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RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL RESEARCH

Untangling the relationship between smoking and systemic sclerosis: an analysis of the EUSTAR cohort

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To cite: Ciaffi J, Liem SIE, Cannegieter S, *et al*. Untangling the relationship between smoking and systemic sclerosis: an analysis of the EUSTAR cohort. *RMD Open* 2024;**10**:e004101. doi:10.1136/rmdopen-2024-004101

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/rmdopen-2024-004101).

JC and SIEL contributed equally.

Preliminary results of this study were presented at the EULAR 2023 Congress in Milan (Italy) and at the ACR Convergence 2023 in San Diego (USA).

Received 10 January 2024
Accepted 12 April 2024

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ABSTRACT

Objectives To untangle the association between smoking and systemic sclerosis (SSc).

Methods In the European Scleroderma Trials and Research cohort, the autoantibody status was compared between ever-smokers and never-smokers. Time until disease progression was assessed using Kaplan-Meier curves. Cox models were built to investigate the influence of smoking over 15 years of follow-up. All analyses were performed for the total cohort and stratified for sex and for positivity of anti-centromere (ACA) and anti-topoisomerase antibodies (ATA).

Results Overall, 12 314 patients were included in the study. Of these, 10 393 were women (84%), 4637 were ACA-positive (38%), 3919 were ATA-positive (32%) and 4271 (35%) were ever-smokers. In men, but not in women, smoking was associated with mortality (HR 1.63, 95% CI 1.23 to 2.16, p=0.001). Ever-smoking women were at higher risk for skin progression (HR 1.10, 95% CI 1.00 to 1.22, p=0.046) and for 'any organ progression' (HR 1.07, 95% CI 1.00 to 1.13, p=0.036). In women, 34% of never-smokers were ATA-positive compared with 21% of ever-smokers (p<0.001). In the group of ever-smokers, higher exposure rates, reflected by the number of pack-years (OR 0.98, 95% CI 0.97 to 0.99, p<0.001) and by smoking duration (OR 0.96, 95% CI 0.95 to 0.97, p<0.001), were associated with lower frequency of ATA. In ACA-positive patients, the risk of mortality (HR 1.29, 95% CI 1.02 to 1.63, p=0.033), cardiac involvement (HR 1.25, 95% CI 1.03 to 1.43, p=0.001), skin progression (HR 1.21, 95% CI 1.03 to 1.42, p=0.018) and 'any organ progression' (HR 1.14, 95% CI 1.05 to 1.24, p=0.002) was increased among smokers. In ATA-positive smoking patients, mortality (HR 1.40, 95% CI 1.10 to 1.78, p=0.006), skin progression (HR 1.19, 95% CI 1.03 to 1.37, p=0.020) digital ulcers (HR 1.17, 95% CI 1.02 to 1.34, p=0.029) and 'any organ progression' (HR 1.11, 95% CI 1.00 to 1.22, p=0.048) occurred more frequently.

Conclusions Our stratified analysis demonstrates that smoking is associated with an increased risk for mortality in male SSc patients but not in women. Strikingly, smoking is associated with lower prevalence of ATA positivity, in particular in women. In both ATA-positive and ACA-positive patients, smoking is a risk factor for mortality, skin progression and 'any organ progression'.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Data about the association between smoking and systemic sclerosis (SSc) are limited.
⇒ Smoking has been hypothesised to contribute to the increased mortality observed in male SSc patients compared with females.
⇒ The available literature suggests a negative association between smoking and positivity of anti-topoisomerase antibodies (ATA).

WHAT THIS STUDY ADDS

⇒ This study confirms that smoking is associated with reduced survival in men with SSc, partially explaining their worse prognosis.
⇒ Smoking is associated with a lower prevalence of ATA in women with SSc and this association is dose-dependent.
⇒ Both in anti-centromere-positive and ATA-positive patients, smoking might increase the risk of mortality, skin progression and 'any organ progression'.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our study highlights the importance of considering smoking as a risk factor for disease progression and mortality in SSc and underlines that smoking cessation programmes should be implemented in the care pathways for all patients.
⇒ For the first time, we describe a negative dose–response relationship between cumulative smoking exposure and the presence of ATA, pointing at a possible etiopathogenetic link between smoking and ATA. This antibody–environment interaction might provide important clues for a better understanding of the SSc pathogenesis.

INTRODUCTION

Systemic sclerosis (SSc) is a rare autoimmune connective tissue disease characterised by vasculopathy and fibrosis of skin and internal

organs.[1 2] The pathogenesis is elusive and incompletely understood.[3] A multifactorial background is postulated in which different endogenous and exogenous triggers contribute to the development of the disease in genetically predisposed individuals.[3–5] With the exception of silica, which is an established risk factor for SSc,[5–7] the nature of these triggers and their effects on the evolution of the disease remain unclear.

With regard to smoking, previous cross-sectional studies showed a correlation between smoking and vascular, gastrointestinal or respiratory symptoms in SSc,[8] but these findings were not confirmed in other cohorts.[9 10] In a longitudinal study on 3319 SSc patients, Jaeger *et al* did not observe a role of smoking on disease-specific cutaneous and pulmonary outcomes.[10] Whether smoking confers a risk for disease progression in SSc is thus debated.[11]

The prevalence of SSc is higher in women, but men have more severe disease and worse outcomes, including the excess mortality observed in male SSc patients compared with females.[12–15] Since men with SSc are more frequently ever-smokers than women,[16] previous studies have hypothesised a role of cigarette smoking as a contributor to this excess mortality.[12 17] Notwithstanding the availability of new treatment strategies[18 19] and of accurate and reliable methods to assess disease-specific manifestations,[20 21] caring for patients with SSc is challenging. Several clinical practice guidelines exist, but a multidisciplinary approach for holistic SSc management is advocated.[22] Elucidating the extent of the impact of smoking on SSc and whether smoking exerts distinct effects in men and women or in patients with different disease characteristics, would have important implications for risk stratification.

Interestingly, two independent groups described a higher proportion of anti-topoisomerase antibodies (ATA) positivity in never-smokers than in ever-smokers in, respectively, 621 and 3319 patients.[10 23] In an analysis of the autoantibody profile of 361 patients of the Leiden Combined Care in SSc cohort, we reported the same finding, but we also showed that smoking might have different effects on autoantibodies in men and women, with never-smoking women being more frequently ATA-positive than ever-smoking women.[24]

In rheumatoid arthritis (RA), smoking has a well-established impact on disease susceptibility and confers a risk for the development of anti-citrullinated peptide antibodies (ACPA) and rheumatoid factor, in particular in patients carrying specific HLA-DRB1 variants.[25] These observations have fuelled the hypothesis that, in a susceptible individual, chronic exposure to specific antigens, for example, in the lungs, can trigger a targeted chronic autoimmune response.[26] Considering that ATA have been traditionally related to aggressive SSc with a higher risk of interstitial lung disease (ILD) and increased mortality,[27 28] the negative association between smoking and ATA is highly intriguing and needs to be further elucidated.

To shed light on the complex interaction between smoking, autoantibodies, sex and disease outcomes in SSc, we performed an in-depth analysis of the EUSTAR cohort, in which we evaluated (1) the impact of smoking exposure on disease progression and mortality in the whole cohort, in men and women; (2) the association between smoking and presence of specific autoantibodies and (3) the intricate interactions among smoking habits and the presence of specific autoantibodies, and their collective impact on the progression of the disease.

This comprehensive approach not only aims to advance our understanding of SSc but also introduces a novel perspective on how lifestyle factors like smoking intersect with autoantibodies to influence disease dynamics.

METHODS

Study design and patients

The research questions were addressed analysing the multinational prospective European Scleroderma Trials and Research (EUSTAR) database. The structure of the EUSTAR database, the definition of each clinical variable and the description of the collected data have been reported elsewhere.[29 30] All patients included in our study fulfilled either the American College of Rheumatology (ACR) 1980 or the ACR/European League Against Rheumatism (EULAR) 2013 classification criteria for SSc[31 32] at the baseline visit (ie, the first visit registered in the database) or during follow-up. Patients were included if they were aged 18 years or older at the baseline visit.

In the EUSTAR database, data have been collected since 2004 and the smoking module was added in 2013. This module assesses patient-reported information about ever smoking, current smoking, number of pack-years and dates of smoking start and cessation. Since the main focus of our study was laid on smoking, patients without any information about smoking status or with inconsistent smoking data during follow-up were excluded. Patients with a first visit before 2013 in which information about baseline smoking status could be derived from data collected during follow-up, were included and categorised as ‘never-smokers’ or ‘ever-smokers’ at the baseline visit. Cases in whom multiple autoantibody positivity for both anticentromere antibodies (ACA) and ATA was recorded during the available follow-up were excluded from the analysis.

Definitions of disease progression

- Mortality: death from all causes.
- SSc-related mortality: death attributed to SSc.
- Development of ILD: evidence, after the baseline visit, of lung fibrosis on standard X-ray or high-resolution CT (HRCT) of the thorax.
- Progression of ILD: forced vital capacity decline>10% or DLCO decline >15% compared with the baseline visit[33] in patients with X-ray or HRCT evidence of ILD.

- Progression of skin involvement: 5-unit and 25% increase in modified Rodnan skin score (mRSS) from the baseline visit[34] or progression of disease subset (from sine scleroderma to limited SSc (lcSSc) or diffuse SSc (dcSSc), or from lcSSc to dcSSc).
- Development of pulmonary hypertension (PH): evidence, after the baseline visit, of pulmonary hypertension confirmed by mean pulmonary arterial pressure (PAP)≥25 mmHg on right heart catheterisation.
- Development of gastrointestinal involvement: onset, after the baseline visit, of gastrointestinal symptoms such as dysphagia, reflux, early satiety, vomiting, diarrhoea, bloating, constipation, paralytic ileus or malabsorption syndrome.
- Development of renal involvement: onset, after the baseline visit, of scleroderma renal crisis or requirement of dialysis.
- Development of cardiac involvement: onset, after the baseline visit, of conduction blocks, arrhythmias, pericardial effusion, diastolic dysfunction, myocarditis, left ventricular ejection fraction <50% or need for cardiac pacemaker implantation.
- Development of digital ulcers or digital ischaemia.
- 'Any organ progression': a composite variable encompassing all of the above organ-specific domains.

Statistical analysis

Data are expressed as mean±SD or median (25th–75th percentile) for continuous variables or number (percentage) for categorical variables.

To investigate the effects of smoking on disease progression in the whole cohort and, separately, in men and women, Kaplan-Meier estimates were used to assess the time until the first diagnosis of the event of interest in ever-smokers and never-smokers. Disease progression was defined as the occurrence of death or disease-specific outcomes within a follow-up period of 15 years since baseline. The starting point was the baseline visit. End time was set at the date of the visit at which the event was first recorded or at the end of follow-up when the event was never observed. Follow-up time was censored at 15 years from the first visit. Kaplan-Meier curves were compared between ever-smokers and never-smokers using a log-rank test. Incidence rates of mortality and 95% CI were calculated in men and women according to their smoking habit, considering never-smoking women as the reference category to determine the relative risk (RR). Additionally, univariable and multivariable Cox regression models, adjusted for age, were used to express the risk of mortality and progression in each disease domain as an HR with its 95% CI.

To assess whether the antibody profile in SSc was different in ever-smokers compared with never-smokers, χ^2 test and univariable logistic regression were applied, with smoking being the independent variable. To investigate a potential role of the intensity of exposure to tobacco on autoantibody occurrence, in the subgroup of ever-smokers, binary logistic regression was used to estimate the association between the number of pack-years or the years of smoking duration (both entered as continuous variables) and the autoantibody positivity, expressed through OR and their 95% CI.

To evaluate how cigarette exposure and ATA or ACA interact and are associated with disease outcomes and mortality, Kaplan-Meier curves were used to compare the time until progression in ever-smokers and never-smokers stratified by autoantibody profile assuming three categories, namely ACA positivity, ATA positivity or negativity of both autoantibodies. Cox regression models were built, adjusted for age and sex after stratification for ACA positivity, ATA positivity or negativity of both autoantibodies. Cox proportional hazards regression analysis was thus used to assess the risk of mortality and disease progression in each category according to the smoking status, expressing the event risk as an HR with its 95% CI.

All analyses were performed by using SPSS Statistics V.28 (IBM) and Stata version V.16 (StataCorp). A $p<0.05$ was considered statistically significant. Forest plots were created using R statistical software, 'ggplot2' package (V.4.3.0; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

EUSTAR population

At the time of data export from EUSTAR (5 July 2022), 21 349 patients were extracted from the database (online supplemental figure S1). Of these, 7 were excluded because sex was unknown and 7640 because information about smoking was either missing (n=7077) or inconsistent during follow-up (n=563). Additionally, of the 14 702 patients with consistent smoking data, 1388 were excluded due to one or more of the following reasons: positivity for both ACA and ATA (n=377), not fulfilling classification criteria (n=1011), age below 18 years at the baseline visit (n=32). Finally, 12 314 patients met the inclusion criteria (online supplemental figure S1). Of these, 4271 (35%) were ever-smokers. Compared with the excluded patients, included patients were more often males (16% vs 14%) and had shorter median disease duration (7.6 (3.0–16.0) vs 8.4 (3.3–16.7) years). Age (55.5±13.8 vs 55.3±14.5 years), prevalence of ACA (38% vs 38%) and limited cutaneous disease subset (56% vs 54%) were similar, whereas included patients were less often ATA-positive (32% vs 33%) and less often had diffuse cutaneous SSc (29% vs 31%). Median follow-up time of the included patients was 30 months (IQR 5–83). For 2848 (23%) included patients, only the baseline visit was available. Demographic and clinical features of the included and excluded patients are shown, respectively, in table 1 and in online supplemental table S3.

Smoking, mortality and disease progression

In females, 807 deaths were observed during 47 162 patient-years of follow-up. In males, 241 deaths were observed over 6878 patient-years. Patients' deaths were

Table 1 Baseline characteristics of the 12 314 patients in the EUSTAR registry included in the study

Demographic and clinical variables		Number of patients with data
Male sex, n (%)	1921 (16)	12 314
Ever-smokers, n (%)	4271 (35)	12 314
Pack-years, median (IQR)	15 (6–30)	2112
Smoking duration, mean±SD, years	23.7±13.2	1686
Age, mean±SD, years	55.5±13.8	12 314
Disease duration, median (IQR), years	7.6 (3.0–16.0)	8773
Anti-centromere antibodies, n (%)	4637 (38)	12 314
Anti-topoisomerase I antibodies, n (%)	3919 (32)	12 314
Anti-RNA polymerase III antibodies, n (%)*	144 (8)	1708
Anti-U1RNP antibodies, n (%)*	85 (5)	1865
Anti-PM/Scl antibodies, n (%)*	37 (4)	833
Anti-SSA antibodies, n (%)*	156 (14)	1093
Extent of skin involvement		8649
No skin involvement, n (%)*	584 (7)	
Only sclerodactyly, n (%)*	831 (10)	
Limited cutaneous involvement, n (%)*	4751(55)	
Diffuse cutaneous involvement, n (%)*	2483 (29)	
Modified Rodnan Skin Score, median (IQR)	4 (2–10)	7948
Digital ulcers, n (%)*	3266 (40)	8266
Pulmonary hypertension, n (%)*	58 (4)	1514
Interstitial lung disease, n (%)*	4472 (44)	10 173
DLCO % predicted, mean±SD	69.9±20.8	9012
FVC % predicted, mean±SD	94.9±22.0	8677
Gastrointestinal symptoms, n (%)*	8189 (67)	12 198

*Percentage is calculated on patients with data available.
DLCO, diffusing capacity of the lungs for carbon monoxide; EUSTAR, European Scleroderma Trials and Research; FVC, forced vital capacity.

attributed to SSc in 448 cases (43%), were not SSc-related in 407 (39%), and the information was missing in 193 (18%). All-cause mortality was slightly higher in ever-smokers compared with never-smokers in the whole cohort (32% vs 30%, p=0.001) (figure 1A) and in male patients (50% vs 40%, p<0.001) (figure 1B), but not in female patients (26% vs 29%, p=0.207) (figure 1B). Incidence rate for mortality was 1.77/100 patient-years in never-smoking women (reference category), 1.56/100 patient-years in ever-smoking women (RR=0.88), 2.53/100 person-years in never-smoking men (RR=1.43), 4.10/100 person-years in ever-smoking men (RR=2.32). Thus, our results outline an increased mortality associated with smoking in men but not in women.

For disease progression in the whole cohort, Kaplan-Meier curves did not show significant differences between ever-smokers and never-smokers in any of the organ domains evaluated, with the exception of skin progression, that occurred in 69% of ever-smokers and 62% of never-smokers (p<0.001) (figure 2A–H).

The same analyses were performed stratified by sex. Here, we observed a higher frequency of development of cardiac involvement among ever-smoking males compared with never-smoking males (78% vs 72%, p=0.025). In ever-smoking females, we found a lower risk of developing ILD (74% vs 78%, p<0.001) and a higher risk of skin progression (66% vs 61%, p=0.020). For all the other outcomes, we did not see an effect of smoking on disease progression in males or females (online supplemental figure S2A–H).

Combining all the above-mentioned domains in a composite 'any organ progression' variable, we noticed a significantly higher progression rate among ever-smokers compared with never-smokers across the entire cohort (figure 3A) and throughout the entire observation period (p<0.001), while no difference was observed in men and women (figure 3B). However, the estimates at the end of follow-up were numerically comparable with 100% of patients showing progression in at least one organ domain.

In multivariable Cox regression models, after adjusting for age, smoking was associated with increased risk of mortality (HR 1.50, 95% CI 1.32 to 1.71, p<0.001), cardiac involvement (HR 1.13, 95% CI 1.04 to 1.23, p=0.003), skin progression (HR 1.22, 95% CI 1.12 to 1.32, p<0.001) and 'any organ progression' (HR 1.10, 95% CI 1.05 to 1.16, p<0.001) in the whole cohort (figure 4A). In women, smoking was associated with a lower risk of developing ILD (HR 0.89, 95% CI 0.82 to 0.96, p<0.001) and with a higher risk for skin progression (HR 1.10, 95% CI 1.00 to 1.22, p=0.046) and for 'any organ progression' (HR 1.07, 95% CI 1.00 to 1.13, p=0.036) (figure 4B). In men, smoking was associated with increased all-cause mortality (HR 1.63, 95% CI 1.23 to 2.16, p=0.001) (figure 4C). The increased mortality risk in ever-smokers was confirmed also analysing only patients who died due to SSc. Having a history of smoking was associated with an increased risk of SSc-related mortality in the whole cohort (HR 1.30, 95% CI 1.06 to 1.59, p=0.011) and in male patients (HR 1.60, 95% CI 1.03 to 2.49, p=0.038).

Smoking and autoantibody profile

Of 12 314 included patients, 4637 were ACA-positive (38%) and 3919 were ATA-positive (32%). We observed a significantly different prevalence of autoantibodies in ever-smokers compared with never-smokers (table 2). Among never-smokers, 35% of patients were ATA-positive, compared with 27% among ever-smokers (p<0.001). When stratified by sex, this difference was accounted for by female patients: 34% of never-smokers were ATA-positive compared with 21% of ever-smokers (p<0.001). Additionally, 39% of never-smoking women were ACA-positive compared with 46% of ever-smoking women (p<0.001). In men, no statistically significant difference in the occurrence of ATA or ACA was observed.

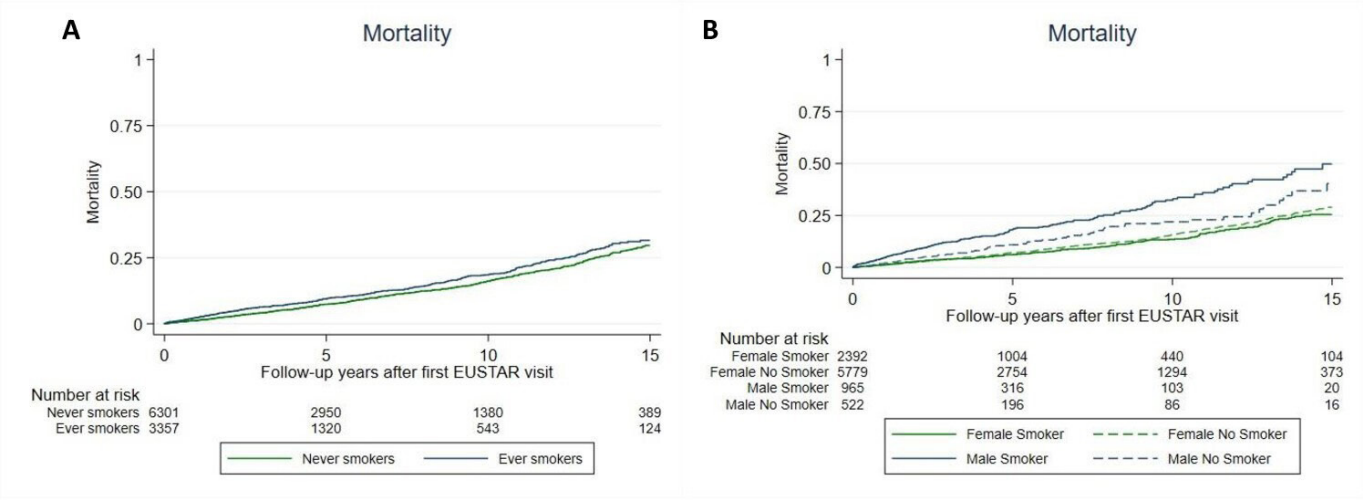

Figure 1 Kaplan-Meier curves of ever-smokers and never-smokers showing mortality rate in the total cohort of EUSTAR patients (A) and after stratification based on sex (B). EUSTAR, European Scleroderma Trials and Research.

No significant difference was found in the prevalence of anti-RNA polymerase III, anti-U1RNP, anti-SSA and anti-PM/Scl antibodies (table 2).

In univariable analysis, being a never-smoker was associated with ATA positivity in the whole cohort (OR 1.46, 95% CI 1.35 to 1.59, p<0.001) and in female patients (OR 1.91, 95% CI 1.73 to 2.11, p<0.001). Moreover, within the group of ever-smoking women, higher exposure rates as reflected by number of pack-years (OR 0.98, 95% CI 0.97 to 0.99, p<0.001) or by years of smoking duration (OR 0.96, 95% CI 0.95 to 0.97, p<0.001), decreased the risk of being ATA-positive.

Smoking and disease progression in ACA and ATA patients

Kaplan-Meier curves stratified for autoantibody profile assuming three categories (ACA positivity, ATA positivity or ACA and ATA negativity) demonstrated that mortality rate was higher in ever-smokers compared with never-smokers for ATA-positive patients (38% vs 30%, p<0.001) and for ACA and ATA-negative patients (36% vs 32%, p=0.041) but not for ACA-positive patients (figure 5A).

The risk of ILD development between ever-smokers and never-smokers was comparable in each autoantibody category (figure 5B). Among patients with ILD at baseline, the risk of decline in lung function during follow-up was higher in ever-smoking than in never-smoking ATA-positive patients (88% vs 77%, p=0.050) (figure 5C).

In ACA-positive patients, development of cardiac involvement (66% vs 61%, p=0.020) (figure 5D) was more frequent in ever-smokers as compared with never-smokers. Skin progression was more common among ever-smokers in both ACA-positive (55% vs 48%, p=0.010), ATA-positive (78% vs 73%, p<0.001) and ACA and ATA-negative patients (75% vs 69%, p=0.014) patients (figure 5E). In ATA-positive smokers, digital ulcers or digital ischaemia occurred more frequently during follow-up (94% vs 90%, p=0.004) (figure 5F). No difference was found in the risk of developing PH (figure 5G), gastrointestinal involvement (figure 5H) or renal complications (figure 5I).

Combining all the above-mentioned organ-specific manifestations in a composite 'any organ progression' variable, we found that 100% of patients were progressors at the end of the follow-up period. However, ever-smokers had a higher progression rate compared with never-smokers for ACA-positive (p=0.007), ATA-positive (p=0.002) and ACA and ATA-negative patients (p=0.014) (figure 5L).

Multivariable Cox regression showed that, after adjusting for age and sex, in ACA-positive patients (figure 6A), smoking was associated with increased risk of mortality (HR 1.29, 95% CI 1.02 to 1.63, p=0.033), cardiac involvement (HR 1.25, 95% CI 1.03 to 1.43, p=0.001), skin progression (HR 1.21, 95% CI 1.03 to 1.42, p=0.018) and 'any organ progression' (HR 1.14, 95% CI 1.05 to 1.24, p=0.002). In ATA-positive patients (figure 6B), smoking was associated with mortality (HR 1.40, 95% CI 1.10 to 1.78, p=0.006), with skin progression (HR 1.19, 95% CI 1.03 to 1.37, p=0.020), with development of digital ulcers or digital ischaemia (HR 1.17, 95% CI 1.02 to 1.34, p=0.029) and with 'any organ progression' (HR 1.11, 95% CI 1.00 to 1.22, p=0.048). Analysing the cases of death attributable to SSc, ever-smoking was associated with an increased risk of SSc-related mortality in ATA-positive patients (HR 1.40, 95% CI 1.00 to 1.96, p=0.047). Cox regression did not demonstrate associations between smoking and any outcome in ACA and ATA-negative patients (figure 6C).

DISCUSSION

In this study, we aimed to evaluate the interplay between smoking, sex, autoantibodies and disease outcomes in patients with SSc. We found that a never-smoking female patient with SSc has an almost twofold higher probability of being ATA-positive. Intriguingly, we observed a

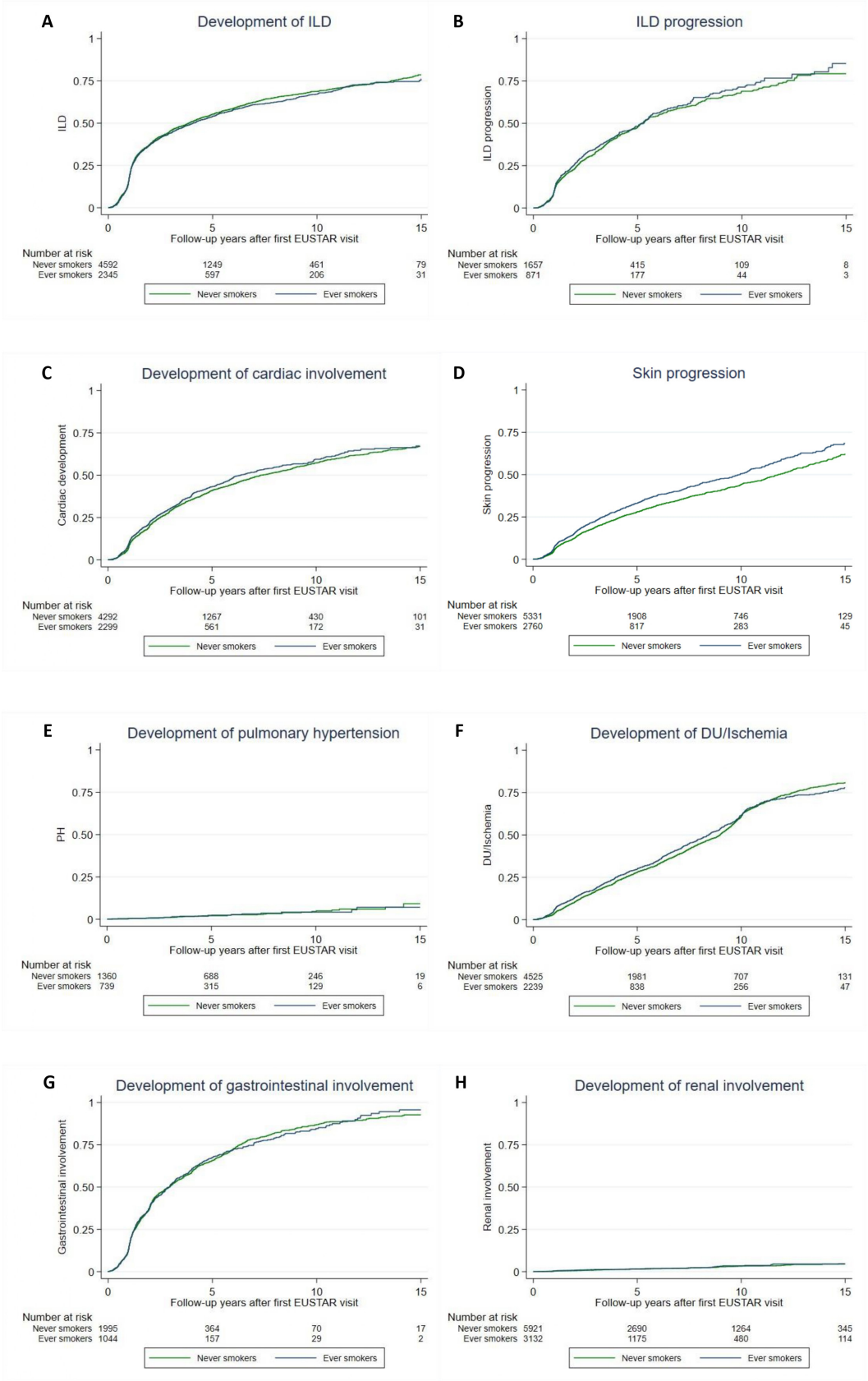

Figure 2 Kaplan-Meier curves of ever-smokers and never-smokers showing development of interstitial lung disease (ILD) (A), progression of ILD (B), development of cardiac involvement (C), progression of skin involvement (D), development of pulmonary hypertension (PH) (E), development of digital ulcers (DU) or digital ischaemia (F), development of gastrointestinal involvement (G) and development of renal involvement (H) in the total cohort of EUSTAR patients. EUSTAR, European Scleroderma Trials and Research.

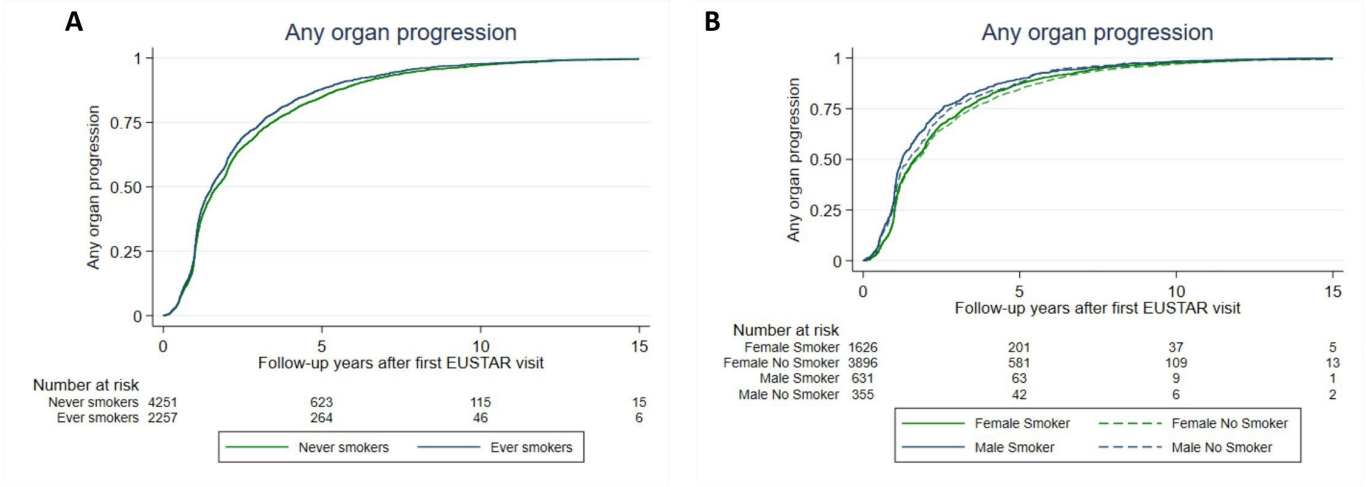

Figure 3 Kaplan-Meier curves of ever-smokers and never-smokers showing 'any organ progression' in the total cohort of EUSTAR patients (A) and after stratification based on sex (B). EUSTAR, European Scleroderma Trials and Research.

negative dose-response effect between smoking exposure and ATA. We demonstrated, for the first time, that smoking is associated with increased mortality in male patients but not in female patients. Ever-smoking women, however, have a higher risk for disease progression. Stratifying the analysis according to the autoantibody status, both ACA-positive and ATA-positive patients have an increased risk for mortality, skin involvement and 'any organ progression' if they are ever-smokers. Additionally, the risk for cardiac involvement is higher in ever-smoking ACA-positive patients while ATA-positive ever-smokers have a higher risk for digital ulcers.

Previous meta-analyses have showed that male sex is associated with increased mortality in SSc (HR of 1.9 for males compared with females).[13 14] In our analysis, smoking was associated with a relative mortality risk of 1.6 in men. Thus, our data suggest that smoking is a factor potentially accounting for the reduced survival of male SSc patients described in the literature.[27 35 36]

Consistent with the findings from the prospective study by Jaeger *et al*, our observations reveal no link between smoking and SSc-specific pulmonary outcomes.[10] Nevertheless, a significant correlation was noted with the advancement of skin involvement, manifesting as either an elevation in mRSS or the progression of the disease subset. Furthermore, we conducted a stratified analysis dissecting the different effects of smoking in males and females and individuals with distinct autoantibody profile.

Interestingly, we observed a lower risk of developing ILD in ever-smoking women than in never-smoking women. This result should be interpreted in the light of the close association between ATA and ILD[37] and of the lower proportion of ATA positivity in ever-smoking women compared with never-smoking women identified by our analysis. Indeed, the apparently higher risk of developing ILD in never-smoking women is entirely mediated by ATA positivity. When ATA positivity is included in the model, smoking loses significance.

In accordance with earlier literature, our study shows that a history of smoking is associated with a lower prevalence of ATA[10 23] and we confirm that this imbalance is found typically in female patients.[24] Additionally, we notice that this association is dose-dependent (ie, the higher the exposure rate, the lower the chance to be ATA-positive), which underlines the hypothesis that, indeed, exposure to cigarette smoke might impact the ATA-specific B cell responses. This finding is unexpected if we consider what happens in other autoimmune diseases such as RA or systemic lupus erythematosus (SLE).

In RA, smoking promotes citrullination in the lung tissue, initiating autoimmunity within the respiratory system and contributing to the formation not only of ACPA, but of multiple autoantibodies involved in the pathophysiological mechanisms of the disease.[38] In SLE, smoking is strongly associated with the risk of anti-dsDNA positivity and with more severe disease manifestations.[39] Smoking can thus impair the immune homeostasis and elicit a broad autoimmune response through different biological pathways.[40] However, in SSc, the apparently protective influence of smoking on the presence of ATA does not correspond to any beneficial clinical outcome for ever-smokers.

The strength of our study is intrinsic to the use of the EUSTAR database, which is the largest available real-life multinational registry of longitudinally collected data on SSc. We were able to comprehensively characterise the disease course of SSc patients according to their smoking habit, investigating the development of multiple clinical outcomes over a follow-up period of 15 years and contributing unique insights into the controversial relationship between smoking and SSc. In addition, we have applied a hypothesis-driven approach to elucidate how smoking interacts with disease progression in SSc, both in different autoantibody groups and in males and females.

Some potential limitations should be acknowledged. The major concern regards the assessment of smoking status, self-reported, and therefore, susceptible to bias.

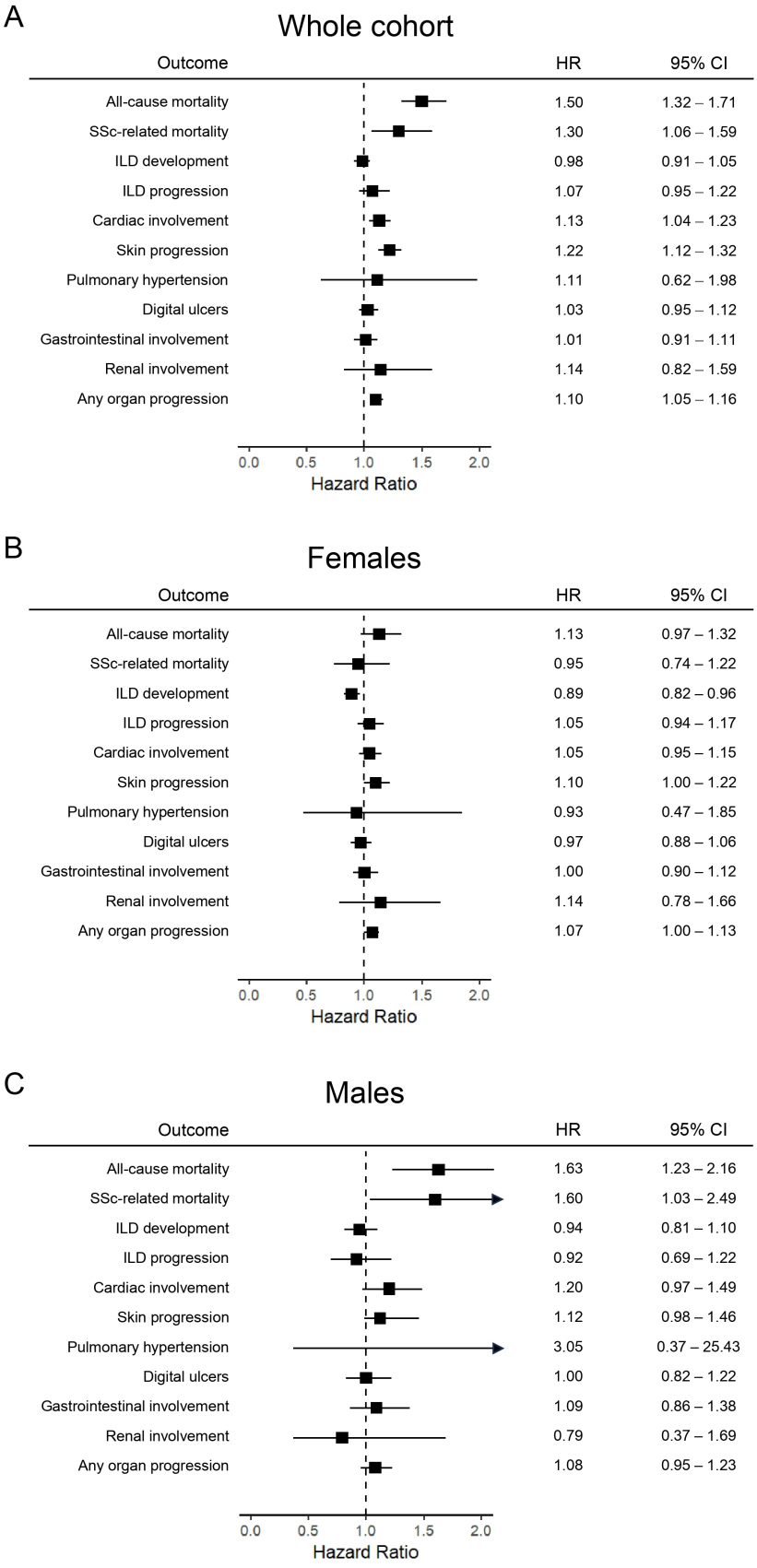

Figure 4 Forest plot of HRs and 95% CIs based on multivariable Cox regression analysis of the effect of cigarette smoking on different systemic sclerosis progression outcomes in the total cohort of EUSTAR patients (A) and after stratification in female patients (B) and male patients (C). All models were adjusted for age. Forest plots were created using R statistical software, 'ggplot2' package (V.4.3.0; R Foundation for Statistical Computing, Vienna, Austria). EUSTAR, European Scleroderma Trials and Research; ILD, interstitial lung disease.

Table 2 Positivity of anti-centromere antibodies (ACA), anti-topoisomerase I antibodies (ATAs), anti-RNA polymerase III antibodies (anti-RNAP-III), anti-U1 ribonucleoprotein antibodies (anti-U1RNP), anti-SSA antibodies and anti-PM/Scl antibodies in the whole EUSTAR cohort, and in men and women according to the smoking status

	Ever-smokers	Never-smokers	P value
	All patients (12 314)		
N	**4271**	**8043**	
Autoantibodies			
ATA, n (%)	1136 (27)	2783 (35)	<0.001
ACA, n (%)	1632 (38)	3005 (37)	0.354
Anti-RNAP-III, n (%)	62/617 (10)*	82/1091 (8)*	0.070
Anti-U1RNP, n (%)	24/684 (4)*	61/1181 (5)*	0.098
Anti-SSA, n (%)	44/368 (12)*	112/725 (15)*	0.119
Anti-PM/Scl, n (%)	14/338 (12)*	23/495 (15)*	0.729
	Men (1921)		
N	**1222**	**699**	
Autoantibodies			
ATA, n (%)	492 (40)	296 (42)	0.372
ACA, n (%)	232 (19)	130 (19)	0.835
Anti-RNAP-III, n (%)	32/193 (17)*	16/116 (14)*	0.512
Anti-U1RNP, n (%)	6/199 (3)*	5/111 (5)*	0.497
Anti-SSA, n (%)	12/115 (10)*	9/67 (13)*	0.541
Anti-PM/Scl, n (%)	5/91 (6)*	3/58 (5)*	0.932
	Women (10 393)		
N	**3049**	**7344**	
Autoantibodies			
ATA, n (%)	644 (21%)	2487 (34%)	<0.001
ACA, n (%)	1400 (46%)	2875 (39%)	<0.001
Anti-RNAP-III, n (%)	30/424 (7)*	66/975 (7)*	0.835
Anti-U1RNP, n (%)	18/485 (4)*	56/1070 (5)*	0.191
Anti-SSA, n (%)	32/253 (13)*	103/658 (16)*	0.253
Anti-PM/Scl, n (%)	9/247 (4)*	20/437 (5)*	0.561

*Number of patients with data available.
EUSTAR, European Scleroderma Trials and Research.

Nevertheless, patients were categorised as never-smokers and ever-smokers to yield the analysis robust against overestimation or underestimation of the smoking exposure. We also recognise that, in the models for disease progression, we were unable to control for factors such as educational level, socioeconomic status and alcohol consumption that might act as confounders in the association between smoking and the evolution of SSc.[41 42] In discussing SSc-related mortality, it is important to note the complexities in determining the exact cause of death, which often relies on the assessment of the attending physician. More than one-third of the patients extracted from the EUSTAR database had to be excluded from the analysis due to missing or inconsistent smoking information, potentially representing a selection bias. Similarly, data about positivity of autoantibodies different from ACA or ATA were missing in a considerable proportion of patients, precluding the possibility to perform exhaustive analyses on less-prevalent autoantibodies. We recognise the complexity of discerning the impact of smoking on the progression of cardiac involvement in SSc, given smoking's established adverse effects on the cardiovascular system. Our analysis did not encompass coronary artery disease or myocardial infarction in defining cardiac involvement. However, it is important to note that conditions such as heart failure, arrhythmias and diastolic dysfunction, which may stem from SSc-related myocardial damage and fibrosis, are prevalent among smokers in the general population.[43 44] To accurately separate the cardiovascular consequences of smoking from the cardiac manifestations intrinsic to SSc, targeted research would be essential. Finally, smoking data were collected

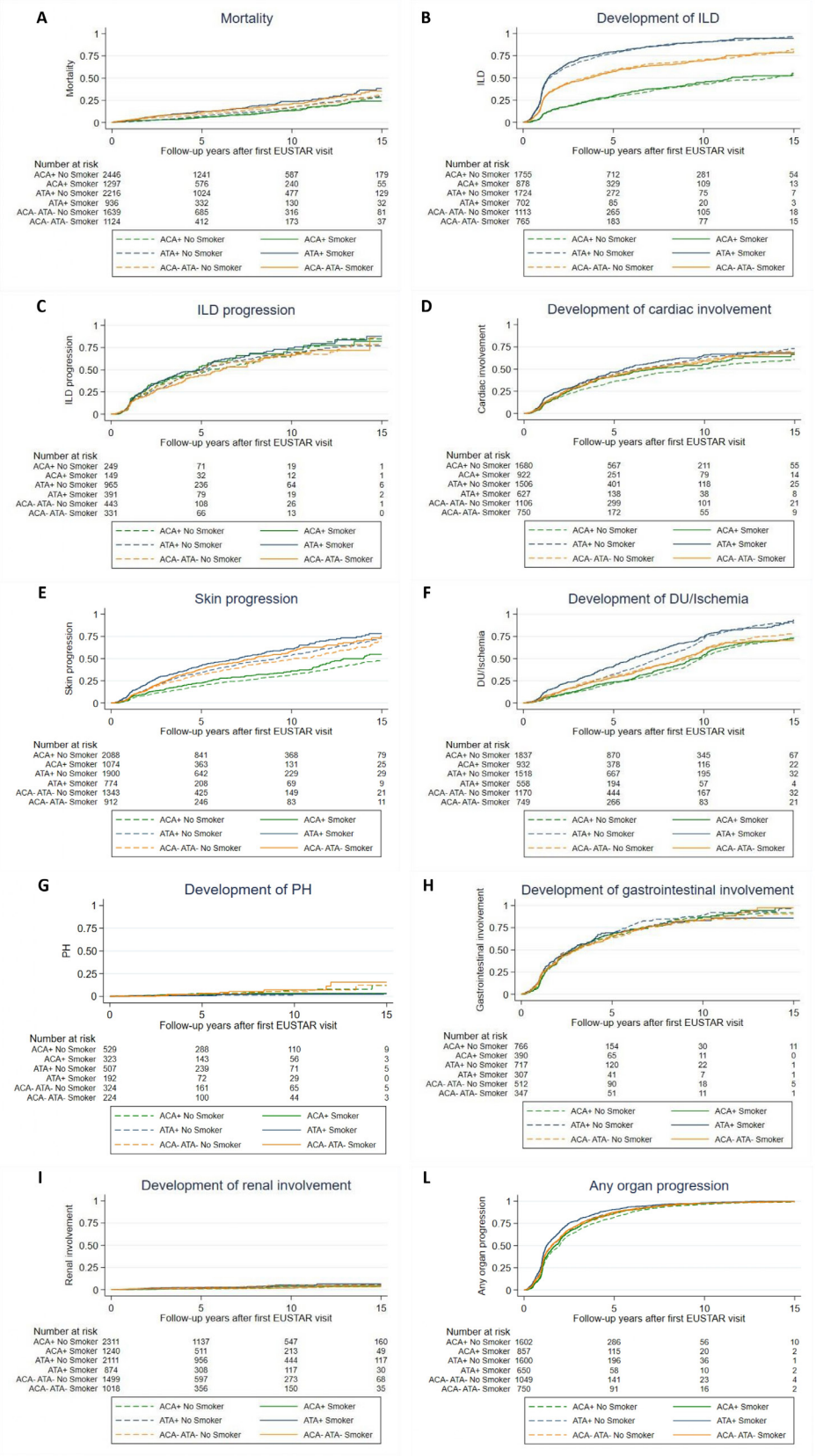

Figure 5 Kaplan-Meier curves of ever-smokers and never-smokers showing mortality rate (A), development of interstitial lung disease (ILD) (B), progression of ILD (C), development of cardiac involvement (D), progression of skin involvement (E), development of digital ulcers (DU) or digital ischaemia (F), development of pulmonary hypertension (PH) (G), development of gastrointestinal involvement (H), development of renal involvement (I) and 'any organ progression' (L) in the cohort of EUSTAR patients stratified by positivity of anti-centromere antibodies (ACA+), positivity of anti-topoisomerase I antibodies (ATA+) or negativity for both antibodies (ACA- ATA-). EUSTAR, European Scleroderma Trials and Research.

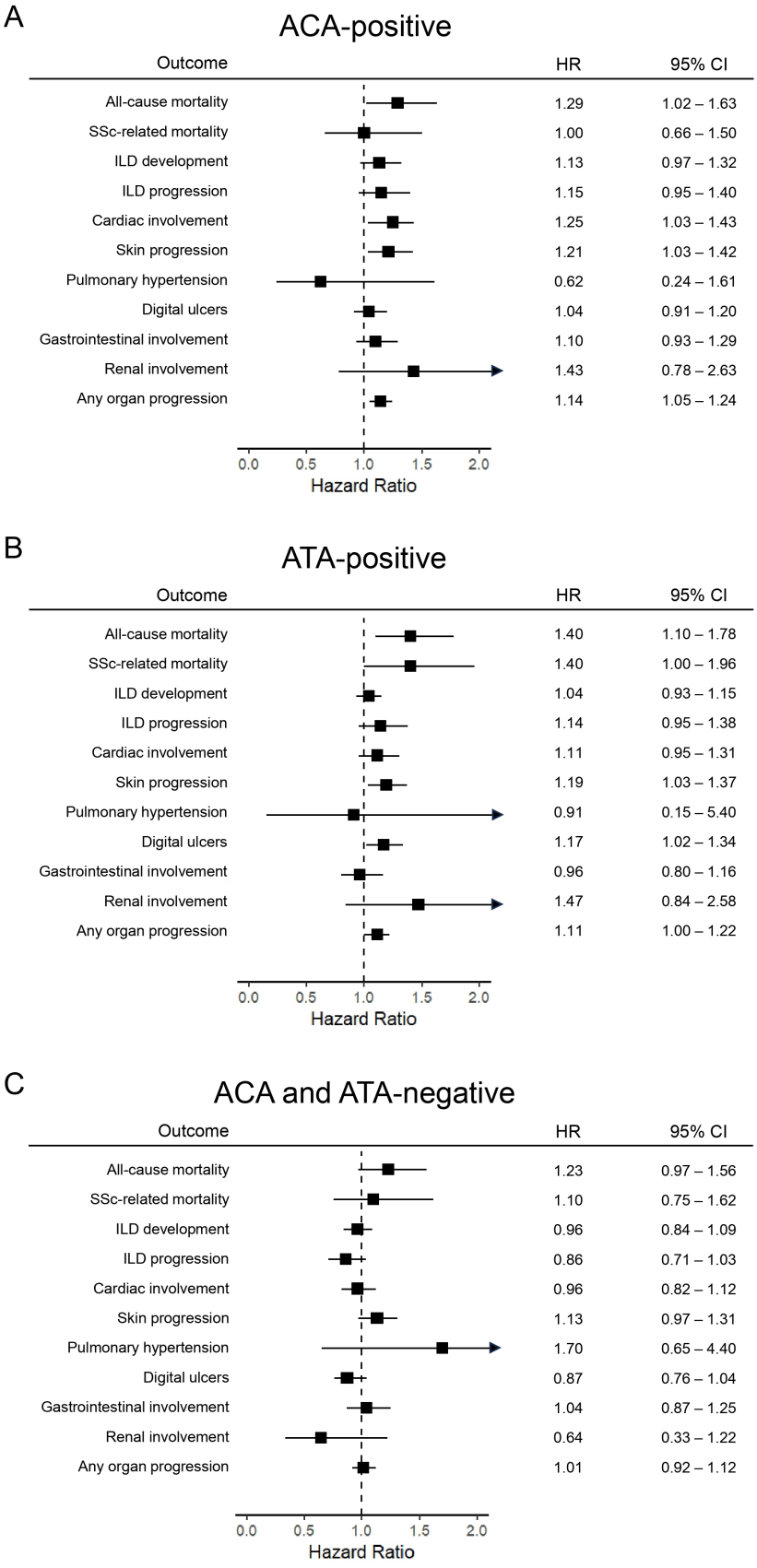

Figure 6 Forest plot of HRs and 95% CIs based on multivariable Cox regression analysis of the effect of cigarette smoking on different systemic sclerosis progression outcomes in the cohort of EUSTAR patients stratified by positivity of anti-centromere antibodies (ACA, A), positivity of anti-topoisomerase I antibodies (ATA, B) or negativity for both antibodies (C). All models were adjusted for age and sex. Forest plots were created using R statistical software, 'ggplot2' package (V.4.3.0; R Foundation for Statistical Computing, Vienna, Austria). EUSTAR, European Scleroderma Trials and Research; ILD, interstitial lung disease.

at the baseline visit only, blurring the interpretation of a time-dependent association between smoking exposure and the development of the assessed outcomes. Since genome-wide association studies have been conducted in SSc,[45 46] it would be interesting to use a Mendelian randomisation method, based on genetic variants, and therefore, robust against bias or confounders, to investigate the association between liability to smoking and risk of SSc, similar to the approach used for other diseases.[47]

In conclusion, we have investigated for the first time the interplay between sex, autoantibodies and smoking on the progression of SSc. Our results corroborate the association between smoking and a reduced prevalence of ATA positivity among SSc patients, particularly notable in females. The impact of smoking on SSc outcomes is heterogeneous and can be modified by factors like sex and autoantibody status. Given the poor prognosis of ATA-positive patients, the negative relationship between smoking and ATA introduces an additional layer of complexity. In males, our data indicate that smoking significantly compromises survival. Additionally, our findings suggest that smoking may influence mortality risk, skin progression and overall organ progression in ACA-positive and ATA-positive individuals, cardiac involvement in ACA-positive patients and the emergence of new digital ulcers in ATA-positive patients.

This study underscores the importance of identifying potential triggers to mitigate disease progression through more precise risk stratification. Nevertheless, the advantages of smoking cessation are unquestionable. We strongly advocate for the implementation of targeted smoking cessation counselling and effective interventions for all individuals with SSc.

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Contributors All authors were involved in drafting and/or critical review of the manuscript and approved the final version for submission. All authors agreed to attest to the accuracy and integrity of the work. Conceptualisation (JC, SIEL, SC and JDV-B), data curation (JC, SIEL, SC and JDV-B), formal analysis (JC, SIEL and JDV-B), investigation (JC, SIEL, SC and JDV-B), methodology (JC, SIEL, SC, JDV-B and TWJH), resources (JC, SC, JDV-B, MM-C), supervision (MM-C, FU, TWJH and JDV-B), validation (SC, JDV-B and TWJH), writing–original draft preparation (JC, SIEL, SA and EMH), writing–review and editing (JC, SIEL, SC, SA, EMH, PW, TA, GS, ABG, LC, EZ, IK, JCH, MM-C, PA, FU, TWJH and JDV-B). Author JC is responsible for the overall content as guarantor.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and each EUSTAR site obtained approval from the respective local ethics committee prior to data inclusion in the registry. All patients included in the EUSTAR database gave written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the EUSTAR board (project number CP 126). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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