Systemic sclerosis: are anti-nuclear antibodies our guiding stars?

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The effect of sex on outcomes in systemic sclerosis: does anti-topoisomerase I status matter? A EUSTAR analysis

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Abstract

Background
Male Systemic Sclerosis (SSc) patients more often express anti-topoisomerase antibodies (ATA) compared to female patients. We present an in-depth analysis on the effects of sex on SSc outcomes, independent of autoantibody status.

Methods
Using Kaplan Meier curves and Cox proportional hazard models, we evaluated the independent effect of sex on mortality and on the incidence of diffuse skin involvement (dcSSc), interstitial lung disease (ILD) and pulmonary hypertension (PH) in SSc in two cohorts: 1. the Leiden Combined Care In SSc cohort (CCISS; n=242) and 2. the EUropean Scleroderma Trial and Research cohort (EUSTAR; n=4263). We profited from the large sample size of the EUSTAR cohort to perform multivariate analyses including adjustment for autoantibody, age and race and accounting for left-truncation.

Results
SSc males more often express ATA than SSc females (CCISS: 40% vs 21%; EUSTAR: 49% vs 38%). EUSTAR based analyses showed that male sex was associated with mortality (HR 2.6 [95% CI 2.0-3.4]) and its effect was stronger than the effect of ATA (HR 1.33 [95% CI 1.0-1.8]). Male sex was also independently associated with development of dcSSc (HR 1.4 [95%CI 1.1-1.8]) and PH (HR 1.5 [95%CI 1.2-2.0]). Only for ILD the effect of ATA (HR 1.9 [95%CI 1.5-2.5]) was stronger than the effect of sex (HR 1.1 [95%CI 0.9-1.3]).

Conclusions
Male sex is strongly associated with mortality in SSc. This association cannot be explained by a higher prevalence of ATA among males.

Introduction

Systemic sclerosis (SSc) is a rare and heterogeneous disease, clinically characterized by Raynaud's phenomenon, skin and pulmonary fibrosis and cardiac and gastro-intestinal dysfunction (1). The disease is characterized by a complex pathophysiology (2). Dysregulation of the immune system is evidenced by the presence of specific antinuclear antibodies that have clinical and prognostic associations (2, 3). For example: anti-topoisomerase I antibodies (ATA) are associated with diffuse cutaneous involvement and occurrence of interstitial lung disease (ILD) (3-6), while anti-centromere antibodies (ACA) are associated with limited cutaneous involvement, gastrointestinal involvement and a lower likelihood of significant ILD (6-8).

While females are overrepresented in SSc (female: male approximately 5 :1), male sex is associated with early and increased mortality and with presence of ILD (5, 9). Interestingly, the prevalence of SSc-specific autoantibodies also differs with sex: In the EUSTAR cohort, prevalence of ACA is 31% among females and 10% among males, while prevalence of ATA is 31% among females and 54% among males (9-11). Based on this sex-specific distribution of SSc specific auto-antibodies, it could be hypothesized that at least part of the differences observed between male and female patients with SSc are explained by differences in autoantibody distribution.

Our aim was to evaluate the effect of sex on mortality and development of diffuse cutaneous skin involvement (dcSSc), ILD and PH, not explained by autoantibody status. To this end, we took advantage of two cohorts: The Leiden Combined Care In Systemic Sclerosis cohort (CCISS) and the EULAR Scleroderma Trials and Research (EUSTAR) prospective multicenter systemic sclerosis cohort. Using Kaplan Meier curves, with stratification of patients into 6 risk-groups according to sex and autoantibody status (i. ACA+ female, ii. ACA+ male, iii. ATA-ACA- female, iv. ATA-ACA-male, v. ATA+ female and vi. ATA+ male) and Cox regression analysis, this study gains insight in the risks of sex and autoantibodies independently. The analyses performed here are unique in the field, as we adjusted for left-truncation to correct for various disease durations at cohort entrance.

Methods

Leiden Combined Care in Systemic Sclerosis
Data from consecutive SSc patients included in the Combined Care in Systemic Sclerosis Cohort (CCISS) of the Leiden University Medical Center, Leiden, The Netherlands between April 1st, 2009 and June 1st, 2016 were analysed. The CCISS cohort comprises annual prospective data collection with local ethics approval, as described previously (12). Unique in this cohort is the standardized and extensive

annual follow-up with high rate of data completeness. Complete results on prevalence of SSc specific autoantibodies including antibodies directed against topoisomerase (ATA) and centromere (ACA) are available in 97% of patients (13).

The EUSTAR cohort
The European Scleroderma Trials and Research group (EUSTAR) database documents a multinational, prospective and dynamic scleroderma cohort with longitudinal follow-up, which started in June 2004. At time of data extraction (March 28th, 2018), data on 14,998 patients were recorded in the database. A detailed description of the cohort is provided elsewhere (4, 14, 15). Participating centers obtained ethics committee approval. The Leiden patients were excluded from the EUSTAR dataset.

Inclusion criteria
From both cohorts, patients meeting the following criteria were included for analysis: 1. fulfilment of the ACR/EULAR 2013 classification criteria for SSc (16), 2. available auto-antibody status (including at least ANA (anti-nuclear antibody), ATA and ACA status), 3. available skin subtyping (as defined by Medsger and Leroy (17), subdivision of patients in limited and diffuse cutaneous SSc), 4. available radiographic assessment of ILD (by either chest X-ray or high resolution computed tomography [HRCT]) at least one time during baseline or follow-up, 5. date of disease onset known (defined as the date of onset of the first non-Raynaud symptom [91% of cases], or when the date of first non-Raynaud symptom was missing, as the date of the first Raynaud symptom [9% of cases]), and 6. no coexisting SSc specific antibodies (ATA, ACA, RNA polymerase III, Pm/Scl, U1RNP, U3RNP). Flowcharts of patient inclusion in both cohorts are shown in Figure 1. Comparing included and excluded patients there were no significant differences.

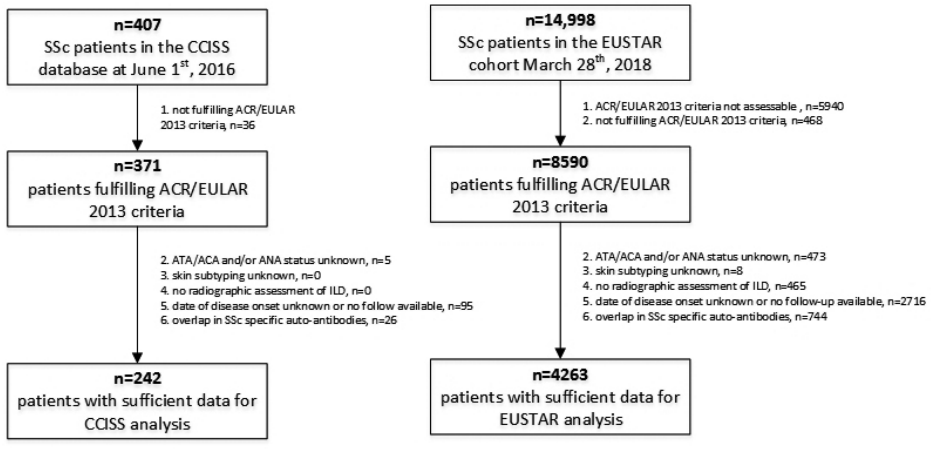

Figure 1. Flowchart of patient inclusion of the Leiden Combined Care in Systemic Sclerosis (CCISS) cohort (left) and EUSTAR cohort (right)

Definitions
Survival time since date of disease onset was registered in each database, including whether death was related to SSc. When a patient once developed dcSSc according to their skin pattern, the patient was classified as dcSSc from that moment onwards, even in case of later improvement to a limited skin pattern. Severe lung involvement was defined as forced vital capacity (FVC) and/or diffusion capacity of the lung for carbon-monoxide (DLCO) of ≤50% of predicted, accompanied by presence of lung fibrosis and/or ground glass opacifications as evaluated by high resolution computed tomography (HRCT). FVC and/or DLCO < 50% was chosen as it corresponds to a score of 2 or higher on the Medsger Disease Severity Scale (18). PH in the CCISS cohort was based on right heart catheterization (RHC); patients were selected for right heart catheterization using the DETECT algorithm (19) and a multidisciplinary team discussion with expert cardiologists, pulmonologists, internal medicine specialists and rheumatologists. In the EUSTAR database PH was registered (yes/no) by the recording physician (based on either echocardiography or RHC).

Statistical analyses
Baseline characteristics of risk-groups (stratified for ATA status, ACA status and sex, i.e., i. ACA+ female, ii. ACA+ male, iii. ATA-ACA- female, iv. ATA-ACA- male, v. ATA+ female and vi. ATA+ male) were compared, testing significancy of differences as appropriate, for both cohorts.

Kaplan Meier methods were used to construct survival curves and in the EUSTAR cohort also for visualization of the development of dcSSc, severe ILD and PH over time. The curves were calculated separately for sex and auto-antibody status and compared using the log rank test. Rates of occurrence of the different outcomes were calculated in different time periods and different risk groups. The Cox proportional hazard model was used to study the effect sex and auto-antibody while adjusting for race and age at disease onset. For all patients, the date of disease onset predated the date of cohort entry (left-truncation). We accounted for this in all analyses to prevent survival bias. Patients were censored at time of last visit or after 10 years of disease duration. The proportional hazards assumption was verified by plotting log minus log survival plots (LML plots) and performance of Schoenfeld's global test. All analyses represent complete case analyses since complete data was an inclusion criterium. Analyses were performed in IBM SPSS Statistics 23. Stata, version 14 (StataCorp LP, College Station, TX USA) was used to account for left-truncation in the survival analyses. Statistical tests were two-sided with an α-level of 0.05.

Table 1. Baseline characteristics and 10-year survival of patients of the Leiden (CCISS) cohort

	overall	females	males	ACA+ female	ACA+ male	ACA-ATA- female	ACA-ATA- male	ATA+ female	ATA+ male
	n=242	**n=190 79%**	**n=52 21%**	**n=83 34%**	**n=10 4%**	**n=67 28%**	**n=21 9%**	**n=40 17%**	**n=21 9%**
median disease duration at cohort entrance (yrs., min-max)	2.4 (0.0-9.8)	2.5 (0.0-9.8)	2.1 (0.0-9.3)	2.5 (0.0-9.8)	1.3 (0.4-8.6)	2.8 (0.1-9.8)	3.0 (0.3-9.2)	1.7 (0.0-9.5)	0.9 (0.0-9.3)
Demographics									
age at disease-onset, mean±SD	51±15	51±15	52±13	53±13	55±14	50±15	49±14	48±17	53±13
caucasian, n (%)	193 (80)	149 (78)	44 (85)	70 (84)	9 (90)	53 (79)	17 (81)	26 (65)	18 (86)
smoking (ever)[1], n (%)	144 (62)	108 (59)	36 (74)	46 (57)	6 (60)	45 (69)	15 (75)	17 (45)	15 (79)
disease features									
dcSSc, n (%)	61 (25)	37 (19)	24 (46)	2 (2)	0 (0)	21 (31)	8 (38)	14 (35)	16 (76)
severe ILD, n (%)	17 (7)	25 (13)	17 (33)	3 (4)	1 (10)	15 (22)	9 (43)	7 (18)	7 (33)
PH, n (%)	17 (7)	12 (6)	5 (10)	8 (10)	0 (0)	4 (6)	2 (10)	0 (0)	3 (14)
renal crisis, n (%)	8 (3)	6 (3)	2 (4)	0 (0)	0 (0)	5 (7)	1 (5)	1 (3)	1 (5)
10-year survival, n (%)	220 (91)	178 (94)	42 (81)	81 (98)	8 (80)	61 (99)	18 (86)	36 (90)	16 (76)

ACA=anti-centromere antibodies; ATA=anti-topoisomerase I antibodies; CCISS=combined care in Systemic Sclerosis; dcSSc=diffuse cutaneous Systemic Sclerosis; ILD=interstitial lung disease; max=maximum; min=minimum; PH=pulmonary hypertension; SD= standard deviation; yrs=years
1 missing in n=9

Results

1. The Leiden Combined Care in Systemic Sclerosis (CCISS) cohort

Of 242 CCISS patients included (Figure 1), 52 were male and 190 were female. This patient population comprised 83 ACA+ females (34%), 10 ACA+ males (4%), 67 ATA-ACA- females (28%), 21 ATA-ACA- males (9%), 40 ATA+ females (17%) and 21 ATA+ males (9%). Baseline characteristics are presented in Table 1. The autoantibody distribution differed significantly between men and women: expression of ATA occurred significantly more often in males compared to females (40 vs. 21%, p<0.01) while ACA expression was significantly more common in females (44 vs. 19% p<0.01). At cohort entry severe ILD (33 vs. 13%, p=0.01) and dcSSc (46 vs. 19%, p=0.01) were more frequent in males compared to females.
During 800 person-years of follow-up (125 for males, 583 for females), 22 patients died (10 males, 12 females). Mortality in males was higher than in females (log-rank p<0.01; data presented in Table 1). After stratification for sex, no significant differences in survival between the three autoantibody groups were observed (log rank in male subgroups analyses p=0.53; female subgroup analyses p=0.16).

2. The EUSTAR cohort

2.1 Male and female distribution of auto-antibodies
To further replicate and deepen the data described above, we next performed a similar analysis in the independent EUSTAR cohort. A total of 4263 patients from the EUSTAR database were included (Figure 1). The included patient set comprised 1380 ACA+ females (32%), 130 ACA+ males (3%), 777

Table 2. Baseline characteristics of EUSTAR Systemic Sclerosis patients

	overall	**male**	**female**	**ACA+ female**	**ACA+ male**	**ACA-ATA- female**	**ACA-ATA- male**	**ATA+ female**	**ATA+ male**
	n=4263	**n=783 18%**	**n=3480 82%**	**n=1380 32%**	**n=130 3%**	**n=777 18%**	**n=272 6%**	**n=1323 31%**	**n=381 9%**
disease duration									
median disease duration at cohort entry (min-max)	3.0 (0.0-10.0)	2.1 (0.0-9.9)	3.2 (0.0-10.0)	3.5 (0.0-10.0)	3.2 (0.0-9.8)	2.7 (0.0-9.9)	1.9 (0.0-9.9)	3.2 (0.0-10.0)	2.0 (0.0-9.9)
demographics									
age at disease-onset, mean±SD	48.8±14.0	49.3±13.3	48.8±14.1	52.5±12.9	53.6±12.8	47.2±14.1	50.3±13.5	45.8±14.4	47.2±13.0
race									
white, n (%)	3592 (84)	699 (89)	2893 (83)	1202 (87)	123 (95)	625 (80)	242 (89)	1066 (81)	334 (88)
black, n (%)	54 (1)	5 (1)	49 (1)	8 (1)	0 (0)	25 (3)	3 (1)	49 (4)	2 (1)
asian, n (%)	94 (2)	9 (1)	85 (2)	15 (1)	0 (0)	21 (3)	3 (1)	16 (1)	6 (2)
other/ undefined, n (%)	523 (12)	70 (9)	453 (13)	155 (11)	7 (5)	106 (14)	24 (9)	192 (15)	39 (10)
smoking (ever), n (%)[a]	1210 (36)	388 (63)	822 (30)	361 (31)	72 (63)	257 (41)	144 (66)	204 (20)	172 (60)
disease-specific features									
dcSSc, n (%)	1638 (39)	422 (55)	1216 (35)	101 (7)	14 (11)	349 (45)	147 (55)	766 (58)	261 (69)

Table 2. Baseline characteristics of EUSTAR Systemic Sclerosis patients *(continued)*

	overall	**male**	**female**	**ACA+ female**	**ACA+ male**	**ACA-ATA- female**	**ACA-ATA- male**	**ATA+ female**	**ATA+ male**
	n=4263	**n=783 18%**	**n=3480 82%**	**n=1380 32%**	**n=130 3%**	**n=777 18%**	**n=272 6%**	**n=1323 31%**	**n=381 9%**
severe ILD[b], n (%)	496 (21)	146 (31)	350 (19)	41 (10)	11 (29)	88 (20)	40 (25)	221 (21)	95 (34)
pulmonary hypertension, n (%)[c]	538 (14)	108 (16)	430 (14)	189 (15)	16 (15)	82 (12)	34 (15)	159 (13)	58 (17)
renal crisis, n (%)	76 (2)	21 (3)	55 (2)	12 (1)	0 (0)	23 (3)	17 (6)	20 (2)	4 (1)

ACA=anti-centromere autoantibody, ATA=anti-topoisomerase I autoantibody; dcSSc=diffuse cutaneous Systemic Sclerosis; ILD=interstitial lung disease

[a] missing in 866 (20%); [b] missing in 1899 (45%) [c] missing in 131 (3%); [d] missing in 39 (1%)

ATA- ACA- females (18%), 272 ATA- ACA- males (6%), 1323 ATA+ females (31%), and 381 ATA+ males (9%). Baseline characteristics are presented in Table 2. Males were more often ATA positive compared to females (49% vs. 38%, p<0.01), and females were more frequently ACA positive compared to males (40% vs. 17%, p<0.01), confirming the findings in the CCISS cohort.

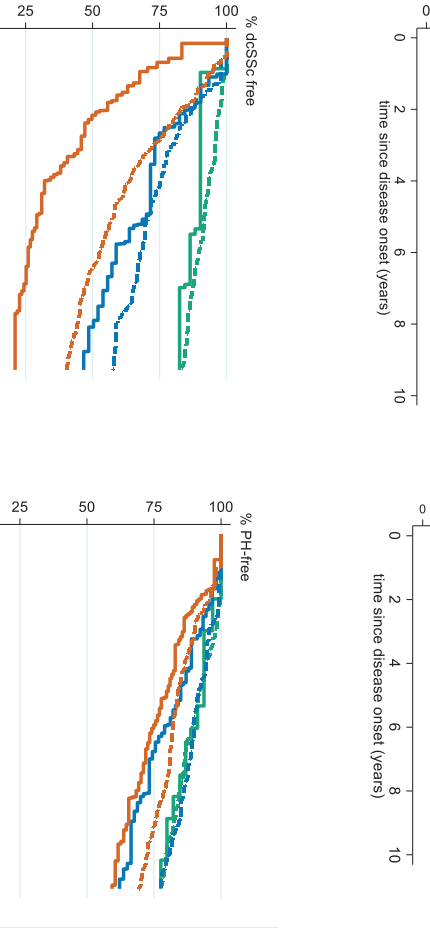

Fig 2. Kaplan Meier curves for survival, development of severe ILD and diffuse cutaneous involvement according to gender and autoantibodies

2.1 Mortality, diffuse cutaneous skin involvement, interstitial lung disease and pulmonary hypertension according to sex and autoantibody derived risk-groups

An overview of available data and events in the EUSTAR cohort is shown in Tables 3. During 15953 person-years of follow-up (2795 for males and 13158 for females) 263 patients died (100 males, 163 females). Kaplan Meier survival curves (Figure 2) show

Table 3. Number of patients developing the outcome of interest and patient years observed in the EUSTAR cohort

	overall	**ACA+ female**	**ACA+ male**	**ACA-ATA- female**	**ACA-ATA- male**	**ATA+ female**	**ATA+ Male**
	n=4263	n=1380	n=130	n=777	n=272	n=1323	n=381
10-year survival analysis							
patient years observed	15953	5382	435	2840	999	4936	1360
deaths, $n/n_{at\ risk}$ (%)	263/4263 (6)	47/1380 (3)	8/130 (6)	34/777 (4)	38/272 (14)	82/1323 (6)	54/381 (14)
development of severe ILD analysis*							
severe ILD at cohort entrance, n (%)[a]	496 (21)	41 (10)	11 (29)	88 (20)	40 (25)	221 (21)	95 (34)
patient years observed	10809	4254	356	1906	688	2870	735
development of severe ILD, $n/n_{at\ risk}$ (%)	337/3064 (11)	40/1137 (4)	5/104 (5)	58/548 (11)	25/192 (13)	161/855 (19)	48/228 (21)
development of dcSSc analysis*							
dcSSc at cohort entrance, n (%)[b]	1638 (39)	101 (7)	14 (11)	349 (45)	147 (55)	766 (59)	261 (69)
patient years observed	8663	4666	367	1324	346	1674	286

Table 3. Number of patients developing the outcome of interest and patient years observed in the EUSTAR cohort *(continued)*

	overall	**ACA+ female**	**ACA+ male**	**ACA-ATA- female**	**ACA-ATA- male**	**ATA+ female**	**ATA+ Male**
	n=4263	n=1380	n=130	n=777	n=272	n=1323	n=381
development of dcSSc, $n/n_{at\ risk}$ (%)	399/2524 (16)	92/1244 (7)	6/108 (6)	75/413 (18)	28/114 (25)	156/528 (30)	42/117 (36)
development of PH analysis							
PH at cohort entrance, n (%)[c]	538 (14)	189 (15)	16 (15)	82 (12)	34 (15)	159 (13)	58 (17)
patient years observed	11251	3888	318	2093	643	3443	866
development of PH, $n/n_{at\ risk}$ (%)	391/3081 (13)	114/1032 (11)	9/87 (10)	57/568 (10)	33/177 (19)	130/962 (14)	48/255 (19)

ACA=anti-centromere autoantibody, ATA=anti-topoisomerase I autoantibody; dcSSc=diffuse cutaneous Systemic Sclerosis; ILD=interstitial lung disease

* $n_{at\ risk}$ and n at cohort entrance do not sum up to n=4263 because of patients lacking baseline or follow-up assessments on skin or pulmonary involvement and therefore exclusion in analysis

[a] missing in 1899 (45%); [b]missing in 39 (1%); [c] missing in 446 (10%)

that survival in males is worse, at all time points within all auto-antibody groups. The same trend was observed for SSc related mortality (Supplementary Material).
The risk to develop dcSSc was highest among ATA+ males, followed by ATA+ females. DcSSc was rare in ACA+ patients, both males and females. Development of ILD was highest in ATA+ males, followed by ATA+ females. Development of pulmonary hypertension is seen most often in ATA-ACA- males and ATA+ subjects, in which males developed PH more often than females (log-rank p=0.03).

2.2 Independent association of sex with SSc outcomes

To evaluate the independent effect of sex on survival and disease outcomes, multivariate left-truncated Cox regression analyses with correction for age, race and gender were performed (Table 4). Interaction between sex and autoantibody status was not statistically significant for any of the outcomes. Both sex and ATA positivity were associated with mortality (male HR 2.6; ATA+ HR 1.3), dcSSc (male HR 1.4; ATA+ HR 1.7) and PH (male HR 1.5; ATA+ HR 1.4) after adjustment for age and race.
Multivariate cox regression analysis confirmed that development of severe ILD is associated with ATA+ (HR 1.9, 95%CI 1.5-2.5), but not with sex (male sex HR 1.1, 95%CI 0.9-1.3).

Table 4. Hazard ratios for mortality, development of severe interstitial lung disease and development of diffuse cutaneous involvement and pulmonary hypertension

	univariate unadjusted HR (95% CI)	p	multivariate model (95%CI)	p
A. MORTALITY				
male	2.9 (2.3-3.8)	<0.01	2.6 (2.0-3.4)	<0.01
ACA-	2.2 (1.6-2.9)	<0.01	2.0 (1.4-2.9)	<0.01
ATA+	1.7 (1.3-2.1)	<0.01	1.3 (<1.0-1.8)	0.06
age at onset (per 10 yrs. increase of age)	1.7 (1.5-1.8)	<0.01	1.8 (1.6-2.0)	<0.01
race (ref=caucasian)				
asian	0.7 (0.2-2.3)	0.59	1.1 (0.3-3.3)	0.92
black	0.7 (0.2-3.0)	0.67	1.4 (0.4-5.8)	0.62
other/undefined	0.6 (0.4-0.9)	0.02	0.7 (0.5-1.1)	0.13
B. SEVERE INTERSTITIAL LUNG DISEASE				
male	1.5 (1.1-1.9)	<0.01	1.1 (0.9-1.3)	0.25
ACA-	4.7 (3.4-6.4)	<0.01	3.3 (2.3-4.8)	<0.01
ATA+	3.2 (2.5-4.0)	<0.01	1.9 (1.5-2.5)	<0.01

Table 4. Hazard ratios for mortality, development of severe interstitial lung disease and development of diffuse cutaneous involvement and pulmonary hypertension *(continued)*

	univariate unadjusted HR (95% CI)	p	multivariate model (95%CI)	p
age at onset (per 10 yrs. increase of age)	1.1 (<1.0-1.2)	0.11	1.2 (1.1-1.3)	<0.01
race (ref=caucasian)				
asian	0.9 (0.3-2.4)	0.8	1.1 (0.4-2.9)	0.88
black	2.4 (1.1-5.4)	0.03	2.4 (1.1-5.6)	0.03
other/undefined	1.4 (1.1-1.8)	0.04	1.3 (0.9-1.7)	0.14
C. DIFFUSE CUTANEOUS INVOLVEMENT				
male	1.7 (1.4-2.2)	<0.01	1.4 (1.1-1.8)	0.01
ACA-	4.2 (3.3-5.2)	<0.01	2.8 (2.1-3.8)	<0.01
ATA+	3.3 (2.7-4.0)	<0.01	1.7 (1.3-2.1)	<0.01
age at onset (per 10 yrs. increase of age)	0.9 (0.8-0.9)	<0.01	1.0 (0.9-1.0)	0.21
race (ref=caucasian)				
asian	1.9 (0.9-3.9)	0.07	2.3 (1.2-4.7)	0.02
black	3.6 (1.5-8.7)	<0.01	3.2 (1.3-7.7)	0.01
other/undefined	1.3 (1.0-1.8)	0.04	1.3 (0.9-1.7)	0.12
D. PULMONARY HYPERTENSION				
male	1.6 (1.2-2.0)	<0.01	1.5 (1.2-2.0)	0.01
ACA-	1.3 (1.1-1.6)	0.01	1.2 (0.9-1.6)	0.13
ATA+	1.4 (1.1-1.6)	<0.01	1.4 (1.1-1.8)	0.01
age at onset (per 10 yrs. increase of age)	1.4 (1.4-1.6)	<0.01	1.5 (1.4-1.7)	<0.01
race (ref=caucasian)				
asian	0.6 (0.2-1.8)	0.34	0.8 (0.2-2.4)	0.65
black	0.8 (0.2-2.4)	0.65	1.3 (0.4-3.9)	0.70
other/undefined	1.0 (0.7-1.3)	0.82	1.1 (0.8-1.5)	0.54

ACA=anti-centromere antibody; ATA=anti-topoisomerase I antibody; HR=hazard ratio
Interactions ('male*ATA+' and 'male*ACA-') were checked, but non-significant.

Discussion

Our data confirms, in two different SSc cohorts, that autoantibody distribution differs with sex, with males being more often ATA+ and less often ACA+ compared to females. Although the population of SSc mainly consists of females, males show increased mortality. In our analyses, we demonstrate that increased mortality among males cannot be explained by a different auto-antibody distribution, being more often ATA+. Notably, the survival of ATA+ females is better than the survival of any of the male risk-groups. Specifically, the presented multivariate analyses show that male sex is the factor with the strongest effect on survival. Additionally, we show that also dcSSc and PH occur more often in SSc males compared to females, independent of autoantibodies. On the contrary, development of ILD is most strongly associated with ATA positivity.

Strikingly, although males comprise only 19% of the total population under study, males account for 39% of all deaths. Currently, the factors underlying this observed morbidity-mortality paradox are not clear. We can only speculate that sex hormones and male-female differences in microcirculation, immunity actors, environmental factors and/or fibroblasts may be involved (20, 21). A morbidity-mortality sex paradox has been observed in several diseases that share features observed in SSc, such as idiopathic PAH (iPAH)(22), interstitial pulmonary fibrosis (IPF) (23) and systemic lupus erythematosus (SLE) (24). In iPAH, hemodynamics are worse in male patients, with higher right arterial pressures and lower cardiac index observed (22). Possibly, more increased endothelial stiffness is present in male SSc. This might affect the lethality of complications such as PAH and ILD in SSc, but may also lead to increased cardiovascular events not directly related to SSc in male subjects. As these sex differences also occur in the bleomycin mouse model for SSc (21), further research in this laboratory setting may help to elucidate the underlying factors explaining more severe disease in males.

In line with our observations, various other studies have indicated that males with SSc have a worse prognosis than females with SSc (4, 5, 25, 26). We confirm the results of Wangkaew et al. (27) and Hoffman-Vold et al. (7), showing that male sex is not influencing the development of ILD, taking into account auto-antibody status. Like our study, a previous EUSTAR analysis evaluating mortality in SSc, also showed a gender gap in SSc survival and PH occurrence (26). However, the investigators hypothesized that the gender gap might reflect increased comorbidity in males, as in their analyses, SSc related mortality was comparable between males and females. The latter might be due to analyses approach taken as the evaluation of SSc-related mortality only included patients that died during follow-up. In addition, the analysis did not account for left-truncation, lead-time and survival bias.

Survival in SSc seems to improve when the time to diagnosis shortens (28). However, we and other authors observed that SSc males have a shorter time to diagnosis than female SSc patients (29, 30). This, therefore, is likely not explaining the sex paradox observed. Nevertheless, this information is important for the interpretation of other studies that identify predictors of mortality: As male patients tend to be diagnosed earlier and die from complications early in the disease course (indicating a higher prevalence of rapidly progressive disease), the chances for males to be included in inception cohorts are increased compared to prevalent cohorts. In incident cohorts, mild SSc cases may be underrepresented, as a delay between first symptoms and confirmation of diagnosis is more likely to occur, while for inclusion the duration of non-Raynaud's may not exceed the defined time period for incident disease. Influenced by this bias, studies identifying predictors of mortality and progressive disease in inception cohorts recognize male sex as a risk factor for mortality (5, 31), while similar studies in prevalent cohorts (not taking into account survival and lead time bias) do not (32, 33). The analysis we present in this study, with survival analysis using adjustment for left-truncation to correct for possible survival bias is therefore additive to the field, providing the opportunity to approach the effects of sex and auto-antibody status on disease outcomes in a more balanced way.

Our analyses have also limitations, which should be taken into account. For the current study, we did not consider other autoantibodies than ATA and ACA. We chose to focus on ACA antibodies and ATA antibodies as these are most prevalent and cover 75% of the population under study. We did not address male-female differences that might be present in other auto-antibody groups (such as RNA polymerase III and Pm/Scl). Presence of other, yet unknown or unmeasured, auto-antibodies in these risk groups cannot be ruled out, but given the rarity of co-expression of different auto-antibodies in SSc it is unlikely to influence the results. Also, in the identification of PH, it is likely some of the identified cases in fact represent false positives, as in EUSTAR PH is defined as a yes/no variable based on echocardiographic findings instead of right heart catheterization. Moreover, although we selected patients fulfilling the ACR/EULAR 2013 classification criteria, aiming to include also milder cases, selection bias might still have occurred by a possible lower inclusion of very mild SSc in the EUSTAR cohort; 50% of the EUSTAR population had diffuse skin involvement. Finally, based on predefined inclusion criteria, we had to exclude 50% of the existing EUSTAR cohort. However, as we specifically chose to focus on the independent effects of sex and antibody status, we preferred a complete case analysis. At the same time, the current study demonstrates the importance and possibilities offered by the EUSTAR database enabling complex survival analyses in a rare and heterogeneous disease. Moreover, in the CCISS cohort the same observations were made, while exclusion in this cohort was mainly based on short follow-up instead of missing data.

To conclude, male sex is an independent and strong risk factor for mortality in SSc and additionally is independently associated with diffuse skin fibrosis and PH. This indicates that sex-factors contribute to the disease phenotype and the lethality of disease complications. These observations therefore point to the possibility to influence sex-related factors for therapy.

Supporting information

Supplementary data can be obtained by contacting the first author.

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