Systemic sclerosis: can we identify patients at risk?

Leeuwen, N.M. van

Part III

Role of microangiopathy and specific autoantibodies in Systemic Sclerosis

Chapter 6

The contribution of sex and auto-antibodies to microangiopathy assessed by nailfold videocapillaroscopy in systemic sclerosis: a systematic review of the literature

Nina M. van Leeuwen, Jacopo Ciaffi, Jan W. Schoones,
Tom W.J. Huizinga, Jeska K. de Vries-Bouwstra

Published Arthritis Care Res (Hoboken)

Objective: Microangiopathy and dysregulation of the immune system play important roles in the pathogenesis of Systemic Sclerosis (SSc). Factors that trigger vascular injury in SSc have not been elucidated so far. To evaluate whether sex or expression of specific antinuclear auto-antibodies might associate with the degree of microangiopathy we performed a systematic review summarizing what is known about these associations.

Methods: Standardized search of PubMed, EMBASE, Web of Science and the Cochrane library were performed to identify studies, that report on auto-antibodies in SSc patients and microangiopathy, and for the second search, that report on sex and microangiopathy.

Result: We included 11 studies that described the relationship between SSc-specific autoantibodies and microangiopathy and 6 studies that reported on the association between sex and microangiopathy. Contradictory results were found on the association between auto-antibodies and microangiopathy, and no association was found between sex and microangiopathy based on the current literature.

Conclusion: Based on this review of the literature, we can conclude that sex does not seem to influence degree of microangiopathy in SSc, while results on association between SSc-specific auto-antibodies and degree of microangiopathy were inconclusive.

INTRODUCTION

Systemic Sclerosis (SSc) is characterised by a triad of microvascular damage, dysregulation of innate and adaptive immunity, and generalized fibrosis that can affect skin and internal organs (1). In SSc, the most frequent symptom of microvascular damage is Raynaud's phenomenon (RP), which is present in up to 96% of patients and often represents the earliest manifestation of the disease. Current concepts indicate that microangiopathy is a key factor in early pathogenesis of SSc. In RP evolving to definite SSc, presence of microvascular damage and SSc specific auto-antibodies indicate a very high probability of developing SSc (2). The frequency of progression is higher with both the presence of SSc auto-antibodies and microvascular damage (79.5%), than with presence of one of these predictors (32.2%) (3). In addition to its diagnostic value, the degree of microangiopathy is also a valuable prognostic marker in SSc patients, as it contributes to predict future organ complications (3-5). The SSc specific auto-antibodies are associated with specific clinical characteristics and therefore are of additional prognostic value. Anti-centromere antibody (ACA) is associated with a decreased risk of lung (OR 0.12) and heart involvement (OR 0.39), while anti-topoisomerase antibody (ATA) + patients have an increased risk for these complications (OR 6.66, OR 2.12) (6, 7). Strikingly, the degree of microangiopathy was comparable between ACA+ and ATA+ patients (late SSc pattern; ACA 33%, ATA 25%). This suggests that presence of a specific antinuclear antibody is independent of the development of microangiopathy.

However, in some studies, an association between microvascular damage and auto-antibodies has been described (8). Antinuclear auto-antibodies (ANA), found in 95% of SSc patients, have been mentioned as one of the possible triggers for vascular injury, by causing acceleration of vascular endothelial cell senescence and therefore inducing RP (9, 10). Other studies suggest that auto-antibody production occurs secondary to vasculopathy, and as such these auto-antibodies should be viewed as a bystander in disease pathogenesis (7, 11, 12).

Vasculopathy in SSc involves all layers of the peripheral blood vessels and is caused by a dysfunction of the endothelium, resulting in an imbalance of vasoactive factors. In particular endothelin-1 plays a prominent role in the regulation of vascular tone through its receptors. RP induces prolonged ischemia-reperfusion injury, which may cause persistent endothelial activation, resulting in apoptosis, microvascular damage, and other toxic stimuli. Recent insights showed that impaired functioning of endothelial progenitor cells could be involved in angiogenic response and in the pathogenesis of SSc. Microvascular tone alterations and cell apoptosis trigger the opening of intercellular junctions in the endothelial barrier. This loss of integrity favors further migration and

homing of inflammatory cells inducing increased microvascular permeability and progressive vascular leak (13). Infective stimuli, environmental exposures, sex, and endocrine disturbances, have all been proposed as contributors to microangiopathy (14, 15).

In SSc there is a marked sex imbalance, with higher prevalence of the disease in females than in males (4:1). Also distribution of ANA is disbalanced with females showing more frequently ACA antibody and males showing more frequently ATA antibody. In general, disease course is more severe in males resulting in lower survival rates (45% vs 23% after 10 years) (16-20). The most frequent disease related causes of death also differ between males and females: interstitial lung disease in males and pulmonary hypertension (PH) in females (21). The higher incidence of PH in females, and the fact that unopposed estrogens replacement therapy has been associated with increased RP, suggests a contribution of hormonal factors to microangiopathic manifestations (22). However, little information is known on the relationship between sex and microangiopathy in SSc.

As microvascular damage is one of the hallmarks of SSc, different imaging techniques have been applied to evaluate structural and functional abnormalities of the finger microcirculation in patients with SSc (23-26) (supplementary file). However, NVC is considered the most reliable tool to distinguish between primary and secondary RP. NVC is widely applied and provides the opportunity to directly visualise the evolving obliterative microangiopathy and nailfold capillary abnormalities characteristic of SSc, that have been classified as scleroderma pattern (27).

Given the role of microangiopathy in the pathogenesis of SSc, insights in the factors responsible for microvascular damage could contribute to our understanding of disease pathophysiology. Therefore, we decided to evaluate and summarize in this comprehensive review what is known about the association between the expression of specific auto-antibodies and microangiopathy, and between sex and microangiopathy in SSc.

METHODS

Literature search

A systematic literature search was performed by J.W.S, including studies published before June 17th 2019. The databases used were Medline (via PubMed), Web of Science, Cochrane and Embase. No restrictions on date were applied and manuscripts published in English or Dutch language were selected. The search strategy intended to include all relevant papers reporting on adult patients with SSc, in which microangiopathy of the hand was evaluated and where association with SSc-specific auto-antibodies was assessed. A second systematic literature search performed the same day intended to include all relevant papers reporting on adult patients with SSc, in which microangiopathy in the hand was evaluated and a comparison between male and female patients was described (see supplementary file for search strategies).

Two reviewers (N.v.L and J.C) independently screened the titles of retrieved articles and, in case one or both reviewers identified a publication as possibly relevant, the study proceeded to abstract screening. In case of discrepancies in agreement, abstracts were reviewed by a third investigator (J.d.V.B). Full text reading was performed for the selected abstracts by N.v.L and J.C.

Screening process and study selection criteria

For the review on auto-antibodies and microangiopathy the following criteria were applied: 1) adult participants (>18 years) with a clinical diagnosis of SSc; 2) fulfilment of either American College of Rheumatology (ACR) 2013, ACR 1980, or LeRoy and Medsger criteria (28, 29); 3) report on prevalence of SSc-related auto-antibodies including at least anti-topoisomerase I antibodies (ATA) or anti-centromere antibodies (ACA) and additionally anti-RNA polymerase III (anti-RNAPIII), anti-RNA polymerase I, anti-fibrillarin, anti-PM/Scl, or anti-Th/To antibodies; 4) assessment of microangiopathy using one or more of the following imaging modalities: nailfold NVC, laser dermoscopy, doppler confocal microscopy, LASCA/video image analysis, and photomicroscopy.

For the review on sex and microangiopathy the following criteria were applied: 1) adult participants (>18 years) with a clinical diagnosis of SSc; 2) fulfilment of either ACR 2013, ACR 1980, or LeRoy and Medsger criteria (28, 29); 3) report on comparison between female and male patients, and with at least n= 3 and 10% males included in the study; 4) assessment of microangiopathy using one or more of the following imaging modalities: NVC, laser dermoscopy, doppler confocal microscopy, LASCA/video image analysis, and photomicroscopy.

Exclusion criteria for both search strategies were: animal studies, editorials, reviews, letters to the editor, unpublished material, case-reports and manuscripts written in languages other than English or Dutch.

Quality assessment

The Newcastle-Ottawa scale was used for assessment of quality of case-control studies, whereas the National Institutes of Health quality assessment tool was used for observational cohort studies (30, 31). Discrepancies in scoring and implications for interpretation of the findings were discussed between N.v.L and J.C.

Evaluation of capillaroscopic descriptions throughout the studies

As in literature a variety of definitions are used to describe NVC. In this review we will report the NVC findings in a standardized way by evaluating the used terminology to describe NVC characteristics per included article. In line with the EULAR recommendations on capillaroscopy, the NVC characteristics can be evaluated quantitatively, qualitatively or semi-quantitively (32). See the supplementary file for a detailed explanation. When available, all these NVC characteristics were extracted throughout the included articles.

RESULTS

Literature search and study description

Figures 1 and 2 show the flowcharts of the systematic review processes. Eleven studies reporting on the association between auto-antibodies and microangiopathy (7, 8, 11, 33-40), and six studies reporting on sex and microangiopathy (33, 37, 40-43), were included. Three studies answered both questions (33, 37, 40). All included articles were cohort or case-control studies, but many were limited by small sample sizes. In the majority of the included articles, except for four (8, 11, 42, 43), the association of interest was not the primary outcome of the study. Characteristics of all included studies are provided in Table 1. In all, these studies reported on 4704 women (83%) and 971 men (17%), with a mean age of 49 years. Subtypes of SSc were specified in all but one article (for diffuse cutaneous SSc (dcSSc) n= 1473, 28%; for limited cutaneous SSc (lcSSc) n= 3746, 72%). Disease duration was defined either as time since onset of RP, as time since onset of first sign or symptom attributable to SSc different from RP, or as time since diagnosis, and ranged between 6 months and 37 years.

Comprehensiveness of reporting

The comprehensiveness of reporting was variable. Although all selected studies used NVC, the parameters to describe microangiopathy and to classify severity of microvascular changes differed between the studies.

Risk of Bias

Study quality is summarized in Table S1 (supplementary file). Three articles were assessed as high quality (7, 8, 33), nine as medium quality (11, 33-39, 42), and two as low quality due to selection bias, performance bias and incomplete outcome data (40, 43). Because of the limited number of studies reporting on the association between auto-antibodies, sex and microangiopathy, we chose to include also medium and low-quality articles.

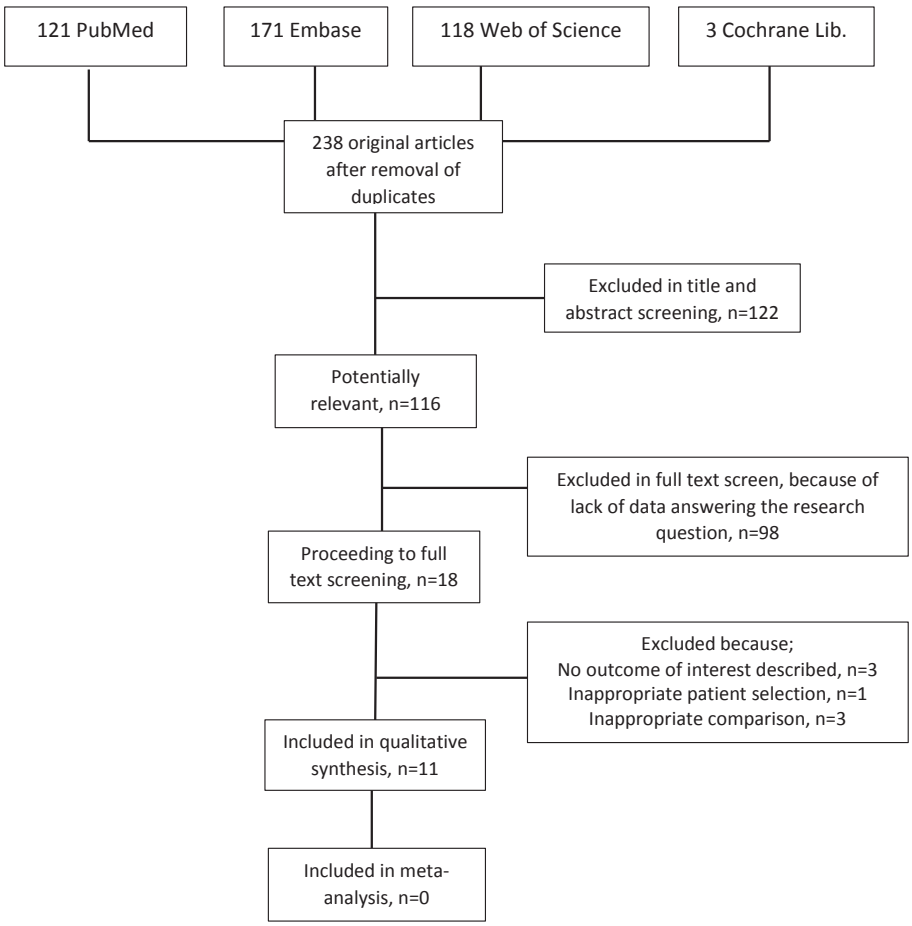

Figure 1. Flowchart association autoantibodies and microangiopathy

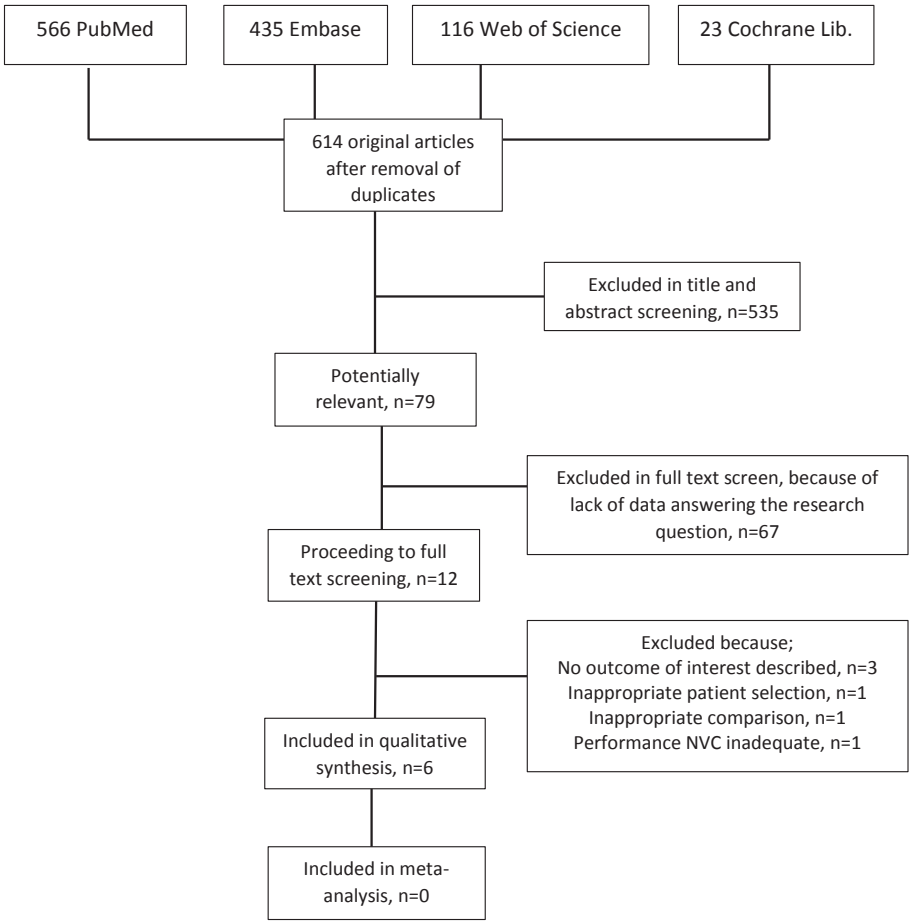

Figure 2. Flowchart association sex and microangiopathy

Baseline characteristics of articles included in the systematic review: association of sex and autoantibodies with the degree of microangiopathy

Study	Country	N	Age, mean years	Sex; f/m	Disease duration*, years since diagnosis
Caramaschi, 2007 (33)	Italy	103	54.3	91/12	7 since diagnosis
De Santis (34)	Italy	44	66	42/2	9 since diagnosis
Fichel (35)	France	88	54.9	81/7	16.5 since onset RP
Ghizzoni (36)	Italy	275	54.9	253/22	36.9 since diagnosis
Markusse (7)	Netherlands	287	53.9	202/85	3.7 since onset RP
Pizzorni (37)	Italy	33	59	28/5	6.6 since diagnosis
Cutolo (8)	Italy	241	57	227/14	5.6 since diagnosis/13.7 since onset RP
Ingegnoli (38)	Italy	2754	54.9	2148/606	7.6 since diagnosis
Sulli (11)	Italy, Belgium	42	47	NA	5 since onset RP
Tieu (39)	Australia	152	43.7	121/31	10.9 since onset RP
Chandran (40)	Australia	148	50	44/8	5 years since onset RP
Simeon (43)	Spain	91	52.5	82/9	6 months and 63 years since RP
Freire (42)	Spain	1506	45.6	1341/165	6.4 since diagnosis
Caramaschi, 2009 (41)	Italy	49	52.4	44/5	8 since diagnosis

Table 1. *Disease duration was defined differently in the articles, either as time since onset RP, time since onset non-RP or time since diagnosis. ANA= anti-nuclear auto-antibody, dcSSc= diffuse cutaneous systemic sclerosis,

	SSc type	Methodological framework	Main topic
	68 lcSSc/ 35 dcSSc	Observation cohort,cross-sectional	NVC pattern and clinical characteristics
	34 lcSSc/ 10 dcSSc	Observational cohort, cross-sectional	Correlation NVC and clinical SSc phenotype
	51 lcSSc/ 15 dcSSc/ 12 non cutaneous	Observational cohort,cross-sectional	Characteristics SSc patients with normal or abnormal NVC
	242 lcSSc/ 33 dcSSc	Observational cohort, longitudinal	Prevalence, evolution of NVC and analysis of characteristics according to capillaroscopic features
	141 lcSSc/ 56 dcSSc	Observational cohort, cross-sectional	Evaluate anti-ENA antibodies in SSc and predictive power of combination of autoantibodies and NVC
	30 lcSSc/ 3 dcSSc	Observational cohort,cross-sectional	Evaluate use of MES assessment with qualitative analysis of NVC and telangiectasia
	148 lcSSc/ 93 dcSSc	Observational cohort,cross-sectional	Relation NVC pattern autoantibodies and subset cutaneous involvement
	1622 lcSSc/ 803 dcSSc	Observatinal cohort,cross-sectional	Frequency of NVC patterns and their disease phenotype
	NA	Observational cohort, longitudinal	Correlation between ANA patterns and NVC stage in SSc
	99 lcSSc/ 30dcSSc	Observational cohort, longitudinal	Investigate possible utility of NVC in predicting survival
	81 lcSSc/ 13 dcSSc	Observational cohort,cross-sectional	Role of NVC in identification and prognostication
	70lcSSc/ 19dcSSc	Observational cohort,cross-sectional	Relationship disease pattern and sex
	1151 lcSSc/ 355 dcSSc	Observational cohort, longitudinal	Influence gender on survival
	31 lcSSc/ 18 dcSSc	Observational cohort, longitudinal	NVC changes after iloprost treatment

ENA= extractable nuclear antigen, lcSSc= limited cutaneous systemic sclerosis, MES= microangiopathy evolution score, NVC= nailfold videocapillaroscopy.

AUTO-ANTIBODIES AND MICROANGIOPATHY

A meta-analysis could not be conducted due to heterogeneity of the studies and use of different outcome measures. In total, 11 studies described associations between auto-antibodies and microangiopathy (Table 2).

Qualitative assessment of NVC

Caramaschi et al. performed NVC in 103 SSc patients and the degree of microangiopathy was defined as early, active or late SSc pattern according to Cutolo et al. (2000) (quality score good) (33) . The distribution of ANA, ACA, and ATA positivity did not differ between patients with early, active, or late SSc pattern. De Santis et al. investigated 44 SSc patients using NVC to identify early, active, or late SSc patterns (quality score medium) (34). No significant differences in the SSc patterns were found between ACA and ATA positive patients. In a study with 287 SSc patients, ACA, ATA, anti-RNP, anti-RNAPIII, anti-fibrillarin, anti-PM/Scl, anti-Th/To and anti-Ku antibodies were evaluated and early, active or late SSc patterns were described on NVC (quality score good) (7). The prevalence of NVC patterns was equally distributed among patients with different specific auto-antibodies. On the contrary, Pizzorni et al. investigated 33 SSc patients and classified the degree of microangiopathy according to the three SSc patterns: early, active or late (quality score medium) (37). ATA positive patients showed more often a late SSc pattern ($p= 0.002$), while in ACA positive patients early or active SSc patterns were more common ($p= 0.03$). Cutolo et al. evaluated NVC patterns and serum auto-antibodies in 241 SSc patients (quality score good) (8). NVC was described as early, active or late SSc pattern. ATA positivity was significantly less frequent in the early (5%) than in the active (25%) or in the late (24%) SSc patterns.

Presence of ATA was shown to be related with earlier expression of the active and late SSc patterns of microvascular damage. On the other hand, ACA positivity was found more frequently, although not significantly, in the early pattern. The authors concluded that specific auto-antibodies do not seem directly linked to the expression of a singular NVC pattern, but that auto-antibodies might be related to the rate of progression of microvascular damage. In a study by Ingegnoli et al. data from the European Scleroderma Trials and Research group (EUSTAR) were used to investigate NVC in 2754 SSc patients (quality score medium) (38). NVC patterns were described as early, active or late SSc pattern. Late pattern was present in 47% of ATA positive and in 28% of ACA positive ($p <0.05$) patients, while early and active patterns were more frequent in ACA positive than in ATA positive patients (44% vs 28%, $p <0.05$). Significant associations were found between ATA positivity and late SSc pattern, and between ACA positivity and early/active SSc pattern ($p= 0.03$). Sulli et al. found that the prevalence of ATA was significantly higher in

patients with the late SSc pattern (n= 42; quality score medium) (11). Fichel et al. described the characteristics of 88 SSc patients with normal, non-specific or SSc-specific NVC pattern (quality score medium) (35). The frequencies of ANA, ACA (p= 0.90) and ATA (p= 0.34) positivity were comparable for normal/nonspecific and SSc-specific NVC patterns. This is in line with the results of Ghizonni et al. who described NVC features, demographic, clinical and serological manifestations of 275 SSc patients (quality score medium) (36). No differences in the percentage of ACA or ATA positivity were found between patients with SSc patterns compared to patients with normal/non-specific NVC patterns (ACA: 15.2% vs 14.6% ATA: 31.8% vs 23.6%; all non-significant).

Quantitative assessment of NVC

Besides the SSc-specific NVC patterns, de Santis et al. also described the amount of giants, neoangiogenesis, avascular areas and the capillary density and compared these characteristics between ACA and ATA positive patients (34). No significant differences were found.

Semi-quantitative assessment of NVC

Tieu et al. included 152 SSc patients and investigated capillary dropout during follow-up (quality score medium) (39). Patients with anti-RNAPIII had a significantly higher nailfold capillary total damage index compared with ACA, ATA and anti-RNP positive patients. Patients with ATA or anti-RNAPIII had greater capillary dropout than patients with ACA, despite a significantly shorter disease duration. Finally, Chandran et al. mentioned that in 52 SSc patients, the ATA positive cases had more severe nailfold changes (quality score low) (40). However, in this study only four ATA positive patients were included and two of them had severe NVC changes, whereas of the 22 ACA positive patients, three had severe NVC changes. Two studies, by Pizzorni et al. and by Sulli et al. (quality score medium) used the MES to semi-quantitatively evaluate the degree of microvascular damage and no significant differences in MES were found between ACA and ATA positive patients (11, 37).

In conclusion, weighing the results of Table 2, the total number of patients in the studies that found an association between auto-antibodies and microangiopathy was 2364, compared to 742 patients in the studies that did not find an association. This would implicate that specific auto-antibodies are associated with the degree of microangiopathy but, when only high-quality studies were evaluated (7, 8, 33), an association was found only in 241 patients, while in 390 patients no association between auto-antibodies and microangiopathy was described.

Association between autoantibodies and microangiopathy

	Study	Patients	Antibodies
Qualitative	Caramaschi, 2007	103	ACA, ATA
	De Santis, 2016 $	44	ACA, ATA
	Markusse, 2017	253	ACA, ATA, RNApol3, RNP, U3RNP, Pm/Scl
	Cutolo, 2004	241	ACA, ATA
	Ingegnoli, 2013	2754	ACA, ATA
	Sulli, 2013 #	42	ACA, ATA
	Pizzorni, 2017 *	33	ACA, ATA
	Ghizzoni, 2014	275	ACA, ATA
	Fichel, 2014	88	ACA,ATA
Semi-quantitative	Tieu, 2018	152	ACA, ATA, RNP, RNApol3
	Chandran, 1995	52	ACA, ATA, RNP
	Pizzorni, 2017 *	33	ACA, ATA
	Sulli, 2013 #	42	ACA, ATA
Quantitative	De Santis, 2016 $	44	ACA, ATA

Table 2. ACA= anti-centromere antibody, ANA= anti-nuclear antibody, ATA= anti-topoisomerase antibody, NVC= nailfold videocapillaroscopy, RNApolIII= anti-RNA polymerase III antibody,

NVC assessment	Significant	Conclusion
Early; Active; Late SSc pattern	Non-significant (not specified)	No significant difference
	P > 0.05	No significant difference
	P > 0.10	No significant difference
	P < 0.01	ATA+ more frequent in Active and Late patterns than in Early
	P < 0.005	ATA more often present in Late pattern compared to Early and Active
	P= 0.03 (OR 8.0 (1.4-47.0))	ATA more frequently present in Late pattern than in Early and Active
	ACA early-active/late p= 0.03, ATA early-active/late p= 0.002	Early-Active pattern is more often present in ACA patients, Late pattern is more often present in ATA patients.
Normal; SSc pattern	Non-significant (not specified)	No significant difference
	ACA normal/ SSc pattern p= 0.90 (OR 0.90 (0.3-2.6)) ATA normal/SSc pattern p= 0.34 (OR 0.50 (0.1-2.6))	No significant difference
Mean capillary damage score; mean capillary dropout score	RNApol3 > capillary damage compared with ACA and RNP (p < 0.001). ATA and RNApol3 > dropout compared with ACA (p= unknown)	Difference found between autoantibodies and capillary damage and capillary dropout.
Moderate loss and enlargement; Extreme capillary dropout; class 1 to 5	Not mentioned	ATA positive patients more severe nailfold changes compared to ACA and RNP+
Microangiopathy evolution score (MES)	ACA MES <6/ > 6 p= 0.72, ATA MES < 6/ > 6 p= 0.43	No significant differences
	ANA vs ACA p= 0.09, ANA vs ATA p= 0.05	No significant differences
Giants, neoangiogenesis, avascular areas, density	P > 0.05	No significant differences

RNP= anti- ribonuclear protein antibody, SSc= systemic sclerosis. $/#/*= same article used two techniques for NVC assessment.

SEX AND MICROANGIOPATHY

In total six studies reported on sex and microangiopathy in patients with SSc (Table 3). A meta-analysis could not be conducted due to heterogeneity of the studies.

Qualitative assessment

Caramaschi et al. investigated 103 SSc patients (12 men, 91 women) and the microvascular alterations were classified as early, active and late SSc patterns (quality score good) (33). In this study no significant differences in NVC patterns were found between male and female patients. Freire et al. studied 1506 SSc patients (165 men, 1341 women) assessing microangiopathy with the use of NVC and describing the degree of microangiopathy as "slow" or "active" pattern (quality score medium) (42). No significant difference in the distribution of patterns was observed between men and women (m/f; 46%-53% for slow pattern and 37% vs 33% for active pattern). Pizzorni et al. evaluated 33 patients, including 5 males, and found no difference in the prevalence of SSc patterns in men or women (37). One out of 6 studies suggested a possible sex difference regarding microangiopathy (41). In 49 SSc patients who were treated with iloprost and underwent two NVC examinations with a 3-year interval, improvement of SSc pattern was found to be associated with male sex (r= 9.07, p= 0.019).

Association between sex and microangiopathy

	Study	Patients	Male/Female
Qualitative	Caramaschi, 2007	103	91 female, 12 male
	Caramaschi, 2009	49	44 female, 5 male
	Pizzorni, 2017*	33	28 women, 5 male
	Freire, 2017	1506	1341 female, 165 male
Semi-quantitative	Simeon, 1996	91	82 female, 9 male
	Chandran, 1995	52	44 female, 8 male
	Pizzorni, 2017*	33	28 women, 5 male

Table 3. NVC= nailfold videocapillaroscopy, SSc= Systemic Sclerosis. * same article used two techniques for NVC assessment.

Quantitative assessment

None of the included studies evaluated the association between sex and quantitative assessment of microangiopathy.

Semi-quantitative assessment

Chandran et al. performed a study on prevalence, subset characteristics and NVC patterns of SSc patients in South-Australia (quality score low) (40). They included 44 females and 8 males, and an equal proportion of males and females had severe capillary changes of class IV (moderate loss of capillaries) and V (extreme capillary dropout). Simeon et al. evaluated 91 SSc patients, of which 9 were men (quality score low). The NVC patterns were described using capillary loss and mega capillaries as parameters. No significant NVC differences were found between male and female patients. In line with these results, Pizzorni et al. compared MES between males and females, and no significant difference was found (37).

In conclusion, of the 6 included articles, 5 studies including 1614 women and 204 men did not show an association between sex and microangiopathy. The only study showing a significant difference included 44 women and 5 men and, importantly, male patients were more often treated with cyclophosphamide, but a multivariate analysis to identify the contribution of sex corrected for the prescribed treatment was not performed (41).

NVC assessment	Significant	Conclusion
Early; Active; Late SSc pattern	Non-significant (not specified)	No significant difference
	P < 0.05	Improvement of NVC was associated with male sex
	P= 0.623	No significant difference
Slow (giants and minimal loss) or Active Pattern (capillary loss and neovascularization)	Slow pattern male/female p= 0.126, Active pattern male/female p= 0.420	No significant difference
Capillary loss and megacapillaries	P= 0.71 for capillary loss, p= 1.00 for megacapilaries	No significant difference
Moderate loss and enlargement; Extreme capillary dropout; class 1 to 5	Not mentioned	No significant difference
Microangiopathy evolution score (MES) score 0 -9, < 6 or > 6 dichotomized	P= 0.625	No significant difference

DISCUSSION

Microangiopathy can be secondary to different causes. Research in different fields shows that many factors can affect microangiopathy, including biological, environmental and socio-economic factors (44, 45). In addition, gender specific factors have been postulated as men and women develop different types of ischemic heart disease with different pathophysiological background (3, 4). Atherosclerosis is more common in men, while in women vasoreactivity prevails, characterized by spasm and endothelial alterations. Microvascular dysfunction with perfusion problems seems to be present more often in women with cardiovascular disease (CVD) and also takotsubo cardiomyopathy, heart failure and stroke are more common in women (46, 47).

Similarly, it has been recognized that there are clinical differences between female and male patients with systemic autoimmune rheumatic diseases in which microangiopathy plays a role, such as systemic lupus erythematosus (SLE) and SSc (48). SLE is rare in men, and males with SLE are more likely to experience cardiovascular complications and myocardial infarction, and less likely to have dermatological manifestations (48). Nevertheless, also for SLE it remains unknown why male SLE differs substantially from SLE in women.

Although there is a growing interest, the exact interplay between auto-antibodies and microangiopathy in autoimmune diseases remains to be elucidated. In SLE, a difference in auto-antibody prevalence has been suggested between men and women. Anticardiolipin antibodies, anti-dsDNA antibodies and lupus anticoagulant were found to be more prevalent in men in a few studies (49). Some studies showed that in lupus nephritis, antiphospholipid antibodies and lupus anticoagulant were more frequently observed in patients with thrombotic microangiopathy of the kidney. In addition, among the auto-antibodies mainly implicated in neuropsychiatric (NP) SLE, anti-β2glycoprotein I (β2GPI) antibodies are preferentially involved in focal NP events which are a consequence of noninflammatory microangiopathy; otherwise, anti-ribosomal P protein antibodies and anti-N-methyl-D-aspartate receptor (NMDAR) antibodies might cause diffuse NP events (49). In dermatomyositis anti-MDa5 auto-antibodies have a strong correlation with vasculopathy (50). Irrespective of these specific cases, little information is available on the association between sex or auto-antibodies and microangiopathy in connective tissue diseases, both for SSc and for other systemic autoimmune diseases.

As the assessment of microangiopathy has an established diagnostic and prognostic role in SSc patients (51), we value possible factors that could influence microangiopathy as relevant. In this review of the literature we focused on the influence of sex and auto-antibodies on microangiopathy in SSc patients. We can conclude that sex does not associate

with degree of microangiopathy in SSc, while the results on association between specific auto-antibodies and degree of microangiopathy were inconclusive. When summarizing the findings of the positive studies for auto-antibodies and microangiopathy, presence of ATA might be associated with more severe microangiopathy as reflected by a late pattern. Indeed, both more severe damage and presence of ATA associate with more severe disease in SSc. However, the degree of microangiopathy can change over time and possible confounders as age, disease duration, comorbidities or medications, were not taken into account in any of the included studies. When evaluating the high-quality studies only, no clear association between ATA and more severe microangiopathy was shown. However, even in these studies the results were not adjusted for confounders. Therefore, we believe that further prospective controlled studies are needed to better explore the association between presence of specific antibodies and the degree of microangiopathy.

Regarding sex and microangiopathy, no clear association was found in the included articles. However, only six studies were retrieved and two evaluated sex differences as primary outcome (42, 43). Besides, a relatively limited number of men was included in the studies. Noteworthy, although several studies focused on sex differences in SSc, a possible difference between males and females in the degree of microangiopathy was disregarded in most studies. To account for the gender gap and disease dissimilarities in SSc, a role of sex hormones has been proposed. Estrogens act as enhancers of the immune system and of cell proliferation, as also demonstrated in cultures of cells harvested from skin biopsies of SSc patients (52-54). A recent study demonstrated a protective effect of estrogens in dermal fibrosis, as estrogens reduce TGF-B dependent activation of dermal fibroblasts, and estrogen inhibition leads to a more severe experimental dermal fibrosis, but their effects on vasculature are largely unknown (55). At macrovascular level, hormone replacement therapy (HRT) might be protective against the risk of pulmonary arterial hypertension, and short- or long-term administration of conjugated estrogens induced flow-mediated dilatation in the brachial artery of SSc patients (56-58). Regarding microvasculature, little is known about the effects of estrogens in patients with SSc (22). A recent study investigated the influence of cumulative endogenous estrogen exposure (CEEE) in patients with SSc on the degree of microvascular damage observed through NVC, and no association between length of CEEE and degree of microvascular impairment was found (59).

We aimed to summarize the available evidence about the association between sex, or specific auto-antibodies, and microangiopathy in SSc, but our review is not without limitations. We could include only a limited number of articles, with variable quality and, due to the heterogeneity of patients and outcomes, a meta-analysis could not be conducted.

Contradictory results were found about the association between auto-antibodies and microangiopathy and no firm conclusions can be drawn. As NVC has prognostic relevance in the global assessment of each single SSc patient, we believe that the identification of factors possibly affecting microangiopathy is of relevance to elucidate the pathophysiology of microangiopathy and also for clinical risk stratification. Therefore, in consideration of the paucity of available data, and especially the lack of data derived from high-quality research, we advocate further prognostic cohort studies to evaluate factors contributing to the degree of microangiopathy in SSc.

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