MS. NINA VAN VAN LEEUWEN (Orcid ID : 0000-0003-4228-012X)

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

Authors: Nina M. van Leeuwen, M.D. [1], Jacopo Ciaffi, M.D.[1,2,3], Jan W. Schoones, drs. [4] , Tom W.J. Huizinga, M.D., Ph.D.[1], Jeska K. de Vries-Bouwstra, M.D., Ph.D.[1]

[1] Leiden University Medical Center, department of Rheumatology, Leiden, The Netherlands

[2] Rheumatology Unit, Azienda Policlinico of Modena, University of Modena and Reggio Emilia, Modena, Italy

[3] Medicine and Rheumatology Unit, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy

[4] Leiden University Medical Center, Walaeus Library, Leiden, The Netherlands

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Correspondence to:

Nina M. van Leeuwen, department of Rheumatology Leiden University Medical Center, C1-R, PO Box 9600, 2300 RC, Leiden, the Netherlands. E-mail: n.m.van_leeuwen@lumc.nl

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ABSTRACT

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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. 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.

Method: 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: Eleven studies were included that report on the relationship between SSc specific auto-antibodies and microangiopathy, and six studies were included that report 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.

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Keywords: Systemic Sclerosis, microangiopathy, sex, auto-antibodies

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Significance and innovations:

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

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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 favours further migration and homing of inflammatory cells inducing increased microvascular permeability and progressive vascular leak (13). Infective stimuli,

92   environmental exposures, sex, and endocrine disturbances, have all been proposed as contributors to

93   microangiopathy (14, 15).

94   In SSc there is a marked sex imbalance, with higher prevalence of the disease in females than in

95   males (4:1). Also distribution of ANA is disbalanced with females showing more frequently ACA antibody

96   and males showing more frequently ATA antibody. In general, disease course is more severe in males

97   resulting in lower survival rates (45% vs 23% after 10 years) (16-20). The most frequent disease related

98   causes of death also differ between males and females: interstitial lung disease in males and pulmonary

99   hypertension (PH) in females (21). The higher incidence of PH in females, and the fact that unopposed

100   estrogens replacement therapy has been associated with increased RP, suggests a contribution of

101   hormonal factors to microangiopathic manifestations (22). However, little information is known on the

102   relationship between sex and microangiopathy in SSc.

103   As microvascular damage is one of the hallmarks of SSc, different imaging techniques have been

104   applied to evaluate structural and functional abnormalities of the finger microcirculation in patients with

105   SSc (23-26) (supplementary file). However, NVC is considered the most reliable tool to distinguish

106   between primary and secondary RP. NVC is widely applied and provides the opportunity to directly

107   visualise the evolving obliterative microangiopathy and nailfold capillary abnormalities characteristic of

108   SSc, that have been classified as "scleroderma pattern" (27).

109   Given the role of microangiopathy in the pathogenesis of SSc, insights in the factors responsible

110   for microvascular damage could contribute to our understanding of disease pathophysiology. Therefore,

111   we decided to evaluate and summarize in this comprehensive review what is known about the association

112   between the expression of specific auto-antibodies and microangiopathy, and between sex and

113   microangiopathy, in SSc.

114

115   **Methods**

116   *Literature search*

117

118   A systematic literature search was performed by J.W.S, including studies published before June 17th 2019.

119   The databases used were Medline (via PubMed), Web of Science, Cochrane and Embase. No restrictions

120   on date were applied and manuscripts published in English or Dutch language were selected. The search

121   strategy intended to include all relevant papers reporting on adult patients with SSc, in which

122   microangiopathy of the hand was evaluated and where association with SSc-specific auto-antibodies was

123   assessed. A second systematic literature search performed the same day intended to include all relevant

124 papers reporting on adult patients with SSc, in which microangiopathy in the hand was evaluated and a

125 comparison between male and female patients was described (see supplementary file for search

126 strategies).

127   Two reviewers (N.v.L and J.C) independently screened the titles of retrieved articles and, in case

128 one or both reviewers identified a publication as possibly relevant, the study proceeded to abstract

129 screening. In case of discrepancies in agreement, abstracts were reviewed by a third investigator (J.d.V.B).

130 Full text reading was performed for the selected abstracts by N.v.L and J.C.

131

132 *Screening process and study selection criteria*

133 For the review on auto-antibodies and microangiopathy the following criteria were applied: 1) adult

134 participants (>18 years) with a clinical diagnosis of SSc; 2) fulfilment of either American College of

135 Rheumatology (ACR) 2013, ACR 1980, or LeRoy and Medsger criteria (28, 29); 3) report on prevalence of

136 SSc-related auto-antibodies including at least anti-topoisomerase I antibodies (ATA) or anti-centromere

137 antibodies (ACA) and additionally anti-RNA polymerase III (anti-RNAPIII), anti-RNA polymerase I, anti-

138 fibrillarin, anti-PM/Scl, or anti-Th/To antibodies; 4) assessment of microangiopathy using one or more of

139 the following imaging modalities: nailfold NVC, laser dermoscopy, doppler confocal microscopy,

140 LASCA/video image analysis, and photomicroscopy.

141 For the review on sex and microangiopathy the following criteria were applied: 1) adult participants (>18

142 years) with a clinical diagnosis of SSc; 2) fulfilment of either ACR 2013, ACR 1980, or LeRoy and Medsger

143 criteria (28, 29); 3) report on comparison between female and male patients, and with at least n=3 and

144 10% males included in the study; 4) assessment of microangiopathy using one or more of the following

145 imaging modalities: NVC, laser dermoscopy, doppler confocal microscopy, LASCA/video image analysis,

146 and photomicroscopy.

147

148 Exclusion criteria for both search strategies were: animal studies, editorials, reviews, letters to the editor,

149 unpublished material, case-reports and manuscripts written in languages other than English or Dutch.

150 *Quality assessment*

151 The Newcastle-Ottawa scale was used for assessment of quality of case-control studies, whereas the

152 National Institutes of Health quality assessment tool was used for observational cohort studies (30, 31).

153 Discrepancies in scoring and implications for interpretation of the findings were discussed between N.v.L

154 and J.C.

155 *Evaluation of capillaroscopic descriptions throughout the studies*

156 As in literature a variety of definitions are used to describe NVC. In this review we will report the NVC

157 findings in a standardized way by evaluating the used terminology to describe NVC characteristics per

158 included article. In line with the EULAR recommendations on capillaroscopy, the NVC characteristics can

159 be evaluated quantitatively, qualitatively or semi-quantitively (32). See the supplementary file for a

160 detailed explanation. When available, all these NVC characteristics were extracted throughout the

161 included articles.

162

163 **Results**

164 *Literature search and study description*

165 Figures 1 and 2 show the flowcharts of the systematic review processes. Eleven studies reporting on the

166 association between auto-antibodies and microangiopathy (7, 8, 11, 33-40), and six studies reporting on

167 sex and microangiopathy (33, 37, 40-43), were included. Three studies answered both questions (33, 37,

168 40). All included articles were cohort or case-control studies, but many were limited by small sample sizes.

169 In the majority of the included articles, except for four (8, 11, 42, 43), the association of interest was not

170 the primary outcome of the study. Characteristics of all included studies are provided in Table 1. In all,

171 these studies reported on 4704 women (83%) and 971 men (17%), with a mean age of 49 years. Subtypes

172 of SSc were specified in all but one article (for diffuse cutaneous SSc (dcSSc) n= 1473, 28%; for limited

173 cutaneous SSc (lcSSc) n=3746, 72%). Disease duration was defined either as time since onset of RP, as

174 time since onset of first sign or symptom attributable to SSc different from RP, or as time since diagnosis,

175 and ranged between 6 months and 37 years.

176 *Comprehensiveness of reporting*

177 The comprehensiveness of reporting was variable. Although all selected studies used NVC, the parameters

178 to describe microangiopathy and to classify severity of microvascular changes differed between the

179 studies.

180

181 *Risk of Bias*

182 Study quality is summarized in Table 4 (supplementary file). Three articles were assessed as high quality

183 (7, 8, 33), nine as medium quality (11, 33-39, 42), and two as low quality due to selection bias,

184 performance bias and incomplete outcome data (40, 43). Because of the limited number of studies

185 reporting on the association between auto-antibodies, sex and microangiopathy, we chose to include also

186 medium and low-quality articles.

187 **Auto-antibodies and microangiopathy**

188 A meta-analysis could not be conducted due to heterogeneity of the studies and use of different outcome

189 measures. In total, 11 studies described associations between auto-antibodies and microangiopathy

190 (Table 2).

191 *Qualitative assessment of NVC*

192 Caramaschi et al. performed NVC in 103 SSc patients and the degree of microangiopathy was defined as

193 early, active or late SSc pattern according to Cutolo et al. (2000) (quality score good) (33) . The distribution

194 of ANA, ACA, and ATA positivity did not differ between patients with early, active, or late SSc pattern. De

195 Santis et al. investigated 44 SSc patients using NVC to identify early, active, or late SSc patterns (quality

196 score medium) (34). No significant differences in the SSc patterns were found between ACA and ATA

197 positive patients. In a study with 287 SSc patients, ACA, ATA, anti-RNP, anti-RNAPIII, anti-fibrillarin, anti-

198 PM/Scl, anti-Th/To and anti-Ku antibodies were evaluated and early, active or late SSc patterns were

199 described on NVC (quality score good) (7). The prevalence of NVC patterns was equally distributed among

200 patients with different specific auto-antibodies. On the contrary, Pizzorni et al. investigated 33 SSc

201 patients and classified the degree of microangiopathy according to the three SSc patterns: early, active or

202 late (quality score medium) (37). ATA positive patients showed more often a late SSc pattern (p=0.002),

203 while in ACA positive patients early or active SSc patterns were more common (p=0.03). Cutolo et al.

204 evaluated NVC patterns and serum auto-antibodies in 241 SSc patients (quality score good) (8). NVC was

205 described as early, active or late SSc pattern. ATA positivity was significantly less frequent in the early (5%)

206 than in the active (25%) or in the late (24%) SSc patterns. Presence of ATA was shown to be related with

207 earlier expression of the active and late SSc patterns of microvascular damage. On the other hand, ACA

208 positivity was found more frequently, although not significantly, in the early pattern. The authors

209 concluded that specific auto-antibodies do not seem directly linked to the expression of a singular NVC

210 pattern, but that auto-antibodies might be related to the rate of progression of microvascular damage. In

211 a study by Ingegnoli et al. data from the European Scleroderma Trials and Research group (EUSTAR) were

212 used to investigate NVC in 2754 SSc patients (quality score medium) (38). NVC patterns were described as

213 early, active or late SSc pattern. Late pattern was present in 47% of ATA positive and in 28% of ACA

214 positive (p <0.05) patients, while early and active patterns were more frequent in ACA positive than in

215 ATA positive patients (44% vs 28%, p <0.05). Significant associations were found between ATA positivity

216 and late SSc pattern, and between ACA positivity and early/active SSc pattern (p=0.03). Sulli et al. found

217 that the prevalence of ATA was significantly higher in patients with the late SSc pattern (n=42; quality

218 score medium) (11). Fichel et al. described the characteristics of 88 SSc patients with normal, non-specific

219 or SSc-specific NVC pattern (quality score medium) (35). The frequencies of ANA, ACA (p=0.90) and ATA

220 (p=0.34) positivity were comparable for normal/nonspecific and SSc-specific NVC patterns. This is in line

221 with the results of Ghizonni et al. who described NVC features, demographic, clinical and serological

222 manifestations of 275 SSc patients (quality score medium) (36). No differences in the percentage of ACA

223 or ATA positivity were found between patients with SSc patterns compared to patients with normal/non-

224 specific NVC patterns (ACA: 15.2% vs 14.6% ATA: 31.8% vs 23.6%; all non-significant).

225 *Quantitative assessment of NVC*

226 Besides the SSc-specific NVC patterns, de Santis et al. also described the amount of giants,

227 neoangiogenesis, avascular areas and the capillary density and compared these characteristics between

228 ACA and ATA positive patients (34). No significant differences were found.

229 *Semi-quantitative assessment of NVC*

230 Tieu et al. included 152 SSc patients and investigated capillary dropout during follow-up (quality score

231 medium) (39). Patients with anti-RNAPIII had a significantly higher nailfold capillary total damage index

232 compared with ACA, ATA and anti-RNP positive patients. Patients with ATA or anti-RNAPIII had greater

233 capillary dropout than patients with ACA, despite a significantly shorter disease duration. Finally,

234 Chandran et al. mentioned that in 52 SSc patients, the ATA positive cases had more severe nailfold

235 changes (quality score low) (40). However, in this study only four ATA positive patients were included and

236 two of them had severe NVC changes, whereas of the 22 ACA positive patients, three had severe NVC

237 changes. Two studies, by Pizzorni et al. and by Sulli et al. (quality score medium) used the MES to semi-

238 quantitatively evaluate the degree of microvascular damage and no significant differences in MES were

239 found between ACA and ATA positive patients (11, 37).

240 In conclusion, weighing the results of Table 2, the total number of patients in the studies that found an

241 association between auto-antibodies and microangiopathy was 2364, compared to 742 patients in the

242 studies that did not find an association. This would implicate that specific auto-antibodies are associated

243 with the degree of microangiopathy but, when only high-quality studies were evaluated (7, 8, 33), an

244 association was found only in 241 patients, while in 390 patients no association between auto-antibodies

245 and microangiopathy was described.

246 **Sex and microangiopathy**

247 In total six studies reported on sex and microangiopathy in patients with SSc (Table 3). A meta-analysis

248 could not be conducted due to heterogeneity of the studies.

249 *Qualitative assessment*

250 Caramaschi et al. investigated 103 SSc patients (12 men, 91 women) and the microvascular alterations

251 were classified as early, active and late SSc patterns (quality score good) (33). In this study no significant

252 differences in NVC patterns were found between male and female patients. Freire et al. studied 1506 SSc

253 patients (165 men, 1341 women) assessing microangiopathy with the use of NVC and describing the

254 degree of microangiopathy as "slow" or "active" pattern (quality score medium) (42). No significant

255 difference in the distribution of patterns was observed between men and women (m/f; 46%-53% for slow

256 pattern and 37% vs 33% for active pattern). Pizzorni et al. evaluated 33 patients, including 5 males, and

257 found no difference in the prevalence of SSc patterns in men or women (37). One out of 6 studies

258 suggested a possible sex difference regarding microangiopathy (41). In 49 SSc patients who were treated

259 with iloprost and underwent two NVC examinations with a 3-year interval, improvement of SSc pattern

260 was found to be associated with male sex (r=9.07, p=0.019).

261 *Quantitative assessment*

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

263 microangiopathy.

264 *Semi-quantitative assessment*

265 Chandran et al. performed a study on prevalence, subset characteristics and NVC patterns of SSc patients

266 in South-Australia (quality score low) (40). They included 44 females and 8 males, and an equal proportion

267 of males and females had severe capillary changes of class IV (moderate loss of capillaries) and V (extreme

268 capillary dropout). Simeon et al. evaluated 91 SSc patients, of which 9 were men (quality score low). The

269   NVC patterns were described using capillary loss and mega capillaries as parameters. No significant NVC

270   differences were found between male and female patients. In line with these results, Pizzorni et al.

271   compared MES between males and females, and no significant difference was found (37).

272   In conclusion, of the 6 included articles, 5 studies including 1614 women and 204 men did not show an

273   association between sex and microangiopathy. The only study showing a significant difference included 44

274   women and 5 men and, importantly, male patients were more often treated with cyclophosphamide, but

275   a multivariate analysis to identify the contribution of sex corrected for the prescribed treatment was not

276   performed (41).

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278

279 **Discussion**

280 Microangiopathy can be secondary to different causes. Research in different fields shows that many

281 factors can affect microangiopathy, including biological, environmental and socio-economic factors (44,

282 45). In addition, gender specific factors have been postulated as men and women develop different types

283 of ischemic heart disease with different pathophysiological background (3, 4). Atherosclerosis is more

284 common in men, while in women vasoreactivity prevails, characterized by spasm and endothelial

285 alterations. Microvascular dysfunction with perfusion problems seems to be present more often in

286 women with cardiovascular disease (CVD) and also takotsubo cardiomyopathy, heart failure and stroke

287 are more common in women (46, 47).

288 Similarly, it has been recognized that there are clinical differences between female and male

289 patients with systemic autoimmune rheumatic diseases in which microangiopathy plays a role, such as

290 systemic lupus erythematosus (SLE) and SSc (48). SLE is rare in men, and males with SLE are more likely to

291 experience cardiovascular complications and myocardial infarction, and less likely to have dermatological

292 manifestations (48). Nevertheless, also for SLE it remains unknown why male SLE differs substantially

293 from female SLE.

294 Although there is a growing interest, the exact interplay between auto-antibodies and microangiopathy in

295 autoimmune diseases remains to be elucidated. In SLE, a difference in auto-antibody prevalence has been

296 suggested between men and women. Anticardiolipin antibodies, anti-dsDNA antibodies and lupus

297 anticoagulant were found to be more prevalent in men in a few studies (49). Some studies showed that in

298 lupus nephritis, antiphospholipid antibodies and lupus anticoagulant were more frequently observed in

299 patients with thrombotic microangiopathy of the kidney. In addition, among the auto-antibodies mainly

300 implicated in neuropsychiatric (NP) SLE, anti-β2glycoprotein I (β2GPI) antibodies are preferentially

301 involved in focal NP events which are a consequence of noninflammatory microangiopathy; otherwise,

302 anti-ribosomal P protein antibodies and anti-N-methyl-D-aspartate receptor (NMDAR) antibodies might

303 cause diffuse NP events (49). In dermatomyositis anti-MDa5 auto-antibodies have a strong correlation

304 with vasculopathy (50). Irrespective of these specific cases, little information is available on the

305 association between sex or auto-antibodies and microangiopathy in connective tissue diseases, both for

306 SSc and for other systemic autoimmune diseases.

307 As the assessment of microangiopathy has an established diagnostic and prognostic role in SSc

308 patients (51), we value possible factors that could influence microangiopathy as relevant. In this review of

309 the literature we focused on the influence of sex and auto-antibodies on microangiopathy in SSc patients.

310 We can conclude that sex does not associate with degree of microangiopathy in SSc, while the results on

311 association between specific auto-antibodies and degree of microangiopathy were inconclusive. When

312 summarizing the findings of the positive studies for auto-antibodies and microangiopathy, presence of

313 ATA might be associated with more severe microangiopathy as reflected by a late pattern. Indeed, both

314 more severe damage and presence of ATA associate with more severe disease in SSc. However, the

315 degree of microangiopathy can change over time and possible confounders as age, disease duration,

316 comorbidities or medications, were not taken into account in any of the included studies. When

317 evaluating the high-quality studies only, no clear association between ATA and more severe

318 microangiopathy was shown. However, even in these studies the results were not adjusted for

319 confounders. Therefore, we believe that further prospective controlled studies are needed to better

320 explore the association between presence of specific antibodies and the degree of microangiopathy.

321   Regarding sex and microangiopathy, no clear association was found in the included articles.

322 However, only six studies were retrieved and two evaluated sex differences as primary outcome (42, 43).

323 Besides, a relatively limited number of men was included in the studies. Noteworthy, although several

324 studies focused on sex differences in SSc, a possible difference between males and females in the degree

325 of microangiopathy was disregarded in most studies. To account for the gender gap and disease

326 dissimilarities in SSc, a role of sex hormones has been proposed. Estrogens act as enhancers of the

327 immune system and of cell proliferation, as also demonstrated in cultures of cells harvested from skin

328 biopsies of SSc patients (52-54). A recent study demonstrated a protective effect of estrogens in dermal

329 fibrosis, as estrogens reduce TGF-B dependent activation of dermal fibroblasts, and estrogen inhibition

330 leads to a more severe experimental dermal fibrosis, but their effects on vasculature are largely unknown

331 (55). At macrovascular level, hormone replacement therapy (HRT) might be protective against the risk of

332 pulmonary arterial hypertension, and short- or long-term administration of conjugated estrogens induced

333 flow-mediated dilatation in the brachial artery of SSc patients (56-58). Regarding microvasculature, little is

334 known about the effects of estrogens in patients with SSc (22). A recent study investigated the influence

335 of cumulative endogenous estrogen exposure (CEEE) in patients with SSc on the degree of microvascular

336 damage observed through NVC, and no association between length of CEEE and degree of microvascular

337 impairment was found (59).

338   We aimed at summarizing the available evidence about the association between sex, or specific

339 auto-antibodies, and microangiopathy in SSc, but our review is not without limitations. We could include

340 only a limited number of articles, with variable quality and, due to the heterogeneity of patients and

341 outcomes, a meta-analysis could not be conducted.

342   Contradictory results were found about the association between auto-antibodies and

343   microangiopathy and no firm conclusions can be drawn. As NVC has prognostic relevance in the global

344   assessment of each single SSc patient, we believe that the identification of factors possibly affecting

345   microangiopathy is of relevance to elucidate the pathophysiology of microangiopathy and also for clinical

346   risk stratification. Therefore, in consideration of the paucity of available data, and especially derived from

347   high-quality research, we advocate further prognostic cohort studies to evaluate factors contributing to

348   the degree of microangiopathy in SSc.

356   **Conflicts of interest**

357   None

358   **References**

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Table 1. 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	SSc type	Methodological framework	Main topic
Caramaschi, 2007 (33)	Italy	103	54,3	91/12	7 since diagnosis	68 lcSSc/ 35 dcSSc	Observation cohort, cross-sectional	NVC pattern and clinical characteristics
De Santis (34)	Italy	44	66	42/2	9 since diagnosis	34 lcSSc/ 10 dcSSc	Observational cohort, cross-sectional	Correlation NVC and clinical SSc phenotype
Fichel (35)	France	88	54.9	81/7	16.5 since onset RP	51 lcSSc/ 15 dcSSc/ 12 non cutaneous	Observational cohort, cross-sectional	Characteristics SSc patients with normal or abnormal NVC
Ghizzoni (36)	Italy	275	54.9	253/22	36.9 since diagnosis	242 lcSSc/ 33 dcSSc	Observational cohort, longitudinal	Prevalence, evolution of NVC and analysis of characteristics according to capillaroscopic features
Markusse (7)	Netherlands	287	53.9	202/85	3.7 since onset RP	141 lcSSc/ 56 dcSSc	Observational cohort, cross-sectional	Evaluate anti-ENA antibodies in SSc and predictive power of combination of autoantibodies and NVC
Pizzorni (37)	Italy	33	59	28/5	6.6 since diagnosis	30 lcSSc/ 3 dcSSc	Observational cohort, cross-sectional	Evaluate use of MES assessment with qualitative analysis of NVC and telangiectasia
Cutolo (8)	Italy	241	57	227/14	5.6 since diagnosis/13.7 since onset RP	148 lcSSc/ 93 dcSSc	Observational cohort, cross-sectional	Relation NVC pattern autoantibodies and subset cutaneous involvement
Ingegnoli (38)	Italy	2754	54.9	2148/606	7.6 since diagnosis	1622 lcSSc/ 803 dcSSc	Observatinal cohort, cross-sectional	Frequency of NVC patterns and their disease phenotype
Sulli (11)	Italy, Belgium	42	47	NA	5 since onset RP	NA	Observational cohort, longitudinal	Correlation between ANA patterns and NVC stage in SSc
Tieu (39)	Australia	152	43.7	121/31	10.9 since onset RP	99 lcSSc/ 30dcSSc	Observational cohort, longitudinal	Investigate possible utility of NVC in predicting survival

Chandran (40)	Australia	148	50	44/8	5 years since onset RP	81 lcSSc/ 13 dcSSc	Observational cohort, cross-sectional	Role of NVC in identification and prognostication
Simeon (43)	Spain	91	52,5	82/9	6 months and 63 years since RP	70lcSSc/ 19dcSSc	Observational cohort, cross-sectional	Relationship disease pattern and sex
Freire (42)	Spain	1506	45.6	1341/165	6.4 since diagnosis	1151 lcSSc/ 355 dcSSc	Observational cohort, longitudinal	Influence gender on survival
Caramaschi, 2009 (41)	Italy	49	52.4	44/5	8 since diagnosis	31 lcSSc/ 18 dcSSc	Observational cohort, longitudinal	NVC changes after iloprost treatment

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

	Study	Patients	Antibodies	NVC assessment	Significant	Conclusion
Qualitative	Caramaschi, 2007	103	ACA, ATA	Early; Active; Late SSc pattern	Non-significant (not specified)	No significant difference
	De Santis, 2016 $	44	ACA, ATA		P > 0.05	No significant difference
	Markusse, 2017	253	ACA, ATA, RNApol3, RNP, U3RNP, Pm/Scl		P > 0.10	No significant difference
	Cutolo, 2004	241	ACA, ATA		P < 0.01	ATA+ more frequent in Active and Late patterns than in Early
	Ingegnoli, 2013	2754	ACA, ATA		P < 0.005	ATA more often present in Late pattern compared to Early and Active
	Sulli, 2013 #	42	ACA, ATA		P=0.03 (OR 8.0 (1.4-47.0))	ATA more frequently present in Late pattern than in Early and Active
	Pizzorni, 2017 *	33	ACA, ATA		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.
	Ghizzoni, 2014	275	ACA, ATA	Normal; SSc pattern	Non-significant (not specified)	No significant difference
	Fichel, 2014	88	ACA, ATA		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
Semi-quantitative	Tieu, 2018	152	ACA, ATA, RNP, RNApol3	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= ?)	Difference found between autoantibodies and capillary damage and capillary dropout.
	Chandran, 1995	52	ACA, ATA, RNP	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+
	Pizzorni, 2017 *	33	ACA, ATA	Microangiopathy evolution score (MES)	ACA MES <6/ > 6 p=0.72, ATA MES < 6/ > 6 p=0.43	No significant differences
	Sulli, 2013 #	42	ACA, ATA		ANA vs ACA p=0.09, ANA vs ATA p=0.05	No significant differences
Quantitative	De Santis, 2016 $	44	ACA, ATA	Giants, neoangiogenesis, avascular areas, density	P > 0.05	No significant differences

Table 2. Association between autoantibodies and microangiopathy

ACA=anti-centromere antibody, ANA=anti-nuclear antibody, ATA=anti-topoisomerase antibody, NVC=nailfold videocapillaroscopy RNApolIII=anti-RNA polymerase III antibody, RNP=anti-ribonuclear protein antibody, SSc= Systemic Sclerosis. */#/$ = same article used two techniques for NVC assessment.

	Table 3. Association between sex and microangiopathy					
	Study	Patients	Male/Female	NVC assessment	Significant	Conclusion
Qualitative	Caramaschi, 2007	103	91 female, 12 male	Early; Active; Late SSc pattern	Non-significant (not specified)	No significant difference
	Caramaschi, 2009	49	44 female, 5 male		P < 0.05	Improvement of NVC was associated with male sex
	Pizzorni, 2017*	33	28 women, 5 male		P=0.623	No significant difference
	Freire, 2017	1506	1341 female, 165 male	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
Semi-quantitative	Simeon, 1996	91	82 female, 9 male	Capillary loss and megacapillaries	P=0.71 for capillary loss, p=1.00 for megacapilaries	No significant difference
	Chandran, 1995	52	44 female, 8 male	Moderate loss and enlargement; Extreme capillary dropout; class 1 to 5	Not mentioned	No significant difference
	Pizzorni, 2017*	33	28 women, 5 male	Microangiopathy evolution score (MES) score 0 -9, < 6 or > 6 dichotomized	P=0.625	No significant difference

NVC=nailfold videocapillaroscopy, SSc=Systemic Sclerosis. * same article used two techniques for NVC assessment.
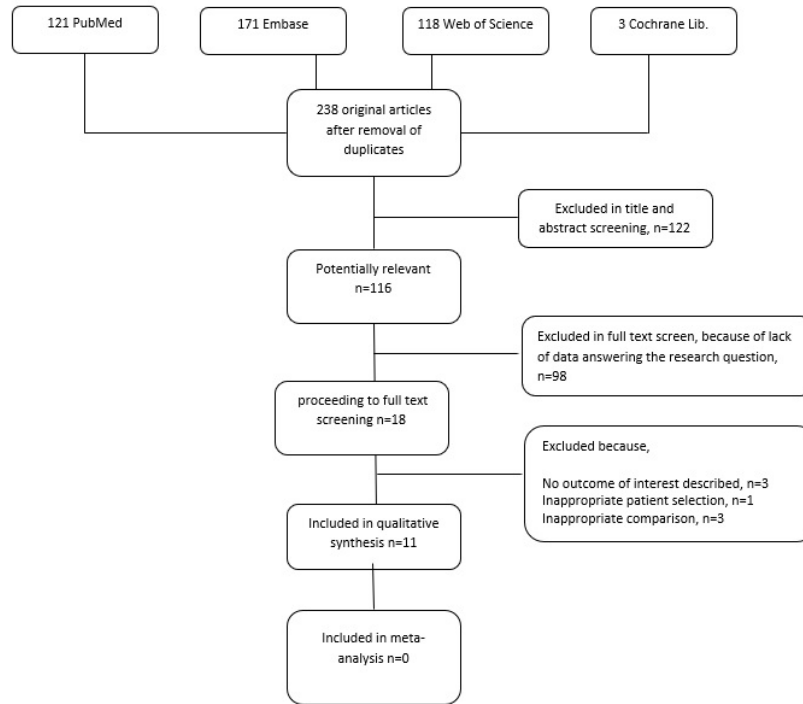
Figure 1. Flowchart association autoantibodies and microangiopathy
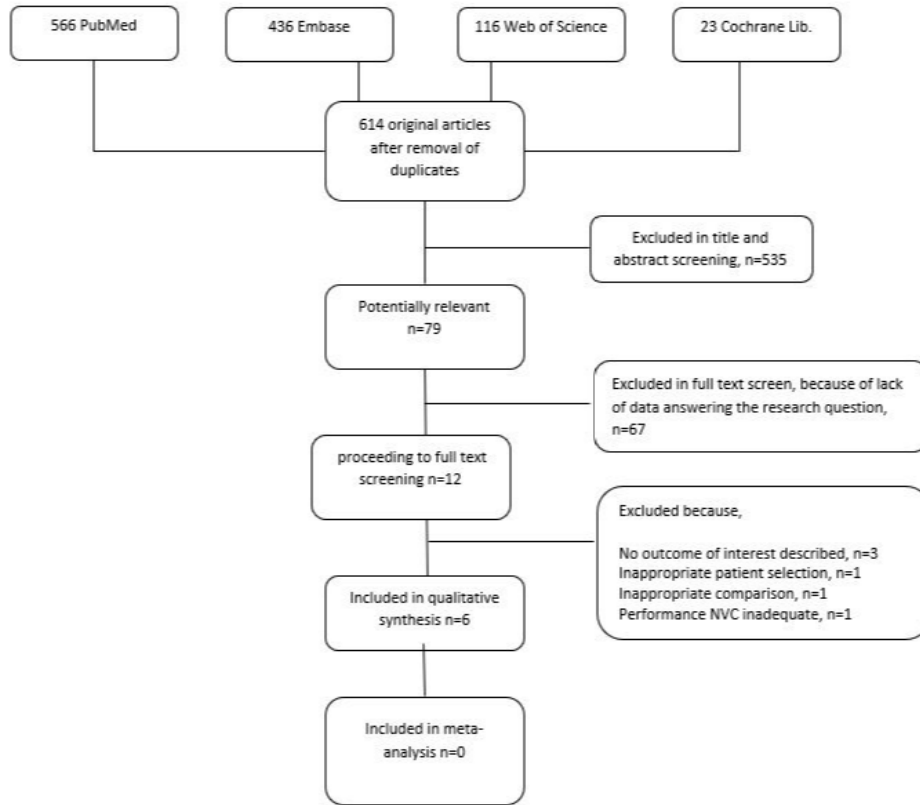
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Figure 2. Flowchart association sex and microangiopathy

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