Systematic analysis of the literature in search of defining systemic sclerosis subsets

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ABSTRACT

Objective. Systemic sclerosis (SSc) is a multisystem disease with heterogeneity in presentation and prognosis. An international collaboration to develop new SSc subset criteria is underway. Our objectives were to identify systems of SSc subset classification and synthesize novel concepts to inform development of new criteria.

Methods. Medline, Cochrane MEDLINE, CINAHL, EMBASE and Web of Science were searched from their inceptions to December 2019 for studies related to SSc sub-classification, limited to humans without language or sample size restrictions.

Results. Of 5686 citations, 102 articles reported original data on SSc subsets. Subset classification systems relied on extent of skin involvement and/or scleroderma-specific autoantibodies (n=61), nailfold capillary patterns (n=29), molecular, genomic and cellular patterns (n=12). While some systems of subset classification confer prognostic value for clinical phenotype, severity, and mortality; only subsetting by gene expression signatures in tissue samples has been associated with response to therapy.

Conclusion. Subsetting on extent of skin involvement remains important. Novel disease attributes including SSc-specific autoantibodies, nailfold capillary patterns and tissue gene expression signatures have been proposed as innovative means of SSc subsetting.

Systemic sclerosis (SSc) is a multi-system autoimmune rheumatic disease characterised by microvascular injury and accumulation of collagen in skin and other organs such as the musculoskeletal system, lungs, kidneys and gastrointestinal tract[1-6]. SSc is associated with poorer patient outcomes and lower quality of life when compared to other rheumatic diseases[7]. The 2013 American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) classification criteria for SSc include skin thickening, fingertip lesions, abnormal nailfold capillaries, and the presence of SSc-related autoantibodies, but do not differentiate subsets of SSc patients[9]. Sub-classification of SSc into a number of pathogenetically homogenous subsets with similar clinical manifestations and outcome would help segregate clearly between prognostically distinct disease subgroups. Despite the complex multiorgan nature of SSc, the subsets are frequently defined as being limited cutaneous (lcSSc) or diffuse cutaneous (dcSSc), based on the location of skin involvement[8]. This classification system gives insight into disease progression, however, within lcSSc and dcSSc the course of disease is highly variable between patients[9, 10]. With a more modern perspective, our understanding of SSc subsets is changing. A combination of different multisystem involvement, antibody profiling, genetic markers, and differences in proteomics may play a role in prognosis and treatment options[11-15]. Further defining subsets of patients with SSc may help to prognosticate, especially in early disease[16].

An international collaboration to develop new criteria to subset SSc is underway.[17] Current perceptions around SSc subset criteria were identified by leading international experts. In a survey of 30 SSc experts from 13 countries, ninety percent of experts use more than two subsets for classifying and treating their patients[18]. Concepts such as progression rates and likely organ involvement are considered for subsetting SSc patients informally in clinical practice.

There is a need for criteria to identify subsets of SSc patients, for both recruitment into clinical trials of novel therapeutic agents, to inform management, and for prognosis in clinical care. Previous attempts of SSc subset classification criteria have mainly relied on clinical

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manifestations[19]. However, in recent years, novel disease attributes including autoantibody profiles, nailfold capillary patterns and gene expression signatures have been proposed as means of subsetting. The objectives of this study were to identify existing systems of subset classification in SSc and synthesize novel concepts in subsetting through a systematic review of the literature.

Materials and Methods

Data sources and search strategy. A search of publications related to systemic sclerosis and subsets was performed using Medline, Cochrane MEDLINE, the Cumulative Index to Nursing and Allied Health Literature (CINAHL), EMBASE and Web of Science from their inceptions to December 2019 (see Supplementary table for search strategy and key terms). The research question was "What are the advantages and disadvantages of existing systems of subset classification in patients with systemic sclerosis?"

Searches were supplemented by hand-searching the bibliographies of relevant articles (including citation searching). Studies were limited to humans without language or sample size restrictions. Non-English language articles were translated by native-language speakers or machine software. EndNoteX9 software was used to check for duplications.

Studies were screened and excluded if they 1) reported localized scleroderma or scleroderma-like syndromes; 2) were abstracts, case reports or review articles; 3) were studies for which updated manuscripts were available. All articles were divided between four research groups (D.K./C.D., J.F./F.V., M.M./J.P./J.S./T.N., M.B./S.J./T.N.) and independently reviewed by investigators from each group using a standardized data abstraction form. Abstracted data included classification schema, number of SSc subsets, number of subjects, country of origin, stated and perceived advantages and disadvantages of the classification system, and external validation. The systematic review conforms to the PRISMA statement. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist was used to assess the reporting quality of the included studies.

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Results

Search results. Our literature review identified 5686 citations, of which 5585 were excluded because they were not relevant (conditions other than SSc, no classification system proposed), had insufficient data, the data were not original, and/or not involving humans. The remaining 102 studies reported schema to subset SSc patients. (Figure 1)

SSc subset criteria. Subset classification systems have relied on clinical manifestations, most commonly extent of skin involvement (n=20) (Table 1)[8-10, 20-36], molecular, genomic and cellular patterns (n=12) (Table 2)[37-48], scleroderma-specific autoantibodies (n=46, including 5 studies exploring both clinical and serological subsets[9, 20, 26, 28, 36]) (Table 3)[9, 20, 26, 28, 36, 49-89], and abnormal nailfold capillary patterns (n=10) (Table 4)[90-99]. Twenty-one studies reporting associations between capillary abnormalities and clinical features or serology were included (Table 5)[66, 93, 98, 100-117]. Using the STROBE checklist, the majority provided clear presentation of what was planned, done and found (Appendix I)[118].

SSc subsets based on the extent of skin involvement. The diffuse versus limited SSc criteria of Le Roy et al.[8] is the most commonly used system of SSc classification. The differences in development of visceral (renal and myocardial) disease and survival were shown for the subsets[8, 10, 24, 25]. The system has a good discriminative value to identify the groups of patients with different dominant features (vascular vs fibrotic), internal organ damage and outcome. It enables identification of early SSc patients with poor prognosis who will need close monitoring, and facilitates comparison of more homogenous groups of patients in epidemiological studies and clinical trials. The LeRoy 1988 classification system has an advantage of comprising only two groups and requires criteria other than cutaneous involvement. To classify as dSSc, the prerequisites are the onset of Raynaud's phenomenon within one year of the onset of skin involvement, early and significant visceral involvement, and the absence of anticentromere antibodies. When using these strict LeRoy criteria, dSSc

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represents only a small portion (8.5%) of the total group with definite SSc.[22] Two SSc-specific autoantibodies were included in the original LeRoy criteria: anti-topoisomerase I (ATA) and anticentromere (ACA).

Acknowledging the important role of autoantibodies and capillary abnormalities, LeRoy updated the classification in 2001, proposing 4 subsets: limited SSc, limited cutaneous SSc, diffuse cutaneous SSc and diffuse fasciitis with eosinophilia. The classification includes lSSc as Raynaud's phenomenon (RP) only in association with serological and/or capillary abnormalities [31]. Considering that SSc is a multistage multiorgan disorder, lSSc is likely an early stage of disease and corresponds to very early SSc in the classification of Avouac et al. [27].

Others have proposed three subset systems based on the extent of cutaneous involvement within the first year of presentation: Type I digital (finger or toe skin involvement), Type II intermediate (skin involvement proximal to MCP, but excluding trunk), and Type III diffuse (truncal sclerosis)[9, 23, 28, 32]. The latter type was characterized by male predominance, shorter RP before skin changes and worse prognosis[10]. The clinical distinctiveness of the types was confirmed by difference in autoantibody profile: ACA was found more frequently in Type I, while ATA in intermediate SSc and dSSc. The authors included into the study only SSc patients with disease duration ≤2 years after the onset of skin lesions and none of the patients had received any treatment which could potentially affect skin sclerosis prior to the enrolment. That ruled out a possibility that intermediate SSc (iSSc) group consisted of SSc patients who would "evolve" into dSSc later or who originally had dSSc with skin regression under the treatment. Compared to the 2-subset LeRoy system, this classification better reflects the clinical heterogeneity of disease and identifies the subgroups with milder or more severe clinic-prognostic evolution.

The simplicity of this 3-subset classification which is based on clinical examination of skin only and does not require special equipment or tests, makes it highly reproducible and suitable for clinical care and research studies. Notably, this classification system includes a time

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determinant reflective of the pace of disease, and, thus, has a prognostic value. Barnett et al emphasized the importance of assessing the extent of skin involvement within the first year of presentation to place a patient into a specific type [9]. Indeed, Type I and II patients had a better prognosis in terms of life-expectancy, compared to type III. However, only slight difference in survival was found between iSSc and lSSc patients.

Patients with iSSc were found to have variable clinical features and represented serologically heterogeneous group. It raises the question about iSSc as a distinct variant. Some authors suggested that further sub-division of iSSc might be necessary to identify the subsets with particular patterns of internal organ damage and outcome. Scussel-Lonzetti et al. divided iSSc into "above and below elbow" groups but found them similar with respect to internal organ involvement, mortality and autoantibody profile[24]. Although the authors supported the concept of iSSc subset, differentiation was shown only between the LeRoy subsets ("normal+limited" vs "intermediate+diffuse") in terms of heart involvement, disease activity (elevated ESR, anemia) and pulmonary fibrosis. The most significant difference in survival rates was found between lSSc and dSSc while the difference between other subsets was absent (lSSc vs iSSc, p=0.2) or very low (iSSc vs dSSc, p=0.03). ATA-positivity was similar between iSSc and dSSc while ACA frequencies gradually decreased from lSSc through iSSc to dSSc (50% - 34% - 3.4%). Supporting the LeRoy system, the skin involvement proximal to metacarpophalangeal (MCP) joints was one of the strong predictors of mortality. In line with those findings, Vayssairat et al. showed the advantages of LeRoy subset system and disutility of adding iSSc as a subset[22]. When SSc patients with proximal SSc were divided into intermediate and truncal subsets, no difference in severity score was found between them.

The patients with CREST syndrome, suspected secondary RP, and/or visceral scleroderma without skin involvement were not acknowledged in the aforementioned two classification systems [9,10]. The recently developed immunoblotting technique to detect SSc-related autoantibodies and nailfold capillary microscopy allow the detection of these probable connective tissue diseases. Expanding the subsets, Maricq et al. added undifferentiated

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connective tissue disorder (UCTD) with scleroderma (SD) features, SD sine SD and CREST[21]. This classification allows inclusion of patients who are in earlier stages of their disease.

Boonstra et al. identified four clinical sub-groups by hierarchical clustering [26] using skin, musculoskeletal, cardiac, pulmonary and gastrointestinal (GI) manifestations, demographics and risk assessment using follow-up data. Subgrouping patients allowed to predict severity and mortality with two subgroups showing higher than average 5-year mortality rates: subgroup 1 (male predominance, dcSSc, higher modified Rodnan skin score (mRSS), scleroderma renal crisis (SRC), ATA, less frequent interstitial lung disease (ILD)) and subgroup 2 (female predominance and non-Caucasians, more frequent pulmonary arterial hypertension (PAH), gastric antral vascular ectasia (GAVE), ILD, lower DLCO and FVC). Low risk clusters (sub-groups 3 and 4) included patients with lcSSc who were predominantly females, had more frequent GI manifestations (dysphagia, diarrhea, constipation) for both sub-groups as well as peripheral vascular involvement (digital ulcers), ACA and Caucasians predominance for Subgroup 3, and less frequent ILD, FVC and DLCO for subgroup 4. Three subgroups (1, 3 and 4) were similar to the clusters (6, 3 and 1, respectively) in another subclassification system developed by Sobanski et al. as a EUSTAR clustering initiative [36]. However, two main clusters A and B in the latter study strongly support the LeRoy 2001 sub-classification into dcSSc and lcSSc.

SSc subsets based on molecular gene expression profiling. Another approach to classify SSc patients into subsets is molecular phenotyping identified through gene expression profiling in tissue samples. Four subsets characterized by distinct molecular pathway signatures have been described and validated in multiple studies: fibroproliferative, inflammatory, normal-like and limited[37-44, 47, 48, 119]. The intrinsic molecular subsets are consistent for each patient, as well as across the different skin biopsy sites, regardless of clinically affected or unaffected status [37, 120]. The subsets are also consistent across the organ systems [37, 38, 41, 120], however highly lung-specific innate immune and cell proliferation processes were shown within the immune–fibrotic axis suggesting there are gene pairs that are more likely to interact in one tissue than the other [121] (Table 2).

SSc subsets according to SSc-related autoantibodies. The classification system according to serum antibodies is based on the findings of mutually exclusive SSc-specific autoantibodies that did not change during the course of disease. The autoantibody subsets are distinguished by patterns of cutaneous involvement, specific clinical features and prognosis (Table 3). SSc-specific autoantibodies were found to be stronger predictors of disease outcome and organ involvement than the extent of skin involvement[26]. The subset of SSc patients positive for ACA represents a clinically homogenous group with distinct clinical features and seems to have a better prognosis: less severity, less frequent ILD, SRC, inflammatory arthritis, inflammatory myositis, and lower GI tract involvement, finger ulcers, digital tuft resorption, or finger contractures; the patients are older at disease onset, predominantly women, more likely to have limited disease, lower skin scores, telangiectasia and PH[9, 20, 28, 50-56, 58, 60-62, 64, 68-70, 72, 73, 83, 85, 88, 122]. ACA status was found to be predictive of the extent of skin involvement over time[58]. Patients with limited disease who were ACA-negative at baseline were more likely to progress to diffuse disease. ACA-negative patients also had a greater extent of cutaneous involvement, worse survival and more severe internal organ involvement [28, 64].

Another study supported sub-division of lcSSc into two serological subtypes (Th/To positive and ACA-positive) with different internal organ involvement and outcome[49]. Compared to the ACA-positive patients, Th/To patients were younger at disease onset, predominantly male, with less PAH development, but more ILD (38% vs 4.5%). The highest mortality was found in "ATA+" and "ATA+, ACA- "subgroups, while "ACA+ATA- "and "Pm/Scl+, RNAP- "patients were classified as low-risk[26]. Some patients are not within described serological subsets, i.e. ACA were commonly found in association with mild skin involvement, but 9% of dcSSc patients with truncal involvement are positive for ACA [9].

Caetano et al. described those patients as a distinct clinical subtype (dcSSc ACA+) who had a more insidious onset of skin and major organ involvement, a lower incidence of ILD and SRC and better survival than expected for dcSSc[69]. Thus, further sub-grouping within each

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autoantibody profile may be promising from a clinical point of view. Indeed, two subgroups of anti-CENPA can explain variable clinical manifestations in an ACA-positive subset[86]. Subgrouping among SSc patients positive for anti-RPC155 antibodies (RNAP III large subunit, 155kDa) revealed that anti-RPA194 was associated with a lower cancer risk and less severe GI disease, while anti-RNAP I/II/III was associated with SRC[74]. Therefore, different autoantibody combinations have utility as tools for organ involvement and cancer risk stratification in SSc.

Patterson et al. reported subgrouping RNAP III-positive patients into two clusters: a strongly positive cluster was associated with an increased risk of GAVE, lower risk of esophageal dysmotility, and shorter disease duration [85]. A strong positivity for anti-RNAPIII (a higher ELISA index) was associated with the development of SRC[74]. Although, three main autoantibodies (ACA, ATA and anti-RNAP III) have strong mutually exclusive relationship, co-expression of other antibodies are relatively common [85, 123-125]. A combination of two SSc-related autoantibodies was revealed in one third of patients in the study of Patterson et al.[85]. Anti-Ro-52 most frequently occurred in combination with other autoantibodies, but co-expressions of ATA with anti-RNAPIII (0.6%) and ACA (3%) were also found in a small proportion of SSc patients [85]. In cases with co-existence of two and more autoantibodies, the autoantibody of highest titer determined the clinical phenotype.

SSc subsets according to nailfold capillary abnormalities. Capillary abnormalities seen on nailfold video capillaroscopy (NVC) can be used to subgroup SSc patients with different clinical manifestations and prognosis. There are 2 classification systems based on the NVC changes (Table 4). First, Maricq et al described two capillary patterns: 'slow' and 'active[126]'. 'Slow' pattern was characterized by capillary telangiectasias and high number of extremely large (giant) capillary loops with a relatively well-preserved capillary distribution. The main feature of 'active' pattern was moderate to extensive capillary loss associated with considerable distortion of the nailfold capillary bed and new blood vessel formation – bushy capillaries. Associations between capillaroscopic findings and disease activity, degree of progression and prognosis were found. SSc patients with 'slow' pattern predominantly had slowly progressive disease (new

symptoms/signs during follow-up were found only in 1 out of 11 patients), longer RP prior to entry and were ACA-positive, while all patients with 'active' pattern were ACA-negative and half showed disease progression. Capillary loss ('active' pattern) reflected disease progression that was confirmed in other publications [97, 113]. The 'active' pattern had more severe disease manifested as extensive skin involvement and greater visceral involvement (muscle, kidney), and patients were ACA-negative in comparison with 'early' pattern[90]. Ostojic et al. found that enlarged capillaries without a significant capillary loss (slow pattern) was more frequently seen in lcSSc, while giant capillaries with advanced capillary loss (active pattern) occurred in dcSSc[102].

The Maricq NVC classification system has been further subdivided within the 'active' pattern into 'active' and 'late', while 'slow' pattern was re-named as 'early' by Cutolo et al.[94, 127]. The principal change was the interpretation of patterns as consecutive phases of progressive obliterative microangiopathy[127]. 'Early' pattern is characterized by a relatively well-preserved capillary distribution and density with a few enlarged/giant capillaries, few capillary microhemorrhages, and no evident loss of capillaries. The following moderate loss of capillaries is a sign of the next 'active' phase with a mildly disturbed architecture of capillaries, frequent giant capillaries and microhaemorrhages, capillary derangement, absence or few ramified capillaries (neoangiogenesis). The capillary changes typical for this phase (haemorrhages and giant capillaries) are closely associated with disease activity. Sambataro et al. showed that NEMO score (cumulative number of micro-haemorrhages and micro-thrombosis) ≥6 was the best predictor of disease activity, followed by the GC score ≥3 (number of giant capillaries)[117]. In the most advanced phase of SSc microangiopathy, represented by 'late' NVC pattern, the disorganization of the normal capillary array is generally seen, with severe loss of capillaries and large avascular areas, irregular enlargement of the capillaries, few or absent giant capillaries, microhemorrhages, and ramified/bushy capillaries. Normal NVC pattern is rarely seen in SSc (4-12%), nearly exclusively in the limited cutaneous subset[102, 128]. Numerous studies confirmed that patients with more advanced NVC patterns had more severe

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disease [90-92, 97, 102, 126, 128]. Significant capillary loss was more common in lcSSc patients who met ACR criteria compared to those who did not[114].

Classifying SSc patients according to the NVC patterns may predict development of a new organ involvement within 1 year[97, 99]. In two studies[97, 99], the odds ratio to develop severe organ involvement (defined as a category 2 or higher in any of the 9 organ systems assessed according to the Medsger Disease severity scale or new PAH or ILD at 18–24 months' follow-up) was stronger according to more severe NVC patterns, adjusting for disease duration, subset, and vasoactive medications. These findings were externally validated in Italian cohort. Associations between certain manifestations and NVC patterns are controversial such as reduced capillary density and PAH [106, 107]. Sample size was sometimes too small to detect possible associations[103].

All three NVC patterns can be observed in both clinical disease subsets (lcSSc and dc SSc)[127], however, 'early' and 'active' patterns are more common in lcSSc, especially early lcSSc[93] whereas the 'late' - in dcSSc[91, 92]. Classifying patients into NVC subsets is important early in the disease course because capillary loss is a reliable indicator of rapidly progressive early disease[24, 93]. Shenavandeh et al. showed that late pattern in early SSc patients was associated with severity of finger contractures and significantly reduced pulmonary function, compared to 'active' and 'early' patterns[93]. Table 4 demonstrates that reduced number of capillaries typical for 'active' and 'late' patterns was more commonly seen in patients with longer disease duration, higher mRSS, more severe lung (including PAH), GI, and peripheral vascular involvement, the higher number of organ affected, and elevated ESR and CRP[66, 93, 100-102, 104, 106, 108-113, 116, 117]. The ACR criteria sensitivity may be improved by adding the NVC patterns [114]. [115]. More severe NVC patterns (active and late) occurred in patients seropositive for ATA and anti-RNAPIII, and negative for ACA[66, 92, 94, 98, 116]. ANA-negative patients[98] and ACA-positive[94] had most favourable 'early' pattern. However, SSc-related autoantibodies are not directly linked with the development of a distinct SSc NVC pattern [128].(Tables 4 and 5).

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The limitations included small proportions of patients with each NVC pattern (especially 'early'), resulting in limited power to detect statistically significant differences, some outcomes were omitted from the analysis (i.e. GI involvement and SRC), while others might be interrelated (i.e. abnormalities in the cardiac parameters might be secondary to pulmonary involvement, rather than present as primary cardiac involvement), the duration of the follow-up in the prospective studies varied and was relatively short. Definition of organ involvement also varied between the studies that made difficult the comparison of the results; the association between reduced capillary density and the extent of skin involvement was not confirmed by Kenik et al. who used "stage of cutaneous disease"[105].

DISCUSSION

SSc subset classification is a rapidly evolving field. This systematic review highlights both the continued importance of skin involvement and the novel role of SSc specific antibodies, abnormal nailfold capillary patterns and molecular profiling in assessing patients to determine a subset.

The diffuse cutaneous subset comprises patients with rapidly progressive disease who require more aggressive treatment. However, disease progression assessed as severity/duration ratio (early significant visceral and skin involvement) suggests disease activity only in early dcSSc[22, 129, 130]. In later stages of disease, patients classified as rapid progressors in the beginning may still have a high disease severity due to the accumulated significant damage, but low disease activity as a result of treatment or spontaneous remission. Some SSc patients first develop severe skin involvement and/or visceral disease late in the disease course. Thus, the limited/diffuse system loses its predictive value in more advanced disease and should be supplemented with a necessary determination of disease activity and severity when it comes to choosing treatment. The recent advances in SSc-specific antibody detection, other SSc-specific autoantibodies could be added to SSc subset classification autoantibody profiling to the skin involvement while determining a subset.

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Based on gene expression profiling, lcSSc patients can be assigned to the limited, inflammatory or normal-like subsets, while fibroproliferative subset is seen in dcSSc patients. The molecular subsets seem to be a universal feature of SSc end-target organ pathology not affected significantly by heterogeneity of skin involvement within a patient and/or fibroblast heterogeneity in tissues [37, 38, 120]. The molecular intrinsic subset assignment could represent a valuable approach for matching SSc patients to appropriate therapies. Molecular phenotyping may aid personalized medicine by identifying therapies with higher potential for success in each individual patient, as well as to select SSc patients who will improve naturally as part of their disease course[46].

Some limitations of subgrouping by molecular phenotyping include relatively small sample sizes of clinical trials due to rarity of disease itself, specific inclusion criteria that misrepresents the full spectrum of SSc, lack of controls, differences in methods of transcript quantification and in the exact list of genes between studies. Moreover, not all therapy- or disease-relevant genes are regulated at the mRNA level. The use of molecular subsetting in clinical practice for individual patients is limited as paired skin samples from each individual are often not available, analyses are not standardized and large numbers of samples in a data set are needed to identify the molecular subset with accuracy. Recently, supervised machine learning algorithms have been developed and may be successfully used to assign single samples to intrinsic gene expression subsets according to pre-defined criteria [46]. The method utilizes a multinomial elastic net classifier and an optimized set of genes. Classifier accuracy in that study was proved using concordance of samples (83.3%), reporting Cohen's kappa coefficient (0.7391), and was externally validated. Further efforts are needed to explore molecular heterogeneity and intrinsic subsets in other tissues and particularly in peripheral blood, given its accessibility.

Attempts to identify SSc subsets considering SSc-specific autoantibodies have faced a variety of challenges. Boonstra et al reported that adding autoantibody status to the cluster process resulted in correct classification of patients with ILD, PAH and SRC[26]. All high-risk patients were correctly identified by taking autoantibodies into account, but the number of patients

incorrectly identified as possibly high-risk increased significantly (by 66%) suggesting limited additional value of autoantibody status for clustering[26]. The limitations of studies on SSc-specific autoantibodies included underestimation of the number of antigens due to either the limitations of the techniques not allowing the identification of membrane proteins, or a loss of proteins at each step, small sample size, a lack of validation groups and limited generalizability (i.e. SRC is rare in Japanese patients; clinical features in each SSc-related ANA-based subgroup appear to vary among populations of different backgrounds). Feasibility is another consideration as some autoantibodies are identified by immunoprecipitation, which is not widely used in clinical laboratories, and/or some detection kits are not commercially available. Limitations of classification systems developed by cluster analysis are exclusion of a significant number of patients due to missing data and/or loss to follow up that affects the extrapolation of the results. Finally, there has been inconsistent definitions of variables between the studies, a lack of analysis of the potential effect of treatment regimens on survival and the influence of disease duration on the clustering process.

In conclusion, modern methods to subset SSc include skin involvement, immunologic profile, molecular signatures, visceral involvement, and age. Classifying on the basis of skin involvement, NVC and autoantibody profile may allow prediction of internal organ involvement early. Molecular subsetting may inform those who are likely to respond to therapy. Longitudinal prospective studies to track subsets are needed to provide insight into disease trajectory, to assess their predictive value, possible transition between subsets and evolution under treatment.

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Appendix II (online-only data supplement) – references 101-131

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Figure 1. Flow diagram of search results

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│ 7142 Medline, Embase, CINAHL and Cochrane records    │
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│ 287 full-text articles       │───────►│ 185 articles excluded     │
│ assessed for eligibility     │        └──────────────────────────┘
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                 │                      ┌──────────────────────────┐
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┌──────────────────────────────┐       │ 6 – classification system │
│ 102 articles included in      │       │ was not original          │
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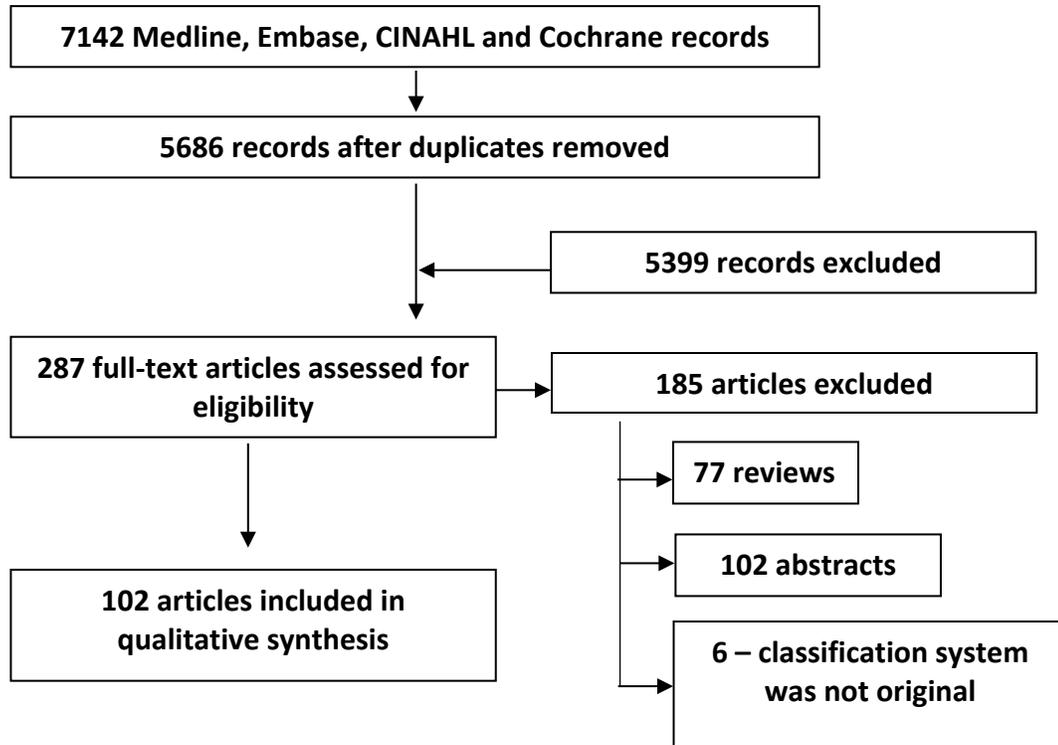
Table 1. Summary of clinical SSc subsets

Citation	Country	STROBE	Number of patients	List of subsets
Ferri 1991[20]	Italy	18	150	Cutaneous: Limited; Intermediate; Diffuse (higher % of men, worse prognosis, shorter RP before skin changes). Serological: ACA (higher % of female, lSSc, calcinosis, telangiectasia); ATA (iSSc and dSSc, GI and heart involvement, myositis, shorter RP duration before skin changes, skin ulcers, hyperpigmentation)
Ferri 2002[10]	Italy	17	1,012	4 subsets: "sine scleroderma SSc" absence of cutaneous involvement with visceral involvement, nailfold capillary changes, and autoantibodies; "limited cutaneous" skin involvement of fingers with or without involvement of neck, face, and axillae; "intermediate cutaneous" skin involvement of upper and lower limbs, neck and face without truncal involvement, "diffuse cutaneous" distal, and truncal skin involvement.
Maricq 2004[21]	USA	18	165	1.Diffuse - Skin involvement proximal to elbows/knees; includes trunk; 2.Intermediate -Skin involvement proximal to MCP/MTP, distal to elbows/knees; trunk not involved; 3.Digital SD - Sclerodactyly only: meets ACR minor criteria, but excludes those without skin involvement.; 4.SD sine SD - capillary pattern or pitting scars and visceral involvement; no ACA; no telangiectasia; 5.UCTD- two out of three of the following SD features: sclerodactyly, pitting scars, or SD capillary pattern or one of these three and another one from the following group: RP, pulmonary fibrosis or other visceral involvement (esophagus, heart, or kidney) but do not meet the criteria of groups III and IV. Those with CREST-type telangiectasia and/or ACA are excluded; 6."CREST" - No skin involvement, or sclerodactyly only; T is required with one or more other acronyms; or ACA is required with any two or more acronyms.
Vayssairat 1992[22]	France	18	164	Comparison of different systems.1. The diffuse versus limited classification according to the criteria of Le Roy; 2," The ARA classification" –diffuse = proximal to MCPs and distal is defined as a combination of two or more of the following: sclerodactyly (sclerodermatous involvement distal to the MCP), digital pitting scars and bibasilar fibrosis as revealed by chest X-ray; 3. digital (finger or toe skin involvement), proximal extremity (proximal extremities but not trunk skin involvement), and truncal. They studied how accurately all these systems reflected disease severity (assessed by severity score).
LeRoy 1988[8]	USA	4	-	Two subsets: "diffuse cutaneous SSc" onset of RP within 1 year; truncal and acral skin involvement; tendon friction rubs; early incidence of ILD, renal failure, diffuse GI disease, myocardial involvement; absence of ACA, abnormal NC; lcSSc RP for years, skin involvement limited to hands, face, feet, and forearms or absent; late incidence of PAH, trigeminal neuralgia, calcinosis, telangiectasia; high incidence of ACA, abnormal NC.
Barnett 1969[23]	Australia	9	61	3 subsets: "limited,""moderate," and "extensive," based on skin involvement of the fingers only, limbs and face, and involvement of the trunk, respectively.
Barnett 1988[9]	Australia	10	177	Type 1 - Sclerodactyly only; Type 2 – sclerosis proximal to MCP, but excluding trunk; Type 3 – diffuse skin sclerosis including trunk

Scussel-Lonzetti 2002[24]	Canada	18	309	SSc without skin involvement, lSSc, intermediate SSc and dSSc. Further, iSSc was divided into "above and below elbow" forms.
Simeon 1997[25]	Spain	19	72	group 1 - sclerosis of fingers and neck; group 2 - sclerosis of face and distal to elbows; group 3 - generalized sclerosis including trunk
Boonstra 2018[26]	Netherlands	19	407	Clinical cluster analysis identified 4 subgroups, with two subgroups showing higher than average 5-year mortality rates. High-risk subgroups: Subgroup 1: male predominance, dcSSc, mRSS, SRC, ATA, less ILD. Subgroup 2: female predominance and non-Caucasians, PAH, GAVE, ILD, lower DLCO and FVC. Subgroup 3: female predominance, Caucasians, lcSSc, GI, reflux, constipation, diarrhea, peripheral vascular involvement (digital ulcers), ACA. Subgroup 4: female predominance, lcSSc, GI, dysphagia, diarrhea, less ILD, FVC and DLCO. Adding autoantibody status to the cluster process resulted in 5 subgroups with 3 showing higher than average mortality.
Avouac 2011[27]	85 EUSTAR centres	19	-	Very early systemic sclerosis (VEDOSS[a]: RP[b], puffy fingers, antinuclear antibodies, AND capillaroscopy OR SSc[c]-specific antibodies
Giordano 1986[28]	Italy		90	6 subsets were studied: I - sclerodactyly only; II - sclerodactyly and skin involvement of neck, lower eyelid or axillae; III - skin involvement of hands and forearms±legs±face; IV - group III and arm and/or thigh skin involvement; V - group III and thorax; VI - group III and/or IV and/or V plus abdomen. Three subsets were designated: "limited" skin involvement of fingers, face, neck, axillae; "intermediate" skin involvement proximal to fingers; "diffuse" truncal skin involvement.
Goetz 1945[29]	USA	5	13	2 subsets: "acrosclerosis" and "diffuse", based on skin thickening limited to extremities or includes trunk.
Holzmann 1987[30]	Germany	5	-	5 subsets (Types I-IV) based on the extent and location of skin sclerosis, presence/absence of RP, extra-cutaneous manifestations, ANA
LeRoy 2001[31]	USA	5	-	4 subsets: LSSc consists of (1) objective RP plus any one of NC changes or SSc selective autoantibodies OR (2) subjective RP plus both NC changes and SSc selective autoantibodies; *lcSSc* criteria for LSSc plus distal cutaneous changes; *dcSSc*[i] criteria for lcSSc plus proximal cutaneous changes; "diffuse fasciitis with eosinophilia" proximal cutaneous changes without criteria for LSSc or lcSSc.
Masi 1988[32]	USA	6	-	3 subsets: digital - skin involvement of fingers or toes but not proximal extremity or trunk; proximal extremity - proximal extremities or face but not trunk; truncal - thorax or abdomen.
Rodnan 1979[33]	USA	6	273	3 subsets: classical disease involving skin of the trunk, face & proximal extremities, and early involvement of esophagus, intestine, heart, lung and kidney; CREST syndrome; and overlap syndromes including sclerodermatomyositis and MCTD
Winterbauer 1964[34]	USA	2	7	*CRST*[k] *syndrome:* calcinosis, RP, sclerodactyly, telangiectasia.

Tuffanelli 1962[35]	USA	9	727	2 subsets: "acrosclerosis"RP, acral skin involvement, "diffuse SSc"no RP, skin involvement beginning centrally.
Sobanski 2019[36]	120 EUSTAR centres	19	6927	2 clusters: A. lcSSc (81%), 2/3 without severe organ damage, ACA+ (54%); B. dcSSc (61%), younger at disease onset, severe organ damage, ATA+ (54%), reduced survival. 6 clusters (increasing mortality from 1 to 6): 1. lcSSc, females, older at disease onset, GI involvement, low frequency of ILD, ACA(79%); 2. lcSSc, PH, ILD, ATA(35%), ACA(24%); 3. lcSSc, rare GI involvement and ILD, ACA(48%), ATA(24%); 4. lcSSc, severe cardiac, lung, GI, musculoskeletal and peripheral vascular involvement; 5. dcSSc, males, GI, cardiac, lung involvement, ATA(50%), ACA(20%); 6. dcSSc, males, high peak mRSS, severe organ damage, ATA(77%), ACA(12%).

SD – scleroderma, lSSc – limited systemic sclerosis, lcSSc – limited cutaneous systemic sclerosis, dSSc – diffuse systemic sclerosis, dcSSc – diffuse cutaneous systemic sclerosis, iSSc – intermediate systemic sclerosis, MCTD – mixed connective tissue disease, UCTD – undifferentiated connective tissue disorder, RP- Raynaud's phenomenon, ILD – interstitial lung disease, SRC – scleroderma renal crisis, PAH – pulmonary arterial hypertension, mRSS – modified Rodnan skin score, GAVE – gastric antral vascular ectasia, GI – gastrointestinal, MCP - metacarpophalangeal joints, MTP -metatarsophalangeal joints, DLCO - diffusing capacity for carbon monoxide, FVC- forced vital capacity, NC – nailfold capillaroscopy, ACA- anticentromere autoantibodies, ATA – antibodies to topoisomerase I, ANA – antinuclear autoantibodies, EUSTAR- European League Against Rheumatism (EULAR) Scleroderma Trials and Research.

Table 2. Molecular, genomic and cellular SSc subsets

Citation	Country	STROBE	Number of patients	List of subsets
Milano 2008[37]	USA	21	24 SSc, 3 morphea, 6 healthy controls (skin)	Normal-like, diffuse-proliferation, inflammatory, limited signatures. Diffuse-proliferation: higher mRSS, all dcSSc, longer disease duration compared to dcSSc pts in the inflammatory and normal-like groups; increased number of proliferating cells in the epidermis. Inflammatory: both lcSSc and dcSSc; increased T-cell infiltration in the dermis. Limited: lcSSc, more severe RP. Normal-like: both dcSSc and lcSSc.
Pendergrass 2012[38]	USA	17	22 dcSSc, 9 healthy controls (skin)	Normal-like, fibroproliferative, inflammatory. The gene-based subsets are reproducible, inherent, stable over time and independent of disease duration. The intensity of the signature is associated with changes in disease duration and mRSS (i.e. high expression fibroproliferative subset – longer disease duration and higher mRSS; low expression inflammatory subset – higher mRSS). No association with SSc-related autoantibodies.
Hinchcliff 2013[39]	USA	18	12 SSc, 10 healthy controls (skin)	Normal-like, fibroproliferative, inflammatory. Stable signatures over time, regardless of treatment. Reproducibility. Independence of autoantibody status. Predicted response to MMF treatment: improvement mapped to inflammatory signature, while non-responders belonged to normal-like and fibroproliferative subgroups.
Mahoney 2015[40]	USA	22	3 SSc patient cohorts from the studies [37-39] (skin)	Normal-like, fibroproliferative, inflammatory. Identified the core sets of genes consistently associated with the intrinsic subsets, and created a gene-gene interaction network across the intrinsic subsets.
Taroni 2015[41]	USA	21	16 SSc, 7 controls (esophageal biopsies)	Inflammatory, non-inflammatory and proliferative. Independent of dcSSc/lcSSc subtypes, serum autoantibodies and esophagitis. Inflammatory: older, a trend towards ILD (reduced DLCO, FVC, TLC).
Chakrovarty 2015[42]	USA	22	13SSc (10 treatment, 3 placebo), 4 healthy controls	Fibroproliferative, inflammatory and normal-like groups. 4 out of 5 improvers mapped to the inflammatory intrinsic subset showed decreased gene expression in inflammatory pathways over 24 weeks. 1 improver had normal-like signature (spontaneous improver?).
Gordon 2018[43]	USA	21	15 patients were assigned to either an inflammatory or a proliferative molecular subset at baseline	Inflammatory, proliferative, normal-like. Molecular subset at baseline was not associated with clinical improvement in the belimumab arm, the placebo arm, or the pooled treatment arms. An overall reduction in inflammatory gene expression and movement toward the normal-

				like subset was associated with improvement in mRSS;8 of 10 improvers were assigned to a normal-like molecular subset posttreatment.
Taroni 2017[44]	USA	16	Patients from multiple clinical trials	Immune and fibrotic signatures. High "inflammatory" signatures represented an active disease state. Epithelial-mesenchymal transition was significantly decreased in improvers from all trials. Different immunomodulatory treatments modulate distinct functional processes, i.e. abatacept had higher scores for vascular- and collagen-related modules, while MMF had higher scores for proliferation and type I interferon modules.
Frost 2019[45]	South Africa, USA	15	8	Two groups co-segregated with clinical features of ILD and/or inflammatory myopathy, or the absence of an inflammation phenotype. These groups showed paradoxical gene expression of the genes TCF7, SOX17, and FRZB in affected and unaffected skin.
Franks 2019[46]	USA	21	297 skin biopsy samples from 102 SSc patients and controls	4 intrinsic molecular subsets of SSc by supervised machine learning algorithms: fibroproliferative, inflammatory, normal-like, and limited.
van der Kroef 2020[47]	Netherlands, USA, Italy	19	19	4 clusters based on the distribution of monocute subsets: Cluster 1: high CD16+ monocytes and low memory B cell subsets, lcSSc; Cluster 2: increased classical monocytes, dcSSc, high mRSS, the strongest increase of CXCL10 and CXCL11 in the plasma; Cluster 3: larger amounts of memory B cells; Cluster 4: lower numbers of circulating classical monocytes, often no skin involvement.
Martyanov 2017 [48]	USA	20	19dc SSc patients (12 at baseline and post-treatment with dasatinib)	Skin-based intrinsic gene expression: fibroproliferative, inflammatory and normal-like

lcSSc – limited cutaneous systemic sclerosis, dcSSc – diffuse cutaneous systemic sclerosis, RP- Raynaud's phenomenon, ILD – interstitial lung disease, mRSS – modified Rodnan skin score, DLCO - diffusing capacity for carbon monoxide, FVC- forced vital capacity, TLC – total lung capacity, MMF -mycophenolate mofetil.

Table 3. Associations between SSc-related autoantibodies and clinical SSc manifestations

Citation	Country	STROBE	Number of patients	Autoantibodies	Associations
Barnett 1988[9]	Australia	10	74	ACA	SSc type: a higher frequency of ACA in type 1 SSc sclerodactyly only (60.8%), followed by type 2 sclerosis proximal to MCP, but excluding trunk (29.7%) and type 3 diffuse skin sclerosis including trunk (9.5%).
Ceribelli 2010[49]	Italy, USA	18	216	anti –Th/To	lcSSc and mild slowly progressive ILD. Compared to ACA"+" subset, anti –Th/Th "+"was associated with higher frequency of pericarditis, lower FVC, male gender, younger SSc patients and less frequent telangiectasia.
Gliddon2011[50]	UK	15	180 lcSSc	ACA, ATA, Anti-Th/To, anti-RNAP I, II, III, anti- U1 RNP, unidentified ANA, ANA negative	ACA: older at disease onset, isolated reduction in DLCO, reduced creatinine clearance, telangiectasia, less frequent ILD; ATA: more extensive skin involvement, lung fibrosis; Anti-U1 RNP: younger at disease onset, rare esophageal involvement, less frequent telangiectasia.
Falkner 2000[51]	USA	19	282	ACA, ATA, Anti-Th/To, anti-RNAP III, anti-fibrillarin, unidentified ANA	ACA and anti-Th/To - lcSSc
Graf 2012[52]	Australia	17	129 for clinical associations 298 for survival analysis	10 serological subtypes studied	dcSSc: ATA: ILD, reduced survival anti-RNAP III: SRC, reduced survival; lcSSc: ACA: no ILD anti-Th/To: PAH anti-Ku: myositis (NS) Overlap: anti-U1-RNP: frequent PAH, reduced survival, younger at disease onset anti-PM/Scl: ILD (NS)
Hamaguchi 2008[53]	Japan	20	203	ACA; ATA; Anti-U1-RNP; Anti-RNAP; Anti-Th/To (small number of pts); Anti-U3 RNP (small number of pts)	ATA: dcSSc, high mRSS, diffuse skin hyperpigmentation, pulmonary fibrosis, decreased survival rate Anti-RNAP: dcSSc, high mRSS, finger contractures ACA: lcSSc, low mRSS, less frequent ILD Anti-U3-RNP: dcSSc, rarely decreased DLCO Anti-U1-RNP: low mRSS Anti-Th/To: low mRSS, rarely decreased DLCO and upper GI involvement Negative ANA: low mRSS

					dcSSc positive for anti-RNAP (compared to dcSSc positive for ATA): rapid skin progression, skin hyperpigmentation, less frequent pitting scars and ILD, lower serum IgG levels.
Hanke 2010[54]	Germany	19	103	anti-CENP-A or anti-CENP-B*	ACA (anti-CENP-A or anti-CENP-B): lSSc; less frequent ILD, cardiac involvement, skin ulcers
Ferri 1991[20]	Italy	18	150	ACA, ATA	ACA: female predominance, lcSSc, calcinosis, telangiectasia ATA: intermediate and diffuse SSc, GI and heart involvement, myositis, skin ulcers, hyperpigmentation, shorter RP duration before skin changes.
Harvey 1999[55]	UK	19	155	ACA, ATA, anti-RNAP I/II/III	ACA: lcSSc, rare renal disease and ILD ATA: ILD, renal involvement (compared to ACA) Anti-RNAP I/II/III: dcSSc
Hesselstrand 2003[56]	Denmark	19	276	ACA, ATA, anti-RNAP I, II, III, anti-U1-RNP, anti-histone.	ACA: less frequent ILD, female predominance, vascular changes (finger systolic pressure), reduced GFR; ATA: dSSc, higher % of men, ILD; anti-RNAP I, II, III: ILD; anti-U1-RNP: younger at disease onset, vasospasm; anti-histone: more frequent cardiac, pulmonary and renal involvement, reduced survival.
Song 2013[57]	China, USA	18	185	ACA*(anti-CENP-B and anti-CENP-Q)	Less frequent ILD
Hudson 2012[58]	Canada	22	802	ACA*	ACA: older at disease onset, women predominance, lcSSc and lower mRSS, pulmonary hypertension, lower overall disease severity, less likely to have finger ulcers, digital tuft resorption, or finger contractures, ILD, SRC, inflammatory arthritis and myositis; ACA status was predictive of the extent of skin involvement over time. lcSSc patients who were CENP-A-negative at baseline were more likely to progress to diffuse disease.
Kuwana 2005[59]	Japan	20	534	anti–RNAP III *	dcSSc, higher maximum mRSS, and increased frequency of tendon friction rubs, SRC.
McCarty 1983[60]	USA	17	27 ACA	ACA*	better prognosis, less frequent major renal, cardiac, pulmonary, and lower GI tract involvement compared to speckled or nucleolar ANA patterns.
Vazquez-Abad 1994[61]	USA	16	611	ACA (CENP-B)*	CREST
Wu 2007[62]	Israel, USA	18	50 CREST 21 other	Anti-CCP3 in combination with ACA*	CREST

Giordano 1986[28]	Italy	13	105	ACA*	ACA: sclerodactyly with/without minimal skin involvement in other areas – armpits, eyelids, neck ACA-negative (most were ATA-positive): arms, legs +/-trunk involvement, lower cumulative survival rate and higher severity of internal organ involvement
Santiago 2007[63]	Canada	19	242	antiRNAP III*	Risk of SRC
Salazar 2015[64]	USA	19	3249	ANA negative*	less frequent vasculopathic manifestations
Satoh 2009[65]	Japan	18	354	Anti-RNAP III *	severe skin and renal involvement
Sato 2009[66]	Japan	20	103	anti-calpastatin antibodies*	higher ESR and inflammatory muscle involvement.
Simon 2009[67]	Hungary	19	293 (59 ATA positive)	ATA fragment F1*	No clinical associations
Iniesta Arandia 2017[68]	Spain	19	209	ACA, ATA and anti-RNAP III positive	ACA: female predominance, less common dcSSc and ILD, longer time from onset to SSc diagnosis; ATA: higher prevalence of ILD, less frequent lcSSc and sine scleroderma subtypes; Anti-RNAPIII: dcSSc, malignancies more frequent, especially synchronous neoplasia. No difference in terms of survival rate at 5 years and 30 years and causes of death.
Boonstra 2018[26]	Netherlands	19	407	5 clusters based on clinical and serological features	Autoantibodies improved detection of lung involvement, PAH and renal crisis, as well as patients with actual severe disease course, when shifting from clinical subgrouping to combined auto-antibody and clinical subgrouping. High-risk (mortality around 10%): Subgroup 1: dcSSc and renal crisis, less often females, ATA+; Subgroup 2: dcSSc, PAH, GAVE, less often Caucasians, ATA+, ACA-. Intermediate (mortality risk 7.2%): Subgroup 5: less frequent ILD and vasculopathy (pitting scars, digital ulcers), anti-RNAPIII+, Pm/Scl-. Low-risk: Subgroup 3: GI, ACA+, ATA- Subgroup 4: miscellaneous, Pm/Scl+, RNAP-.
Caetano 2018[69]	UK	20	1313	ACA+dcSSc, ACA+lcSSc and ACA-dcSSc	dcSSc ACA+ : insidious onset of skin and major organ involvement, a lower incidence of ILD and SRC and better survival than expected for dcSSc.

Caramaschi 2015[70]	Italy	5	178	ACA, ATA, Anti-RNAPIII, Th/To,PM/Scl	ACA: older, longer disease duration from RP onset; ATA: ILD; anti-RNAPIII: SRC.
Coppo 2013[71]	France	19	199 individuals, including patients suffering from various autoimmune disorders (Group I, n = 145) and non autoimmune diseases (Group II, n = 44 patients) as well as healthy individuals (Group III, n = 30).	anti-HP1 positive*	CREST
Igusa 2018[72]	USA	19	2383	ACA, anti-RNAP III dcSSc and anti-RNAP lcSSc	Anti-RNAPIII+, ATA-, ACA-, anti-RNAPII - had increased risk of cancer; ACA+: lowest cancer risk; dcSSc anti-RNAPIII: breast cancer; lcSSc anti-RNAPIII : lung cancer.
Foocharoen 2017[73]	Thailand	20	285	ATA, ACA (CENP A, CENP B), anti-PM/Scl-100, anti-PM/Scl-75, anti-Ku, anti-Ro52, anti-RNAP III (RP11 and RP155), anti-fibrillarin (U3RNP), anti-NOR-90, anti-Th/To, anti-PDGFR.	ATA: female, dcSSc, high peak mRSS, RP, hand deformity; ACA: negative association with hand deformity; Anti-Ku: overlap syndrome SSc/PM.

Hamaguchi 2015[74]	Japan	20	583	anti-RNAPIII	anti–RNAP III: SRC, in particular, co-existence of anti–RNAP II and anti–RNAP I/III (anti–RNAP I/II/III) and a higher ELISA index for anti–RNAP III.
Haddon 2017[75]	USA	21	24	anti-PM/Scl-100 as a part of the signature*, also based on levels of CD40 ligand, chemokine (C-X-C motif) ligand 4 (CXCL4)	clinical improvement.
Foocharoen 2016 [73]	Thailand	17	294	ATA, ACA	ATA: hand deformity; ACA: negative association with hand deformity; ATA+dcSSc: earlier ILD vs ATA-; ATA-lcSSc: RP.
Hoa 2016[77]	Canada, Australia, USA, Mexico	20	2140	anti-Ku*	Anti-Ku: ILD, increased creatine kinase levels. No difference in survival
Terras 2016[78]	Germany	16	158 (11)	anti-RNAP III*	dcSSc, higher mRSS, renal involvement.
Perosa 2013[79]	Italy	21	121 (75 ACA positive)	ACA cross reacting with FOXE3p53-62*	Less likely to develop active disease.
Wodkowski 2015[123]	Canada, Australia, USA	17	1574 (103)	Monospecific anti-Ro52/TRIM21 antibodies*	Less likely Caucasians, ILD, poor survival.
Shah 2010[81]	USA	19	23 (6)	anti-RNAP I/III*	Temporal relationship with the onset of cancer.
Sánchez-Montalvá 2014[82]	Spain	19	132	Anti-SSA/Ro52*	No clinical associations
Shah 2019[83]	USA	18	168	anti-RPA194 (subgrouping among anti-RPC155 antibodies)*	Cancer, less severe GI disease
Shayakhmetova 2019[84]	Russia	18	330 positive for a-U1RNP	anti-U1RNP*	lSSc (91%), digital ulcers/scars (50%), ILD (63%). Often joint (65%) and muscle (43%) involvement. 1/3 Sjogren syndrome

Patterson 2015[85]	Australia	18	505	ACA, anti-RNAP III strong, anti-RNAP III weak, ATA, anti-RNAP III, anti-NOR-90, anti-fibrillarin, anti-Th/To, anti-PM/Scl-75, anti-PM/Scl-100, anti-Ku, ATA, anti-Ro 52, anti-PDGFR	lSSc: ACA; dcSSc: RNAPIII, ATA; Anti- Th/To: less likely joint contractures and reflux esophagitis; Anti-fibrillarin: digital amputation and a trend toward GAVE; anti-TRIM-21/Ro 52: telangiectasia, dry eyes, PAH, and calcinosis; Anti -PM/Scl-75/100: a history of digital ulcers and a trend toward lcSSc, no history of smoking; RNAPIII- dcSSc, joint contractures, SRC; a strong RNAPIII cluster with increased risk of GAVE, lower risk of esophageal dysmotility, shorter disease duration;
Perosa 2016[86]	Italy	21	84 anti-CENPA positive	Subspecificities of anti-CANPA:anti-pc4.2 antibodies, anti-pc14.1 antibodies	anti-pc4.2 antibodies: sPAP and inversely associated with DLCO; anti-pc14.1 antibodies: inversely sPAP and positively DLCO.
Wuttge 2015[87]	Denmark	19	95	ACA, ATA, anti-RNAP	Specific cell-free plasma miRNA profiles: ACA- higher MiR-409-3p expression levels; ATA, anti-RNAPIII – higher MiR-184; ATA, anti-RNP: lower MiR-92a.
Wodkowski 2015[80]	Canada	17	16 monospecific anti PM75 and 11 anti-PM100	anti-PM75 and anti-PM100	Both anti-PM75 and anti-PM100: myositis; anti-PM75: ILD, calcinosis; Anti-PM100: calcinosis, better survival.
Liaskos 2017[88]	Greece, Germany, USA	19	131	ATA, ACA, a-RNAP III (RP11, RP155), anti-fibrillarin, anti-Ku, anti-NOR90, anti-PM-Scl100, anti-PM-Scl75.	ATA: dcSSc, ILD, PH and ILD-PH, digital ulcers (NS). ACA (anti-CENPB): lcSSc, negatively ILD. anti-RP11: male gender; anti-NOR90 – male gender, ILD; anti-Ro52 – arthritis.

lcSSc – limited cutaneous systemic sclerosis, dcSSc – diffuse cutaneous systemic sclerosis, RP- Raynaud's phenomenon, ILD – interstitial lung disease, SRC – scleroderma renal crisis, PAH – pulmonary arterial hypertension, PH- pulmonary hypertension, sPAP- systolic pulmonary artery pressure, mRSS – modified Rodnan skin score, GAVE – gastric antral vascular ectasia, GI – gastrointestinal, MCP - metacarpophalangeal joints, DLCO - diffusing capacity for carbon monoxide, FVC- forced vital capacity, GFR - glomerular filtration rate, ESR – erythrocyte sedimentation rate, ACA- anticentromere autoantibodies, ATA – antibodies to topoisomerase I, ANA – antinuclear autoantibodies, a-RNAP – antibodies to RNA polymerase, NS- not significant

Table 4. Associations between nailfold capillary patterns and clinical manifestations of SSc

Citation	Country	STROBE	No of pts	Classification	Associations with clinical picture, SSc-related autoantibodies or outcome
Chen 1984[90]	USA, China	18	68 SSc	Slow and Active	'slow' capillary pattern: ACA 'Active': extensive skin involvement and greater visceral involvement (muscle, kidney), more often hypertension
Caramaschi 2007[91]	Italy	21	103 SSc	Early, Active, Late	Severity of skin, lung, heart and peripheral vascular involvement, as well as homocysteine plasma levels progressively increased across the patterns, from 'early' to 'late'. 'Early' and 'active' patterns were more common in lcSSc, whereas the 'late' in dcSSc. 'Late': increased risk of active disease, digital ulcers and moderate to severe skin (mRSS ≥15), heart and lung (lowest DLCO and FVC) involvement, risk of ILD.
Ingegnoli 2013[92]	EUSTAR	21	2754SSc	Early, Active, Late	Severity for skin involvement and number of systemic manifestations progressively increased across the patterns. 'Early' and 'active': mild/moderate skin involvement and a low number of disease manifestations 'Late': more severe disease - ATA positive cases with diffuse cutaneous involvement.
Shenavandeh 2017[93]	Iran	19	70 SSc	Normal, Early, Active, Late, Non-specific.	'Early': early (<5 years) lcSSc versus the early dcSSc (>3 years). 'Late' and 'Active': skin telangiectasia, pitting scars, and pulmonary rales compared to those with 'early' pattern. 'Late': limitation of the finger-to-palm range of motion, FEV1 < 70% compared to 'active' and 'early' (only in the early SSc subgroup and lcSSc subtype).
Cutolo2004[94]	Italy	19	241 SSc	Early, Active, Late	'Early' and 'Active': lcSSc, ACA+ 'Late': dcSSc, longer duration of RP and SSc, more advanced age, ACA-. 'Active' and 'Late" – ATA.
Cutolo 2016[95]	Europe, multicentre	22	623 SSc from 59 centers (14 countries)	Normal, Early, Active, Late	
Bruni 2015[96]	Italy	17	110 SSc	Early, Active, Late	'Early' and 'active': digital ulcers (96%) compared to patients without a history or present digital ulcers (66%). 'Early': presence or/and history of digital ulcers.
Smith 2012[97]	Italy	18	66 SSc	Normal, Early, Active, Late.	The Odds ratio of future severe peripheral vascular and lung involvement at 18–24 months (defined as category 2–4 DSS per organ) rose steadily throughout the patterns.
Sulli 2013[98]	Belgium, Italy	15	42 SSc	Early, Active, Late	ANA-negative patients had a slower progression of nailfold microangiopathy characterized by the 'early' pattern. Progression to the 'late' pattern was associated with a different autoantibody pattern on IIF (fine-speckled + nucleolar pattern being the most prevalent). 'Late': ATA.
Smith 2013[99]	Belgium,	17	148	Normal, Early, Active,	The Odds Ratio to develop novel future severe organ involvement (in any of 9 organ systems,

	Italian			Late	defined as category 2 to 4 per organ of the DSS at 18-24 month) was stronger according to more severe NVC patterns and similar in both cohorts.

lcSSc – limited cutaneous systemic sclerosis, dcSSc – diffuse cutaneous systemic sclerosis, RP- Raynaud's phenomenon, ILD – interstitial lung disease, mRSS – modified Rodnan skin score, DSS – disease severity scale, DLCO - diffusing capacity for carbon monoxide, FVC- forced vital capacity, FEV1- forced expiratory volume in one second, NVC – nailfold video capillaroscopy, ACA- anticentromere autoantibodies, ATA – antibodies to topoisomerase I, ANA – antinuclear autoantibodies.

Table 5. Association between particular capillary abnormalities and clinical manifestations in SSc patients

	Country	STROBE	No of pts	Classification	Associations with clinical picture, SSc-related autoantibodies or outcome
Houtman 1985[100]	Netherlands	16	107: 39 isolated RP and 68 CTD (15 SSc, 9 CREST, 15-MCTD)	Total number of capillary loops, number of enlarged capillaries	Decreased number of capillary loops: sclerodactyly, digital ulcers or pitting, tuft resorption, telangiectasia, the higher number of organs affected, severe RP, oesophagus and lung involvement (x-ray), increased fibrinogen level (>3 mg%) and ESR. Increased number of enlarged loops: lung involvement, arthralgia, elevated CRP. Decreased capillary density AND an increased number of enlarged loops: a positive Rose-Waaler, latex agglutination test, ANA, and CIC.
Bredemeier 2004[101]	Brazil	20	91 SSc	The **severity of capillary loss** was evaluated on each digit according to the score described **by Lee** et al (0 - no avascular areas; 1 - one or 2 discrete areas of vascular deletion; 2- > 2 discrete areas of vascular deletion; 3 - large confluent avascular areas). Severity score ≥ 1 were considered as severe capillaroscopic alterations. **The mean avascular score (MAS)** was calculated by dividing the sum of the scores by the number of digits examined. **The number of megacapillaries.**	MAS: higher mRSS, severity of sclerodactyly, signs of peripheral ischaemia (pitting scars, finger amputation), esophageal dysfunction, ATA, ground-glass opacities, longer disease duration (a confounder due to end organ damage). A higher number of megacapillaries per finger: ACA, ANA+. Among patients with ≤5 years of disease duration, a greater number of megacapillaries per finger was in those with esophageal dysfunction; patients with ground-glass opacities had higher avascular scores and a tendency to a greater number of megacapillaries per finger.
Ostojić 2006[102]	Yugoslavia	16	105: 50 lcSSc 55 dcSSc	Dilated capillaries without capillary loss; severe capillary damage/loss	Enlarged capillaries without a significant capillary loss: lcSSc Very enlarged capillaries with advanced capillary loss: dcSSc
Shenavandeh 2017[93]	Iran	19	70 SSc	Giant capillaries, capillary elongation, tortuosity, neoangiogenesis, reduced capillary density, avascular areas, abnormal blood flow and haemorrhages	Neoangiogenesis, reduced capillary density, avascular area, and haemorrhages: limitation of the finger-to-palm range of motion. Neoangiogenesis: pitting scars. Avascular area: GI problems (any of dysphagia, heart burn, difficulty swallowing, the feeling of being full, vomiting, diarrhea, and constipation). Giant loops: dysphagia. Abnormal blood flow: positive CRP. Capillary elongation: an inverse association with pitting scars. Capillary tortuosity: an inverse association with peripheral vascular manifestations.
Lefford 1986[103]	UK	16	42 with CTD (14	Capillary parameters (apex, loop and limb widths, loop length), number of capillaries,	Greater apex, loop and limb widths in SSc, compared to controls and RA. Shorter loop length and less capillaries, longer interpeak capillary distance,

			RA, 19 SLE, 9 SSc).	interpeak capillary distance.	greater degree of variation in interpeak distances in SSc, compared to controls. No association with clinical manifestations and serological data.
Lovy 1985[104]	USA	15	42	Capillary loss, capillary enlargement, telangiectasias	Extreme capillary loss: longer disease duration. No significant correlation was found between the presence or severity of capillary enlargement (and capillary loss) and the extent/number of organ involvement. Telangiectasias correlated with the presence and severity of nailfold capillary enlargement: all patients with extremely enlarged capillary loops had telangiectasias.
Kenik 1981[105]	USA	14	24 with CTD (18 SSc)	Not detailed	No association between the degree of capillary changes and the stage of cutaneous disease.
Hofstee 2009[106]	Netherlands	18	21 healthy controls 20 idiopathic PAH 40 SSc	Capillary density and loop dimensions	Low capillary density: SSc-related PAH compared with those without PAH, while loop dimensions were equal. Capillary density: severity of PAH in both SSc-related and Idiopathic PAH.
Sato 2009[66]	Brazil	20	92 SSc	(1) number of capillary loops/mm, (2) vascular deletion score assessed according to Lee's method, (3) number of enlarged loops, and (4) number of giant capillary loops.	Higher vascular deletion: mRSS, ATA+, finger pad lesions, ≥3 internal organs involved, dcSSc, compared to lcSSc, sine scleroderma SSc, and overlap syndrome.
Greidinger 2001[107]	USA	20	37 PPH, 15 SSc, 13 healthy controls	Capillary loop enlargement, dropout, density, bushy and tortuous capillaries.	No difference between SSc patients with and without PAH.
Alivernini 2009[108]	Italy	20	130 SSc	Avascular areas	Avascular areas – a major risk factor for the development of skin ulcers with a negative impact on healing.
Sebastiani 2009[109]	Italy	16	120 SSc	total number of capillaries in the distal row (N), maximum loop diameter (D), number of megacapillaries (M), and the M:N ratio.	The CSURI (D x M:N^2) at the cutoff value of 2.94 represents a novel tool with the ability to predict the development of digital ulcers in SSc patients.
Sebastiani 2013[111]	Italy	14	170 SSc	CSURI	CSURI showed good sensitivity, specificity, positive and negative predictive value.
Sebastiani 2012[110]	Italy	15	229 SSc,	CSURI	High specificity (81.4%), sensitivity (92.98%) at the cut-off value of 2.96 and reproducibility (κ-statistic measure of interrater agreement of 0.8514) of CSURI.

					for the persistence and/or appearance of new digital ulcers.
Manfredi 2015[112]	Italy	17	219 SSc	CSURI	altered CSURI is one of the factors associated with appearance of digital ulcers. A prediction risk chart of the development of digital ulcers within 6 months with four risk classes were built on the basis of CSURI, male gender, history of digital ulcers, and ESR.
Avouac 2017[113]	France, Italy	21	140 SSc	Number of capillaries, giant capillaries	Increased number of giant capillaries: less risk to develop new digital ulcers. Loss of capillaries within a follow-up: overall disease progression, appearance of new digital ulcers, progression of pulmonary vascular involvement, skin fibrosis and worsening of the Medsger severity score.
Lonzetti 2001[114]	Canada	7	259 SSc	Capillary dilatation (0 = normal; 1 = borderline [<2× normal diameter]; 2 = definitely dilated [≥2× but ≤4× normal diameter]; 3 = extremely dilated [>4× normal diameter]); Avascular areas (A = no capillary loss; B = rare avascular areas; C = moderate capillary loss; D = extensive capillary loss).	Severe capillary loss (grade C or D avascular areas): lcSSc ACR criteria + versus the lcSSc ACR− group. The sensitivity of ACR criteria was improved from 33.4% to 74.3% by adding grade 2 or 3 dilated capillaries, and further to 82.9% by grade C or D avascular areas, and to 88.8% with clinically visible capillary telangiectasias.
Hudson 2007[115]	Canada	18	101 SSc	Nailfold capillary abnormalities defined as the presence or absence of any dilated loops, giant capillary loops and/or avascular areas for each digit. No scoring was done.	The sensitivity of the ACR criteria in lcSSc was improved from 67% to 99% by adding nailfold capillary abnormalities and clinically visible telangiectasias.
Herrick 2010[116]	UK	18	176 SSc	Capillary width, distance between capillaries, density, tortuosity and derangement	Both automated and manually measured distance between capillaries: severe digital ischemia, ACA+. Reduced density: ACA-. Wider capillaries: moderate/severe telangiectasias.
Sulli 2013[98]	Belgium, Italy	15	42 SSc		A slight reduction of capillary number at baseline: either the nucleolar or the fine-speckled + nucleolar pattern on IIF.
Sambataro 2014[117]	Italy	19	107 SSc	Number of micro-haemorrhages (MHE), micro-thrombosis (MT), giant capillaries with a diameter over 50 µm (GC), and normal/dilated capillaries (Cs) in NVC; NEMO score (number of micro-haemorrhages) - the cumulative number of MHE and MT observed in the images obtained from eight fingers in each patient. The GC and Cs scores - the total number of GC and the mean number of normal or	**NEMO score**: ESSG index scores, mRSS, scleredema, worsening of skin, cardio-pulmonary, and vascular features, current digital ulcers, and ESR over 30 mm/h. **GC score:** ESSG index score, mRSS, scleredema, digital ulcers and worsening of cutaneous, vascular, and cardio-pulmonary features. **Cs score:** negatively with ESSG index and mRSS, lower in patients with scleredema, digital ulcers, and DLCO <80%. A NEMO score ≥6 is the best predictor of disease activity, followed by a GC score

				slightly dilated Cs observed in the same NVC fields counted in each patient.	≥3, and a Cs score ≤6 with the most balanced performance in terms of sensitivity/specificity ratio and the best accuracy.

lcSSc – limited cutaneous systemic sclerosis, dcSSc – diffuse cutaneous systemic sclerosis, MCTD – mixed connective tissue disease, CTD – connective tissue disease, RA – rheumatoid arthritis, CREST – calcinosis, Raynaud's phenomenon, esophageal involvement, sclerodactyly, telangiectasia, RP- Raynaud's phenomenon, PAH – pulmonary arterial hypertension, mRSS – modified Rodnan skin score, GI – gastrointestinal, DLCO - diffusing capacity for carbon monoxide, ACA- anticentromere autoantibodies, ATA – antibodies to topoisomerase I, ANA – antinuclear autoantibodies, ESR – erythrocyte sedimentation rate, CRP- C-reactive protein, CIC – circulating immune complexes, MAS – mean avascular score, CSURI- capillaroscopic skin ulcer risk index, ESSG index- European Scleroderma Study Group index, IIF- Indirect immunofluorescence.