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Progression of patients with Raynaud's phenomenon to Systemic Sclerosis classified according to the 2013 ACR/EULAR criteria: five year analysis of the EUSTAR multicentre prospective study for Very Early Diagnosis Of Systemic Sclerosis (VEDOSS)

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Abstract

Background: Preliminary criteria for the Very Early Diagnosis of Systemic Sclerosis (VEDOSS) have been previously proposed to identify signs and symptoms of very early disease in patients with Raynaud's Phenomenon (RP). Patients with all VEDOSS signs/symptoms already fulfil the 2013 ACR/EULAR classification criteria for Systemic Sclerosis (SSc). However, prospective data on the evolution to fulfilling these criteria are lacking. The identification of clusters at high-risk of progression among patients with RP and one or more additional VEDOSS criteria may support a stratified approach for both clinical management and prevention trials.

Methods: RP patients were enrolled in a prospective, multicentre, observational registry with yearly follow-up visits: VEDOSS criteria (ANA positivity, puffy fingers, specific SSc antibodies and abnormal nailfold capillaroscopy) were recorded. The endpoint of this study was the fulfilment of 2013 ACR/EULAR classification criteria for SSc. Proportion of progressors and criteria interaction were reported descriptively. Predictors of progression of the distinct criteria interactions were determined based on the point prevalence at 5 years. To investigate the intermediate course of progression of distinct criteria and their combinations, Kaplan-Meier analysis was performed.

Results: 1150 patients were enrolled to October 2018. After excluding patients without confirmed RP signs and symptoms (n=35), patients who already fulfilled 2013 criteria at inclusion (n=350) or had missing information (n=1) and patients without follow-up visits (n=209), 553 patients were finally included in the analysis [507 females - 91.7%, mean age 45.9 years, median RP duration 4.0 (IQR 1.7;10.0) years]. Four hundred-one patients were ANA positive (73.7%), of which 39.5% had SSc specific antibodies (SSc-Ab). Nailfold capillaroscopy abnormalities were present in 36% of patients. Puffy fingers were detected in 17.4%. 1885 follow up visits were recorded. Two hundred fifty-four patients (45.9%) completed the study with progression or a follow-up over the 5 years. A total of 133 (52.4%) completers met the end point (progressors). Lack of ANA at baseline was the factor most strongly associated with lack of progression within 5 years (89.2%; 95%C.I 75.3-95.7). Conversely, positivity at baseline for SSc-Ab and puffy fingers was the combination carrying the highest risk of progression (94.1%; C.I. 73-99.7).

Interpretation: The results of a 5-year analysis of the VEDOSS study offers a useful tool for a stratified risk approach to RP patients. The absence of ANA is a strong protective factor which identifies patients with very low risk of developing SSc. The presence of one or two VEDOSS criteria in patients with RP confers a progressively higher risk of fulfilling classification criteria for SSc over time. This stratification tool can be used both for clinical management and to inform early interventional trials.

Key words: Raynaud's phenomenon, Systemic Sclerosis, Puffy Fingers, antinuclear antibodies, nailfold videocapillaroscopy

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Background

Systemic sclerosis (SSc) is an autoimmune disease characterized by high clinical heterogeneity in morbidity and mortality.[1,2] The disease represents ~~a~~ great challenge for the rheumatologist because of its unpredictable course. Despite the advances in understanding the pathogenesis of SSc[1] and the development of new-targeted therapies,[3-5] SSc represents a heavy socio-economic burden. This is particularly true in patients where complications might be avoided if an early diagnosis could have been made.[6-7] In fact, an early diagnosis of SSc is of particular importance so that therapy can be started before the skin progresses and other organs are damaged.[8] Population-based studies showed that mild SSc occurs more frequently than previously suspected[9] and that organ involvement is already present in the "pre-clinical" stage. [10-13]

In 1980, the first classification criteria were published and widely used as diagnostic criteria [14] despite a very poor sensitivity for an early classification of SSc patients.[15] In 1988, LeRoy et al proposed new criteria that included clinical features, autoantibodies and capillaroscopy, underlying the differences between the two main SSc subsets.[16]

In 2001, LeRoy and Medsger proposed a revision of the classification criteria to include `early' cases of SSc, making use of nail fold capillary SSc pattern and SSc-specific autoantibodies (SSc-Ab).[6] These criteria have been validated by Koenig and reveal the positive predictive value of the presence of SSc specific antibodies and SSc pattern on capillaroscopy.[17] Finally, the revised ACR/EULAR

classification criteria were published in 2013, showing an increased sensitivity to classify SSc patients with less apparent skin involvement.[8]

Raynaud's phenomenon (RP) is a fundamental sentinel sign identifying patients at higher risk of developing SSc or other connective tissue diseases.[17,18] In line with the previous proposal from LeRoy and Medsger from 2001,[6] the preliminary criteria for the very early diagnosis of SSc (VEDOSS) were developed in 2011 as a result of a multicentre web-based Delphi exercise among SSc experts.[19] The study identified RP, antinuclear antibodies (ANA) positivity and puffy fingers (PF) as "red flags" or level 1 signs to raise suspicion for the very early diagnosis of SSc (18,19). In the presence of these "red flags", in the "level 2" assessment, the positivity of SSc specific antibodies (anticentromere (ACA), anti-scleroderma-70 (anti-Scl-70) and RNA polymerase III (RNAPOLIII)) and/or the detection of abnormal nailfold capillaroscopy (NVC)) could allow the identification of a patient affected by very early SSc.[15,19]

In order to validate this approach, patients with RP, not fulfilling the ACR/EULAR classification criteria for SSc, were enrolled in a prospective, multicentre, longitudinal registry (VEDOSS project), supported by the European Scleroderma Trial and Research group (EUSTAR). Here we aimed to determine, in patients with RP, the clinical value of the VEDOSS criteria to identify patients progressing within 5 years to 2013 ACR/EULAR criteria defined SSc.

Patients and methods

The VEDOSS project was performed in 42 EUSTAR centres following local Ethical Committee approval and data were recorded in a centralised database within the EUSTAR database infrastructure. A detailed list of the centres and relative PIs holding the local Ethical Committee approval documents is attached in supplementary file.

Patients with RP, defined as a history of at least 2 of 3 color changes (white, blue, red), usually induced by cold exposure, and involving at least 1 finger of each hand, were eligible.[19] The fulfilment of the ACR 1980 [14] and/or 2013 ACR/EULAR classification [8] criteria for SSc , as well as of any other ACR or EULAR classification criteria for other definite connective tissue diseases (i.e. overlap syndromes) constituted the main exclusion criteria. Patient enrolment was opened in March 2010; database export for the current analysis was through October 04, 2018. Data recorded in the database included demographics, medical history and clinical/serological data, results from laboratory and imaging examinations, current medications and capillaroscopic characteristics (as per the Clinical Research form).

The other 4 VEDOSS criteria were included in the analysis: ANA positivity (according to local laboratory customs), puffy fingers, specific SSc-Ab and abnormal NVC. The SSc-Ab were defined by recording of ACA, Scl-70 or RNAPOLIII positivity alone or in combination. The NVC abnormalities (defined as NVC) were based on the ACR 2013 definition: either giant capillaries or capillary loss with or without haemorrhages.[8] To this end, capillaroscopic evaluations which had been described in the VEDOSS database by giants ("rare", "moderate" or "severe") or any capillary loss ("rare", "moderate" or "severe") were categorised as "abnormal NVC" (potentially comprising also aspecific images with "rare" loss of capillaries).[20-21] Primary endpoint was fulfilment of the ACR/EULAR 2013 classification criteria, defined in this study as "progression".[8] Frequency of single and combined items at inclusion and at follow-up was descriptively analysed. The analysis was limited to 5 years follow up. For the calculation of 5-year event rates, patients not reaching the endpoint and/or duration of observation of less than 5 years were not included. Accordingly, progression events after 5 years were censored.

In order to answer various questions about the disease course of VEDOSS patients, it was planned to include 1000 patients in the study. The aim of this study was to provide first estimates of 5-year progression rates of VEDOSS patients. Indeed, the data collection was started as a registry without pre-defined termination rules. At the point of intermediate data base freeze for this analysis, at least one follow-up was available for 72% of the enrolled patients. Among the 211 patients without follow-up, 93% had a baseline visit at least 3 years before database freeze, so the chance to ever see these patients again was low. Of the remaining patients with follow-up, the 5-year observation criterion or progression was fulfilled in more than 50%. Therefore, we considered 764 patients a reasonable number to describe baseline characteristics of our VEDOSS cohort and 533 with follow-up a reasonable basis to provide first estimates for progression rates of this patient population. This decision was not driven by case number calculations of pre-defined hypotheses.

To investigate the course of progression based on the fully available follow-up data, the time to fulfilling classification as SSc was evaluated with Kaplan-Meier analysis; the Breslow tests accounting for decreasing case numbers and thereby censored data was used. To identify baseline predictors of progression to definite SSc for different combinations of the 4 baseline criteria in the same cohort, missing values for the 4 criteria were imputed with 10 repetitions. Logistic regression on the 5-year events and Cox regression on the fully available follow-up data were run for all combinations of baseline criteria; pooled results of the imputed data are reported. For continuous data, mean and standard deviation (SD) or median and interquartile range (IQR) are shown, for categorical data counts and percentages. Due to the exploratory character of the study no adjustment for multiple testing was done. IBM SPSS Statistics version 24 was used for analysis.

Results

Based on the available baseline data, 1,150 RP patients were included: 764 fulfilled the intended VEDOSS criteria (ANA, puffy fingers SSc-Ab, NVC): 553 patients (507 females - 91.7%, mean age 45.9 years) had at least one available follow up visit and the median duration of follow up was 3.6 years (IQR 1.7-5.8). The flow chart summarizing patient selection is shown in Figure 1A. Median time since RP onset was 4.0 years (IQR 1.7-10), not significantly different from the 211 patients without follow-up (Table 1).

Complete 5-year follow-up data were available for 254 (45.9%) patients. The specific number of patients with data on each VEDOSS criterion are available in supplementary table 1.

At baseline, of the 553 patients with follow-up, 401/544 (73.7%) RP patients had detectable ANA, with 208/527 (39.5%) positive for SSc-Ab. The majority was ACA positive, followed by Scl-70 and RNA-Pol III positivity (Table 1 and Figure 1B). NVC abnormalities were present in 182/505 patients (36%). Puffy fingers were detected in 96 patients of the 539 with data available (17.8%).

In patients with more than one VEDOSS criteria in addition to RP, case number with available information on the respective item combinations varied between 505 and 535. The summary of criteria interactions are shown in UpSet plots, where intersection of a matrix of interactions are plotted in a bar graph (figure 1B). The most common combination was the presence of ANA and NVC abnormalities, recorded in 29.3% of cases. The second most common combination was among SSc-Ab and NVC abnormalities (15.6%). The third most common was the combination of ANA and puffy fingers (12.8%). Specific SSc antibodies and puffy fingers were recorded in 5%, NVC abnormalities and puffy fingers in the absence of SSc-Ab was noted in 5.6% of cases (Figure 1B).

During follow up, ANA data were available for 1695 visits, with 1630 events recorded for SSc-Ab. The NVC evaluation was available for 1146 visits and the presence of puffy fingers in 1823 visits (Figure 1C). The distribution of the VEDOSS criteria during follow up visits showed that prevalence of ANA was stable with a marginal 3% increase compared to baseline prevalence (Figure 1D). Similarly, NVC abnormalities remained largely stable, with a 5.6% increase. On the contrary, puffy fingers were recorded in 25.8% of observations at baseline, with a 40% increase in prevalence during follow-up. SSc-Ab were also reported more frequently during follow up (45.6% of observations), with a relative increase of 15.4% over baseline prevalence. This increase was associated with a nearly 300% increase in prevalence of observations including all of ANA, SSc-Ab and puffy fingers; there was about a 60% increase in the combination of ANA and puffy fingers or NVC abnormalities and puffy fingers (Figure 1B-C).

The increase in detection of SSc Abs over time was interesting and never reported before. To determine whether this observation was linked to a bias in obtaining SSc Ab specific information we have analysed the proportion of unknown or missing SSc Ab over time. ACA and Scl-70 were reported as missing or unknown in 9.4 and 7.8% at baseline, respectively. In all cases, this proportion did not decrease over time. On the contrary we observed a slight increase in the proportion of missing data in subsequent visits, to 15.0 and 15.7%, respectively. We speculate that this increase could be explained by a diminishing eagerness to document Ab status once this had been documented before. Regardless of the putative explanation, the observation of increased prevalence of SSc specific antibodies over time remained supported by the data.

In our population of RP patients, the analysis of progression over 5 years by baseline criteria offered the opportunity to examine risk stratification. The proportion of progressors divided by VEDOSS criteria and their interaction is summarised in the plots shown in figure 2, 3A and 3B.

Follow-up data beyond or progression within 5 years were available for 254 (45.9%) patients, of whom 133 reached the primary endpoint, resulting in an overall progression rate of 52.4%. One-hundred twenty-six (94.7%) of the progressors were ANA positive, which corresponded to 58.9% of the ANA positive patients (126/214).

The absence of ANA at baseline was a strongly protective factor against progression, with only 11% of ANA negative patients progressing. This is a relative risk of 0.18. Seventy percent of patients with SSc-Ab at baseline progressed, against 31% progression in patients that did not have SSc-Ab at baseline. The presence of NVC abnormalities or puffy fingers at baseline conferred a similar risk, with 70% and 71% of patients progressing, respectively (Figure 2 and 3, Supplementary Table 2).

When looking at combinations of VEDOSS criteria, RP patients with SSc specific antibodies and puffy fingers at baseline represented the highest proportion of progressors (94%). The coexistence of RP, SSc-Ab and NVC abnormalities carried the second highest frequency of progression (82%), (Figure 3A). A colour coded distribution of progression risk, according to presence or absence of one or two VEDOSS criteria in combination is summarised in Figure 3B. Here, the risk of progression is reported on a green to red scale according to low (green) or progressively higher risk of progression. For comparison of the predictive value of different criteria combinations, missing baseline values were imputed: 9 values were missing for ANA (1.6%), 13 values were missing for puffy fingers (2.4%), 26 values were missing for SSc-Ab (4.7%), and 48 values were missing for NVC (8.7%).

When focusing on the percentage of correct prediction of 5-year progression, univariable logistic regression provided the best prediction for SSc-Ab, with 69.2% correct predictions overall comparing both non-progression and progression. Among the significant singles/combinations the best

prediction of progression was for ANA with 96.2%, though with a low correct rate for non-progressions (27.3%) (Supplementary Table 3). When testing the 4 single criteria in a multivariable logistic regression model, with stepwise backward selection, the pooled results confirmed all criteria with significant predictive value: ANA with a risk ratio (RR) of 6.21 (95% CI: 1.57-24.6, p=0.010), puffy fingers with a RR of 5.83 (2.40-14.2, p<0.001), SSc-Ab with a RR of 4.20 (2.21-7.99, p<0.001), and NVC with a RR of 2.52 (1.35-4.72, p=0.004).

In univariable Cox regression, incorporating the fully available follow-up, SSc-Ab again was the best predictor for progression (Supplementary Table 4). When testing the 4 single criteria in a multivariable Cox-regression model, with stepwise backward selection, the pooled results confirmed all criteria with significant predictive value: ANA with a RR of 5.05 (1.71-14.95, p=0.004), SSc-Ab with a RR of 3.02 (2.00-4.55, p<0.001), puffy fingers with a RR of 2.92 (1.91-4.46, p<0.001), and NVC with a RR of 1.70 (1.18-2.46, p=0.004).

Overall comparison of time to fulfil ACR /EULAR classification criteria for all combinations is displayed in figure 4A. Subgroup analyses comparing progression with single or combined criteria versus those without progression are shown in Figure 4B-C and Supplementary Figure 1. Consistent with the baseline data analysis, the lowest risk category was patients without ANA. Comparison of groups with or without ANA showed a significantly different progression course (p<0.001, Figure 4B). Similarly, the group with the highest progression rate was the one displaying SSc Ab and puffy fingers (comparison of the population with this combination versus those with either only a single or none of the 2 features showed a remarkably steeper progression, p<0.001, Figure 4C). The presence of any single criteria or dual combination was always associated with a significantly higher progression than in the comparison group without the single or dual feature, though by differing magnitudes. While the largest 5-year difference was seen for the presence versus absence of SSc-Ab and puffy fingers (91.3% vs. 32.8%)), the smallest gap was seen for the presence versus absence of NVC (46% vs. 28.3%) (Figure 4B-C, Supplementary figure 1).

The course of progression analysis also offered the opportunity to determine the proportion of progression at each of the earlier time points. The combination of puffy fingers with ANA or SSc-Ab, or the combination of SSc-Ab and NVC abnormalities were associated with a 35 to 40% rate of progression at 24 months, rising to close to 50% at 36 months. This latter observation may be extremely useful in the context of powering studies aimed at prevention to fulfil 2013 ACR/EULAR criteria in VEDOSS patients.

Discussion

The present analysis has evaluated the clinical, laboratory and imaging features of a large, prospective, multicentre cohort of RP patients. Its purpose was to identify, on top of RP, those signs that may best predict progression to the fulfillment of SSc classification criteria. Our results confirm the clinical value of RP, puffy fingers and ANA positivity[17-19] among the pivotal signs and biomarkers to raise the suspicion of SSc. These signs together with the positivity of SSc-Ab and/or abnormal NVC may identify those RP patients with a very early SSc characterized by the highest risk to evolve to definite SSc, as defined by the 2013 classification criteria.[8] The present data offer also a clinical validation of the VEDOSS criteria since each one of these increases the risk of fulfilling the ACR/EULAR classification criteria for SSc within 5 years.

In 2008, Koenig et al found, in a 20-year single centre prospective study on 586 RP patients, that RP patients with SSc-Ab and/or SSc specific NVC abnormalities without any other SSc clinical manifestation had an increased risk to develop SSc, classified according to 1980 ACR classification criteria, compared to other RP patients.[17] At 5 years of follow-up they found that 47% of the patients with RP were classified as definite SSc, according to the 1980 ACR criteria. The data from the study of Koenig had used SSc specific NVC changes and pinpoints the relevance of the combination of SSc specific antibodies and NVC in centres specialized in NVC analysis. Our data show a similar proportion of patients fulfilling the newest ACR criteria at 5 years (52.4%). Consistent with the registry approach, our data reflect closely daily practice, and are based mainly on clinical signs and antibody detection. There was no central analysis of NVC images, neither monitoring of NVC execution or interpretation. The definition of abnormal NVC changes in our analysis is broader than the one used in the Koenig study. This may have under evaluated the role of NVC interpretation by including some nonspecific abnormalities.

On the other hand it revealed the importance of ANA as a sign of evolving autoimmunity in this at-risk population and role of puffy fingers.[22,23]

The strong negative predictive value of the absence of ANA could be considered when monitoring patients for the risk of progression to SSc. It is also interesting to note that ANA did not change over time. *Notably, the absence of Th/To and U3-RNP in the ACR/EULAR 2013 criteria may underestimate the impact of these specific autoantibodies in our analysis of "progression". Nevertheless, patients with these specific autoantibodies still cannot be classified as SSc with the current criteria and therefore there was no specific sub analysis in this study."*

Similarly, the prevalence of NVC abnormalities were rather stable, increasing only 5.6% over 5 years, thus making both ANA and NVC abnormalities solid stratification points for clinical management.

In contrast, the prevalence of PF increased by 40% over the 5 years. Similarly, the prevalence of SSc-Ab increased as well as the increasing prevalence of SSc-Ab over time is analogous to the increasing

prevalence of anti-CCP and RF in rheumatoid arthritis (which is thought to reflect epitope spreading).[24]

These observations may lead to speculate that SSc- Ab and PF may reflect antigen spreading and progressive vascular damage[25] in patients with autoimmunity (ANA) and initial capillary abnormalities. *Interestingly, while the higher risk of progression was in patients with the highest 2013 ACR/EULAR score at baseline (8 points in patients with SSc ab and either PF or NVC), the presence of ANA, while not adding any points to the score, increased the relative risk to each of the other criteria (Figure 3B).* Further studies, where only SSc specific NVC changes will be evaluated, will elucidate the role of specific SSC NVC pattern in this RP population.

Our data are in agreement with Ricciardi et al, who reported similar results in a population of 102 UCTD-SSc-at-risk patients, a condition characterized by RP, and either SSc-Ab and distinct NVC alterations or both (26). In this group, patients showed a low risk of progression to any condition other than SSc, suggesting that SSc-risk is perhaps more appropriate than a diagnosis of UCTD for this cohort of patients.

One of the major strengths of the present study is that it is the first prospective, multi-centre, international study on this at-risk population, indicating that the data are generalizable. Furthermore, the data have been obtained from specialised tertiary referral centres for SSc, suggesting that patients were likely to be correctly and uniformly evaluated.

Koenig et al reported from a single centre that the mean time between the onset of RP and the first non-RP symptom or sign was 4.8 years in limited and 1.9 years in diffuse cutaneous SSc (Arthritis Rheum 2008; 58:3902-3912) This observation, if confirmed in our population may lead to a bias towards the diagnosis of lcSSc in our population. Despite the historical clinical subset definition has been recently challenged by the community (Scand J Rheumatol. 2018 Jan;47(1):62-70.), a detailed sub-analysis of the VEDOSS criteria more predictive of progression to diffuse subset vs limited subsets is currently an important point in the research agenda.

Our study also has some limitations, inherent to any large international registry study with a large number of centers. Perhaps the most important one is the low frequency of patients with follow-up (<50%). From the analysis of **table 1**, it is apparent that patients with no ANA and normal nailfold capillaroscopy were more likely lost at follow-up. This observation could reflect the lack of clinical need for follow up in these patients but might have resulted in an overestimation of the proportion of progressors.

Of particular importance were the difficulties relating to measuring NVC changes. This very study had been initiated in an era before the consensus on how to fast track categorise images as SSc pattern or not. Hence nonspecific as well as SSc specific changes in capillaroscopic characteristics have been

taken into account in this study analysis reflecting daily practice where rheumatologists with any capillaroscopy training level had participated. Further studies taking the recently published SCTC/EULAR consensus on evaluation of capillaroscopy and concerning classification of an image as scleroderma pattern or not will elucidate the role of SSc specific changes in such a cohort.[27] Another limitation may be the potential differences in ANA and SSc-specific antibodies detection. Since these were done locally, we cannot exclude a variability in sensitivity of the different methods and the potential bias in unknown RNA pol III status, in the majority of centres. Nevertheless, this effect is likely to be minor as methods for doing these tests are generally uniform, although not exactly alike. Further, the measures were used as dichotomous variables, so that titer differences are not of concern and limits of detection should not be a major methodological issue, given the large number of patients.

An important limitation of this study is that, again typical for an observational long-term registry, there was wide heterogeneity in the follow-up rates. In fact, complete 5-year follow-up data were available for 45.9% of patients and among patients without follow-up, 45% were without specific features at baseline, showing that, at least in part, the missing follow-up could reflect a lack of progression bias and be due to patients having a mild disease course (or no progression at all). Despite this, the number of patients was sufficient for an appropriate analysis.

In conclusion, our data show that a significant proportion of patients, diagnosed as VEDOSS, progress to a definite classification of SSc according to ACR/EULAR 2013 criteria within 5 years of follow up. The results of this study provide a simple and effective risk stratification model which may be relevant in clinical practice thus allowing an earlier therapeutic intervention to stop SSc progression to fibrosis. In fact, the direct reference scale we propose in Figure 3B may help the physician to stratify the risk and tailor regularity of follow up in patients with higher vs lower risk of progression focusing frequent and expensive screening procedures on the patients at higher risk. The implementation of this risk stratification model in a clinical trial setting may also inform sample size calculations for early therapeutic trials aimed at preventing the progression of VEDOSS patients to SSc, at improving long-term outcome and at preventing organ damage.

REFERENCES

1. Varga *J,* Trojanowska M, Kuwana M. Pathogenesis of systemic sclerosis: recent insights of molecular and cellular mechanisms and therapeutic opportunities J Scleroderma Rel Dis 2017; 2(3): 137-52
2. Moore DF, Steen VD. Overall mortality. J Scleroderma Rel Dis, June 2020. doi:10.1177/2397198320924873.

3. van Laar JM, Farge D, Sont JK, Naraghi K, Marjanovic Z, Larghero J, et al., EBMT/EULAR Scleroderma Study Group Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. JAMA. 2014 Jun 25;311:2490-8
4. Distler O, Highland KB, Gahlemann M, Azuma A, Fischer A, Mayes MD, et al ; SENSCIS Trial Investigators Nintedanib for Systemic Sclerosis-Associated Interstitial Lung Disease. N Engl J Med. 2019 Jun 27;380(26):2518-28
5. Kowal-Bielecka O, Fransen J, Avouac J, Becker M, Kulak A, Allanore Y, et al. Update of EULAR recommendations for the treatment of systemic sclerosis Ann Rheum Dis. 2017 Aug;76(8):1327-33
6. LeRoy EC, Medsger TA Jr. Criteria for the classification of early systemic sclerosis. J Rheumatol 2001;28:1573–6
7. Al-Sheikh H, Ahmad Z, Johnson SR. Ethnic Variations in Systemic Sclerosis Disease Manifestations, Internal Organ Involvement, and Mortality. J Rheumatol. 2019. pii: jrheum.180042.
8. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A et al 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Ann Rheum Dis 2013 Nov;72(11):1747-55. doi: 10.1136/annrheumdis-2013-204424.
9. Jordan S, Maurer B, Toniolo M, Michel B, Distler O. Performance of the new ACR/EULAR classification criteria for systemic sclerosis in clinical practice Rheumatology (Oxford). 2015;54(8):1454-8.
10. Czirjak L, Matucci-Cerinic M. Beyond Raynaud's phenomenon hides very early systemic sclerosis: the assessment of organ involvement is always mandatory. Rheumatology 2011;50:250–251
11. Valentini G, Cuomo G, Abignano G et al. Early systemic sclerosis: assessment of clinical and preclinical organ involvement in patients with different disease features. Rheumatology 2011;50:317–23
12. Lepri G, Guiducci S, Bellando-Randone S, Giani I, Bruni C, Blagojevic J et al Esophageal and anorectal involvement in patients with very early diagnosis of systemic sclerosis (VEDOSS): report from a single EUSTAR centre. Ann Rheum Dis 2015;74:124-8
13. Bruni C, Guiducci S, Bellando-Randone S, Lepri G, Braschi F, Fiori G, Bartoli F, Peruzzi F, Blagojevic J, Matucci-Cerinic M. Digital ulcers as a sentinel sign for early internal organ involvement in very early systemic sclerosis. Rheumatology (Oxford). 2015 Jan;54(1):72-6. doi: 10.1093/rheumatology/keu296. Epub 2014 Jul 26. PMID: 25065009.
14. Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum. 1980;23(5):581-90.

15. Lonzetti LS, Joyal F, Raynauld JP, Roussin A, Goulet JR, Rich E. et al. Updating the American College of Rheumatology preliminary classification criteria for systemic sclerosis: addition of severe nailfold capillaroscopy abnormalities markedly increases the sensitivity for limited scleroderma. Arthritis Rheum. 2001;44(3):735-6
16. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988;15:202–5
17. Koenig M, Joyal F, Fritzler MJ, Roussin A, Abrahamowicz M, Boire G et al. Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis. Arthritis Rheum 2008; 58:3902-3912
18. Matucci-Cerinic M, Allanore Y, Czirják L, Tyndall A, Müller-Ladner U, Denton C et al. The challenge of early systemic sclerosis for the EULAR Scleroderma Trial and Research group (EUSTAR) community. It is time to cut the Gordian knot and develop a prevention or rescue strategy. Ann Rheum Dis 2009; 68:1377-80
19. Avouac J, Fransen J, Walker UA Riccieri V, Smith V, Muller C, et al. Preliminary criteria for the very early diagnosis of systemic sclerosis (VEDOSS): results of a Delphi Consensus Study from EULAR Scleroderma Trials and Research Group (EUSTAR). Ann Rheum Dis. 2011 Mar;70(3):476-81
20. Cutolo M, Sulli A, Secchi ME, Pizzorni C. Capillaroscopy and rheumatic diseases: state of the art. Rheumatology. 2006 ;65(4):290-6
21. Smith V, Herrick AL, Ingegnoli F, Damjanov N, De Angelis R, Denton CP,et al; EULAR Study Group on Microcirculation in Rheumatic Diseases and the Scleroderma Clinical Trials Consortium Group on Capillaroscopy. Standardisation of nailfold capillaroscopy for the assessment of patients with Raynaud's phenomenon and systemic sclerosis. Autoimmun Rev. 2020;19(3):102458. doi: 10.1016/j.autrev.2020.10245
22. Minier T, Guiducci S, Bellando-Randone S, et al: Preliminary analysis of the Very Early Diagnosis of Systemic Sclerosis (VEDOSS) EUSTAR multicentre study: evidence for puffy fingers as a pivotal sign for suspicion of systemic sclerosis. Ann Rheum Dis. 2014 ;73:2087-93
23. Blaja E., Jordan S., Mihai C.M. , Dobrota R, Becker M.O., Maurer B., Matucci-Cerinic M., Distler O. The challenge of Very Early Systemic Sclerosis: a Combination of Mild and Early Disease? J Rheumatol. 2020 Mar 15. pii: jrheum.190976. doi: 10.389
24. Kongpachith S, Lingampalli N, Ju CH, Blum LK, Lu DR, Elliott SE, Mao R, Robinson WH. Affinity Maturation of the Anti-Citrullinated Protein Antibody Paratope Drives Epitope Spreading and Polyreactivity in Rheumatoid Arthritis. Arthritis Rheumatol. 2019;71(4):507-517. doi: 10.1002/art.40760

25. Bruni C., Frech T., Manetti M., Rossi FM , D.E., De Pauli A. Vascular Leaking, a Pivotal and Early Pathogenetic Event in Systemic Sclerosis: Should the Door Be Closed? Front Immunol 2018 Sep 7;9:204
26. Riccardi A, Marcoccia A, Borgia A, Guastafierro T, Bondanini F, Fasano S, et al Undifferentiated connective tissue disease at risk of systemic sclerosis: A weighted score to identify patients who will evolve Autoimmun Rev. 2019;18(9):102358
27. Smith V, Vanhaecke A, Herrick AL, Distler O, Guerra MG, Denton CP, et al ; EULAR Study Group on Microcirculation in Rheumatic Diseases. Fast track algorithm: How to differentiate a "scleroderma pattern" from a "non-scleroderma pattern". Autoimmun Rev. 2019;18(11):102394. doi: 10.1016/j.autrev.2019.102394

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Legend:

Figure 1. Patient selection and Distribution of VEDOSS criteria with relative interactions. A. Patient selection flow chart from the 1150 recorded in database export of October 4 2018 to the 553 patients analysed at baseline. **B-D UpSet plots of VEDOSS criteria and their interactions grouped by frequency at Baseline and during Follow Up.** Total sample size is 553 patients at baseline and 6067 observations during follow up. Each bar graph represents the frequency (% Raynaud's or observation as noted on Y axis), which is displayed as % number on top of each bar. Criteria present at the same time are shown as black dots connected by vertical lines in the table and their size of interaction is described in the horizontal bars. **B**. Baseline distribution and interaction in the 553 patients included in the analysis **C**. Follow up distribution of the criteria by event recorded. **D**. Bar graph summarising the change of interaction size between Baseline (B) and Follow up (C) . White

Bars represent same interaction prevalence as shown in A. Grey areas show the % increase of that interaction over Baseline, described by the number on each column.

Figure 2. Progression to fulfill ACR/EULAR 2013 classification of SSc by VEDOSS criteria at baseline. Only patients with 5 years follow up or progression to the endpoint were included (n=254). Each Pie Chart represents proportion of patients presenting at baseline observation with (Positive – Black) or without (Negative – White) Antinuclear Antibodies (ANA, **A) ,** SSc specific ANA (SSc Ab**, B);** abnormal Nailfold video capillaroscopy **(NVC, C)** and Puffy Fingers, **(PF, D)**. Bar graphs on each side represent proportion of progressors (red) and non-progressors (blue) within the given subgroup**.**

Figure 3. Proportion of patients with 5 years complete follow up or progressing to fulfill ACR/EULAR 2013 criteria (n=254) according to VEDOSS criteria at baseline. **A.** Upset plots showing proportion of progressor according to VEDOSS criteria and their interaction. Sample size numerosity is shown on bottom left graph. **B.** Frequency of progression as calculated on the presence (upper table) or absence (lower table) of VEDOSS criteria alone or in combination. Absence for combinations comprises none or just one of two criteria. Frequencies are colour-ranked according to low and high risk of progression. **C** Diagram summarizing the progressively higher or lower proportion of patients progressing to fulfill criteria within 5 years as per table in B.

Figure 4. Progression to fulfill ACR/EULAR 2013 classification of SSc by different single or combinations of VEDOSS criteria at baseline (A), comparing progression of SSc specific antibody negative patients with puffy fingers versus those with only single or none of these features (B), and comparing progression of patients with or without ANA positivity (C).

Supplementary figure 1: Progression to fulfill ACR/EULAR 2013 classification of SSc by different combinations of VEDOSS criteria at baseline, comparing progression of patients A) with versus without SSc specific antibody positivity, (B) with versus without puffy fingers, C) with or without abnormalities on NVC, D) with SSc specific antibodies and abnormalities on nailfold capillaroscopy versus those with only a single or none of these features, E) with ANA antibodies and puffy fingers versus those with only single or none of these features, and F) with ANA positivity and abnormalities on NVC versus those with only single or none of these three features.

RESEARCH IN CONTEXT

Evidence before this study: In the past 15 years there have been numerous single centre studies evaluating the relative risk of ANA or Nailfold capillaroscopic abnormalities for progression to Scleroderma. While the topic has generated quite some attention in the recent years, evidence has remained limited to retrospective analysis of single centre cohorts. The preliminary validation of the VEDOSS criteria has contributed to inform the 2013 ACR/EULAR classificaiton criteria for Systemic Sclerosis, but the relative risk of the criteria, single or in combination has never been analysed prospectively in patients with Raynaud's Phenomenon.

Added value of this study

Here we analyse the relative risk of progression to fulfill 2013 ACR/EULAR criteria for Systemic Sclerosis at 5 years and within the 5 years. This is the first multicentre study of this nature capturing real life progression within the context of a registry.

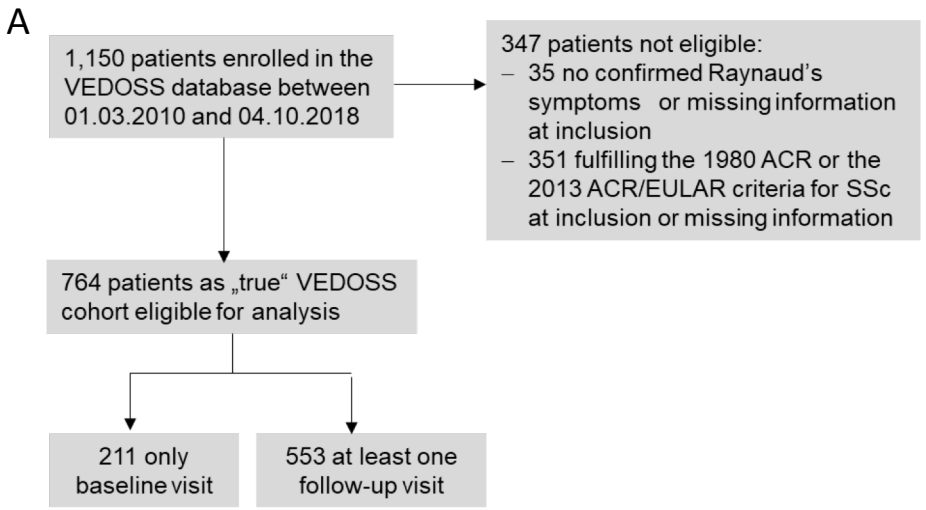
Implications of all the available evidence The confirmation in a multicentre prospective setting of the high negative predictive value for future Scleroderma of the absence of ANA is a very important factor in assessing risk and programming extensinve screening and clinical follow up in patients with Raynaud's. Further, the "riskmeter" offered within our results can be a valuable tool both for stratified medicine approaches and for translational studies aimed at understanding disease progression. Further, the longitudinal analysis offers insights in the stable vs. progressive nature of the criteria contributing to classificaiton of SSc. This latter may be useful in the identification of surrogate endpoints in prevention studies.

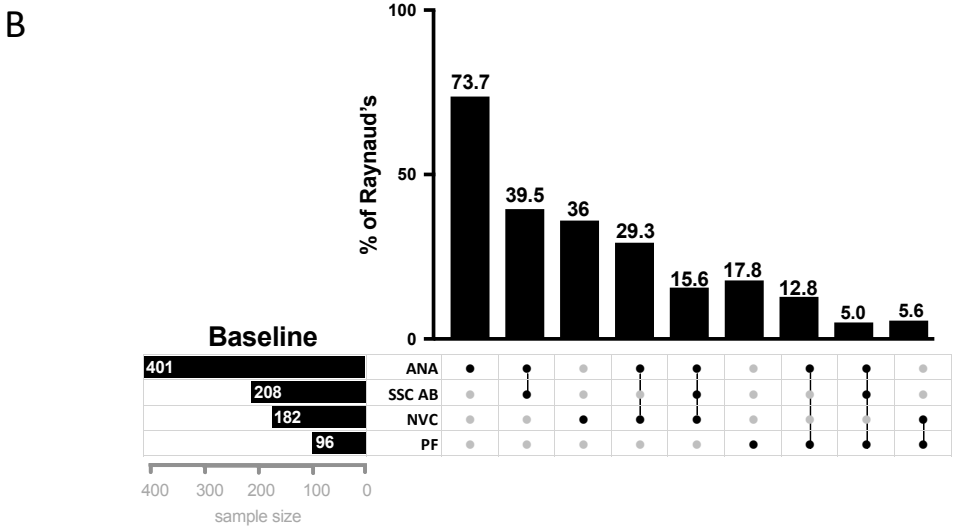
Contributors:

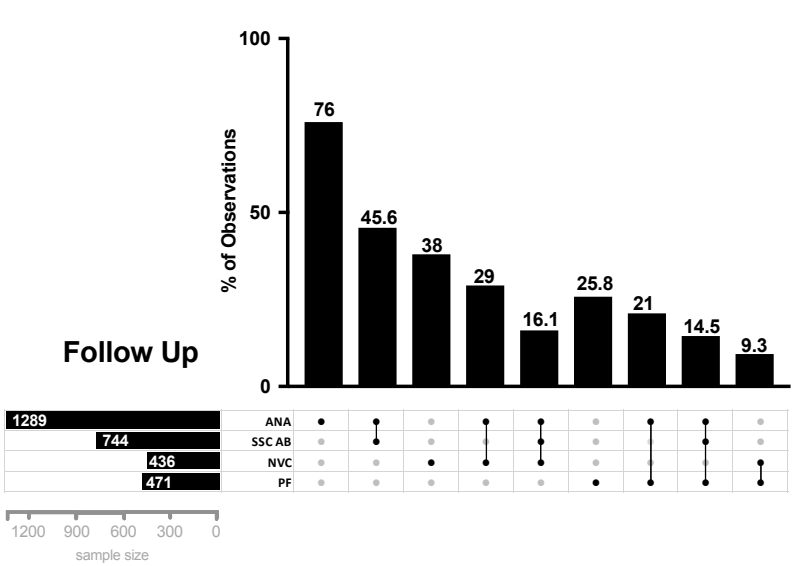
SBR and FDG conceived the analysis plan and drafted manuscript and figures; DH performed all statistical analysis and contributed to manuscript draft; GL,TM, LC,CM,SG,JA,MC,VS contributed to data interpretation and revised the manuscript; DEF, YA, OD, MMC conceived and contributed to all aspects of the study.

Data sharing statement

Data dictionary will be made available upon request. De-identified individual participant data could be made availble upon Clinical Project Request following WSF/EUSTAR standard operatic procedures. (https://eustar.org/wp-content/uploads/EUSTAR-APPLICATION-FILE.pdf)


A
1,150 patients enrolled in the VEDOSS database between 01.03.2010 and 04.10.2018
347 patients not eligible:
– 35 no confirmed Raynaud's symptoms or missing information at inclusion
– 351 fulfilling the 1980 ACR or the 2013 ACR/EULAR criteria for SSc at inclusion or missing information
764 patients as „true" VEDOSS cohort eligible for analysis
211 only baseline visit
553 at least one follow-up visit


B
% of Raynaud's
100
50
0
73.7
39.5
36
29.3
15.6
17.8
12.8
5.0
5.6
Baseline
401
208
182
96
ANA
SSC AB
NVC
PF
400 300 200 100 0
sample size


C
% of Observations
100
50
0
76
45.6
38
29
16.1
25.8
21
14.5
9.3
Follow Up
1289
744
436
471
ANA
SSC AB
NVC
PF
1200 900 600 300 0
sample size

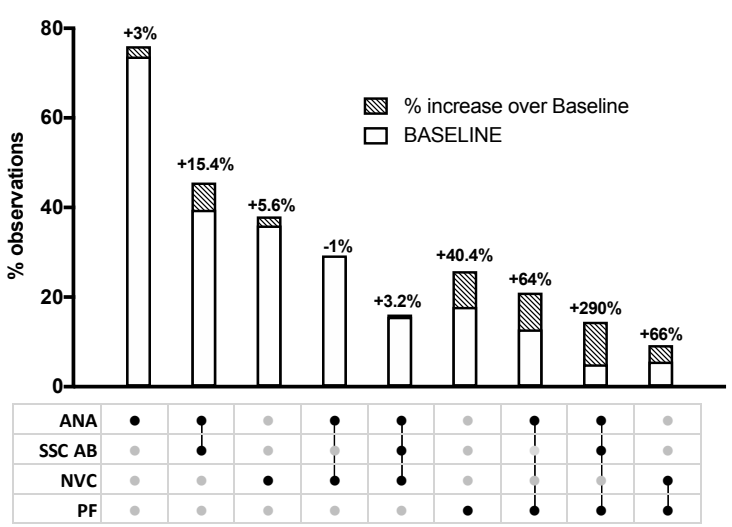

D
% observations
80
60
40
20
0
+3%
+15.4%
+5.6%
-1%
+3.2%
+40.4%
+64%
+290%
+66%
% increase over Baseline
BASELINE
ANA
SSC AB
NVC
PF

Figure 1

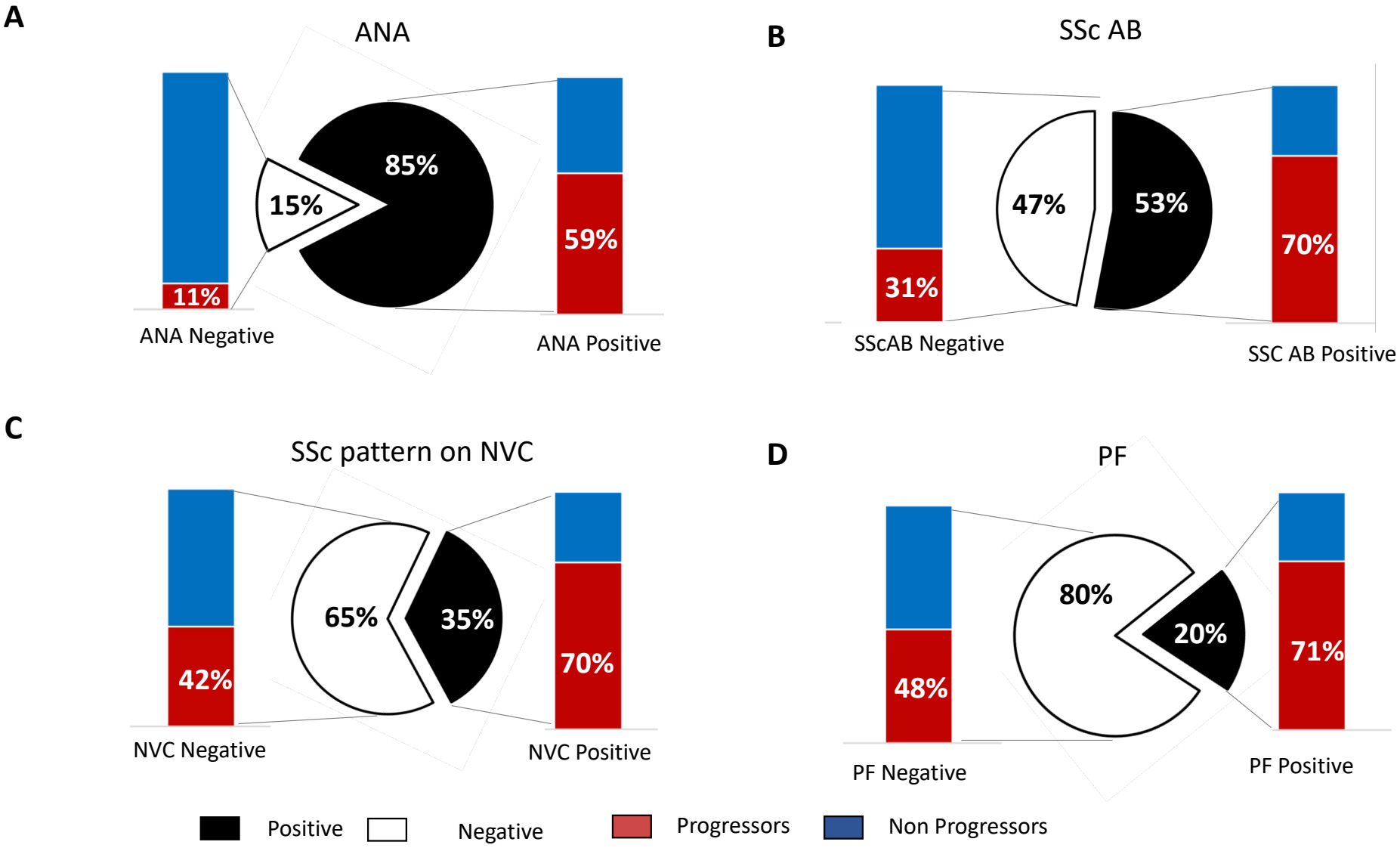
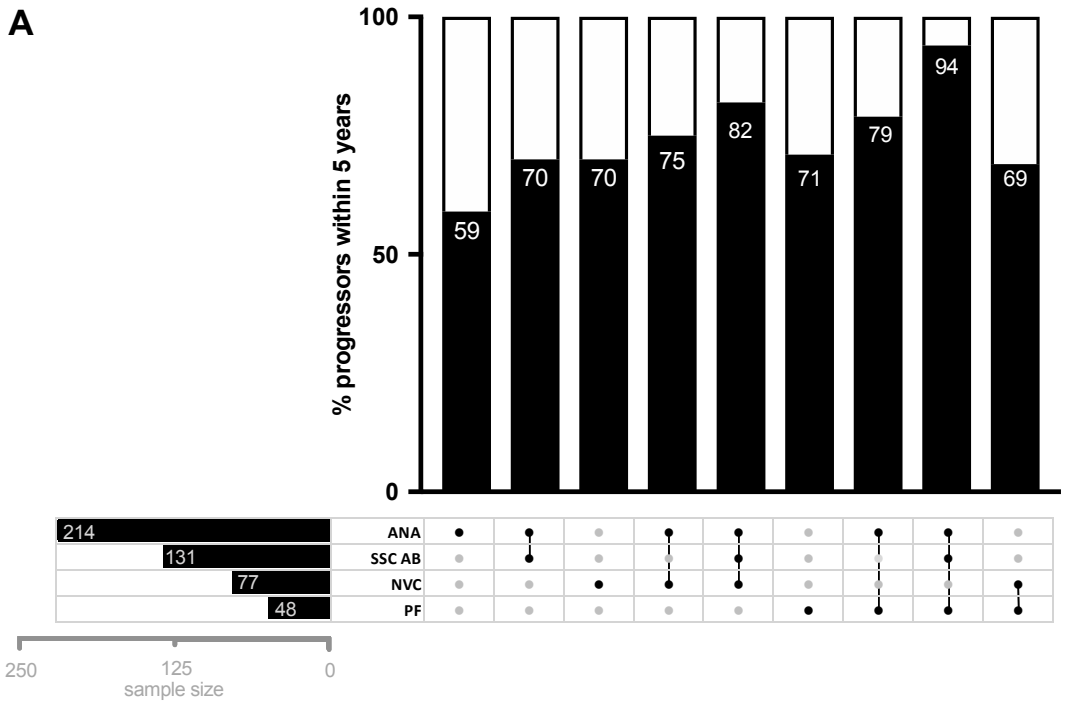

A
ANA
85%
15%
11%
59%
ANA Negative
ANA Positive
B
SSc AB
47%
53%
31%
70%
SScAB Negative
SSC AB Positive
C
SSc pattern on NVC
65%
35%
42%
70%
NVC Negative
NVC Positive
D
PF
80%
20%
48%
71%
PF Negative
PF Positive
Positive
Negative
Progressors
Non Progressors

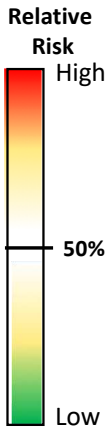
Figure 2

A



B

Proportion fulfilling 2013 criteria		ANA	Ssc Ab	SSc pattern on NVC	Puffy fingers
in the **presence** of	ANA	**58.9%**	70.2%	75.0%	79.0%
	SSc Ab	70.2%	**70.2%**	82.2%	94.1%
	SSc pattern on NVC	75.0%	82.2%	**70.1%**	69.2%
	Puffy fingers	79.0%	94.1	69.2%	**70.8%**
in the **absence** of	ANA	**10.8%**	31.0%	40.4%	47.5%
	SSc Ab	31.0%	**31.0%**	41.9%	49.6%
	SSc pattern on NVC	40.4%	41.9%	**41.5%**	50.9%
	Puffy fingers	47.5%	49.6%	50.9%	**47.9%**



C

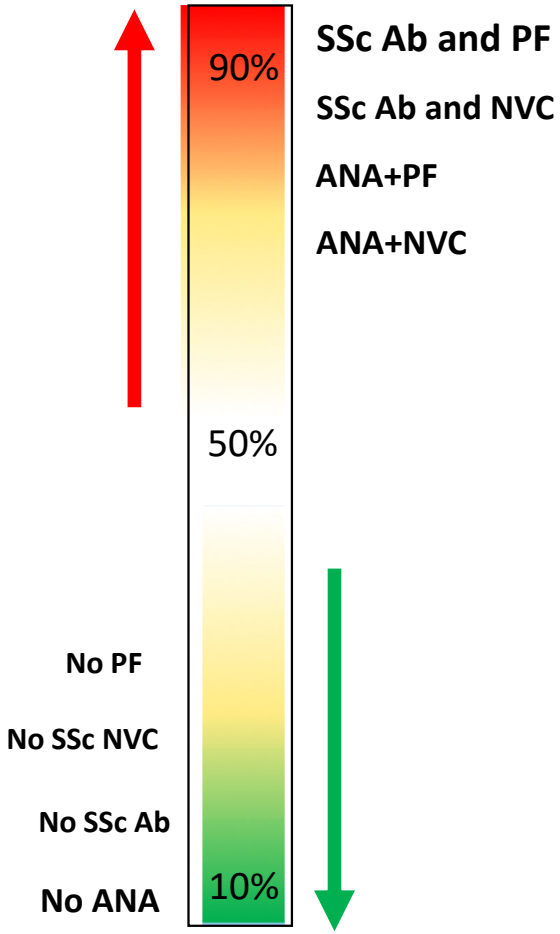


Figure 3

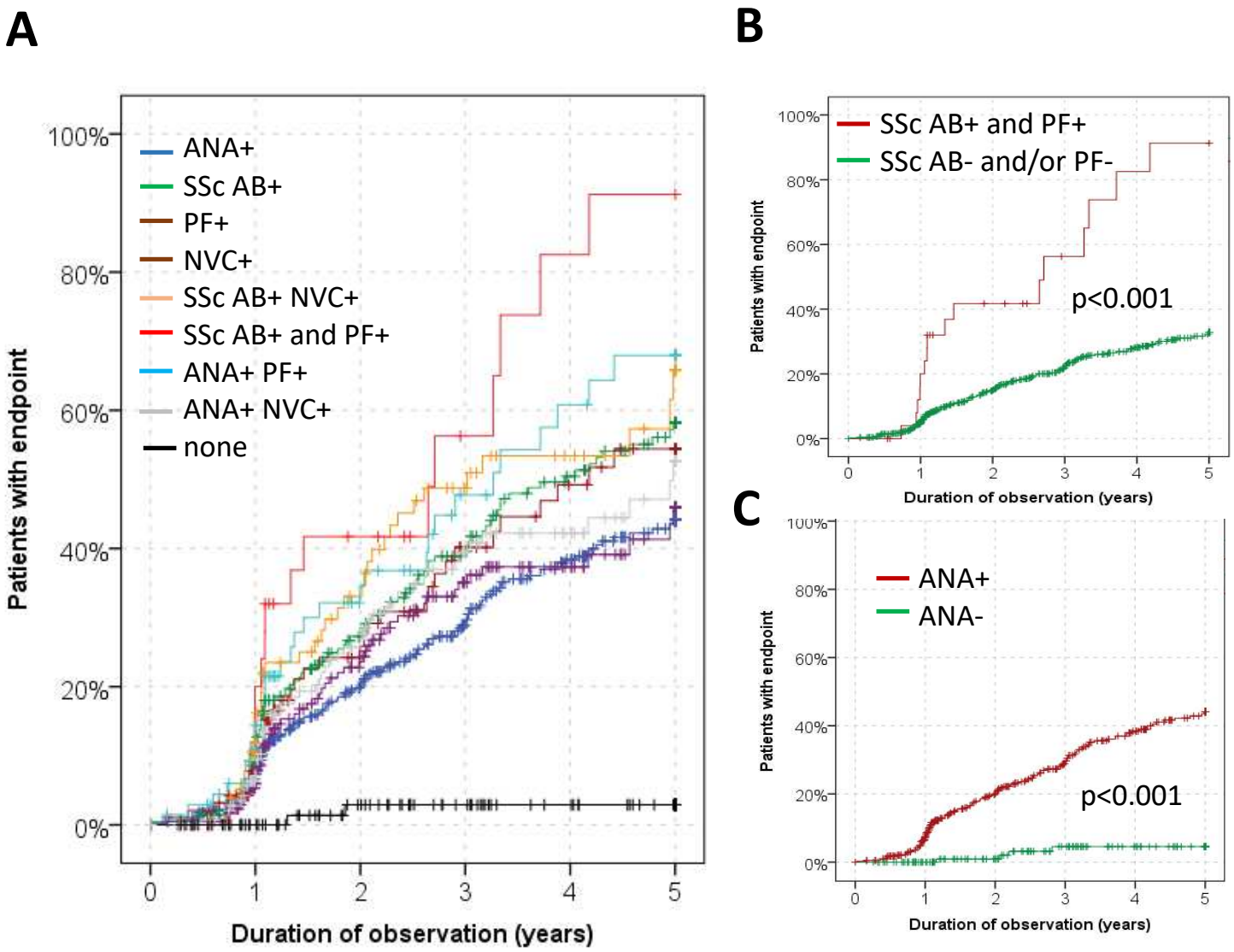

A
B
C
ANA+
SSc AB+
PF+
NVC+
SSc AB+ NVC+
SSc AB+ and PF+
ANA+ PF+
ANA+ NVC+
none
Patients with endpoint
Duration of observation (years)
SSc AB+ and PF+
SSc AB- and/or PF-
p<0.001
ANA+
ANA-
p<0.001

Figure 4