

Contribution of Sex and Autoantibodies to Microangiopathy Assessed by Nailfold Videocapillaroscopy in Systemic Sclerosis: A Systematic Review of the Literature

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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. We undertook this study to evaluate whether sex or expression of specific antinuclear autoantibodies might associate with the degree of microangiopathy through performance of a systematic review that summarizes what is known about these associations.

Methods. A standardized search of PubMed, Embase, Web of Science, and the Cochrane Library were performed to identify studies that described autoantibodies in SSc patients and microangiopathy and, for the second search, those that described sex and microangiopathy.

Results. 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 SSc-specific autoantibodies 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 autoantibodies and degree of microangiopathy were inconclusive.

INTRODUCTION

Systemic sclerosis (SSc) is characterized 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 that is evolving to definite SSc, presence of microvascular damage and SSc-specific autoantibodies indicate a very high probability of developing SSc (2). The frequency of progression is higher with both the presence of SSc autoantibodies and microvascular damage (79.5%) than with the presence of 1 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 the

prediction of future organ complications (3–5). The SSc-specific autoantibodies are associated with specific clinical characteristics and therefore are of additional prognostic value. Anticentromere antibodies (ACAs) are associated with a decreased risk of lung (odds ratio [OR] 0.12) and heart (OR 0.39) involvement, while patients who are anti-topoisomerase I antibody (ATA) positive have an increased risk for these complications (OR 6.66 and OR 2.12, respectively) (6,7). Strikingly, the degree of microangiopathy was comparable between ACA+ and ATA+ patients (late SSc pattern; ACA 33%, ATA 25%), which suggests that the presence of a specific antinuclear antibody (ANA) is independent of the development of microangiopathy.

In some studies, however, an association between microvascular damage and autoantibodies has been described (8). ANAs, found in 95% of patients with SSc, have been mentioned as 1 of the possible triggers for vascular injury by causing acceleration of vascular endothelial cell senescence and therefore inducing

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SIGNIFICANCE & INNOVATIONS

- Degree of microangiopathy is used as a diagnostic and prognostic tool in systemic sclerosis (SSc).
- Factors that influence microangiopathy are not completely elucidated.
- Based on the current literature in SSc, there is no association between sex and degree of microangiopathy, but for SSc-specific autoantibodies, the results are contradictory, advocating further evaluation.

RP (9,10). Other studies suggest that autoantibody production occurs secondary to vasculopathy, and as such these autoantibodies 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 women than in men (4:1). Also, distribution of ANA is disbalanced, with women showing more frequently ACA positivity and men showing more frequent ATA positivity. In general, disease course is more severe in men, resulting in lower survival rates (45% versus 23% after 10 years) (16–20). The most frequent disease-related causes of death also differ between men and women, with interstitial lung disease in men and pulmonary hypertension (PH) in women (21). The higher incidence of PH in women and the fact that unopposed estrogen replacement therapy has been associated with increased RP suggest a contribution of hormonal factors to microangiopathic manifestations (22); however, little information is known about 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) (see Supplementary Appendix A and Supplementary Table 1, available on the *Arthritis Care & Research* website at <http://onlinelibrary.wiley.com/doi/10.1002/acr.24149/abstract>). However, nailfold videocapillaroscopy (NVC)

is considered the most reliable tool to distinguish between primary and secondary RP. NVC is widely applied and provides the opportunity to directly visualize 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 autoantibodies and microangiopathy, and between sex and microangiopathy in SSc.

MATERIALS AND METHODS

Literature search. A systematic literature search was performed (JWS), including studies published before June 17, 2019. The databases used were Medline (via PubMed), Web of Science, the Cochrane Library, and Embase. No restrictions on date were applied, and only manuscripts published in English or Dutch were selected. The search strategy intended to include all relevant reports describing adult patients with SSc, in which microangiopathy of the hand was evaluated and where association with SSc-specific autoantibodies was assessed. A second systematic literature search performed on the same day intended to include all relevant reports describing adult patients with SSc, in which microangiopathy of the hand was evaluated and a comparison between male and female patients was described (for search strategies, see Supplementary Appendix A, available on the *Arthritis Care & Research* website at <http://onlinelibrary.wiley.com/doi/10.1002/acr.24149/abstract>).

Two reviewers (NMvL and JC) independently screened the titles of retrieved articles and, in the case that 1 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 (JKdV-B). Full text reading was performed for the selected abstracts (NMvL and JC).

Screening process and study selection criteria. For the review on autoantibodies and microangiopathy, the following criteria were applied: 1) adult participants (ages >18 years) with a clinical diagnosis of SSc; 2) fulfillment of either American College of Rheumatology (ACR) 2013, ACR 1980, or LeRoy and Medsger criteria (28,29); 3) report on prevalence of SSc-related autoantibodies, including at least ATAs or ACAs, and additionally, anti-RNA polymerase III (anti-RNAP III), anti-RNAP I, antifibrinogen, anti-PM/Scl, or anti-Th/To antibodies; and 4) assessment of microangiopathy using ≥ 1 imaging modality, including NVC, laser dermoscopy, Doppler confocal microscopy, laser speckle contrast analysis (LASCA)/video image analysis, and photomicroscopy.

For the review on sex and microangiopathy, the following criteria were applied: 1) adult participants (ages >18 years) with a

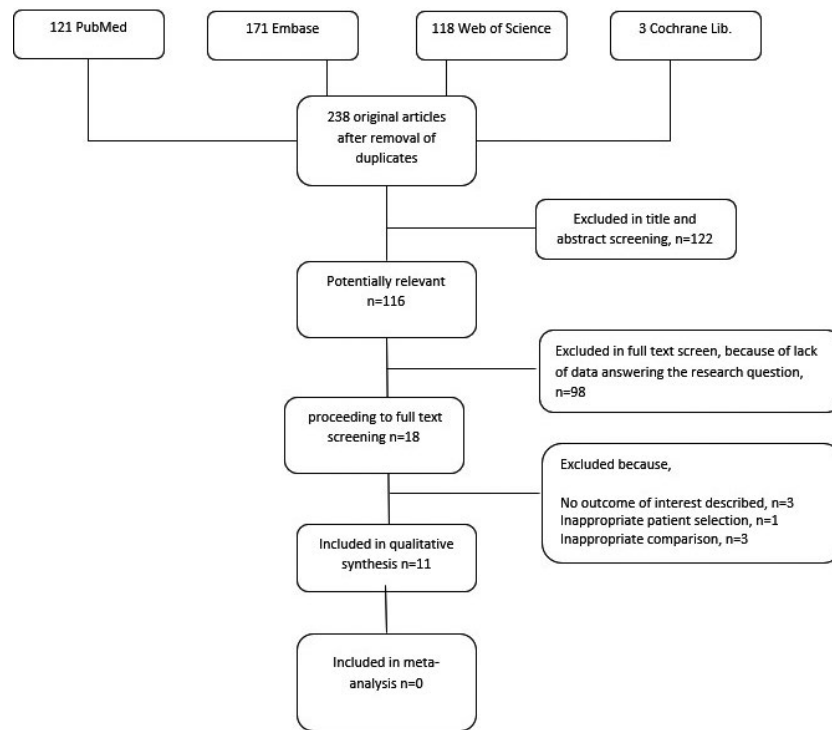


Figure 1. Flow chart of the association of autoantibodies and microangiopathy.

clinical diagnosis of SSc; 2) fulfillment of either ACR 2013, ACR 1980, or LeRoy and Medsger criteria (28,29); 3) report on the comparison between female and male patients, with at least $n = 3$ and 10% male patients included in the study; and 4) assessment of microangiopathy using ≥ 1 imaging modality, including 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 (NMvL and JC).

Evaluation of capillaroscopic descriptions throughout the studies. In the 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 European League Against Rheumatism recommendations on capillaroscopy, the NVC characteristics can be evaluated quantitatively, qualitatively, or semi-quantitatively (32) (see Supplementary Appendix A and Supplementary Table 1, available online at <http://onlinelibrary.wiley.com/doi/10.1002/acr.24149/abstract>). When

available, all these NVC characteristics were extracted throughout the included articles.

RESULTS

Literature search and study description. Figures 1 and 2 show flow charts of the systematic review processes. Eleven studies that demonstrated the association between autoantibodies and microangiopathy (7,8,11,33–40) and 6 studies that demonstrated the association between sex and microangiopathy (33,37,40–43) were included. Three studies addressed both associations (33,37,40). All of the 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 4 (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 included 4,704 women (83%) and 971 men (17%), with a mean age of 49 years. Subtypes of SSc were specified in all but 1 article (diffuse cutaneous SSc [$n = 1,473$ (28%)] and limited cutaneous SSc [$n = 3,746$ (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.

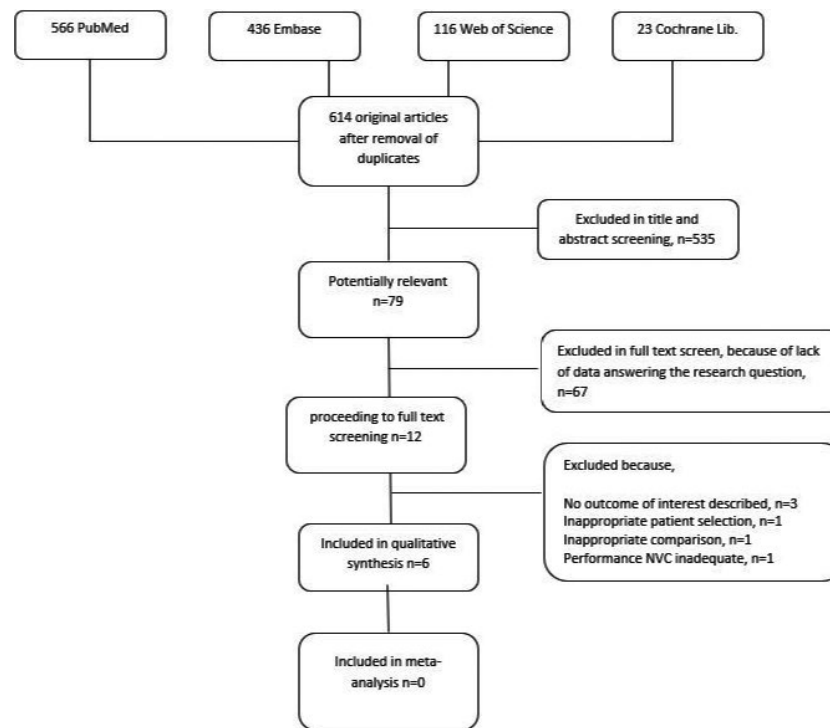


Figure 2. Flow chart of the association of sex and microangiopathy.

Risk of bias. Study quality is summarized in Supplementary Table 1, available online at <http://onlinelibrary.wiley.com/doi/10.1002/acr.24149/abstract>. Three articles were assessed as high quality (7,8,33), 9 as medium quality (11,33–39,42), and 2 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 autoantibodies, sex, and microangiopathy, we chose to also include medium- and low-quality articles.

Autoantibodies and microangiopathy. A meta-analysis could not be conducted due to heterogeneity of the studies and the use of different outcome measures. In total, 11 studies described the associations between autoantibodies 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 (quality score good) (32,33). The distribution of ANA, ACA, and ATA positivity did not differ between patients with early, active, or late SSc patterns. 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+ patients. In a study that included 287 SSc patients, ACA, ATA, anti-RNP, anti-RNAP III, anti-fibrillar, 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 autoantibodies. On the contrary, Pizzorni et al investigated 33 SSc patients and classified the degree of microangiopathy according to the 3 SSc patterns: early, active, or late (quality score medium) (37). ATA+ patients showed a late SSc pattern ($P = 0.002$) more frequently, while in ACA+ patients early or active SSc patterns were more common ($P = 0.03$). Cutolo et al evaluated NVC patterns and serum autoantibodies 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 SSc patterns (5%) than in the active (25%), or late (24%) SSc patterns.

Presence of ATA was shown to be related to 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 autoantibodies do not seem directly linked to the expression of a singular NVC pattern, but that autoantibodies 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 were used to investigate NVC in 2,754 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+ and in 28% of ACA+ ($P < 0.05$) patients, while early and active patterns were more frequent in ACA+ than in ATA+ patients (44% versus 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

Table 1. Baseline characteristics of articles included in the systematic review: association of sex and autoantibodies with the degree of microangiopathy*

Study	Country	Patients, no.	Age, mean years	Sex, female/male	Disease duration, years since diagnosis	Ssc type	Methodological framework	Main topic
Markusse et al, 2017 (7)	Netherlands	287	53.9	202/85	3.7 since onset of RP	141 lcSSc/56 dcSSc	Observational cohort, cross-sectional	Evaluate anti-ENA antibodies in SSc and predictive power of combination of autoantibodies and NVC
Cutolo et al, 2004 (8)	Italy	241	57	227/14	5.6/13.7 since onset of RP	148 lcSSc/93 dcSSc	Observational cohort, cross-sectional	Relation NVC pattern autoantibodies and subset cutaneous involvement
Sulli et al, 2013 (11)	Italy, Belgium	42	47	NA	5 since onset of RP	NA	Observational cohort, longitudinal	Correlation between ANA patterns and NVC stage in SSc
Caramaschi et al, 2007 (33)	Italy	103	54.3	91/12	7	68 lcSSc/35 dcSSc	Observation cohort, cross-sectional	NVC pattern and clinical characteristics
De Santis et al, 2016 (34)	Italy	44	66	42/2	9	34 lcSSc/10 dcSSc	Observational cohort, cross-sectional	Correlation NVC and clinical SSc phenotype
Fichel et al, 2014 (35)	France	88	54.9	81/7	16.5 since onset of RP	51 lcSSc/15 dcSSc/12 noncutaneous	Observational cohort, cross-sectional	Characteristics SSc patients with normal or abnormal NVC
Ghizzoni et al, 2015 (36)	Italy	275	54.9	253/22	36.9	242 lcSSc/33 dcSSc	Observational cohort, longitudinal	Prevalence, evolution of NVC and analysis of characteristics according to capillaroscopic features
Pizzorni et al, 2017 (37)	Italy	33	59	28/5	6.6	30 lcSSc/3 dcSSc	Observational cohort, cross-sectional	Evaluate use of MES assessment with qualitative analysis of NVC and telangiectasia
Ingegnoli et al, 2013 (38)	Italy	2,754	54.9	2,148/606	7.6	1,622 lcSSc/803 dcSSc	Observational cohort, cross-sectional	Frequency of NVC patterns and their disease phenotype
Tieu et al, 2018 (39)	Australia	152	43.7	121/31	10.9 since onset of RP	99 lcSSc/30 dcSSc	Observational cohort, longitudinal	Investigate possible utility of NVC in predicting survival
Chandran et al, 1995 (40)	Australia	148	50	44/8	5 since onset of RP	81 lcSSc/13 dcSSc	Observational cohort, cross-sectional	Role of NVC in identification and prognosis
Caramaschi et al, 2009 (41)	Italy	49	52.4	44/5	8	31 lcSSc/18 dcSSc	Observational cohort, longitudinal	NVC changes after ioproprost treatment
Freire et al, 2017 (42)	Spain	1,506	45.6	1,341/165	6.4	1,151 lcSSc/355 dcSSc	Observational cohort, longitudinal	Influence sex on survival
Simeon et al, 1996 (43)	Spain	91	52.5	82/9	6 months and 63 years since onset of RP	70 lcSSc/19 dcSSc	Observational cohort, cross-sectional	Relationship disease pattern and sex

* Disease duration was defined differently in the articles, either as time since onset of Raynaud's phenomenon (RP) or non-RP, or time since diagnosis. ANA = antinuclear autoantibody; dcSSc = diffuse cutaneous systemic sclerosis; ENA = extractable nuclear antigen; lcSSc = limited cutaneous systemic sclerosis; MES = microangiopathy evolution score; NA = not available; NVC = nailfold videocapillaroscopy; SSc = systemic sclerosis.

Table 2. Association between autoantibodies and microangiopathy*

Study, type	Patients, no.	Antibodies	NVC assessment	Significance	Conclusion
Markusse et al, 2017 (7); qualitative†	253	ACA, ATA, RNAPIII, RNP, U3 RNP, Pm/Scl	Early; active; late SSc pattern	$P > 0.10$	No significant difference
Cutolo et al, 2004 (8); qualitative	241	ACA, ATA	Early; active; late SSc pattern	$P < 0.01$	ATA+ more frequent in active and late patterns than in early
Sulli et al, 2013 (11); qualitative	42	ACA, ATA	Early; active; late SSc pattern	$P = 0.03$ (OR 8.0 [1.4-47.0])	ATA more often present in late pattern than in early and active
Sulli et al, 2013 (11); semiquantitative	42	ACA, ATA	MES	ANA vs. ACA, $P = 0.09$, ANA vs. ATA, $P = 0.05$	No significant differences
Caramaschi et al, 2007 (33); qualitative	103	ACA, ATA	Early; active; late SSc pattern	Nonsignificant (not specified)	No significant difference
De Santis et al, 2016 (34); qualitative	44	ACA, ATA	Early; active; late SSc pattern	$P < 0.05$	No significant difference
De Santis et al, 2016 (34); quantitative	44	ACA, ATA	Giants, neoangiogenesis, avascular areas, density	$P > 0.05$	No significant differences
Fichel et al, 2014 (35); qualitative	88	ACA, ATA	Normal; SSc pattern	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
Ghizzoni et al, 2015 (36); qualitative	275	ACA, ATA	Normal; SSc pattern	Nonsignificant (not specified)	No significant difference
Pizzorni et al, 2017 (37); qualitative	33	ACA, ATA	Early; active; late SSc pattern	ACA early and active/late, $P = 0.03$; ATA early and active/late, $P = 0.02$	Early-active pattern more often present in ACA patients; late pattern more often present in ATA patients.
Pizzorni et al, 2017 (37); semiquantitative†	33	ACA, ATA	MES	ACA MES $<6/ >6$, $P = 0.72$; ATA MES $<6/ >6$, $P = 0.43$	No significant differences
Ingegnoli et al, 2013 (38); qualitative	2,754	ACA, ATA	Early; active; late SSc pattern	$P < 0.05$	ATA more often present in late pattern than in early and active
Tieu et al, 2018 (39); semiquantitative	152	ACA, ATA, RNP, RNAPIII	Mean capillary damage score; mean capillary dropout score	RNAPIII $>$ capillary damage compared with ACA and RNP ($P < 0.001$); ATA and RNAPIII $>$ dropout compared with ACA ($P = \text{unknown}$)	Difference found between autoantibodies and capillary damage and capillary dropout
Chandran et al, 1995 (40); semiquantitative	52	ACA, ATA, RNP	Moderate loss and enlargement; extreme capillary dropout; class 1 to 5	Not mentioned	ATA+ patients more severe nailfold changes compared to ACA and RNP+

* ACA = anticentromere antibody; ANA = antinuclear antibody; ATA = anti-topoisomerase 1 antibody; MES = microangiopathy evolution score; NVC = nailfold videocapillaroscopy; RNAP III = RNA polymerase III; SSc = systemic sclerosis.

† Same article used 2 techniques for NVC assessment.

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, nonspecific, 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 Ghizzoni et al who described NVC features, demographic, clinical, and serologic 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/nonspecific NVC patterns (ACA: 15.2% versus 14.6%, ATA: 31.8% versus 23.6%; all nonsignificant).

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+ patients (34). No significant differences were found.

Semiquantitative assessment of NVC. A study by Tieu et al included 152 SSc patients and investigated capillary dropout during follow-up (quality score medium) (39). Patients with anti-RNAP III had a significantly higher nailfold capillary total damage index compared with ACA+, ATA+, and anti-RNP+ patients. Patients with ATA or anti-RNAP III had greater capillary dropout than patients with ACA, despite a significantly shorter disease duration. Finally, a study by Chandran et al demonstrated that in 52 SSc patients, the ATA+ cases had more severe nailfold changes (quality score low) (40). However, in this study only 4 ATA+ patients were included and 2 of them had severe NVC changes, whereas of the 22 ACA+ patients, 3 had severe NVC changes. Two studies, by Pizzorni et al and by Sulli et al (quality score medium) used the microangiopathy evolution score (MES) to semiquantitatively evaluate the degree of microvascular damage. No significant differences in the MES were found between ACA+ and ATA+ patients (11,37).

In conclusion, weighing the results shown in Table 2, the total number of patients in the studies that found an association between autoantibodies and microangiopathy was 2,364, compared to 742 patients in the studies that did not find an association. This would implicate that specific autoantibodies are associated with the degree of microangiopathy; however, 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 autoantibodies and microangiopathy was noted.

Sex and microangiopathy. In total, 6 studies reported on sex and microangiopathy in patients with SSc (Table 3). A meta-analysis could not be conducted due to the heterogeneity of the studies.

Qualitative assessment. A study by Caramaschi et al included 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 1,506 SSc patients (165 men, 1,341 women) and assessed microangiopathy with the use of NVC and described 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 (46% versus 53% for slow pattern and 37% versus 33% for active pattern). Pizzorni et al evaluated 33 patients, including 5 men, and found no difference in the prevalence of SSc patterns in men or women (37). One of 6 studies suggested a possible sex difference regarding microangiopathy (41). In 49 SSc patients who were treated with iloprost and underwent 2 NVC examinations with a 3-year interval, improvement of SSc pattern was found to be associated with male sex ($r = 9.07$, $P = 0.019$).

Quantitative and semiquantitative assessment. None of the included studies evaluated the association between sex and quantitative assessment of microangiopathy. Chandran et al performed a study on prevalence, subset characteristics, and NVC patterns of SSc patients in South Australia (quality score low) (40). The study included 44 men and 8 women, and an equal proportion of men and women 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) (43). The NVC patterns were described using capillary loss and megacapillaries as parameters. No significant NVC differences were found between male and female patients. In line with these results, Pizzorni et al compared

Table 3. Association between sex and microangiopathy*

Study, type	Patients, no.	Sex, female/male	NVC assessment	Significance	Conclusion
Caramaschi et al, 2007 (33); qualitative	103	91/12	Early; active; SSc pattern	Nonsignificant (not specified)	No significant difference
Pizzorni et al, 2017 (37); qualitative†	33	28/5	Early; active; late SSc pattern	$P = 0.623$	No significant difference
Pizzorni et al, 2017 (37); semiquantitative†	33	28/5	MES 0–9, <6 or >6 dichotomized	$P = 0.625$	No significant difference
Chandran et al, 1995 (40); semiquantitative	52	44/8	Moderate loss and enlargement; extreme capillary dropout; class 1 to 5	Not mentioned	No significant difference
Caramaschi et al, 2009 (41); qualitative	49	44/5	Early; active; late SSc pattern	$P < 0.05$	Improvement of NVC associated with male sex
Freire et al, 2017 (42); qualitative	1,506	1,341/165	Slow (giants and minimal loss) or active pattern (capillary loss and nonvascularization)	$P = 0.126$ (slow pattern male/female); $P = 0.420$ (active pattern male/female)	No significant difference
Simeon et al, 1996 (43); semiquantitative	91	82/9	Capillary loss and megacapillaries	$P = 0.71$ (capillary loss); $P = 1.00$ (megacapillaries)	No significant difference

* MES = microangiopathy evolution score; NVC = nailfold videocapillaroscopy; SSc = systemic sclerosis.

† Same article used 2 techniques for NVC assessment.

MES between men and women, and no significant difference was found (37).

In conclusion, of the 6 included articles, 5 studies including 1,614 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).

DISCUSSION

Microangiopathy can be secondary to different causes. Research in different fields shows that many factors can affect microangiopathy, including biological, environmental, and socioeconomic factors (44,45). In addition, sex-specific factors have been postulated as men and women develop different types of ischemic heart disease with different pathophysiologic 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, and 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 men with SLE are more likely to experience cardiovascular complications and myocardial infarction and are less likely to have dermatologic manifestations (48). Nevertheless, it remains unknown why SLE in men differs substantially from SLE in women.

Although there is a growing interest, the exact interplay between autoantibodies and microangiopathy in autoimmune diseases remains to be elucidated. In SLE, a difference in autoantibody prevalence has been suggested between men and women. Anticardiolipin antibodies, anti-double-stranded DNA 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. Additionally, among the autoantibodies mainly implicated in neuropsychiatric (NP) SLE, anti- β_2 -glycoprotein I antibodies are preferentially involved in focal NP events that are a consequence of noninflammatory microangiopathy; otherwise, anti-ribosomal P protein antibodies and anti-N-methyl-D-aspartate receptor antibodies might cause diffuse NP events (49). In dermatomyositis, anti-MDa5 autoantibodies have a strong correlation with vasculopathy (50). Irrespective of these specific cases, little information is available on the association between sex or autoantibodies

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 autoantibodies 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 autoantibodies and degree of microangiopathy were inconclusive. When summarizing the findings of the positive studies for autoantibodies 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 such 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 6 studies were retrieved and 2 evaluated sex differences as primary outcome (42,43). Also, a relatively limited number of men was included in the studies. 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 sex 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 transforming growth factor β -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 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 estrogen 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 autoantibodies,

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 autoantibodies 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 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.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. van Leeuwen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Van Leeuwen, Ciaffi, Schoones, de Vries-Bouwstra.

Acquisition of data. Van Leeuwen, Ciaffi, de Vries-Bouwstra.

Analysis and interpretation of data. Van Leeuwen, Ciaffi, Huizinga, de Vries-Bouwstra.

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