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Sex-specific risk of anti-topoisomerase antibodies on mortality and disease severity in systemic sclerosis: 10-year analysis of the Leiden CCISS and EUSTAR cohorts

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Summary

Background We aimed to evaluate sex-specific risk of anti-topoisomerase I antibodies (ATA) on mortality, diffuse cutaneous systemic sclerosis, interstitial lung disease, and pulmonary hypertension in two cohorts of people with systemic sclerosis.

Methods This study was a 10-year analysis of the prospective Leiden Combined Care in Systemic Sclerosis (CCISS) cohort in the Netherlands and the international European Scleroderma Trials and Research (EUSTAR) cohort. We included participants with systemic sclerosis according to the 2013 American College of Rheumatology–European League Against Rheumatism (ACR–EULAR) classification criteria; available autoantibody status; available skin subtyping; at least one available radiographic assessment of interstitial lung disease; and with a known date of disease onset. People with systemic sclerosis were categorised in six risk groups by sex and autoantibody status (anti-centromere antibody [ACA]-positive female, ACA-positive male, ACA and ATA-negative female, ACA and ATA-negative male, ATA-positive female, and ATA-positive male). We constructed Kaplan-Meier curves and Cox proportional hazard models, accounting for left-truncated survival to prevent bias because the date of disease onset (first non-Raynaud's symptom) preceded the date of cohort entry for all patients. The primary outcome was all-cause mortality and the secondary outcomes were diffuse cutaneous systemic sclerosis, interstitial lung disease, and pulmonary hypertension.

Findings 445 (63%) of 708 participants between April 1, 2009, and Jan 1, 2022, in CCISS (101 [23%] male and 344 [77%] female) and 4263 (50%) of 8590 between June 1, 2004, and March 28, 2018, in EUSTAR (783 [18%] male and 3480 [82%] female) were eligible for this study. In both cohorts, ATA expression occurred significantly more often in males than in females (39 [39%] of 101 males *vs* 67 [19%] of 344 females in CCISS; p<0·0001 and 381 [49%] of 783 males *vs* 1323 [38%] of 3480 females in EUSTAR; p<0·0001). According to estimated survival rates, 30% of ATA-positive males versus 12% of ATA-positive females died in the CCISS cohort and 33% versus 15% died in the EUSTAR cohort within 10 years. After adjustment for age, race, and autoantibody status, male sex remained the most important risk factor for all-cause mortality (HR 2·9 [95% CI 1·5–5·5] in CCISS, p=0·0018; and HR 2·6 [2·0–3·4] in EUSTAR, p<0·0001).

Interpretation We show that the association between male sex and increased mortality in systemic sclerosis cannot be explained by higher ATA prevalence. However, additional research on the effect of sex-specific characteristics on people with systemic sclerosis is required.

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Introduction

Systemic sclerosis is a rare and heterogeneous disease clinically characterised by Raynaud's phenomenon, skin and pulmonary fibrosis, and cardiac and gastrointestinal dysfunction.[1] The disease has a complex pathophysiology.[1] Dysregulation of the immune system is evidenced by presence of specific antinuclear antibodies that have clinical and prognostic association.[2,3] For example, anti-topoisomerase I antibodies (ATA) have been identified as a risk factor for increased disease severity with progressive skin and lung involvement (ie, interstitial lung disease),[4–6] whereas anti-centromere antibodies (ACA) are associated with limited cutaneous systemic sclerosis, gastrointestinal involvement, and a lower likelihood of pronounced interstitial lung disease.[3,4]

Although systemic sclerosis is less frequent in males and few longitudinal studies have focused on the differential prognostic factors since disease onset between males and females with systemic sclerosis, the risk of severe outcomes is higher in males than females.[7,8] Males have higher ATA prevalence than females with systemic sclerosis,[9–12] which is intriguing given that the disease is predominant in females. Based on this sex-specific distribution of systemic sclerosis-specific autoantibodies, we hypothesised that the differences in outcomes observed between males and females with systemic

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For the **EUSTAR collaborators** see https://eustar.org

Research in context

Evidence before this study

We systematically searched MEDLINE (via PubMed), Web of Science, Cochrane, and Embase on the prognostic value of anti-topoisomerase I antibodies (ATA) and anti-centromere antibodies for outcomes in patients with systemic sclerosis including all original research published from database inception to Nov 13, 2020. We included the terms "anti-topoisomerase", "anti-centromere", "prognosis", and "systemic-sclerosis". We found that in most studies males with systemic sclerosis expressed ATA more often than females, male patients had worse disease outcomes, and presence of ATA was associated with worse disease outcomes. None of the longitudinal studies examining prognostic factors in people with systemic sclerosis adjusted for confounding caused by different autoantibody distribution among sexes or accounted for possible survival and lead time bias.

Added value of this study

In this study, we have examined the sex-specific risks of ATA in people with systemic sclerosis on mortality in two independent prospective cohorts over 10 years of follow-up. Diffuse skin involvement, interstitial lung disease, and pulmonary hypertension were evaluated as secondary outcomes. Contrary to previous prognostic cohort studies in people with systemic sclerosis, we performed left truncation to prevent survival bias because time of disease onset (defined as time since developing the first non-Raynaud symptom) predated cohort entry for all patients. As anticipated, males with systemic sclerosis expressed ATA more frequently and showed increased mortality compared with females in the Leiden CCISS and EUSTAR cohorts. We show that increased mortality among males cannot be explained by a higher frequency of ATA positivity. Male sex remained the most important risk factor for mortality after adjusting for autoantibody status, age at disease onset, and race. Additionally, we show that diffuse cutaneous systemic sclerosis and pulmonary hypertension occur more frequently in males than females with systemic sclerosis. We show that development of severe interstitial lung disease is strongly associated with ATA positivity, whereas the effect of sex is small.

Implications of all the available evidence

We show that the strong association between male sex and increased mortality in systemic sclerosis cannot be explained by a higher ATA prevalence. This finding is relevant for clinical practice to underline that survival of females with ATA expression is higher than in any of the male subgroups. Additional research investigating the effect of sex-specific characteristics on systemic sclerosis is required. ATA positivity is associated with the development of severe interstitial lung disease, which underlines the notion that the topoisomerase I-specific immune response is associated with progression of systemic sclerosis-associated interstitial lung disease. This finding might indicate a link between the ATA response and the presence and severity of interstitial lung disease. The sex-specific effect on severity of systemic sclerosis observed in this study will affect disease prognostication in clinical practice and might affect the design of clinical cohort studies and trials in people with systemic sclerosis.

sclerosis are partly explained by autoantibody expression. No previous studies have evaluated a possible interaction between presence of ATA, sex, and disease outcomes including mortality, interstitial lung disese, diffuse cutaneous skin involvement, and pulmonary hypertension. Additionally, cohort studies in people with systemic sclerosis might contain survival and lead time bias if patients are included after disease onset because rapid progression can result in disease-related death within a few months. This is particularly relevant for ATA-positive patients, in whom rapid progression is more frequently observed. Therefore, we aimed to evaluate sex-specific risk of ATA in two different cohorts (the Combined Care in Systemic Sclerosis [CCISS] cohort and European Scleroderma Trials and Research [EUSTAR] cohort) of people with systemic sclerosis.

Methods

Study design and participants

This study was a 10-year analysis of the prospective Leiden CCISS and EUSTAR cohorts. Data were analysed from consecutive people with systemic sclerosis included in the CCISS of the Leiden University Medical Center in the Netherlands between April 1, 2009, and Jan 1, 2022. The CCISS cohort included prospective data, standardised and extensive annual follow-up, and high degree of data completeness. ATA samples were measured by fluorescence ELISA using a Phadia250 system (ThermoFisher Scientific, Waltham, MA, USA). All patients provided written informed consent. The Leiden CCISS cohort was approved by the Leiden Ethics Committee (REU 043/SH/sh).[13]

The EUSTAR database is a multinational, prospective, cohort with longitudinal follow-up that occurred between June 1, 2004, and March 28, 2018.[3,14] At the time of data extraction, 14 998 patients were recorded in the database. The Leiden CCISS patients were excluded from the EUSTAR dATAet. Presence of ATA was determined as part of routine clinical care at participating centres. Ethical approval was obtained by the participating centres. All patients provided written informed consent.

Participants from both cohorts meeting the following criteria were included in the analysis: fulfilment of the 2013 American College of Rheumatology–European League Against Rheumatism (ACR–EULAR) classification criteria for systemic sclerosis;[15] available autoantibody status (anti-nuclear antibody [ANA], ATA, and ACA); available skin subtyping (defined by Medsger and Leroy[16]

as a subdivision of people with limited and diffuse cutaneous systemic sclerosis); at least one available radiographic assessment of interstitial lung disease by chest x-ray or high-resolution CT at baseline or during follow-up; and with a known date of disease onset defined as time since developing the first non-Raynaud symptom (4614 [98%] of 4708 patients) or first Raynaud symptom (when the date of first non-Raynaud was missing; 94 [2%] of 4708). Patients were excluded if they simultaneously had more than one type of systemic sclerosis-specific autoantibodies (including ATA, ACA, RNA polymerase III, Pm-Scl, U1RNP, or U3RNP), had no available follow-up data after baseline, or with disease duration of more than 10 years at inclusion.

Procedures

People with systemic sclerosis were categorised in six risk groups by sex and autoantibody status (ACA-positive female, ACA-positive male, ACA and ATA-negative female, ACA and ATA-negative male, ATA-positive female, and ATA-positive male). Survival time from the date of disease onset was registered in each database, including whether the death was related to systemic sclerosis in EUSTAR. Severe lung involvement was defined as a threshold of 50% or lower predicted forced vital capacity or diffusion capacity of the lung for carbon-monoxide, or both (chosen because this threshold corresponds with a score of ≥2 on the Medsger Disease Severity Scale),[17] accompanied by the presence of lung fibrosis or ground-glass opacities, or both, as evaluated by high-resolution CT. Patients were classified as having diffuse cutaneous systemic sclerosis according to their skin pattern, even in cases of later improvement to a limited skin pattern. Pulmonary hypertension in the CCISS cohort was based on right heart catheterisation with patients selected using the DETECT algorithm[18] and a multidisciplinary discussion between cardiologists, pulmonologists, internal medicine specialists, and rheumatologists. In the EUSTAR database, presence of pulmonary hypertension was registered by the recording physician using echocardiography or right heart catheterisation.

For the primary outcome, we evaluated all-cause mortality. For secondary outcomes, we focused on the development of severe interstitial lung disease, diffuse skin involvement (ie, diffuse cutaneous systemic sclerosis), and pulmonary hypertension.

Statistical analysis

Baseline characteristics of the six risk-groups were compared using χ^2 tests or ANOVA, as appropriate, for both cohorts. Follow-up started at date of disease onset (first non-Raynaud's symptom) instead of cohort entry to prevent survival bias in the analyses. Patients were censored at the time of the last visit or after 10 years of disease duration. Because the date of disease onset predated the date of cohort entry for all patient's, left truncation was performed in the survival analyses.

Kaplan-Meier analyses were used to construct survival curves and visualise the development of diffuse cutaneous systemic sclerosis, severe interstitial lung disease, and pulmonary hypertension over time. Curves were calculated separately for the six risk groups and compared using the log-rank test, and separately for sex and autoantibody status.

The Cox proportional hazard model was used to study the effect of sex and autoantibody status on mortality and time until development of severe interstitial lung disease,

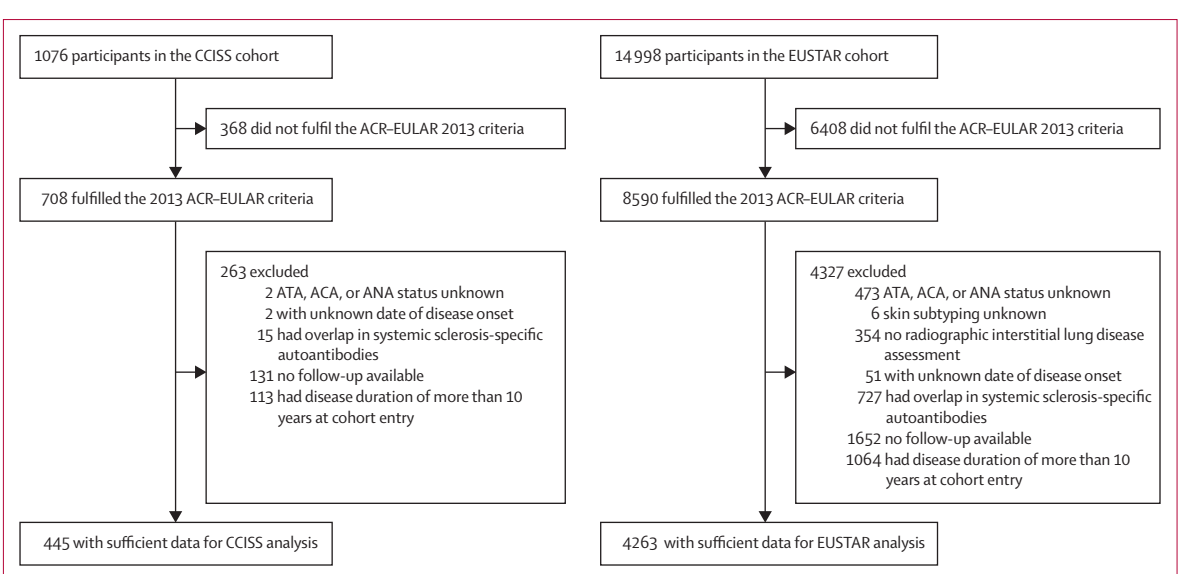

Figure 1: **Participant inclusion of the Leiden CCISS and EUSTAR cohorts**
ACA=anti-centromere antibodies. ACR/EULAR=European League Against Rheumatism and the American College of Rheumatology. ANA=anti-nuclear antibody. ATA=anti-topoisomerase I antibodies. CCISS=Combined Care in Systemic Sclerosis. EUSTAR=European Scleroderma Trials and Research group.

	ACA-positive female	ACA-positive male	ACA and ATA-negative female	ACA and ATA-negative male	ATA-positive female	ATA-positive male
Leiden CCISS cohort (n=445)						
Median disease duration at cohort entry, years	1·7 (0·0–9·7)	2·7 (0·0–9·9)	1·5 (0·0–9·8)	1·6 (0·0–8·7)	1·7 (0·0–9·6)	0·6 (0·0–9·2)
Mean age at disease onset, years	52 (14)	52 (13)	50 (15)	51 (12)	47 (16)	50 (12)
Race						
White	147/165 (89%)	13/15 (87%)	99/112 (88%)	41/47 (87%)	50/67 (75%)	36/39 (92%)
Black	0	0	3/112 (3%)	2/47 (4%)	2/67 (3%)	1/39 (3%)
Asian	5/165 (3%)	1/15 (7%)	2/112 (2%)	3/47 (6%)	3/67 (4%)	1/39 (3%)
Other or undefined	13/165 (8%)	1/15 (7%)	8/112 (7%)	1/47 (2%)	12/67 (18%)	1/39 (3%)
Smoking (ever)	94/165 (57%)	10/15 (67%)	62/112 (55%)	29/47 (62%)	29/67 (43%)	30/39 (77%)
Diffuse cutaneous systemic sclerosis	2/165 (1%)	1/15 (7%)	33/112 (29%)	26/47 (55%)	28/67 (42%)	21/39 (54%)
Severe interstitial lung disease	2/165 (1%)	0	20/112 (18%)	10/47 (21%)	14/67 (21%)	10/39 (26%)
Pulmonary hypertension	4/165 (2%)	0	3/112 (3%)	2/47 (4%)	2/67 (3%)	0
Renal crisis	0	0	7/112 (6%)	1/47 (2%)	2/67 (3%)	2/39 (5%)
EUSTAR cohort (n=4263)						
Median disease duration at cohort entry, years	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)
Mean age at disease onset, years	53 (13)	54 (13)	47 (14)	50 (13)	46 (14)	47 (13)
Race						
White	1202/1380 (87%)	123/130 (95%)	625/777 (80%)	242/272 (89%)	1066/1323 (81%)	334/381 (88%)
Black	8/1380 (1%)	0	25/777 (3%)	3/272 (1%)	49/1323 (4%)	2/381 (1%)
Asian	15/1380 (1%)	0	21/777 (3%)	3/272 (1%)	16/1323 (1%)	6/381 (2%)
Other or undefined	155/1380 (11%)	7/130 (5%)	106/777 (14%)	24/272 (9%)	192/1323 (15%)	39/381 (10%)
Smoking (ever)*	361/1149 (31%)	72/114 (63%)	257/632 (41%)	144/217 (66%)	204/1000 (20%)	172/285 (60%)
Diffuse cutaneous systemic sclerosis	101/1380 (7%)	14/130 (11%)	349/777 (45%)	147/272 (54%)	766/1323 (58%)	261/381 (69%)
Severe interstitial lung disease†	41/417 (10%)	11/38 (29%)	88/438 (20%)	40/161 (25%)	221/1034 (21%)	95/276 (34%)
Pulmonary hypertension‡	189/1256 (15%)	16/110 (15%)	82/679 (12%)	34/228 (15%)	159/1202 (13%)	58/342 (17%)
Renal crisis§	12/1372 (1%)	0/127	23/771 (3%)	17/269 (6%)	20/1307 (2%)	4/378 (1%)

Data are median (min–max), mean (SD), or n (%). ACA=anti-centromere antibodies. ATA=anti-topoisomerase I antibodies. CCISS=Combined Care in Systemic Sclerosis. EUSTAR=European Scleroderma Trials and Research group. *Missing in 866 (20%) of 4263 patients in the EUSTAR cohort. †Missing in 1899 (45%). ‡Missing in 446 (10%). §Missing in 39 (1%).

Table 1: **Baseline characteristics**

diffuse skin involvement (ie, diffuse cutaneous systemic sclerosis), and pulmonary hypertension, while adjusting for potential confounders. The hypothesis was that immune dysregulation underlying autoantibody expression might contribute to disease complications and there might be a statistically significant interaction with sex. Possible confounders in this model were factors that influence antibody expression (exposure) and outcome (mortality); therefore, sex, race, and age were identified as possible confounders. No data are available to suggest possible confounders for other lifestyle factors, including smoking and obesity. An interaction term between sex and autoantibody group was added to evaluate whether the effect of ATA is different in males versus females. These models were performed for both cohorts, except for pulmonary hypertension in the CCISS cohort due to low numbers of pulmonary hypertension events. The proportional hazard assumption was verified by log-minus-log survival plots and use of Schoenfeld's global test. Based on Schoenfeld residuals assessment, no deviations in proportionality were detected for all outcomes in both cohorts. Because we specifically chose to focus on the independent effects of sex and antibody status, complete case analysis was preferred. Missing data were handled by treating data availability as an inclusion criterion. All statistical tests were two-sided with an α of 0·05 and analyses were performed in Stata (version 16).

Role of the funding source
There was no funding source for this study.

Results

From the Leiden CCISS cohort, 708 participants with systemic sclerosis fulfilled the ACR–EULAR criteria[15] between April 1, 2009, and Jan 1, 2022, of whom 445 (63%) were eligible for this study. From the EUSTAR cohort, 8590 participants with systemic sclerosis fulfilled the ACR–EULAR criteria[15] between June 1, 2004, and March 28, 2018, of whom 4263 (50%) were eligible for this study (figure 1). In both cohorts, patients who were included had diffuse cutaneous systemic sclerosis more and ACA positivity less often at baseline than those who were excluded.

Of 445 CCISS cohort participants, 101 (23%) were male and 344 (77%) were female. This cohort included 165 (37%) ACA-positive females, 15 (3%) ACA-positive males, 112 (25%) ACA and ATA-negative females, 47 (11%) ACA and ATA-negative males, 67 (15%) ATA-positive females, and 39 (9%) ATA-positive males. Baseline characteristics are shown in table 1 and the appendix (p 1). ATA occurred significantly more often in males than in females (39 [39%] of 101 males *vs* 67 [19%] of 344 females; p<0·0001), whereas ACA was significantly more common in females (165 [48%] of 344 females *vs* 15 [15%] of 101 males; p<0·0001).

An overview of available data and events in the CCISS cohort is shown in the appendix (p 2). Kaplan-Meier survival curves show that mortality in all male risk groups was higher than in the female risk groups (figure 2A; log-rank p<0·0001). For development of severe interstitial lung disease, there were no clear statistically significant differences between males and females (figure 2B). Development of severe interstitial lung disease and diffuse cutaneous systemic sclerosis was rare in ACA-positive males and females (figure 2C).

After adjustment for age, race, and autoantibody status, all-cause mortality was still associated with male sex in the CCISS cohort (HR 2·9 [95% CI 1·5–5·5]; p=0·0018; table 2). The effect of ATA expression did not change after adjustment (ATA-positive HR 0·7 [95% CI 0·3–1·3]; p=0·24; and ACA-positive HR 0·2 [0·1–0·5]; p=0·0001). No significant interaction between ATA and sex for all-cause mortality was found (p=0·45). For severe interstitial lung disease, the HR was 0·9 (95% CI 0·4–2·1; p=0·88) for male sex, 1·5 (0·8–3·2; p=0·23) for ATA expression, and 0·1 (0·05–0·4; p=0·0001) for ACA expression.]; p=0·0001). Sex or ATA expression were not significantly associated with diffuse cutaneous systemic sclerosis in multivariable analyses, but a significant interaction was seen between sex and autoantibody status (p=0·0083).

Of 4263 EUSTAR cohort participants, 783 (18%) were male and 3480 (82%) were female. This cohort included 1380 (32%) ACA-positive females, 130 (3%) ACA-positive males, 777 (18%) ACA and ATA-negative females, 272 (6%) ACA and ATA-negative males, 1323 (31%) ATA-positive females, and 381 (9%) ATA-positive males. Baseline characteristics are shown in table 1 and the appendix (p 3). ATA expression occurred significantly more often in males than in females (381 [49%] of 783 males *vs* 1323 [38%]

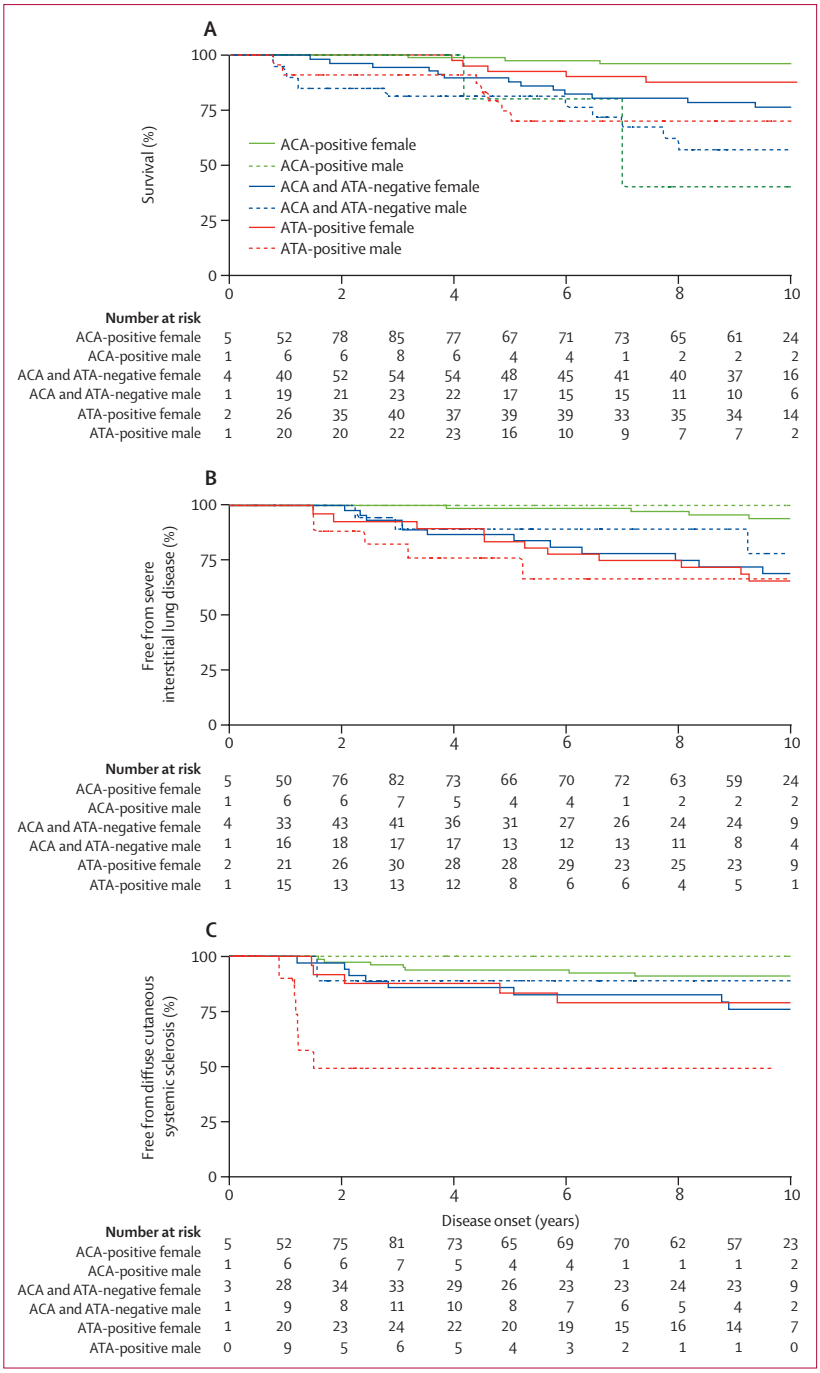

Figure 2: **Kaplan-Meier curves according to sex and autoantibody status in the Leiden CCISS cohort**
(A) Survival (mortality log-rank p<0·0001 for sex, p=0·00019 for the six risk groups, and p=0·0017 for autoantibody status). (B) Development of severe interstitial lung disease (log-rank p=0·37 for sex, p=0·0007 for the six risk groups, and p<0·0001 for autoantibody status). (C) Development of diffuse cutaneous systemic sclerosis (log-rank p=0·066 for sex, p<0·0001 for the six risk groups, and p=0·0039 for autoantibody status). ACA=anti-centromere antibodies. ATA=anti-topoisomerase I antibodies. CCISS=Combined Care in Systemic Sclerosis.

See **Online** for appendix

	Univariable unadjusted hazard ratio (95% CI)	p value	Multivariable model without interaction after adjustment (95% CI)	p value	Multivariable model with interaction after adjustment (95% CI)	p value
All-cause mortality						
Male (*vs* female)	3·4 (1·8–6·5)	<0·0001	2·9 (1·5–5·5)	0·0018	2·2 (0·9–5·3)	0·073
Autoantibody status (*vs* ACA and ATA-negative)						
ACA-positive	0·2 (0·1–0·5)	0·0011	0·2 (0·1–0·5)	0·0001	0·1 (0·04–0·5)	0·0017
ATA-positive	0·7 (0·3–1·4)	0·28	0·7 (0·3–1·3)	0·24	0·5 (0·2–1·5)	0·24
Age at onset, years*	2·3 (1·7–3·2)	<0·0001	2·6 (1·9–3·6)	<0·0001	2·6 (1·9–3·5)	<0·0001
White (*vs* non-White)	2·1 (0·6–6·7)	0·22	1·0 (0·3–3·3)	0·98	1·0 (0·3–3·4)	0·97
Male and autoantibody status (*vs* ACA and ATA-negative)	..	..	..	..	..	0·45†
ACA-positive	..	..	..	..	2·8 (0·4–21·4)	0·31
ATA-positive	..	..	..	..	1·5 (0·4–6·2)	0·58
Severe interstitial lung disease						
Male (*vs* female)	1·4 (0·6–3·2)	0·37	0·9 (0·4–2·1)	0·88	0·6 (0·2–2·1)	0·43
Autoantibody status (*vs* ACA and ATA-negative)						
ACA-positive	0·2 (0·1–0·5)	0·0015	0·1 (0·05–0·4)	0·0001	0·1 (0·04–0·4)	0·0006
ATA-positive	1·4 (0·7–2·9)	0·33	1·5 (0·8–3·2)	0·23	1·2 (0·5–2·8)	0·63
Age at onset, years*	1·2 (0·9–1·5)	0·17	1·4 (1·1–1·9)	0·011	1·4 (1·1–1·9)	0·0090
White (*vs* non-White)	0·7 (0·3–1·6)	0·35	0·4 (0·2–1·1)	0·067	..	..
Male and autoantibody status (*vs* ACA and ATA-negative)	..	..	..	..	..	0·41†
ACA-positive	..	..	..	..	0	..
ATA-positive	..	..	..	..	2·6 (0·5–13·5)	0·27
Diffuse cutaneous systemic sclerosis						
Male (*vs* female)	2·2 (0·9–5·3)	0·073	1·7 (0·7–4·2)	0·23	0·4 (0·1–3·6)	0·45
Autoantibody status (*vs* ACA and ATA-negative)						
ACA-positive	0·4 (0·1–1·1)	0·068	0·4 (0·2–1·2)	0·11	0·4 (0·1–1·0)	0·055
ATA-positive	1·8 (0·7–4·3)	0·20	1·8 (0·7–4·3)	0·21	0·8 (0·3–2·6)	0·76
Age at onset, years*	0·9 (0·7–1·2)	0·52	1·0 (0·7–1·3)	0·93	1·0 (0·7–1·3)	0·80
White (*vs* non-White)	0·8 (0·3–2·3)	0·68	0·8 (0·3–2·2)	0·61	0·6 (0·2–1·8)	0·34
Male and autoantibody status (*vs* ACA and ATA-negative)	..	..	..	..	..	0·0083†
ACA-positive	..	..	..	..	0	..
ATA-positive	..	..	..	..	11·8 (1·0–134·1)	0·047

ACA=anti-centromere antibodies. ATA=anti-topoisomerase I antibodies. CCISS=Combined Care in Systemic Sclerosis. *Per 10 years increase of age. †Likelihood ratio test between the multivariable model without interaction versus with interaction.

Table 2: **Hazard ratios for all-cause mortality, development of severe interstitial lung disease, and diffuse cutaneous systemic sclerosis in the Leiden CCISS cohort**

of 3480 females; p<0·0001), whereas ACA expression occurred less often in males (130 [17%] males *vs* 1380 [40%] females; p<0·0001), confirming the findings from the CCISS cohort.

According to estimated survival rates, 30% of ATA-positive males versus 12% of ATA-positive females died in the CCISS cohort and 33% versus 15% died in the EUSTAR cohort within 10 years. An overview of available data and events in the EUSTAR cohort is shown in the appendix (p 5). In EUSTAR, all-cause mortality was greater in males than in females (log-rank p<0·0001; figure 3) with clear differences between the risk groups (log-rank p<0·0001) and autoantibodies (log-rank p<0·0001). Similar findings were observed for systemic sclerosis-related mortality (appendix p 5). The development of severe interstitial lung disease (figure 3B) and diffuse cutaneous systemic sclerosis (figure 3C) was highest in ATA-positive males, followed by ATA-positive females. Development of pulmonary hypertension was most often seen in ACA and ATA-negative males and ATA-positive participants, in which males developed pulmonary hypertension more often than females (figure 3D).

After adjustment for age, race, and autoantibody status, male sex remained the most important risk factor for all-cause mortality in EUSTAR (male HR 2·6 [95% CI 2·0–3·4], p<0·0001; ATA-positive HR 1·3 [1·0–1·8], p=0·059; ACA-positive HR 0·5 [0·3–0·7], p=0·00011; table 3). The development of severe interstitial lung disease was not significantly associated with sex, but the adjusted ATA expression had significantly increased (ATA-positive HR 1·9 [95% CI 1·5–2·5], p<0·0001; ACA-positive HR 0·3 [0·2–0·4]; p<0·0001). For the other secondary outcomes, males still had a higher risk to develop diffuse cutaneous systemic sclerosis after adjustment (male HR 1·4 [95% CI 1·1–1·8]; p=0·0064) and pulmonary hypertension (HR 1·5 [1·2–2·0]; p=0·00051). Diffuse cutaneous systemic sclerosis and pulmonary hypertension remained significantly associated with ATA expression after adjustment for age, sex, and race (table 3). In the EUSTAR cohort, interaction between sex and autoantibody status was not statistically significant for any of the outcomes.

Discussion

Our 10-year analysis of two different systemic sclerosis cohorts shows that autoantibody distribution differs according to sex, with males being predominantly ATA-positive and less frequently ACA-positive than females. Although most people with systemic sclerosis are female, males show increased mortality. In our analyses adjusted for survival bias, we show that increased mortality among males cannot be explained by a different autoantibody distribution, such as ATA-positive status. This finding is underlined by longer survival of ATA-positive females than that of any male risk group in this study and emphasised by multivariable analyses, which showed that male sex is the strongest risk factor for mortality. Additionally, we show that diffuse cutaneous systemic sclerosis and pulmonary hypertension occur primarily in males with systemic sclerosis after adjument for autoantibodies. Contrary to these findings, the development of severe interstitial

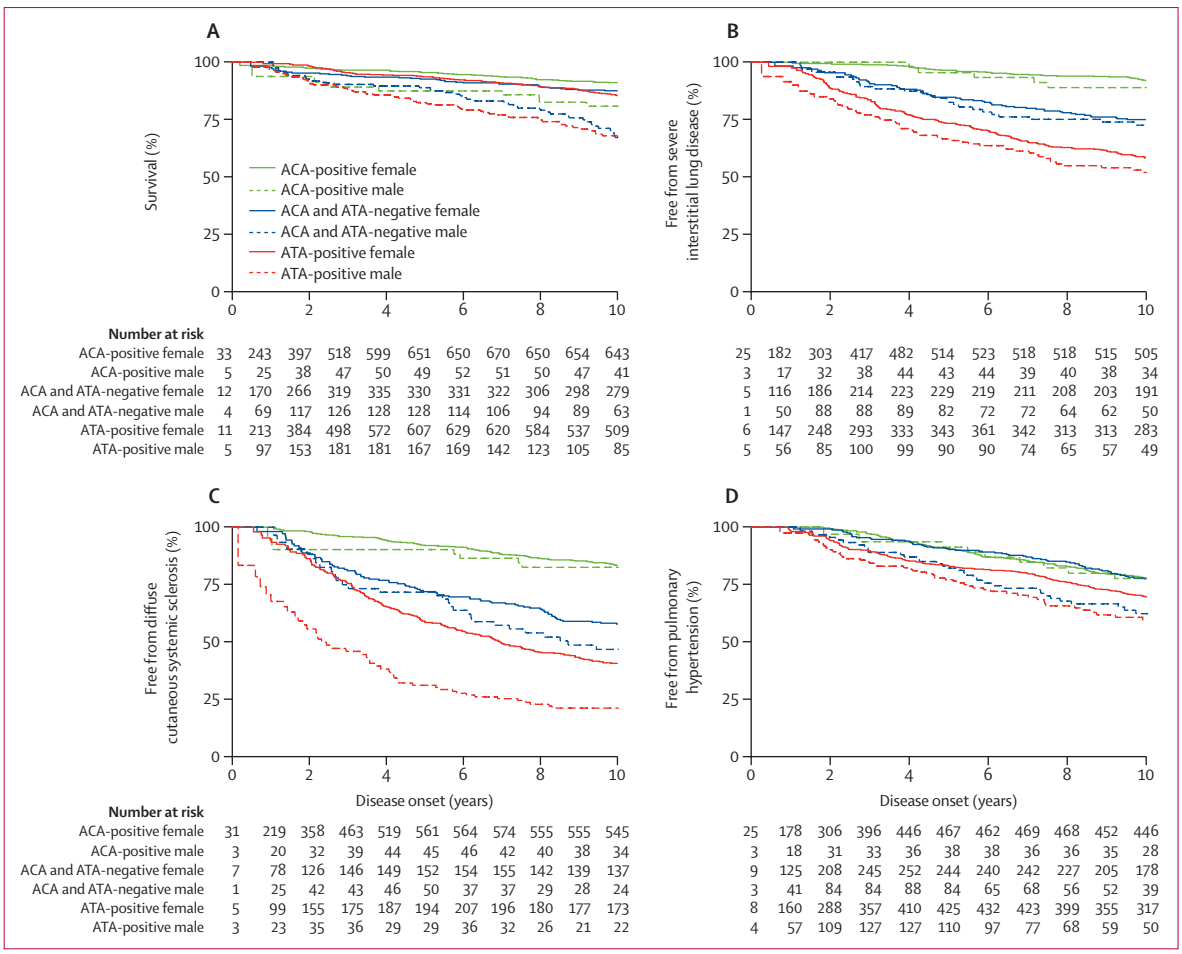

Figure 3: **Kaplan Meier curves according to sex and autoantibody status in the EUSTAR cohort**
(A) Survival (mortality log-rank p<0·0001 for sex, p<0·0001 for the six risk groups, and p<0·0001 for autoantibody status). (B) Development of severe interstitial lung disease (log-rank p=0·0027 for sex, p<0·0001 for the six risk groups, and p<0·0001 for autoantibody status). (C) Development of diffuse cutaneous systemic sclerosis (log-rank p<0·0001 for sex, p<0·0001 for the six risk groups, and for autoantibody status p<0·0001). (D) Development of pulmonary hypertension (log-rank p=0·0021 for sex, p=0·00020 for the six risk groups, and p=0·0086 for autoantibody status). ACA=anti-centromere antibodies. ATA=anti-topoisomerase I antibodies. EUSTAR=European Scleroderma Trials and Research group.

lung disease after adjustment for potential confounders was associated with ATA positivity, but not with sex.

Various other studies have indicated that males with systemic sclerosis have a worse prognosis than females.[3,7,19,20] The primary outcome of this study was all-cause mortality. To increase the understanding of disease-specific outcomes, we evaluated mortality related to systemic sclerosis; however, this investigation comes with important limitations[21] and thus was performed as a sensitivity analysis (appendix pp 5–7). In accordance with our results for all-cause mortality, systemic sclerosis-related mortality was higher in males than females in the EUSTAR cohort. These findings are in line with the RESCLE cohort[8] but not with the previous EUSTAR analysis,[20] in which all cause-mortality was higher in males and systemic sclerosis-related mortality was similar between males and females. In the EUSTAR analysis,[20] the investigators hypothesised that this difference might reflect increased comorbidity in males. However, this observation might also be explained by the chosen approach—ie, the fact that evaluation of systemic sclerosis-related mortality only included participants who died during follow-up and thus, did not account for survival bias. The survival analysis in our study used left truncated data to correct for possible survival bias and thus, adds to the field of systemic sclerosis.

Survival of people with systemic sclerosis seems to improve when the time to diagnosis is shorter.[22] However, males with systemic sclerosis have a shorter time to diagnosis than females;[23,24] therefore, the observed sex prevalence and severity paradox probably cannot be explained by time to diagnosis. Nevertheless, this aspect is important for the interpretation of other studies that identify predictors of mortality. Males are more likely to be included in inception cohorts than in prevalent cohorts because they tend to be diagnosed earlier and are more

	Univariable unadjusted hazard ratio (95% CI)	p value	Multivariable model without interaction after adjustment (95% CI)	p value	Multivariable model with interaction after adjustment (95% CI)	p value
All-cause mortality						
Male (*vs* female)	2·9 (2·3–3·8)	<0·0001	2·6 (2·0–3·4)	<0·0001	3·2 (0·4–1·3)	0·35
Autoantibody status (*vs* ACA and ATA-negative)						
ACA-positive	0·5 (0·4–0·7)	<0·0001	0·5 (0·3–0·7)	0·00011	0·6 (0·4–0·9)	0·011
ATA-positive	1·2 (0·9–1·5)	0·31	1·3 (1·0–1·8)	0·059	1·5 (1·0–2·2)	0·045
Age at onset, years*	1·7 (1·5–1·8)	<0·0001	1·8 (1·6–2·0)	<0·0001	1·8 (1·6–2·0)	<0·0001
Race (*vs* White)						
Asian	0·7 (0·2–2·3)	0·59	1·1 (0·3–3·3)	0·92	1·0 (0·3–3·3)	0·94
Black	0·7 (0·2–3·0)	0·67	1·4 (0·4–5·8)	0·62	1·5 (0·4–5·9)	0·59
Other or undefined	0·6 (0·4–0·9)	0·020	0·7 (0·5–1·1)	0·13	0·7 (0·5–1·1)	0·13
Male and autoantibody status (*vs* ACA and ATA-negative)	..	..	..	..	..	0·55†
ACA-positive	..	..	..	..	0·7 (0·3–1·6)	0·37
ATA-positive	..	..	..	..	0·8 (0·4–1·3)	0·35
Severe interstitial lung disease						
Male (*vs* female)	1·5 (1·1–1·9)	0·0029	1·2 (0·9–1·5)	0·25	1·2 (0·5–1·7)	0·89
Autoantibody status (*vs* ACA and ATA-negative)						
ACA-positive	0·3 (0·2–0·5)	<0·0001	0·3 (0·2–0·4)	<0·0001	0·3 (0·2–0·4)	<0·0001
ATA-positive	1·8 (1·4–2·4)	<0·0001	1·9 (1·5–2·5)	<0·0001	1·9 (1·4–2·6)	<0·0001
Age at onset, years*	1·1 (1·0–1·2)	0·11	1·2 (1·1–1·3)	<0·0001	1·2 (1·1–1·3)	<0·0001
Race (*vs* White)						
Asian	0·9 (0·3–2·4)	0·80	1·1 (0·4–2·9)	0·88	1·1 (0·4–2·9)	0·88
Black	2·4 (1·1–5·4)	0·034	2·5 (1·1–5·6)	0·030	2·5 (1·1–5·6)	0·030
Other or undefined	1·4 (1·1–1·8)	0·036	1·3 (0·9–1·7)	0·14	1·2 (0·9–1·7)	0·14
Male and autoantibody status (*vs* ACA and ATA-negative)	..	..	..	..	..	0·85†
ACA-positive	..	..	..	..	1·3 (0·5–3·6)	0·64
ATA-positive	..	..	..	..	1·0 (0·5–1·7)	0·89
Diffuse cutaneous systemic sclerosis						
Male (*vs* female)	1·7 (1·4–2·2)	<0·0001	1·4 (1·1–1·8)	0·0064	1·5 (0·9–2·3)	0·090
Autoantibody status (*vs* ACA and ATA-negative)						
ACA-positive	0·3 (0·2–0·4)	<0·0001	0·4 (0·3–0·5)	<0·0001	0·4 (0·3–0·5)	<0·0001
ATA-positive	1·6 (1·3–2·1)	<0·0001	1·7 (1·3–2·1)	<0·0001	1·7 (1·3–2·2)	0·00034
Age at onset, years*	0·9 (0·8–0·9)	<0·0001	1·0 (0·9–1·0)	0·21	1·0 (0·9–1·0)	0·21
Race (*vs* White)						
Asian	1·9 (0·9–3·9)	0·070	2·3 (1·2–4·7)	0·018	2·3 (1·1–4·7)	0·020
Black	3·6 (1·5–8·7)	0·0046	3·2 (1·3–7·7)	0·011	3·1 (1·3–7·7)	0·012
Other or undefined	1·3 (1·0–1·8)	0·036	1·3 (0·9–1·7)	0·12	1·3 (0·9–1·7)	0·11
Male and autoantibody status (*vs* ACA and ATA-negative)	..	..	..	..	..	0·34†
ACA-positive	..	..	..	..	0·6 (0·2–1·5)	0·25
ATA-positive	..	..	..	..	1·1 (0·6–1·9)	0·78

(Table 3 continues on next page)

likely to die from complications early in the disease course (indicating a higher prevalence of rapidly progressive disease).[25] People with mild systemic sclerosis might be underrepresented in incident cohort studies because a delay between first symptoms and confirmation of diagnosis is more likely, whereas for inclusion, the

	Univariable unadjusted hazard ratio (95% CI)	p value	Multivariable model without interaction after adjustment (95% CI)	p value	Multivariable model with interaction after adjustment (95% CI)	p value
(Continued from previous page)						
Pulmonary hypertension						
Male (vs female)	1·6 (1·2–2·0)	0·00022	1·5 (1·2–2·0)	0·00051	1·9 (1·3–3·0)	0·0025
Autoantibody status (vs ACA and ATA-negative)						
ACA-positive	0·9 (0·7–1·2)	0·38	0·8 (0·6–1·1)	0·13	0·9 (0·7–1·3)	0·56
ATA-positive	1·3 (1·0–1·6)	0·079	1·4 (1·1–1·8)	0·011	1·5 (1·1–2·1)	0·010
Age at onset, years*	1·5 (1·4–1·6)	<0·0001	1·5 (1·4–1·7)	<0·0001	1·5 (1·4–1·7)	<0·0001
Race (vs White)						
Asian	0·6 (0·2–1·8)	0·34	0·8 (0·2–2·4)	0·65	0·8 (0·2–2·4)	0·63
Black	0·8 (0·2–2·4)	0·65	1·3 (0·4–3·9)	0·70	1·3 (0·4–4·0)	0·68
Other or undefined	1·0 (0·7–1·3)	0·82	1·1 (0·8–1·5)	0·54	1·1 (0·8–1·5)	0·54
Male and autoantibody status (vs ACA and ATA-negative)	..	..	..	..	..	0·23†
ACA-positive	..	..	..	..	0·5 (0·2–1·1)	0·098
ATA-positive	..	..	..	..	0·8 (0·5–1·4)	0·39

ACA=anti-centromere antibodies. ATA=anti-topoisomerase I antibodies. EUSTAR=European Scleroderma Trials and Research group. *Per 10 years increase of age. †Likelihood ratio test between the multivariable model without interaction versus with interaction.

Table 3: **Hazard ratios for all-cause mortality, development of severe interstitial lung disease, and diffuse cutaneous systemic sclerosis, and pulmonary hypertension in the EUSTAR cohort**

duration of non-Raynaud's symptoms might not exceed the defined timeframe for incident disease. Influenced by this bias, studies identifying predictors of mortality and progressive disease in inception cohorts recognise male sex as a risk factor for mortality,[7,26] whereas similar studies in prevalent cohorts do not (except for survival and lead time bias).[27,28] Although our results show that male sex remains a strong risk factor for mortality, diffuse cutaneous systemic sclerosis, and pulmonary hypertension in systemic sclerosis (after adjustment for ATA expression), we do not want to undermine the importance of autoantibodies specific to systemic sclerosis. We found that development of severe interstitial lung disease and diffuse cutaneous systemic sclerosis occur most frequently among ATA-positive males and females. Our results that the presence of ATA (adjusted for sex, age, and race) is associated with the development of severe interstitial lung disease, are in line with the findings by Wangkaew and colleagues[29] and Hoffman-Vold and colleagues.[6] ATA overrules sex as a risk factor, which could underline the notion that the topoisomerase I-specific immune response might be implicated in the development of fibrotic disease complications. Indeed, ATA has the most evidence in favour of a pathogenic role in disease progression of systemic sclerosis, and there are studies[30,31] suggesting that ATA has direct effect on fibroblasts. We observed that the presence of ATA and sex for the development of diffuse cutaneous systemic sclerosis in the CCISS cohort might further support this hypothesis and needs to be evaluated in other cohorts.

Furthermore, in the multivariable model without interaction after adjustment, we observe that pulmonary hypertension is significantly associated with male sex as well as ATA positivity in the EUSTAR cohort, but not with ACA positivity. This finding might be because we did not separate participants with pulmonary hypertension related to interstitial lung disease from those with pulmonary hypertension without interstitial lung disease.[32] We need to emphasise that the definition of pulmonary hypertension comes with important limitations. In EUSTAR, this definition does not necessarily include a right heart catheterisation and therefore, we could not separate pulmonary arterial hypertension from pulmonary hypertension related to interstitial lung disease. We still included pulmonary hypertension as a secondary outcome because it is one of the most severe complications of systemic sclerosis with high clinical relevance.

Another limitation includes the fact that we did not consider other autoantibodies. We chose to focus on ACA and ATA because these autoantibodies have the highest prevalence in people with systemic sclerosis. The presence of other unknown or unmeasured autoantibodies in these risk groups cannot be ruled out but was unlikely to influence results given the rarity of concurrent expression of different autoantibodies in people with systemic sclerosis. Additionally, we cannot exclude that some individuals had a false positive test for ATA.[33] However, because all patients included in this study were ANA positive and had a clinical diagnosis of systemic sclerosis, we do not think that our findings are driven by

false positive ATA results. Although we used left-truncated data to minimise the risk of survival bias, this method also comes with some limitations. For example, alive patients with follow-up of less than 5 years are included but depending on disease duration might not have developed disease complications yet, which is balanced by the fact that patients who die rapidly (<5 years of follow-up) are also considered. Additionally, wider 95% CIs show estimates with more uncertainty because less data are available.

Finally, selection bias should be considered when interpreting results on the basis of predefined inclusion criteria in our study, because we excluded 37% of participants with systemic sclerosis fulfilling ACR–EULAR 2013 criteria[15] in the Leiden CCISS cohort and 50% in the EUSTAR cohort. The main reason for exclusion was disease duration at cohort entry of more than 10 years, which explains why excluded participants had limited cutaneous systemic sclerosis and were ACA positive more often than included participants, because these people tend to have milder disease with longer survival compared with people with limited cutaneous systemic sclerosis. This observation underlines the importance of correction for survival bias in analyses. Many participants were excluded (53 [40%] of 131 in the CCISS cohort and 329 [20%] of 1652 in EUSTAR) due to absence of follow-up, because they joined the study less than 1 year before the end of the inclusion period, so their follow-up visits were scheduled after the inclusion period. Another reason for the observed differences between included and excluded participants seems to be the exclusion of those with combinations of systemic sclerosis-specific autoantibodies.[34] Therefore, these results should not be extrapolated to patients with autoantibody combinations. Our study shows the importance and possibilities offered by the EUSTAR database, enabling complex survival analyses for a rare and heterogeneous disease, which is underlined by the fact that exclusion in the CCISS cohort was based on short follow-up instead of missing data and similar observations were made between these two studies. By performing the same analyses in two independent cohorts, each with different strengths (sample size in EUSTAR *vs* high degree of data reliability and completeness in CCISS), and both showing the strong association between male sex and worse outcomes and between ATA positivity and interstitial lung disease, this study is unique in the evaluation of risk factors in people with systemic sclerosis.

To conclude, male sex is a strong risk factor for mortality in systemic sclerosis and is associated with diffuse skin fibrosis and pulmonary hypertension, even after adjustment for autoantibody status. The presence of ATA is associated with development of severe interstitial lung disease after adjustment for potential confounders, which might indicate that the topoisomerase I-specific immune response is implicated in the development or progression of systemic sclerosis-associated interstitial lung disease. Importantly, sex-specific factors probably contribute to the systemic sclerosis phenotype and disease complications. The increased male mortality and correlation of ATA-positivity with interstitial lung disease are relevant factors to consider when designing future cohort studies and clinical trials in people with systemic sclerosis. Reasons underlying this finding are still unclear and warrant further research. Ultimately, clinicians need to consider that mortality in males with systemic sclerosis is higher than in females, independent of ATA positivity.

Contributors

MB, TWJH, REMT, HUS, and JKdV-B developed the research question. All members of the EUSTAR collaboration contributed to the data collection. MB and SIEL have accessed and verified all the data in the study. SIEL and MB wrote the statistical analysis plan and analysed the data. SIEL, SLC, and MB performed the statistical analyses. TWJH, REMT, HUS, and JKdV-B helped with the interpretation of results. SIEL and MB wrote the manuscript with input from SLC, AR, PA, OD, MM-C, CC, ES, YA, TWJH, REMT, HUS, and JKdV-B for the final manuscript. All authors had full access to all the data in the study and accept responsibility to submit for publication.

Declaration of interests

SIEL received a grant from ZonMw and Nationale vereniging voor mensen met lupus, APS, sclerodermie en MCTD to study physical therapy in primary care in systemic sclerosis. PA received consulting fees from Bristol Myers Squibb; payments or honoraria from Bristol Myers Squibb, Bohringer Ingelheim, and Novartis; and support for attending meetings and travel from CSL Behring, Janssen, Roche, and Bristol Myers Squibb. OD was a consultant, received research funding, or speaker fees, or both, from 4P-Pharma, 4P-Science, Abbvie, Acceleron, Alcimed, Altavant Sciences, Amgen, AnaMar , Arxx, AstraZeneca, Blade Therapeutics, Bayer, Boehringer Ingelheim Pharma, Corbus Pharmaceuticals, CSL Behring, Galapagos, Glenmark, Horizon, Inventiva, Janssen, Kymera, Lupin, Medscape, Miltenyi Biotec, Mitsubishi Tanabe Pharma, MSD, Novartis, Prometheus Biosciences, Pfizer, Redxpharma, Roivant, Sanofi, and Topadur. JKdV-B received research grants from Roche, Galapagos, and Janssen; and consulting fees from Abbvie, Janssen, and Boehringer Ingelheim. All other authors declare no competing interests.

Data sharing

A data dictionary will be made available upon request. For the data from the Leiden CCISS cohort, deidentified individual participant data could be made available upon reasonable request. Data sharing will have to follow appropriate regulations by the Dutch law. The deidentified individual participant data from the EUSTAR cohort can be made available upon a clinical project request, following the standard operating procedures of EUSTAR.

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