort. Microvascular damage was qualitatively assessed based on presence of normal findings, aspecific/secondary pattern, or distinct “early”, “active”, or “late” scleroderma patterns, according to the classification proposed by Cutolo *et al.* (13). Information about smoking status was collected, but data regarding intensity and duration of tobacco exposure were not available for all individuals. Patients were thus categorised as “never-smokers” or, if current or former smokers, as “ever-smokers”. The presence of ATA and of anti-centromere (ACA) and anti-RNA

Table I. Demographic, clinical, and videocapillaroscopic patterns in the whole SSc population, in ever-smokers and never-smokers.

	All patients (n=361)	Ever-smokers (n=208)	Never-smokers (n=153)	p-value
Demographic				
females/males, n (%)	279/82 (77/23)	146/62 (70/30)	133/20 (87/13)	
<0.001FAge (years), mean (SD)	55.9 (14.6)	56.3 (13.5)	55.4 (15.9)	0.54
Duration RP (years), median (IQR)	10.1 (2.9-20.6)	8.7 (2.7-19.4)	11.8 (3.3-21.5)	0.14
Duration non-RP (years), median (IQR)	3.3 (0.9-10.1)	3 (0.8-8)	3.9 (1.1-12)	0.06
Disease subset				
lcSSc, n (%)	228 (63)	126 (60)	102 (67)	0.24
dcSSc, n (%)	71 (20)	43 (21)	28 (18)	0.57
ssSSc, n (%)	62 (17)	39 (19)	23 (15)	0.35
Autoantibodies				
ATA, n (%)	81 (22)	41 (20)	40 (26)	0.15
ACA, n (%)	156 (43)	90 (43)	66 (43)	0.98
ARA, n (%)	16 (4)	11 (5)	5 (3)	0.36
NVC pattern				
scleroderma pattern early, n (%)	40 (11)	26 (12)	14 (9)	0.32
scleroderma pattern active, n (%)	164 (45)	91 (44)	73 (48)	0.45
scleroderma pattern late, n (%)	89 (25)	44 (21)	45 (29)	0.07
normal pattern, n (%)	4 (1)	4 (2)	0	0.08
aspecific/secondary pattern, n (%)	64 (18)	43 (21)	21 (14)	0.09

lcSSc: limited cutaneous systemic sclerosis; dcSSc: diffuse cutaneous systemic sclerosis; ssSSc: sine-scleroderma systemic sclerosis; SD: standard deviation; IQR: interquartile range; ATA: anti-topoisomerase I antibody; ACA: anti-centromere antibody; ARA: anti-RNA polymerase III antibody.

polymerase III (ARA) antibodies was investigated as part of the screening for anti-extractable nuclear antigens (anti-ENA) antibodies, measured by fluorescence enzyme-linked immune sorbent assay (FEIA), using Phadia250® system (Thermo Fisher Scientific, Nieuwegein, The Netherlands).

Statistical analysis

Baseline characteristics of all SSc patients were summarized using descriptive statistics. For comparison between ever-smokers and never-smokers, Pearson's chi-squared test was used for categorical variables and two-sample t-test or Mann-Whitney U-test were used to compare differences between, respectively, normally or non-normally distributed continuous variables. In order to control for possible confounders, we also used multivariable logistic regression to investigate the influence of smoking on microvascular damage and on autoantibody positivity. Three distinct analyses were performed. Presence of late scleroderma pattern, indicative of the worst degree of microangiopathy, and positivity of ATA or ACA, were respectively entered as dependent variables. Odds ratio (OR) and 95%

confidence intervals (CI) are reported. SPSS v. 23 was used to conduct all analyses, with $p < 0.05$ considered significant.

Results

Patients characteristics

Of the 762 patients enrolled in the CCISS cohort until 31st December 2018, 401 were excluded because of not meeting the 2013 ACR/EULAR classification criteria or lack of baseline NVC. Therefore, the studied population was composed of 361 patients (279 women and 82 men). Mean age at inclusion in the CCISS cohort was 55.9 ± 14.6 years (Table I), median time since onset of RP was 10.1 years (IQR 2.9-20.6) and median time since onset of first non-Raynaud SSc-related sign or symptom was 3.3 years (IQR 0.9-10.1). Concerning the disease subset, 228 patients (63%) had limited SSc, 71 (20%) had diffuse SSc, and 62 (17%) were classified as having the sine-scleroderma form. In our cohort, 208 (58%) patients were current or past smokers and 153 (42%) had never smoked. A higher prevalence of male patients was observed among smokers ($p < 0.001$).

Table II. Association of smoking history, in multivariable logistic regression, with positivity of anti-topoisomerase I antibodies (ATA).

	ATA positivity	
	OR (95% CI)	p-value
Female sex	0.36 (0.20 – 0.64)	<0.001
Being a never-smoker	1.77 (1.04 – 2.99)	0.034
Age	0.99 (0.97 – 1.01)	0.261

Table III. Autoantibody positivity and videocapillaroscopy patterns of ever-smokers and never-smokers in the gender-based sub-cohorts.

	Men			Women		
	Ever-smokers	Never-smokers	p-value	Ever-smokers	Never-smokers	p-value
n (%)	62 (76)	20 (24)		146 (52)	133 (48)	
Autoantibodies						
ATA, n (%)	25 (40)	4 (20)	0.10	16 (11)	36 (27)	<0.001
ACA, n (%)	10 (16)	6 (30)	0.17	80 (55)	60 (45)	0.11
ARA, n (%)	2 (3)	0	0.42	9 (6)	5 (4)	0.36
NVC pattern						
scleroderma early, n (%)	5 (8)	0	0.19	21 (14)	14 (10)	0.33
scleroderma active, n (%)	24 (39)	11 (55)	0.20	67 (46)	62 (47)	0.90
scleroderma late, n (%)	13 (21)	5 (25)	0.70	31 (21)	40 (30)	0.09
normal, n (%)	2 (3)	0	0.42	2 (1)	0	0.18
aspecific, n (%)	15 (24)	3 (15)	0.43	20 (14)	14 (10)	0.31

ATA: anti-topoisomerase I antibody; ACA: anti-centromere antibody; ARA: anti-RNA polymerase III antibody.

Influence of smoking on NVC patterns and on the expression of auto-antibodies in the studied population

No statistically significant difference was found comparing the disease characteristics and the NVC patterns of ever-smokers and never-smokers (Table I). ACA, ATA, and ARA were found, respectively, in 90 (43%), 41 (20%), and 11 (5%) ever-smokers, and in 66 (43%), 40 (26%) and 5 (3%) never-smokers (all $p>0.05$). Scleroderma patterns early, active, and late, were present respectively in 12%, 44% and 21% of ever-smokers, and in 9%, 48%, and 29% of never-smokers (all $p>0.05$).

In the first multivariable logistic regression, smoking history was not associated with presence of late scleroderma pattern at NVC. This analysis was adjusted for sex, age, disease duration since onset of RP, BMI, positivity of ACA or ATA. In the second analysis, adjusted for sex and age, being a never-smoker was significantly associated with ATA positivity (OR 1.77, 95% CI 1.04–2.99, $p=0.034$) (Table II). In the third analysis, the expression of ACA was not influenced by smoking history.

Differences in NVC patterns and expression of autoantibodies in the gender-based sub-cohorts

When evaluating differences between ever-smoking and never-smoking female and male patients (Table III), we again observed non-significant differences of NVC patterns in smokers compared to sex-matched non-smokers but, regarding autoantibodies, 36 (27%) female patients who never smoked were ATA positive, compared to 16 (11%) ever-smoking women ($p<0.001$). The opposite trend of ATA positivity was found in men, although not reaching statistical significance ($p=0.10$), with 25 (40%) ATA-positive ever-smokers, and 4 (20%) ATA-positive never-smokers. No difference in presence of ACA or ARA between ever-smokers and never-smokers was found either in men or women.

Discussion

The aim of our study was to assess differences in autoantibody profiles and patterns of microvascular damage between ever-smokers and never-smokers in a cohort of SSc patients. We did not

observe a detrimental effect of smoking on microangiopathy in our patients and the prevalence of NVC patterns was comparable between ever-smokers and never-smokers, with results not changing when gender-based sub-cohorts were considered separately. Regarding autoantibody positivity, in the studied population we found a significant association between negative history of smoking and presence of ATA. The prevalence of ATA was lower in ever-smokers and this disparity was largely contributed by the statistically significant higher frequency of ATA positivity in women who never smoked compared to ever-smokers.

Smoking plays different roles in autoimmunity (5), but its association with autoantibody production has been reported both in RA (6, 7) and in SLE (8, 9). Interestingly, in SSc the available evidence seems to point in a different direction and two previous studies (10, 11) described a higher risk of ATA positivity in never-smokers. Our results confirm these findings.

Although it might be a spurious association until not replicated in other cohorts, we also outline the possibility that in SSc smoking might have a different effect on autoantibody expression in men and women. Further studies in larger populations could help to elucidate the interplay between sex, smoking, and autoantibody profile but, regarding our data, we can postulate a hypothesis. No association was found between cumulative endogenous oestrogen exposure and severity of microangiopathy in SSc (14), but oestrogens, acting as immune system enhancers (15), have been suggested to be implicated in autoantibody production (16). A role of oestrogens has also been hypothesised in the pathogenesis of SSc (17) and smoking has been shown, although not unanimously (18), to exert modulatory effects on sex hormones' metabolism. Therefore smoking, with its inhibitory action on oestrogens, might dampen immunoreactivity and autoantibody production. However, it is not clear why only ATA, and not ACA or ARA, should be selectively affected by this phenomenon, and further considerations in this regard appear specu-

lative given the explorative nature of our study and the presence of some limitations. Specifically, individual-level intensity and duration of cigarette smoking were not available in most of the patients and we were not able to calculate pack-years in our cohort. We believe that, for future studies, integrating the evaluation process with thorough assessment of tobacco exposure, could be of great value to elucidate the role of smoking in SSc, with particular interest in autoantibody expression. Other possible limitations are that we did not take into account second-hand smoke, and that we had no means of verifying whether information reported by the patients was accurate or not. However, we present data on a relatively large and well-characterised SSc population. All patients underwent NVC examinations as part of the standard assessment which also includes a questionnaire regarding smoking habits, and NVC images were evaluated by certified and trained readers.

In summary, according to our data, in SSc patients there is no association between smoking and NVC pattern, nor between sex and NVC pattern but, intriguingly, we observed an association between smoking and autoantibody expression. However, firm conclusions cannot be drawn. Given the potential consequences on risk stratification in clinical practice, but also to gain a better insight in the possible drivers of antibody expression, confirmation in other SSc cohorts is warranted to determine the robustness of our results.

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