USING AUTOANTIBODIES AND CUTANEOUS SUBSET TO DEVELOP OUTCOME-BASED DISEASE CLASSIFICATION IN SYSTEMIC SCLEROSIS

Running head: Scleroderma antibodies and disease classification

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ABSTRACT

Objective

To describe the associations between autoantibodies, presentation and outcome among systemic sclerosis (SSc) patients. We propose a new SSc classification incorporating antibodies and cutaneous subset.

Methods

Survival analysis was used to assess the effect of antibodies on organ disease and death.

Results

The study included 1325 subjects. The ACA+ limited cutaneous (lc)SSc group (n=374) had the highest 20-year survival (65.3%), lowest incidence of clinically-significant pulmonary fibrosis (csPF, 8.5%) and scleroderma renal crisis (SRC, 0.3%), low cardiac SSc incidence (4.9%), while pulmonary hypertension (PH) frequency was similar to the cohort average.

The anti-Scl70+ lcSSc (n=138) and diffuse cutaneous (dc)SSc groups (n=149) had the highest csPF incidence (86.1% and 84% at 15 years). The dcSSc group had the lowest survival (32.4%) and the second highest incidence of cardiac SSc (12.9%) at 20 years, while in the lcSSc group other complications were rare, demonstrating the lowest incidence of PH (6.9%) and second highest survival (61.8%).

The anti-RNA polymerase+ group (n=147) had the highest incidence of SRC (28.1%). The anti-U3RNP+ group (n=56) had the highest PH (33.8%) and cardiac SSc incidence (13.2%).

Among lcSSc patients with other autoantibodies (n=295), risk of SRC and cardiac SSc was low, while other outcomes were similar to the cohort average. DcSSc patients with other

antibodies (n=166) had poor prognosis, with the second lowest survival (33.6%) and frequent organ complications.

Conclusion

We highlight the importance of autoantibodies, cutaneous subset and disease duration when assessing SSc morbidity and mortality. Our classification may benefit disease monitoring and clinical trial design.

Autoantibody testing has become an essential part of systemic sclerosis (SSc) patient assessment. The most commonly observed and strongly scleroderma-specific antibodies are the anti-centromere antibody (ACA), anti-topoisomerase I antibody (ATA, anti-Scl70) and anti-RNA polymerase antibody (ARA), which together are found in 50-80% of SSc patients (1, 2). While ATA positivity predicts development of pulmonary fibrosis (PF) and ARA predicts severe skin disease and scleroderma renal crisis (SRC), ACA reduces the risk of lung- and kidney-based organ disease (3). Much rarer, but still very disease specific are the anti-fibrillarin antibody (anti-U3RNP), anti-Th/To and anti-U11/U12RNP, while anti-PmScl and anti-Ku associate with scleroderma overlap syndromes (4-10).

The majority of studies that report associations between autoantibodies and organ disease are cross-sectional and provide sparse information on timing of organ complication development (11). In a previous publication we demonstrated time-dependent effect of ATA on the hazard of clinically significant PF (csPF) (12). Some studies have suggested that ARA+ patients tend to develop PF later in the disease course, in contrast to those with ATA antibodies, in whom PF is an early complication (13, 14). A major caveat for this type of analyses would be different disease duration at study entry, which could significantly bias the estimation of time to event (15).

Similarly, skin thickness and the change in modified Rodnan skin score (mRSS) over the disease course varies substantially (16, 17). While several studies describe mRSS change over time and its role in SSc risk stratification, those tend to use small number of skin assessments, often at fixed time points (17-19). Even when multiple mRSS assessments are analysed, the mathematical modelling approaches generally assume constant change in skin thickness, i.e. linear association between time and mRSS (20, 21). Since it is widely accepted

that mRSS trajectory is non-linear, timing of assessment is very likely to be an important predictor of both absolute mRSS and subsequent change (22). Autoantibodies associate strongly with mRSS changes and frequency of the SSc hallmark antibodies are very different in diffuse cutaneous (dc)SSc patients with high initial mRSS compared to those with milder skin disease (16, 23).

SSc is a rare disorder with substantial clinical and serological heterogeneity. Several authors have proposed classification schemes for the disease, based on varying extent of skin involvement (24, 25), although patients are still most commonly classified into diffuse or limited cutaneous (lc)SSc, as proposed by LeRoy and colleagues in 1988 (26). Subdividing SSc cases into more than two cutaneous subsets does not improve risk stratification (27). Conversely, autoantibodies are a very strong predictor of organ involvement and a combination of antibodies and skin subset could substantially refine risk stratification (17, 28, 29).

We have used a large well-characterized SSc cohort to describe the associations between autoantibodies and skin thickness changes over time, frequency and timing of organ complications and survival among SSc patients. We propose a simple classification of SSc, incorporating antibodies and disease subset.

PATIENTS AND METHODS

Cohort selection

All patients fulfilled the ACR/EULAR Classification Criteria for Systemic Sclerosis (30). We included patients with disease onset between 1st January 1995 and 31st December 2007 for all analyses of time to organ complications and death. As the definition of csPF included pulmonary function test (PFT) results, to avoid bias in the estimation of timing of this

complication we included only subjects who had at least one PFT within the first 3 years from disease onset. For the analysis of the changes in skin thickness score over time we focused on patients with dcSSc and at least one modified Rodnan skin score (mRSS) assessment. In order to increase the number of subjects, we did not set any restrictions on the time of disease onset for this group.

This project was conducted in compliance with the Declaration of Helsinki. Data used were obtained through two studies, involving routine data and sample collection from patients seen in our centre, which have been approved by the London-Hampstead and the London-Fulham Research Ethics Committees.

Disease characteristics and outcome definitions

Disease onset was defined as the time of first non-Raynaud's symptom of SSc as recalled by the patient or observed by a physician. Skin thickness was assessed using mRSS (range 0-51). Cutaneous subset was defined as limited when skin thickening did not extend proximally to elbows and knees and as diffuse otherwise (26). Patients were recorded as having pulmonary fibrosis (PF) if this was confirmed on HRCT. PF was considered clinically significant (csPF) if one of the following criteria were fulfilled: 1) forced vital capacity (FVC)<70% predicted; 2) FVC≤80% and a documented absolute decline in FVC of ≥15%; 3) diffusing capacity for carbon monoxide (DLCO)<70% with no history of PH or development of PH in the following 3 years; 4) DLCO≤80% and a documented decline in DLCO of ≥15% with no history of PH or development of PH in the following 3 years. Pulmonary hypertension (PH) was defined as mean pulmonary artery pressure ≥25 mmHg at rest with pulmonary artery wedge pressure ≤15 mmHg on right heart catheter. This included group 1 (connective tissue disease-associated pulmonary arterial hypertension, PAH) and group 3

(interstitial lung disease-associated PH). Cardiac scleroderma was defined as haemodynamically significant arrhythmias, pericardial effusion, or congestive heart failure (left ventricular ejection fraction below 50%), requiring specific treatment in the absence of other known cardiac causes. Scleroderma renal crisis (SRC) was defined as a new-onset systemic hypertension >150/85mmHg and a documented decrease in eGFR>30%, or confirmed SRC features on renal biopsy. Details on autoantibody assessment are available in the supplement.

Autoantibody associations

For the analysis of associations between antibodies and morbidity/mortality, we focused on SSc-specific antibodies (ACA, ATA, ARA, anti-U3RNP, anti-PmScl). Where a patient was positive for more than one antibody, they were included in the group of the antibody that was SSc-specific. ANA+ENA- patients formed a separate group. Patients who carried all other defined antibodies (anti-U1RNP, Th/To, SL, Ku, Jo1, Ro, La, XR, PL7, hnRNP and Sm) as well as ANA negative ones were included in the “Other” group.

Classification development

For the development of SSc classification, subjects were divided into 14 initial subgroups by antibodies (ACA, ATA, ARA, anti-U3RNP, anti-PmScl, anti-nuclear antibody (ANA) positive, but extractable nuclear antigen (ENA) negative and other) and skin subset (diffuse and limited). The endpoints of interest were survival and cumulative incidence of organ complications at 5, 10, 15 and 20 years from disease onset and those were calculated for each subgroup. Within each endpoint, subgroups were ranked in terms of survival/cumulative incidence of organ disease estimates and subgroups, which showed similar ranking in multiple endpoints, were merged.

Statistical analysis

Survival was analysed using Kaplan-Meier (KM) estimation, while organ complication incidence was calculated using both 1-KM and the cumulative incidence function (CIF), accounting for competing risks. The KM method works under the assumption of non-informative censoring, which does not hold in the cases where death occurs before an organ complication has developed and may result in overestimation (31). For that reason, 1-KM and CIF, accounting for death as a competing risk, were compared. Discreet time hazard rates were calculated within intervals of 12 months over the follow-up in order to assess the timing of highest rates of death and organ complication development. The effect of antibody specificities on the hazards was assessed using Cox proportional hazard regression analysis. Proportionality of hazards assumption was tested using plots and through incorporating time-varying effects in the models. Linear mixed effects models were used to assess associations between autoantibody specificities and changes in mRSS over time.

RESULTS

Cohort description

Of the 1354 SSc patients that fitted the inclusion criteria, 29 did not have information on antibody specificity and were excluded from the analysis. Demographic and clinical characteristics of the remaining 1325 subjects are summarised in Table 1. For 10 (0.8%) subjects there was missing information on presence of PF. In 115 (8.7%) patients we found multiple autoantibody specificities and 9 had dual SSc hallmark antibodies. Three were ACA+ and ATA+, two ACA+ and anti-U3RNP+ and one ACA+ and anti-PmScl+ and those were classified into the respective non-ACA groups, as in all cases ACA was not detected on first serology testing. Two subjects had switched antibody from ATA to ARA at a later stage of

their disease, so those were classified as ATA+. One patient was ARA+ and anti-PmScl+, but the anti-PmScl positivity was very weak, therefore the patient was classified as ARA+.

Autoantibodies and survival

Survival for the cohort at 5, 10, 15 and 20 years from onset was 91.8%, 82.2%, 67.5% and 53.8%, with much lower survival among dcSSc patients (84.4%, 72%, 53.9% and 39.7%) compared to lcSSc ones (95.8%, 87.7%, 74.6% and 61%), $p<0.001$. The subjects who carried ACA had the highest survival, while the group that was ANA+ENA- had the lowest (Table 2, Figure 1A). The hazard for death appeared to gradually increase over time for the majority of antibody groups, although among anti-U3RNP+ patients this went down over time (Figure 1B). For that reason, we fitted an extended Cox model, allowing for time-varying effect of antibodies (Table 3). This showed that even though anti-U3RNP patients had higher hazard of death in the earlier years of disease compared to other antibody groups, long-term survival was better, and hazard of death was lower in the later years of disease. On the other hand, anti-PmScl+ subjects appeared to be at a very low risk of death in the first 10 years of disease, while this increased significantly and became higher than in other antibody groups in the second decade of the disease.

Autoantibodies and cumulative incidence and timing of organ complications

Clinically-significant pulmonary fibrosis

A subgroup of 654 patients, who had available PFT results within the first 3 years from disease onset, was included in the csPF analysis (Table 1). Of those, 308 (47.1%) developed csPF, the majority within the first 5 years (1-KM estimate 44.8%) with much lower incidence rate thereafter (48.3% and 50.3% at year 10 and 15 respectively, and no additional cases after 15 years). Cutaneous subset was strongly associated with csPF development, with 5,

10 and 15 year cumulative incidence for csPF of 37.4%, 39.3% and 41.2% in lcSSc patients compared to 52.5%, 58.0% and 60.1% among dcSSc patients ($p<0.001$).

Analysis within autoantibody subgroups confirmed the very low risk of csPF among ACA+ subjects and the remarkably high risk among ATA+ patients, where csPF ultimately occurred in most cases (Table 2). Rates of csPF development among ARA+ patients were higher than those in ACA+ patients, but still much lower than ATA+ ones and even after 20 years of follow-up, the cumulative incidence of csPF among them was about half of that among ATA+ patients (Table 2, Figure 1C). Comparison between 1-KM estimates and CIF, accounting for death as a competing risk, revealed some small differences, with 1-KM overestimating the incidence of csPF by approximately 2% in the later stages of the disease among ATA+ and ARA+ patients and by 3% among anti-U3RNP+ and ANA+ENA- subjects (Table 2).

The hazard of csPF development for the overall cohort peaked in the second year from disease onset and this observation was replicated in the subgroup analysis by antibodies (Figure 1D). Among ATA+ patients hazard of csPF was 30% in year 1, 45.7% in year 2, peaked at 57.4% in year 3 and went down sharply thereafter. Although much lower among ACA+ patients, the hazard of csPF was highest in the second year from disease onset (1.5%, 5.5% and 0.8% in years 1, 2 and 3). The hazard for csPF in ARA+ patients also peaked in year 2 (12.3%, 13.1% and 5% at year 1, 2 and 3 respectively) and declined thereafter. For the remaining antibody subgroups, the hazard of csPF development at 1, 2 and 3 years from SSc onset was 3%, 10% and 3.9% for U3RNP; 11.8, 34.2 and 6.3 for PmScl; 17.1%, 26% and 25.9% for ANA+ENA- patients; and 15%, 27.7% and 17.8% for the combined group of other antibodies. Cox regression confirmed that compared to ATA, other antibodies lowered the hazard of csPF and presence of ACA was associated with the greatest reduction (Table 3).

Pulmonary hypertension

Cumulative incidence of PH at 5, 10, 15 and 20 years from onset was 4%, 9.2%, 16.2% and 22.6% for the cohort. Incidence of PH in the two cutaneous subsets was nearly identical (4.1%, 9.5%, 16.5% and 22.7 for lcSSc and 3.7%, 8.5%, 15.6% and 22.3% for dcSSc patients at 5, 10, 15 and 20 years from onset, p=0.981).

Autoantibody specificity associated strongly with PH risk and the highest incidence was observed among anti-U3RNP+ patients, while there was very little difference among patients with other antibodies (Table 2, Figure 1E). Death as a competing risk had an effect on the estimates of PH incidence in most antibody subgroups, with the greatest overestimation seen among anti-U3RNP+ and ANA+ENA- subjects, where 1-KM overestimated this by approximately 5% at 15 and 20 years from onset, compared to CIF. Hazard of PH was very low in the first years from onset and for most patients it varied between 1 and 2% per year from year 3 onwards with some gradual increase for the later stages of disease, generally after 10 years (Figure 1F). ATA+ and anti-PmScl+ patients had the lowest hazard of PH development, while hazard among ACA+ patients was similar to the average for the cohort (Table 3). Equivalent results were obtained when reanalysing the data including only PAH (Group 1 PH) as an endpoint (Supplement).

Cardiac scleroderma

Cardiac involvement was a rare complication, affecting less than 5% of the cohort with 1-KM estimates of cumulative incidence at 5, 10, 15 and 20 years of 2.9%, 4.1%, 5.4% and 6.8%. DcSSc patients had significantly higher incidence of cardiac SSc (1-KM 6.2%, 8.6%, 10% and 10% at 5, 10, 15 and 20 years) compared to 1.2%, 1.8%, 3.1% and 4.9% among lcSSc patients ($p<0.001$). Autoantibodies associated significantly with cardiac SSc development and the

two with strongest positive association were anti-U3RNP and ATA (Table 2). Comparison between 1-KM and CIF estimates of cardiac SSc development did not reveal any substantial differences (Table 2). Except for ATA, all other antibodies were associated with significantly reduced hazard of cardiac SSc development compared to U3RNP+ patients (Table 3, Figure S2). There was no clear association between disease duration and cardiac SSc development and hazards fluctuated over time with cardiac complications developing both in early and late disease.

Scleroderma renal crisis

Over 90% of the subjects with SRC developed this within 5 years from onset (1-KM estimates at 5, 10 and 15 years- 6.5%, 7.1% and 7.6% respectively, with no cases after 14 years) and 9/94 had SRC at presentation. SRC was much more common among dcSSc patients (1-KM estimates at 5, 10 and 15 years were 14.1%, 15.5% and 17.4%) compared to lcSSc patients (2.4% and 2.5% at 5 and 10 years, with no SRC cases after year 7, $p<0.001$).

Autoantibodies demonstrated significant associations with SRC with the highest hazard seen in ARA+ and the lowest in ACA+ subjects (Table 3, Figure S3). As this was a very early complication, cumulative incidence estimation was not affected by death as a competing risk (Table 2). For all antibody groups hazards were highest in the first year of disease, except for the anti-PmScl group, where the majority of cases developed in years 5 and 6.

Autoantibodies and skin changes over time

The cohort included in the analysis of skin score changes over time consisted of 581 dcSSc subjects (Table 1). Three or more mRSS assessments were available for 413 of the subjects (71.1%), two for 88 (15.2%) and one for 80 (13.8%). First mRSS assessment was made within 3 years from disease onset for 383 (65.9%) of the patients.

The average mRSS at 12 months from disease onset was 24.2 (SD 9.2; 95%CI 23.3, 25.2) and this gradually declined following a non-linear trajectory (mRSS=24.2-2.5*years+0.13*years^2-0.002*years^3; p<0.001 for all parameters). Thus, skin improvement was greater in earlier disease with average drop in mRSS of 2.3 between years 1 and 2, 2.1 between years 2 and 3, 1.9 between years 3 and 4, 1.6 between years 4 and 5, and 0.8 between years 9 and 10 (Figure 2A, Table S6). There was a moderately strong, negative association between mRSS at 1 year and drop in mRSS over time (correlation coefficient=-0.6), suggesting that higher initial skin scores are associated with greater subsequent improvement.

Autoantibodies showed significant association with both baseline mRSS and changes in mRSS over time. At 1 year from disease onset, highest average mRSS was observed in ANA+ENA- patients (25.5) and ARA+ patients (25). Compared to ANA+ENA- ones, mRSS in ATA+ patients was 24.3, p=0.421; in anti-U3RNP+ this was 21, p=0.041, anti-PmScl+ 19.6, p=0.022 and other antibodies 23.3, p=0.216. Over subsequent years, the greatest improvement was observed in ANA+ENA- and ARA+ patients with respective average drop in mRSS of 9.4 and 9.1 units between year 1 and 5. Drop was 6.4 in ATA+ (p=0.004 for ATA*time interaction, compared to ANA+ENA-*time), 6 in anti-U3RNP+ (p=0.029), 6.9 in PmScl+ (p=0.206) and 7.9 in patients with other antibodies (p=0.265), following a non-linear trajectory with greater reduction in earlier years (Figure 2B, Table S8).

Proposed classification of systemic sclerosis, using subset and autoantibodies

Table S10 presents 1-KM estimates of endpoint incidence for the original 14 groups. Merging all subgroups that had similar rankings within different endpoints resulted in 7 final classification groups. Five of those included patients with SSc-specific antibodies, while the

last two had all remaining patients with other antibodies, including ANA+ENA- and ANA- subjects (Table 4, Figure 2C-F).

ACA+ lcSSc group

This group was the largest (n=374, 28.2% of the cohort), with the highest survival, lowest incidence of csPF and SRC, very low incidence of cardiac SSc and incidence of PH similar to the cohort average.

ATA+ lcSSc group

This group consisted of 138 subjects (10.4% of the cohort). Although incidence of csPF among those patients was extremely high, other complications were rare and they had the lowest incidence of PH and second highest survival of all 7 groups.

ATA+ dcSSc group

The subjects from this group (n=149, 11.3% of the cohort) had the worst prognosis with the lowest survival and the second highest incidence of cardiac SSc of all groups. The incidence of csPF was almost identical to that among ATA+ lcSSc patients.

ARA+ group

As expected, this group (n=147, 11.1% of the cohort) had the highest incidence of SRC. On the other hand, it had the lowest incidence of cardiac scleroderma, while other organ complications and survival were similar to the cohort average.

U3RNP+ group

Only 4.2% of the cohort (n=56) was included in this group. Although long-term survival among those subjects was higher than the cohort average, they had the highest PH and cardiac SSc incidence.

Other antibodies lcSSc group

This group (n=295, 22.3%) had a low overall risk of SRC and cardiac SSc, while other outcomes were similar to the cohort average.

Other antibodies dcSSc group

Conversely, this group (n=166, 12.5%) had poor prognosis, with the second lowest survival and above average rates of csPF, cardiac scleroderma and SRC.

DISCUSSION

We describe a large single-centre SSc cohort, focusing on the effect of autoantibodies on timing of organ complication development and disease prognosis. We confirm that double SSc-specific autoantibody positivity is extremely rare (<1%) and highlight the importance of careful and accurate antibody analysis to inform patient monitoring and prognosis.

Although it is often suggested that ACA positivity strongly predicts development of PH, there is in fact very little evidence in the literature to support this and studies are either based on enriched cohorts or do not use robust definitions for PH (6, 32-34). We found no evidence for association between ACA and PH. Incidence of PH in the ACA+ group was similar to the cohort average. We confirm the strong association between ACA and low incidence rates of major organ-based complications and mortality, suggesting that ACA positivity in SSc patients is a good prognostic sign.

Similarly good outcome was observed in anti-PmScl positive subjects, with overall low PH, SRC and cardiac SSc incidence, although approximately half of those patients did develop csPF within the first 15 years of disease (35). Mortality rates, although low in the first 10

years of disease, appeared to increase faster than among other antibody groups in the second decade, possibly related to csPF progression or development of malignancies (36).

As expected, ATA positivity was associated with a substantial risk of csPF, with no difference in incidence between cutaneous subsets. Despite that, long-term prognosis was strikingly different between lcSSc and dcSSc patients with this antibody, with much better survival and low risk of other organ complications among lcSSc patients (29).

Anti-U3RNP+ patients had the highest incidence of both PH and cardiac scleroderma, in line with previously published studies (4, 5). They also had very high mortality rates in the early stages of the disease, although long-term survival was among the highest, suggesting that patients with this antibody are at much higher risk in the first 10 years of disease.

Our analysis clearly demonstrated that there is no difference in the timing of csPF development between patients with different antibodies. In all groups hazards peaked within the first 3 years and rapidly declined thereafter. Hazards for SRC peaked even earlier, within the first year of disease. Conversely, hazard of PH was very low early on and gradually increased, especially in the second decade. For all antibody subgroups, except anti-U3RNP, the hazard of death was low initially and gradually increased over time. In anti-U3RNP+ patients this showed an early peak suggesting that patients with this antibody would require more active management early in the disease course.

Skin involvement is an important aspect of SSc morbidity and a large proportion of clinical trials have utilised mRSS as a primary endpoint. Spontaneous improvement in mRSS occurs in the majority of dcSSc patients and we demonstrate that at a group level, mRSS declines over time. The greatest improvement is early in the disease, while during later stages there

is little change. Similar to previous studies, we found negative association between change in mRSS and both baseline mRSS and disease duration (21). We also confirm that skin change is significantly associated with autoantibodies, with higher skin scores in early disease observed in ARA+ and ANA+ENA- patients, while at the same time those groups experienced greater improvement and had lower average mRSS compared to other antibodies in the later stages of disease. Antibody specificities only partly explained the changes in mRSS with considerable residual variance even after accounting for antibodies and their interaction with time (Table S8).

Our study has several important limitations. There was a relatively small number of patients that had mRSS assessment within the first 12 months of disease, which could explain why an initial increase in mRSS was not observed for the overall cohort and indeed only a very small number of patients had deterioration in mRSS. Nevertheless, sensitivity analysis demonstrated that the results held when excluding subjects with first mRSS done over 3 years after onset (Supplement).

We could not use HRCT scans to assess severity of PF as the imaging for a number of patients was performed prior to the introduction of electronic imaging storage or was done in another hospital. However, since severity of PF was based on PFTs, even when HRCT had not been done, absence of csPF could be reasonably assumed, based on preserved and stable lung function. When HRCT information was not available to confirm presence of PF and the PFT results showed abnormalities, we considered this missing data. As a result, it is likely that we underestimate the overall presence of PF (any degree) among the study subjects, but the estimate of csPF incidence should be comparatively accurate.

To avoid immortal time bias, we included an incident cohort with disease onset during a fixed time window. In addition, the tendency for severe cases to be referred early means that it is unlikely for our cohort to be biased towards milder cases, who survive for long enough to be seen in a specialist centre.

Some of the autoantibodies (anti-U3RNP, anti-Th/To) were defined, based on IIF with no confirmatory test and SSc-specific antibody testing may not be available in some hospitals, where physicians may only receive a result reporting nucleolar pattern on IIF. For that reason we repeated the analysis of associations between autoantibodies and endpoints, using sub-classification of ACA, ATA, ARA, ANA nucleolar pattern and Other (Supplement).

By combining autoantibody specificity and extent of skin involvement, we propose a simple classification for SSc patients into 7 groups. This enables more precise risk stratification of patients, compared to the simple division into dcSSc and lcSSc, and reflects widespread opinion that the current subset classification fails to take account of the variability of organ-based complications (37). Testing for the more common and SSc-specific antibodies is available to most rheumatologists and cutaneous subset is easy to determine, which makes this classification easy to apply in everyday clinical practice. Patients could be classified at their initial visit and would remain in the same group, even if other characteristics of the disease subsequently change. Once validated in other cohorts, this classification could be used to inform prognosis and disease monitoring in routine practice and for cohort enrichment in event-driven clinical trials.

REFERENCES

1. Kuwana M. Circulating Anti-Nuclear Antibodies in Systemic Sclerosis: Utility in Diagnosis and Disease Subsetting. Journal of Nippon Medical School = Nippon Ika Daigaku zasshi. 2017;84(2):56-63.
2. Mecoli CA, Casciola-Rosen L. An update on autoantibodies in scleroderma. Current opinion in rheumatology. 2018;30(6):548-53.
3. Nihtyanova SI, Denton CP. Autoantibodies as predictive tools in systemic sclerosis. Nature reviews Rheumatology. 2010;6(2):112-6.
4. Tormey VJ, Bunn CC, Denton CP, Black CM. Anti-fibrillarin antibodies in systemic sclerosis. Rheumatology (Oxford). 2001;40(10):1157-62.
5. Aggarwal R, Lucas M, Fertig N, Oddis CV, Medsger TA, Jr. Anti-U3 RNP autoantibodies in systemic sclerosis. Arthritis Rheum. 2009;60(4):1112-8.
6. Mitri GM, Lucas M, Fertig N, Steen VD, Medsger TA, Jr. A comparison between anti-Th/To- and anticentromere antibody-positive systemic sclerosis patients with limited cutaneous involvement. Arthritis Rheum. 2003;48(1):203-9.
7. Fertig N, Domsic RT, Rodriguez-Reyna T, Kuwana M, Lucas M, Medsger TA, Jr., et al. Anti-U11/U12 RNP antibodies in systemic sclerosis: a new serologic marker associated with pulmonary fibrosis. Arthritis Rheum. 2009;61(7):958-65.
8. McMahan ZH, Domsic RT, Zhu L, Medsger TA, Casciola-Rosen L, Shah AA. Anti-RNPC3 (U11/U12) antibodies in systemic sclerosis are associated with moderate to severe gastrointestinal dysmotility. Arthritis care & research. 2018.
9. D'Aoust J, Hudson M, Tatibouet S, Wick J, Mahler M, Baron M, et al. Clinical and serologic correlates of anti-PM/Scl antibodies in systemic sclerosis: a multicenter study of 763 patients. Arthritis & rheumatology (Hoboken, NJ). 2014;66(6):1608-15.
10. Hoa S, Hudson M, Troyanov Y, Proudman S, Walker J, Stevens W, et al. Single-specificity anti-Ku antibodies in an international cohort of 2140 systemic sclerosis subjects: clinical associations. Medicine. 2016;95(35):e4713.
11. Steen VD. Autoantibodies in systemic sclerosis. Seminars in arthritis and rheumatism. 2005;35(1):35-42.
12. Nihtyanova SI, Schreiber BE, Ong VH, Rosenberg D, Moinzadeh P, Coghlan JG, et al. Prediction of pulmonary complications and long-term survival in systemic sclerosis. Arthritis & rheumatology (Hoboken, NJ). 2014;66(6):1625-35.
13. Foocharoen C, Suwannachat P, Netwijitpan S, Mahakkanukrauh A, Suwannaroj S, Nanagara R. Clinical differences between Thai systemic sclerosis patients with positive versus negative anti-topoisomerase I. International journal of rheumatic diseases. 2016;19(3):312-20.
14. Hoffmann-Vold AM, Midtvedt O, Tennoe AH, Garen T, Lund MB, Aalokken TM, et al. Cardiopulmonary Disease Development in Anti-RNA Polymerase III-positive Systemic Sclerosis: Comparative Analyses from an Unselected, Prospective Patient Cohort. J Rheumatol. 2017;44(4):459-65.
15. Nihtyanova SI, Denton CP. Scleroderma Lung Involvement, Autoantibodies, and Outcome Prediction: The Confounding Effect of Time. J Rheumatol. 2017;44(4):404-6.
16. Shand L, Lunt M, Nihtyanova S, Hoseini M, Silman A, Black CM, et al. Relationship between change in skin score and disease outcome in diffuse cutaneous systemic sclerosis: application of a latent linear trajectory model. Arthritis Rheum. 2007;56(7):2422-31.

17. Perera A, Fertig N, Lucas M, Rodriguez-Reyna TS, Hu P, Steen VD, et al. Clinical subsets, skin thickness progression rate, and serum antibody levels in systemic sclerosis patients with anti-topoisomerase I antibody. Arthritis Rheum. 2007;56(8):2740-6.
18. Maurer B, Graf N, Michel BA, Muller-Ladner U, Czirjak L, Denton CP, et al. Prediction of worsening of skin fibrosis in patients with diffuse cutaneous systemic sclerosis using the EUSTAR database. Ann Rheum Dis. 2015;74(6):1124-31.
19. Dobrota R, Maurer B, Graf N, Jordan S, Mihai C, Kowal-Bielecka O, et al. Prediction of improvement in skin fibrosis in diffuse cutaneous systemic sclerosis: a EUSTAR analysis. Ann Rheum Dis. 2016;75(10):1743-8.
20. Amjadi S, Maranian P, Furst DE, Clements PJ, Wong WK, Postlethwaite AE, et al. Course of the modified Rodnan skin thickness score in systemic sclerosis clinical trials: analysis of three large multicenter, double-blind, randomized controlled trials. Arthritis Rheum. 2009;60(8):2490-8.
21. Merkel PA, Silliman NP, Clements PJ, Denton CP, Furst DE, Mayes MD, et al. Patterns and predictors of change in outcome measures in clinical trials in scleroderma: an individual patient meta-analysis of 629 subjects with diffuse cutaneous systemic sclerosis. Arthritis Rheum. 2012;64(10):3420-9.
22. Medsger TA, Jr. Natural history of systemic sclerosis and the assessment of disease activity, severity, functional status, and psychologic well-being. Rheumatic diseases clinics of North America. 2003;29(2):255-73, vi.
23. Herrick AL, Pan X, Peytrignet S, Lunt M, Hesselstrand R, Mouthon L, et al. Treatment outcome in early diffuse cutaneous systemic sclerosis: the European Scleroderma Observational Study (ESOS). Ann Rheum Dis. 2017;76(7):1207-18.
24. Maricq HR, Valter I. A working classification of scleroderma spectrum disorders: a proposal and the results of testing on a sample of patients. Clinical and experimental rheumatology. 2004;22(3 Suppl 33):S5-13.
25. Giordano M, Valentini G, Migliaresi S, Picillo U, Vatti M. Different antibody patterns and different prognoses in patients with scleroderma with various extent of skin sclerosis. J Rheumatol. 1986;13(5):911-6.
26. 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(2):202-5.
27. Cottrell TR, Wise RA, Wigley FM, Boin F. The degree of skin involvement identifies distinct lung disease outcomes and survival in systemic sclerosis. Ann Rheum Dis. 2014;73(6):1060-6.
28. Srivastava N, Hudson M, Tatibouet S, Wang M, Baron M, Fritzler MJ. Thinking outside the box--The associations with cutaneous involvement and autoantibody status in systemic sclerosis are not always what we expect. Seminars in arthritis and rheumatism. 2015;45(2):184-9.
29. Boonstra M, Mertens BJA, Bakker JA, Ninaber MK, Ajmone Marsan N, van der Helm-van Mil AHM, et al. To what extent do autoantibodies help to identify high-risk patients in systemic sclerosis? Clinical and experimental rheumatology. 2018;36 Suppl 113(4):109-17.
30. 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. Arthritis Rheum. 2013;65(11):2737-47.
31. Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. Stat Med. 2007;26(11):2389-430.

32. Coghlan JG, Denton CP, Grunig E, Bonderman D, Distler O, Khanna D, et al. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. Ann Rheum Dis. 2014;73(7):1340-9.
33. Walker UA, Tyndall A, Czirjak L, Denton C, Farge-Bancel D, Kowal-Bielecka O, et al. Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials And Research group database. Ann Rheum Dis. 2007;66(6):754-63.
34. Hudson M, Mahler M, Pope J, You D, Tatibouet S, Steele R, et al. Clinical correlates of CENP-A and CENP-B antibodies in a large cohort of patients with systemic sclerosis. J Rheumatol. 2012;39(4):787-94.
35. Hanke K, Bruckner CS, Dahnrich C, Huscher D, Komorowski L, Meyer W, et al. Antibodies against PM/Scl-75 and PM/Scl-100 are independent markers for different subsets of systemic sclerosis patients. Arthritis research & therapy. 2009;11(1):R22.
36. Bruni C, Matucci Cerinic M, Lages A, Patel H, Harvey J, Nihtyanova SI, et al. Resolution of paraneoplastic PM/Scl-positive systemic sclerosis after curative resection of a pancreatic tumour. Rheumatology. 2016;56(2):317-8.
37. Johnson SR, Soowamber ML, Fransen J, Khanna D, Van Den Hoogen F, Baron M, et al. There is a need for new systemic sclerosis subset criteria. A content analytic approach. Scandinavian journal of rheumatology. 2018;47(1):62-70.

FIGURE LEGENDS

Figure 1. Associations between autoantibodies and organ complications in systemic sclerosis patients; (A) Kaplan-Meier estimates of survival in subgroups by antibodies; (B) Smoothed hazard of death over time in subgroups by antibodies; (C) 1-Kaplan-Meier estimates of cumulative incidence of clinically-significant pulmonary fibrosis in subgroups by antibodies; (D) Smoothed hazard of clinically-significant pulmonary fibrosis over time in subgroups by antibodies; (E) 1-Kaplan-Meier estimates of cumulative incidence of pulmonary hypertension in subgroups by antibodies; (F) Smoothed hazard of pulmonary hypertension over time in subgroups by antibodies.

Figure 2. Association between modified Rodnan skin score and time for the cohort as a whole (A) and in subgroups by autoantibodies (B); thick lines represent model-predicted average skin score; thin lines and dots are observed individual patient skin scores for patients with multiple and single skin score assessments, respectively. Survival (C) and time to organ complications (D, E, F) in the seven classification subgroups; legend for panels C, D, E and F is in panel D.

Table 1. Cohort characteristics

	Overall cohort		csPF analysis subgroup		Skin cohort	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**
Total number	1325	(100.0)	654	(100.0)	581	(100.0)
Follow-up (years), mean ± SD	12.3±5.6		11.2±5.8		12.2±7.9	
Age at onset (years), mean ± SD	46.8±13.6		47.8±13.1		44.0±13.8	
Male	222	(16.8)	112	(17.1)	131	(22.6)
Diffuse cutaneous subset	476	(35.9)	329	(50.3)	581	(100.0)
Overlap syndromes	262	(19.8)	114	(17.4)	102	(17.6)
Autoantibodies						
Anti-centromere	391	(29.5)	139	(21.3)	17	(2.9)
Anti-topoisimerase I	287	(21.7)	157	(24.0)	178	(30.6)
Anti-RNA polymerase	149	(11.3)	113	(17.3)	161	(27.7)
Anti-U3RNP	56	(4.2)	34	(5.2)	39	(6.7)
Anti-PmScl	56	(4.2)	27	(4.1)	27	(4.7)
Other, including anti-U1RNP, Th/To, SL, Ku, Jo1, Ro, La, XR, PL7, hnRNP and Sm	214	(16.2)	95	(14.5)	79	(13.6)
ANA+ ENA-	196	(14.8)	103	(15.8)	95	(16.4)
ANA negative	58	(4.4)	28	(4.3)	24	(4.1)
Organ complications						
Pulmonary fibrosis, any	*575	(43.4)	326	(49.9)	**316	(54.4)
Clinically-significant pulmonary fibrosis	*520	(39.3)	308	(47.1)	--	--
Pulmonary hypertension (group 1 & 3)	172	(13.0)	84	(12.8)	54	(9.3)
Pulmonary arterial hypertension	134	(10.1)	64	(9.8)	34	(5.9)
Cardiac scleroderma	63	(4.8)	41	(6.3)	43	(7.4)
Renal crisis	94	(7.1)	63	(9.6)	84	(14.5)
Death	441	(33.3)	257	(39.3)	189	(32.5)

*Missing pulmonary fibrosis data for 10 (0.8%) of the patients; **Missing pulmonary fibrosis data for 11 (1.9%) of the patients;

Table 2. Organ complication incidence and survival within antibody subgroups - comparison between Kaplan-Meier estimates and cumulative incidence function estimates, accounting for death as a competing risk.

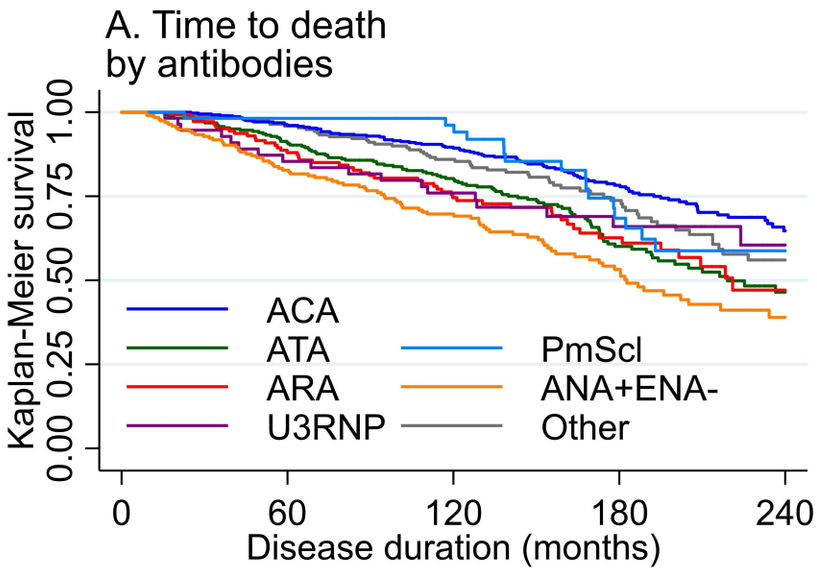
Time (months)	Clinically-significant pulmonary fibrosis		Pulmonary hypertension		Cardiac scleroderma		Scleroderma renal crisis		Survival
	1-KM	CIF	1-KM	CIF	1-KM	CIF	1-KM	CIF	KM
Anti-centromere antibody									
60	7.5%	7.5%	5.0%	5.0%	1.3%	1.3%	0.8%	0.8%	96.1%
120	8.4%	8.3%	10.2%	10.0%	1.6%	1.6%	0.8%	0.8%	89.3%
180	8.4%	8.3%	14.6%	14.0%	2.4%	2.3%	0.8%	0.8%	78.3%
240	8.4%	8.3%	22.4%	20.9%	5.3%	4.8%	0.8%	0.8%	64.7%
Anti-topoisimerase I antibody									
60	80.3%	79.3%	1.1%	1.1%	3.6%	3.6%	4.7%	4.6%	91.0%
120	85.0%	83.2%	6.1%	5.5%	6.2%	6.0%	5.6%	5.5%	80.3%
180	87.0%	84.7%	11.4%	9.8%	10.1%	9.0%	6.4%	6.1%	60.1%
240	87.0%	84.7%	13.3%	11.1%	11.5%	10.0%	6.4%	6.1%	46.5%
Anti-RNA polymerase antibody									
60	34.1%	33.4%	4.6%	4.4%	1.4%	1.4%	23.3%	23.3%	88.0%
120	44.0%	42.1%	10.0%	9.4%	2.3%	2.2%	25.1%	24.9%	74.6%
180	46.9%	44.5%	16.0%	14.6%	2.3%	2.2%	29.0%	28.1%	62.6%
240	46.9%	44.5%	26.8%	23.3%	2.3%	2.2%	29.0%	28.1%	47.1%
Anti-U3RNP antibody									
60	19.2%	17.9%	6.0%	5.7%	9.2%	9.1%	11.3%	11.0%	85.4%
120	19.2%	17.9%	19.9%	17.7%	13.8%	13.2%	11.3%	11.0%	76.0%
180	24.3%	21.5%	38.6%	33.8%	13.8%	13.2%	11.3%	11.0%	66.0%
240	24.3%	21.5%	38.6%	33.8%	13.8%	13.2%	11.3%	11.0%	60.5%
Anti-PmScl antibody									
60	40.7%	40.7%	2.0%	2.0%	1.9%	1.9%	3.8%	3.8%	98.2%
120	40.7%	40.7%	4.1%	4.0%	1.9%	1.9%	5.8%	5.8%	96.1%
180	50.6%	49.8%	11.1%	10.1%	1.9%	1.9%	5.8%	5.8%	68.5%
240	50.6%	49.8%	11.1%	10.1%	1.9%	1.9%	5.8%	5.8%	58.8%
ANA+ ENA-									
60	56.8%	53.4%	4.7%	4.3%	3.9%	3.7%	10.6%	10.4%	82.8%
120	56.8%	53.4%	7.6%	6.8%	4.7%	4.3%	10.6%	10.4%	69.7%
180	56.8%	53.4%	20.3%	16.3%	6.0%	5.2%	10.6%	10.4%	53.2%
240	56.8%	53.4%	27.0%	20.8%	6.0%	5.2%	10.6%	10.4%	39.0%
Other antibodies, including anti-U1RNP, Th/To, SL, Ku, Jo1, Ro, La, XR, PL7, hnRNP, Sm and ANA negative									
60	51.5%	51.5%	4.7%	4.6%	3.7%	3.6%	3.5%	3.5%	95.9%
120	55.8%	55.6%	10.7%	10.2%	4.3%	4.2%	4.1%	4.1%	86.0%
180	57.8%	57.4%	18.8%	17.5%	5.1%	4.9%	4.1%	4.1%	73.7%
240	57.8%	57.4%	27.6%	24.7%	5.1%	4.9%	4.1%	4.1%	56.0%

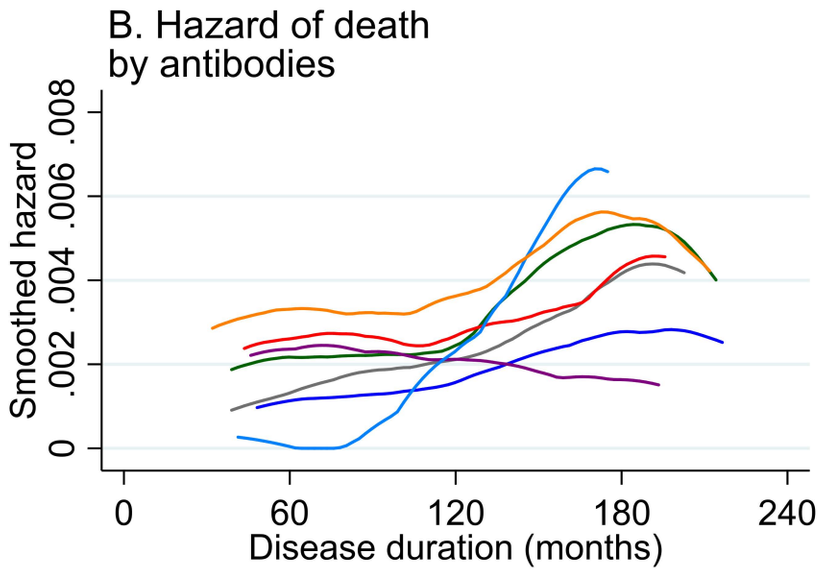
Table 3. Cox proportional hazards models for the associations between antibody and outcomes. The group with the highest associated hazard has been used as a reference.

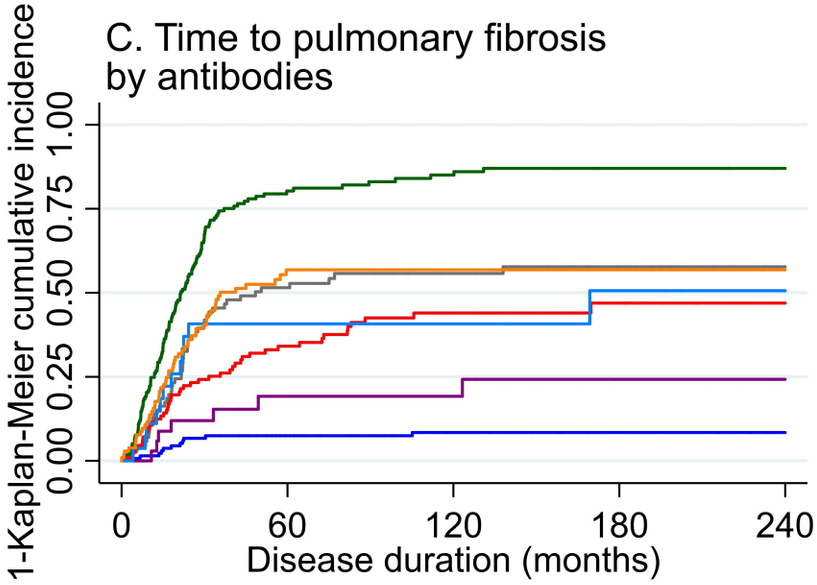
Autoantibody group	HR	95% CI	
Clinically-significant pulmonary fibrosis			
ATA	reference group		
ACA	0.048	(0.026 , 0.089)	<0.001
ARA	0.303	(0.216 , 0.425)	<0.001
U3RNP	0.141	(0.066 , 0.301)	<0.001
PmScl	0.350	(0.193 , 0.633)	0.001
ANA+ENA-	0.487	(0.354 , 0.669)	<0.001
Other	0.458	(0.328 , 0.639)	<0.001
Pulmonary hypertension			
U3RNP	reference group		
ACA	0.420	(0.237 , 0.742)	0.003
ATA	0.271	(0.141 , 0.523)	<0.001
ARA	0.499	(0.254 , 0.983)	0.044
PmScl	0.221	(0.073 , 0.665)	0.007
ANA+ENA-	0.473	(0.247 , 0.906)	0.024
Other	0.562	(0.305 , 1.036)	0.065
Cardiac scleroderma			
U3RNP	reference group		
ACA	0.171	(0.069 , 0.426)	<0.001
ATA	0.535	(0.238 , 1.202)	0.130
ARA	0.149	(0.040 , 0.562)	0.005
PmScl	0.114	(0.014 , 0.915)	0.041
ANA+ENA-	0.351	(0.135 , 0.911)	0.031
Other	0.294	(0.114 , 0.763)	0.012
Scleroderma renal crisis			
ARA	reference group		
ACA	0.025	(0.008 , 0.080)	<0.001
ATA	0.187	(0.104 , 0.335)	<0.001
U3RNP	0.367	(0.155 , 0.869)	0.023
PmScl	0.178	(0.055 , 0.578)	0.004
ANA+ENA-	0.363	(0.211 , 0.624)	<0.001
Other	0.132	(0.062 , 0.283)	<0.001
Death			
U3RNP	reference group		
ACA	0.172	(0.065 , 0.454)	<0.001
ATA	0.424	(0.166 , 1.085)	0.074
ARA	0.609	(0.223 , 1.666)	0.334
PmScl	0.078	(0.016 , 0.382)	0.002
ANA+ENA-	0.909	(0.358 , 2.306)	0.840
Other	0.206	(0.073 , 0.580)	0.003
ACA*Time(months)	1.013	(1.004 , 1.022)	0.005
ATA*Time(months)	1.010	(1.001 , 1.019)	0.023
ARA*Time(months)	1.007	(0.998 , 1.017)	0.136
PmScl*Time(months)	1.020	(1.008 , 1.032)	0.001
ANA+ENA-*Time(months)	1.007	(0.998 , 1.015)	0.148
Other*Time(months)	1.014	(1.004 , 1.023)	0.004

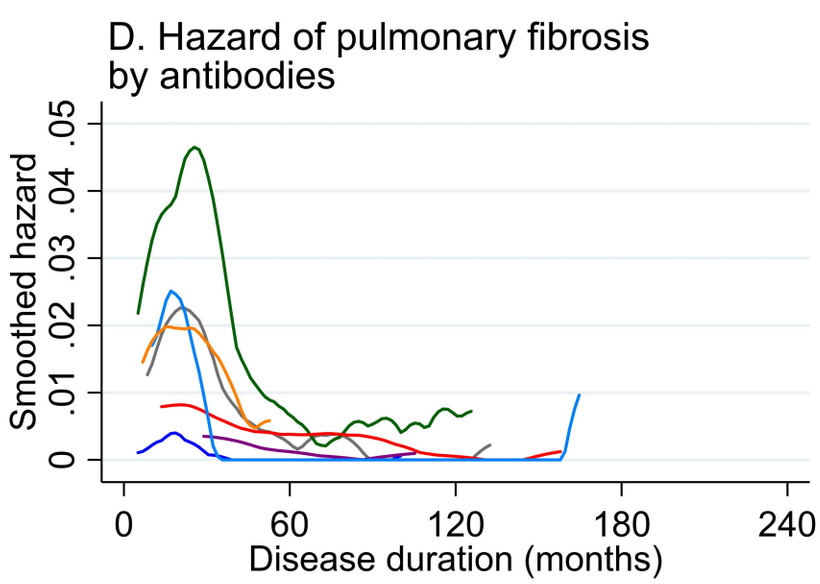
Table 4. Cumulative incidence function estimates, accounting for death as a competing risk, for pulmonary fibrosis, pulmonary hypertension, cardiac scleroderma and scleroderma renal crisis, as well as Kaplan-Meier estimates of all-cause mortality in the final 7 classification groups

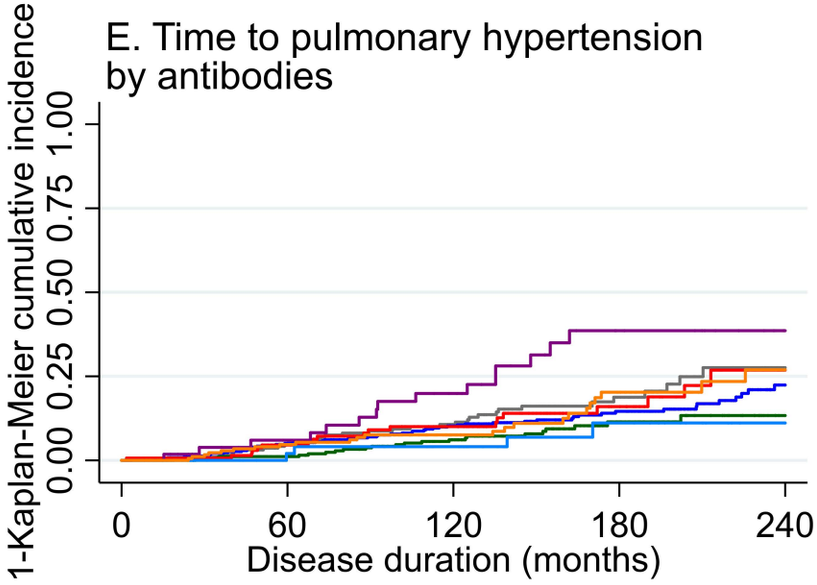
	Time	Classification groups						
Endpoints	**(months)**	**ACA+ L**	**ATA+ L**	**ATA+ D**	**ARA+**	**U3RNP+**	**Other L**	**Other D**
Clinically-significant pulmonary fibrosis	**60**	7.7%	82.2%	77.7%	33.4%	17.9%	49.9%	50.2%
	120	8.5%	82.2%	84.0%	42.1%	17.9%	52.8%	50.2%
	180	8.5%	86.1%	84.0%	44.5%	21.5%	53.9%	53.8%
	240	8.5%	86.1%		44.5%	21.5%	53.9%	53.8%
Pulmonary hypertension	**60**	5.1%	0.8%	1.4%	4.4%	5.7%	4.2%	3.9%
	120	10.3%	4.1%	7.0%	9.4%	17.7%	8.6%	6.1%
	180	13.9%	6.9%	12.5%	14.6%	33.8%	18.5%	11.1%
	240	20.4%	6.9%	15.3%	23.3%	33.8%	24.3%	16.4%
Cardiac scleroderma	**60**	1.4%	0.0%	7.0%	1.4%	9.1%	1.0%	7.6%
	120	1.7%	1.6%	10.1%	2.2%	13.2%	1.4%	8.4%
	180	2.4%	5.0%	12.9%	2.2%	13.2%	2.4%	8.4%
	240	4.9%	7.0%	12.9%	2.2%	13.2%	2.4%	8.4%
Scleroderma renal crisis	**60**	0.3%	3.0%	6.2%	23.3%	11.0%	2.7%	14.1%
	120	0.3%	3.8%	7.0%	24.9%	11.0%	2.7%	15.6%
	180	0.3%	3.8%	8.3%	28.1%	11.0%	2.7%	15.6%
	240	0.3%	3.8%	8.3%	28.1%	11.0%	2.7%	15.6%
Mortality	**60**	4.1%	3.0%	14.6%	12.0%	14.6%	4.2%	18.5%
	120	10.7%	10.9%	28.0%	25.4%	24.0%	12.9%	31.7%
	180	20.9%	26.4%	51.9%	37.4%	34.0%	28.4%	52.1%
	240	34.7%	38.2%	67.6%	52.9%	39.5%	43.8%	66.4%

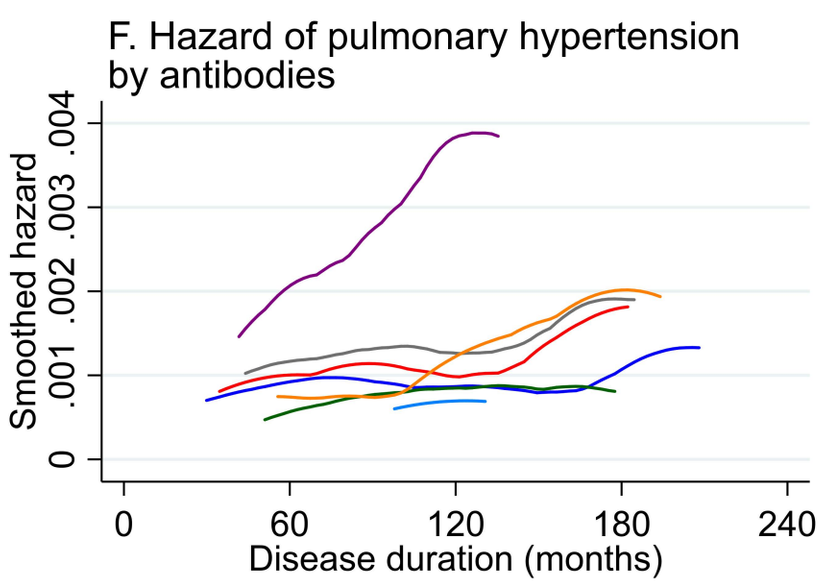

A. Time to death
by antibodies
Kaplan-Meier survival
0.00 0.25 0.50 0.75 1.00
ACA
ATA
ARA
U3RNP
PmScl
ANA+ENA-
Other
0
60
120
180
240
Disease duration (months)

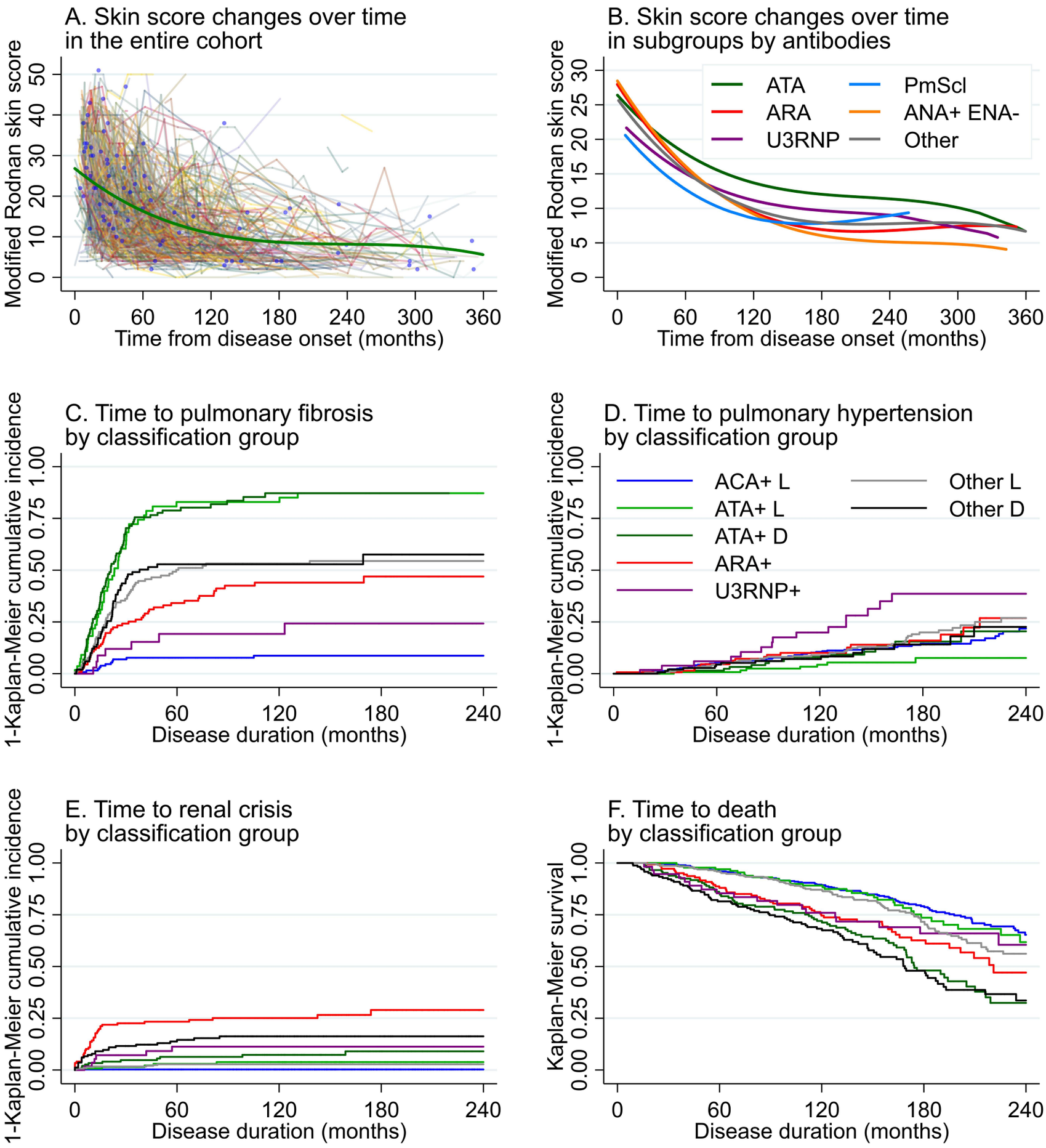

B. Hazard of death
by antibodies
Smoothed hazard
0 .002 .004 .006 .008
0
60
120
180
240
Disease duration (months)


C. Time to pulmonary fibrosis
by antibodies
1-Kaplan-Meier cumulative incidence
0.00 0.25 0.50 0.75 1.00
0
60
120
180
240
Disease duration (months)


D. Hazard of pulmonary fibrosis
by antibodies
Smoothed hazard
0 .01 .02 .03 .04 .05
0
60
120
180
240
Disease duration (months)


E. Time to pulmonary hypertension
by antibodies
1-Kaplan-Meier cumulative incidence
0.00 0.25 0.50 0.75 1.00
0
60
120
180
240
Disease duration (months)


F. Hazard of pulmonary hypertension
by antibodies
Smoothed hazard
0 .001 .002 .003 .004
0
60
120
180
240
Disease duration (months)


A. Skin score changes over time in the entire cohort
Modified Rodnan skin score
0 10 20 30 40 50
0 60 120 180 240 300 360
Time from disease onset (months)
B. Skin score changes over time in subgroups by antibodies
Modified Rodnan skin score
0 5 10 15 20 25 30
ATA
ARA
U3RNP
PmScl
ANA+ ENA-
Other
0 60 120 180 240 300 360
Time from disease onset (months)
C. Time to pulmonary fibrosis by classification group
1-Kaplan-Meier cumulative incidence
0.00 0.25 0.50 0.75 1.00
0 60 120 180 240
Disease duration (months)
D. Time to pulmonary hypertension by classification group
1-Kaplan-Meier cumulative incidence
0.00 0.25 0.50 0.75 1.00
ACA+ L
ATA+ L
ATA+ D
ARA+
U3RNP+
Other L
Other D
0 60 120 180 240
Disease duration (months)
E. Time to renal crisis by classification group
1-Kaplan-Meier cumulative incidence
0.00 0.25 0.50 0.75 1.00
0 60 120 180 240
Disease duration (months)
F. Time to death by classification group
Kaplan-Meier survival
0.00 0.25 0.50 0.75 1.00
0 60 120 180 240
Disease duration (months)