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Autoantibody status in systemic sclerosis patients defines both Cancer risk and Survival with ANA-negativity in cases with concomitant cancer having a worse survival

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Running title: Cancer among SSc-patients.

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Abstract

Background: A higher rate of cancer in systemic sclerosis (SSc) is recognised but the role of SSc-linked autoantibodies status (positive/negative and autoantibody specificities) in the survival of SSc-patients with cancer remains poorly understood.

Methods: We utilized the Clalit-Health-Services medical database in a case-control study to evaluate the autoantibody status and specificities of SSc-patients with age- and sex-matched controls with regard to the prevalence of different cancer-subtypes and their impact on mortality. SSc-linked autoantibodies (ANA, anti-centromere, anti-RNP, anti-RNA polymerase III (RNAPIII) and anti-Scl-70) status was assessed in terms of cancer risk and outcome.

Results: 2,431 SSc-patients and 12,377 age- and sex-matched controls were included. SSc-patients had a relative risk of cancer of 1.90 (95%CI 1.62-2.24, $p<0.0001$) and tended to develop malignancies earlier than controls. RNAPIII and Scl-70 autoantibody were associated with an increased overall cancer risk and after SSc diagnosis risk of cancer, respectively. As expected, SSc-patients with cancer had a risk of death of 2.15 (1.65-2.79) in comparison to SSc-patients without cancer. ANA positive SSc-patients with cancer had a better prognosis than ANA negative cases ($p=0.0001$). Despite the benefit of ANA positive status on survival, the anti-Scl-70-positive subgroup with cancer had a significant negative impact on the survival compared to Scl-70-positive cases without cancer, whereas anti-RNAPIII and anti-centromere had no significant impact.

Conclusion: ANA positivity is an independent predictor of favourable prognosis in SSc-patients with cancer, possibly suggesting that humoral autoimmunity in SSc with cancer may have some benefit. However, no survival benefit was discernible with the common autoantibodies.

Introduction

Systemic sclerosis (SSc) is a complex autoimmune disease, of unknown aetiology in which there is abnormal activation of fibroblasts and overproduction and accumulation of extracellular matrix in the skin but also in different internal organs that may culminate in end stage organ failure[1]. The role of autoimmunity as the cardinal underlying driver in SSc is being increasingly appreciated with the recognition of shared genetic pathways with other autoimmune diseases from GWAS studies and molecular studies, especially of type-I interferon responses [2, 3].

Analogous to other autoimmune diseases, most notably dermatomyositis, SSc is associated with an increased age- and sex-adjusted risk of malignancy development [4, 5], commonly in the lung, breast, liver, hematologic system, bladder and ovary [6-8]. The high risk of cancer in such conditions was originally attributed to various factors including disease related chronic inflammation, genetic predisposition for both autoimmunity and malignancy, and as a treatment complication [9].

Several SSc-specific autoantibodies have been linked to specific demographic, clinical, organ system [10], risk of cancer, and survival features which first emerged with the description of anti-Scl-70 [11]. Striking advances have been made in recent years in elucidating the mechanisms linking cancer and SSc with, cancer expression of RNA polymerase III (RNAPIII) been linked to serum anti-RNAPIII autoantibodies in SSc [12-14]. Furthermore, an evidence of a genetic abnormality at the POLR3A locus (somatic mutations and/or loss of heterozygosity) has been reported in 6 of 8 SSc patients, but only 3 had somatic mutations[15]. The shorter disease interval reported between RNAPIII and cancer onset powerfully supports the idea of adaptive immunity to tumours may underscore some SSc cases via humoral autoimmune paraneoplastic

mechanisms [15] and that other autoantibodies beyond RNAPIII might contribute to this as a mechanisms of disease [16].

Despite the literature concerning the risk of cancer in SSc, the role of some autoantibodies in the risk of cancer among SSc-patients is still controversial with conflicting findings in relationship to key SSc-linked autoantibodies including anti-Scl-70 [17]. Moreover, there is a dearth of knowledge on the outcome of SSc-patients and with respect to their autoantibody status and cancer. Also, the impact of different cancer subtypes on the mortality of SSc-patients has not been defined. Thus, we conducted a large-scale population-based study to evaluate both cancer risk and impact on survival in SSc. In particular, we sought to determine whether humoral autoimmunity as determined by ANA and autoantibody specificity, in general, impacted on patient survival, the hypothesis being that SSc associated autoimmunity might be associated with a better survival.

Results

Study population

The entire population comprised of 15,141 subjects (12,710 controls and 2,431 SSc-patients). Being an age- and gender-matched case-control study, cases and controls did not differ for age (either age at study production - 63.4±18.1 years in the controls versus 62.7±17.9 years in the cases - or at the diagnosis/beginning of the follow-up - 54.5±18.6 versus 54.8±18.7 in controls and in cases, respectively) and gender (females, representing 81.7% of the sample both for cases and controls): they differed for BMI ($p<0.001$), socioeconomic status (with low socioeconomic status being more represented in cases - 44.4% versus 39.7% in controls, $p<0.001$), occurrence of cancer (higher among cases, 23.1% versus 15.1%, $p<0.001$) and all-cause mortality (being higher among cases, 26.2% versus 12.5%, $p<0.001$). Further details are shown in Table 1.

Independent predictors of cancer occurrence

At the multivariate logistic regression assessing covariates associated with malignancy, independent predictors of occurrence of cancer were age (OR 1.05 [95%CI 1.04-1.05], p<0.0001), socioeconomic status (medium, OR 1.25 [95%CI 1.12-1.41], p=0.0001; high, OR 1.40 [95%CI 1.23-1.60], p<0.0001), SSc (OR 1.90 [95%CI 1.62-2.24], p<0.0001), and smoking (OR 1.25 [95%CI 1.12-1.39], p=0.0001) (Table 1S).

Interaction between SSc and cancer in terms of death: independent predictors of mortality at the univariate analysis

Interaction between SSc and cancer had significant impact on the risk of death. At the Kaplan-Meier survival analysis, controls without cancer and the SSc-patients with cancer had the best and the worst survival curves, respectively (chi-squared=1,213.43; degrees of freedom=3; p<0.0001; Figure 1B). Indeed, in comparison to controls without cancer, controls with cancer (crude HR 3.86 [95%CI 3.39-4.39], p<0.05), SSc-patients without cancer (crude HR 2.63 [95%CI 2.31-3.00], p<0.05) and SSc-patients with cancer (crude HR 5.65 [95%CI 4.45-7.17], p<0.05) exhibited higher risk of death (Table 2S).

SSc-patients without cancer had a lower risk of death (crude HR 0.68 [95%CI 0.58-0.81], p<0.05) in comparison with controls with cancer. SSc-patients with cancer had a higher risk of death (crude HR 1.46 [95%CI 1.13-1.90], p<0.05) compared to controls with cancer. Finally, with respect to SSc without cancer, SSc with cancer had a higher risk of death (crude HR 2.14 [95%CI 1.65-2.79], p<0.05) (Table 2S).

Interaction between SSc and cancer in terms of death: independent predictors of mortality at the multivariate analysis

At the Cox multivariate survival analysis, independent risk factors of death were higher age (HR 1.06 [95%CI 1.05-1.06], p<0.0001), diagnosis of SSc (HR 2.16 [95%CI 1.89-2.48], p<0.0001), presence of malignancy (HR 2.47 [95%CI 2.24-2.72], p<0.0001), BMI < 20 vs 20-24.9 kg/m^2 (HR 1.35 [95%CI 1.15-1.60], p=0.0003). Independent protective factors for death were BMI 25-30 vs 20-24.9 kg/m^2 (HR 0.80 [95%CI 0.71-0.91], p=0.0007), female gender (female vs male, HR 0.78 [0.69-0.87], p<0.0001), and higher socioeconomic status (high vs low, HR 0.66 [0.57-0.75], p<0.0001) (Table 3S).

Multivariate logistic regression analysis of types of SSc-related cancers

At the multivariate logistic regression assessing risk of different cancer subtypes in SSc in comparison to controls after adjustment for age (Table 2), oesophagus cancer (OR 5.32 [95%CI 1.37-20.55], p=0.0154), lung cancer (OR 2.12 [95%CI 1.25-3.60], p=0.0053), vagina and vulva cancers (OR 9.85 [4.51-21.50], p<0.0001), multiple myeloma (OR 3.03 [95%CI 1.31-7.03], p=0.0097), myelodysplastic syndrome (OR 8.10 [95%CI 2.11-31.08], p=0.0023), non-Hodgkin's lymphoma (OR 2.75 [1.70-4.45], p<0.0001), stomach cancer (OR 2.60 [95%CI 1.13-6.00], p=0.0249), and malignancy of unknown primary (OR 4.32 [95%CI 3.16-5.91], p<0.0001) were significantly higher. Chronic leukaemia resulted, instead, associated in a borderline way (OR 2.62 [95%CI 0.99-6.96], p=0.0530). The reported OR is referred to the overall risk of cancer regardless its period of onset (before or after SSc diagnosis).

SSc tended to develop malignancies earlier than controls (p<0.0001, Figure 1A).

Interaction between SSc and SSc-related cancers in terms of death: independent predictors of mortality at the multivariate analysis

Assessing the impact of different cancer subtypes on the survival of SSc-patients after adjustment for SSc, the following cancers exhibited a high risk of death: lung cancer (HR 4.59

[95%CI 3.65-5.76], p=0.0064), oesophagus cancer (HR 3.62 [95%CI 1.87-6.99], p=0.0001), stomach cancer (HR 3.41 [95%CI 2.29-5.07], p<0.0001), liver cancer (HR 5.30 [95%CI 3.37-8.34], p<0.0001), pancreas cancer (HR 5.86 [95%CI 4.22 -8.14], p<0.0001), vagina and vulvar cancer (HR 3.23 [95%CI 1.96-5.30], p<0.0001), Hodgkin's lymphoma (HR 3.72 [95%CI 2.23-6.20], p<0.0001), and multiple myeloma (HR 3.55 [95%CI 2.30-5.48], p<0.0001). Further details are reported in Table 3.

Impact of Autoantibody status on Cancer risk: subgroup analyses

In this cohort, 1651 patients were tested for at least one autoantibody, namely 78.7% were tested for ANA, 61.1% for anti-Scl-70, 49.9% for anti-centromere, anti-RNP 41.5%, and only 10% for anti-RNAPIII. Among these, 84.1% were ANA positive, 39.4% were positive for anti-Scl-70, 32.0% were anti-centromere positive, 15.0% were anti-RNAPIII positive and 3.3% were anti-RNP positive. Double positivity at any time of study period (not necessarily at the same time) was low and reported in Table 4S.

Among the negative ANA group, 31 patients were found to be positive for anti-Scl-70, 3 were positive for anti-centromere, one was positive for anti-RNP and one was positive for RNAPIII. After the exclusion of these "false negative ANA" patients, the percentage of ANA positivity increased to 86%. In this cohort, only Scl-70 and RNAPIII auto-antibodies were associated with cancer risk. Specifically, Scl-70 was found to confer risk after SSc diagnosis (HR 1.41 [95%CI 1.05-1.90], P=0.0224) whereas RNAPIII conferred an overall risk (HR 1.94 [1.00-3.73], p=0.0488) (Table 4).

Impact of Autoantibody status on Survival in Cancer in SSc: subgroup analyses

Negativity of ANA was significantly associated with worse survival (chi-squared=16.12, degrees of freedom=1, p=0.0001) (Figure 2). After the exclusion of ANA negative patients but

positive for other SSc-linked autoantibodies, the p-value became even more significant ($p<0.0001$).

Concerning the impact of different SSc-linked autoantibodies on SSc-patients with cancer survival, anti-Scl-70 (chi-squared=4.23, degrees of freedom=1, p=0.0398), anti-RNP (chi-squared=9.90, degrees of freedom=1, p=0.0017) were associated with a worse survival (Figure 2). Anti-centromere (chi-squared 0.82, degrees of freedom=1, p=0.37) and RNAPIII (chi-squared 0.22, degrees of freedom=1, p=0.64) had no significant impact on the survival of SSc-patients (Figure 2). The HR for death (adjusted for confounders) was statistically significant only for ANA (HR of 0.64, 95%CI 0.50-0.83, p=0.0007) and Scl-70 (HR of 1.39, 95%CI 1.08-1.80, p=0.0106). To assess the interplay between of anti-Scl-70 and cancer in terms of mortality in SSc, we stratified SSc-patients with positive anti-Scl-70 according to malignancy status and we found that SSc-patients with cancer and positive for anti-Scl-70 had a higher risk of death (HR of 1.93, 95%CI, 1.21-3.09, p=0.0058) than those positive to this antibody but without cancer. However, stratifying patients with positive anti-RNP according to malignancy status, no significant differences were found in terms of survival rate (HR of 4.38, 95%CI, 0.86-22.18, p=0.0743).

Discussion

This study is the first to test the hypothesis that humoral autoimmunity as determined by autoantibody status in SSc cases with cancer might impact on patient survival. Indeed, SSc-patients with cancer and ANA negativity both by immunofluorescence and the common performed autoantibody specificities had a worse survival than those exhibiting ANA positivity. This points towards a potential survival benefit at the population level in SSc cases with cancer and discernible autoimmunity compared to the patient group with SSc and cancer without discernible autoimmunity. Despite, this, the “cardinal” autoantibody specificities

within ANA including Scl-70 and RNAPIII antibodies were not linked to a better survival and indeed the former had a worse survival.

At the population level, these novel findings on the presence of ANA being linked to a better survival may represent effective immune system and a better anti-tumour immune reaction. In agreement with this interpretation, one study has found that positivity of ANA in lung cancer, not linked to autoimmune disease, was associated with a prolonged survival [18]. Furthermore, some patients develop ANA after immune checkpoint inhibitors such as PD-1 inhibitors, representing an enhanced immune activity against cancer [19].

In our study, we were not able to link any ANA-specific autoantibodies to the better survival noted in the ANA positive group. In the group negative for ANA by immunofluorescence we identified over 30 cases who had SSc-linked autoantibodies and when these were included in the ANA positive group the link with ANA positivity and cancer survival remained strong.

The possibilities include the presence of other ANA subtypes that are linked to a worse survival in the so-called ANA negative SSc-patients that are yet to be defined. A second possibility is of a cell mediated autoimmunity mechanism accounting for paraneoplastic SSC autoimmunity but poor anti-tumour immune responses. .

Whilst ANA positive status in general was linked to a better survival over ANA negative status, the Scl-70 link to poor survival in cancer is noteworthy. Indeed, the present study showed that SSc-patients with cancer and anti-Scl-70 positivity had a worse prognosis than SSc-patients without this antibody. This finding remains difficult to explain and could relate to the impact of therapy of more severe SSc or an adverse effect of the tumour in aggravating autoimmune responses in other organs, particularly the lungs but defining specific mechanisms of death were beyond the scope of this study. It will be important to determine in future studies whether the mortality in ANA negative cases was due directly to tumour mortality rather than the SSC disease process itself.

Our data confirm earlier observations that SSc-patients positive for RNAPIII are at higher risk of cancer [12, 20]. A recent and important study by Igusa and collaborators [13] have found increased risk of cancer at SSc onset among anti-RNAPIII positive and those negative for all three anti-centromere/RNAPIII/Scl-70 antibodies patients. It is generally known that the relationship between cancer and autoimmunity is complex and bidirectional. Indeed, a study form Joseph et al. [15] has shown that genetic alterations in the POLR3A gene, encoding for RNAPIII polypeptide A, and humoral and cell-mediated immune response against this mutated antigen were demonstrated in patients who are positive for anti-RNAPIII, but not in patients with other SSc-specific antibodies and cancer [15]. Given the link between ANA positivity and cancer survival in SSc and the emergent biological understanding of RNAPIII in cancer in SSc then it was surprising that putative RNAPIII directed anti-tumour immunity did not translate into better survival in this antibody subgroup.

We also found that positivity of Scl-70 antibody is associated with the risk of cancer after SSc diagnosis. Similar results regarding anti-Scl-70 were reported previously, in particular with lung cancer [21, 22]. However, other studies have not reported such an anti-Scl-70 association with cancer [12, 13]. The variability and heterogeneity of findings regarding the role of SSc-related autoantibodies in the risk of cancer might be related to the complex interplay between genetic predisposition, environmental factors and epigenetic modifications in different geographical regions resulting in different rates of cancer in SSc generally and, in autoantibodies-related SSc subgroups in particular.

Surprisingly, there is very little data regarding the impact of different cancer subtypes on the outcome of SSc-patients, and to the best of our knowledge this is the first study to address this outcome. Whilst many cancer subtypes have high rates of mortality in SSc-patients such as pancreas, liver and bile ducts, oesophagus, and lung cancer, the substantial increased mortality of haematological malignancies such as Hodgkin's lymphoma is not completely understood.

This may be attributed to the high comorbidity and low performance status in SSc-patients preventing them of undergoing intensive chemotherapy or deleterious effects of tyrosine kinase inhibitors or immune check point inhibitors given to these individuals, which resulted in enhancement of the autoimmune disorders.

The estimated risk of cancer in SSc-patients varies from one report to another even though most of the studies reported a relative risk (RR) for all sites malignancy of (1.5-2-fold) [4, 23, 24] which is similar to the RR obtained in the current study (1.9-fold). Other cohorts reported a relative risk of cancer in SSc above 4 [25, 26]. We evaluated cancer subtypes and the leading cancer subtypes in our patient cohort were vagina and vulva, oesophagus, lung and haematological system. In our cohort, vagina and vulva cancers were found to be with highest RR in the region of 10 (CI 95% 4.51-21.5). Genital organs malignancies in SSc-patients are not well described in the literature. There are more reports regarding cervical cancer rather than vagina and vulva in SSC and it has been found that atypical cytological findings on pap smears of SSc-patients is higher than in the general population [27]. In our study, the risk of lung cancer was significantly higher with a RR of 2.12 although it is slightly lower to what previously has been reported [28]. We also found a higher prevalence of stomach as well as oesophagus cancer in SSc-patients. The higher risk of oesophageal cancer in SSc-patients is well reported with a variable RR that ranges between 2.86 to 35.0 [29, 30] although, others reported no significant increased risk [30]. A plausible mechanism that may explain the increase rate of oesophageal cancer is the higher prevalence of peptic disease and Barrett's oesophagus in SSc-patients, both known to be linked with oesophageal cancer [31]. Concerning haematological malignancies in SSc, variable RR have been reported according to the study design and population, yet, a metanalysis has showed an overall RR of haematological cancer in SSc of 2.2 [30]. In our study, in terms of specific haematological cancer, the highest RR was found for myelodysplastic syndrome, multiple myeloma and non-Hodgkin's lymphoma.

Our study has several strengths, mainly the sample size and its population-based design, which avoids the potential referral bias that often afflicts centre-based studies. However, there are limitations that need acknowledgement including the inability to explore different SSc phenotypes including interstitial lung disease, cause of death and effect of therapies on cancer and on survival. Finally, as some of these serological tests such as anti-RNP and anti-RNAPIII are not clinically routine and relatively recent tests, they were not available for the entire study population and therefore the data needs to be interpreted with caution. It is also important to mention the possibility of misclassification of SSc related to the big data real-life data based studies.

In conclusion, our study confirms earlier observation on the increased rate of cancer in SSc-patients, especially for those positive for RNAPIII and Scl-70 antibodies. In terms of cancer subtypes, genital organs, lung, oesophagus, stomach, and haematological malignancies were the most common SSc related malignancy and tended to appear earlier during the course of life in comparison to the general population. SSc-patients with cancer and ANA negativity seem to have less favourable outcome than those positive for this antibody. Moreover, the mortality of cancer in SSc may be different than that in the general population. These findings show that the association between SSc and cancer and autoimmunity extends beyond disease risk but also has a complex effect on disparate factors including age of onset and types of cancers and the impact of autoimmunity at the population and cancer survival.

Material and methods

Design, sample and procedures

This study is based on the chronic diseases registry of the Clalit-Health-Services (CHS), the largest healthcare maintenance organization in Israel which provides services for approximately half of the Israeli population where data on SSC in routinely collected.

With the use of massive data-mining techniques, patient data can be automatically retrieved and extracted from the database, enabling scholars to perform a wide-scale epidemiological study on a real-time heterogeneous population in an effective and accurate manner. Using the CHS's computerized database, we extracted a cohort consisting of SSc-patients and compared them with age- and sex-matched controls. The data drawn from the database were recorded continuously since the beginning of the utilization of computerized systems in the CHS, approximately from the year 2000 until the year 2017.

Measures

SSc-patients were defined as such if they had at least one documented diagnosis of SSc in their medical records as an outpatient, either by a primary care physician or a specialist, or if they were diagnosed with SSc in their hospital discharge papers. All SSc-patients detected in the CHS database were considered eligible and, as such, enrolled in this study. Controls were randomly selected from the CHS database, with the exclusion of SSc-patients (that is to say, they may have other diseases and not necessarily healthy controls). Approximately five controls were matched by age and gender for each SSc patient. Data available from the CHS database included an array of variables, such as age, sex, socioeconomic status, body mass index (BMI), smoking status (ever smokers, or never smokers by time of entry in the study), and diagnoses of chronic diseases. More in detail, socioeconomic status was defined according to the poverty index of the member's residence area as defined during the 2008 National Census. More specifically, the poverty index was computed based on household income, education, crowding, material conditions, and car ownership, among others. This composite

index can range from 1 to 20, based on cluster analysis, with 1 as the lowest socioeconomic status and 20 as the highest. We divided the population into 3 categories according to their socioeconomic status, based on tertile distribution.

Concerning BMI, in order to reflect a nonlinear relation between BMI and dependent variables, BMI was classified into 4 categories: <20, 20-24.9, 25-30, and >30 kg/m^2. The normal category (BMI 20-24.9 kg/m^2) was used as a reference category.

The definition of malignancy, similar to that of SSc, was based on a documented diagnosis of malignancy in medical records, as registered in the CHS database. The validity and reliability of the diagnoses in the registry were found to be high, as shown in our previously published studies [32-35].

Serum samples were taken and analysed in SSc-patients by indirect immunofluorescence or enzyme-linked immunosorbent assay to identify SSc-specific autoantibodies during the diagnosis approach or follow-up and were available for the years 2010-2017. These tests were performed as part of routine clinical and were not research assays. In the current study, the following autoantibodies were considered and assessed: ANA, anti-centromere, anti-Scl-70 (topoisomerase-I), anti-RNAPIII and anti-RNP. Tests positivity was defined as supplied by the kit assay insert and manufacturer's instructions. The tests could have been performed any time point during the study period regardless SSc disease onset. In case of multiple/serial assessment of autoantibodies (exams performed at different time-points during the study period), patients were considered positive for an autoantibody if they were ever positive based on clinically obtained assays.

Statistical analyses

Before commencing any statistical analysis and data manipulation, figures were visually inspected for potential outliers. The normality of data distribution was checked using the D'Agostino-Pearson omnibus test.

Rates of malignancies (overall and stratified for single disease) were compared between SSc-patients and controls in the study sample group. For overall we mean the rate of having at least one malignant condition either solid or haematological. The Chi-squared test was used to assess the distribution of categorical socio-demographic and clinical parameters, such as socioeconomic status and gender, between SSc-patients and controls. The Student's t-test and one-way analysis of variance (ANOVA) or their non-parametric versions, were applied for continuous parameters, such as age at study production or age at diagnosis/beginning of the follow-up (between two and more groups, respectively), based on the normality of data distribution.

The association between SSc and malignancies was evaluated by a standard unconditional multivariate logistic regression model, in that matching was loose, that is to say performed on a small number of demographic variables (namely, age and gender). In this situation, Mantel–Haenszel matched-pair conditional regression logistic analyses are not necessary[36, 37] and may result in inaccurate and non-robust estimates. Performed multivariate logistic regression analyses were adjusted for possible confounders, including age and calendar time. The former adjustment was carried out considering that matching for age was done at study entry, but throughout the study period SSc patients tended to develop malignancies earlier than controls. As such, it was necessary to adjust for age in order to minimize the risk of underestimation[36, 38]. The latter adjustment (for calendar time) was performed to reduce the bias due to changes of cancer incidence over time or changes in the screening methods.

Dates of registration in the medical records of SSc (or alternatively, start of follow-up for controls), malignancy and death, as well as anthropometric information and medical co-morbidities, were extracted from the database when available.

Survival analysis using Kaplan-Meier curves, log-rank test and multivariate Cox proportional hazards method was performed to detect variables associated with an increased risk of all-cause mortality, adjusting for possible risk factors and confounders, including SSc disease duration.

Multivariate Cox proportional-hazard regression analysis was used to assess risk of cancer and death stratified according to autoantibody positivity for three different time-points (overall risk, after SSc diagnosis, and 36 months prior and after SSc onset). The HR was computed after adjusting for age, gender, BMI, SES, and smoking status.

All statistical analyses were performed on the entire sample, except for the analyses concerning autoantibody positivity, which were carried out as sub-group analyses.

All statistical analyses were carried out with the commercial software "Statistical Package for the Social Sciences" (SPSS version 24.0, IBM, USA). Graphs were obtained with the commercial software MedCalc Statistical Software (version 17.9.7).

All figures with a P-value of less than 0.05 were considered statistically significant.

- **Competing Interest:** Cohen AD received research grants from Janssen, Novartis, AbbVie, Janssen and Sanofi. Cohen AD served as a consultant, advisor or speaker to AbbVie, Amgen, Boehringer Ingelheim, Dexcel pharma, Janssen, Kamedis, Lilly, Neopharm, Novartis, Perrigo, Pfizer, Rafa, Samsung Bioepis, Sanofi, Sirbal and Taro.
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- **Ethical statement:** Authors confirm that their study's involvement with human subjects complies with the Declaration of Helsinki.

Tables.

Table 1. Overall population, systemic sclerosis (SSc) patients and age-and-sex matched controls – basic characteristics.

Characteristic	All population (n=15,141)	Controls (n=12,710)	SSc-patients (n=2,431)	Statistical significance (p-value)
Age (mean±SD; median)	63.32±18.06; 66	63.44±18.08; 66	62.69±17.90; 66	NS
Age at diagnosis or at the beginning of the follow-up (mean±SD; median)	54.57±18.64; 57	54.54±18.63; 57	54.77±18.67; 57	NS
Gender (female; %)	12,377 (81.7%)	10,390 (81.7%)	1,987 (81.7%)	NS
BMI (n; %)[a]				<0.001
<20 kg/m^2	1,283 (9.2%)	1,098 (8.6%)	185 (15.6%)	
20-24.9 kg/m^2	4,189 (30.1%)	3,803 (29.9%)	386 (32.5%)	
25-30 kg/m^2	4,380 (31.5%)	4,055 (31.9%)	325 (27.4%)	
>30 kg/m^2	4,045 (29.1%)	3,754 (29.5%)	291 (24.5%)	
SES (n; %)[b]				<0.001
Low	5,763 (40.4%)	4,769 (39.7%)	994 (44.4%)	
Medium	5,364 (37.6%)	4,543 (37.8%)	821 (36.7%)	
High	3,122 (22.0%)	2,699 (22.5%)	423 (18.9%)	
Smoking (n; %)	4,332 (28.6%)	3,628 (28.5%)	704 (29.0%)	NS
Cancer (n; %)	2,480 (16.4%)	1,919 (15.1%)	561 (23.1%)	<0.001
All-cause mortality (n; %)	2,226 (14.7%)	1,589 (12.5%)	637 (26.2%)	<0.001

[a] Available for 91.8% of data; [b] Available for 94.1% of data.

Table 2. Multivariate logistic regression assessing the overall risk of different cancers in systemic sclerosis in comparison to controls.

Variable	Overall number of cancers N (%)	Cancer in SSc-patients N (%)	Coefficient	Std. Error	Wald	P	Odds ratio	95% CI
CNS cancer	34 (0.2%)	7 (0.3%)	-0.83	1.02	0.66	0.4180	0.44	0.06 to 3.24
Oropharyngeal cancer	29 (0.2%)	10 (0.4%)	0.65	0.63	1.06	0.3033	1.92	0.56 to 6.61
Larynx cancer	20 (0.1%)	4 (0.2%)	0.28	0.77	0.13	0.7148	1.33	0.29 to 6.00
Thyroid cancer	112 (0.7%)	26 (1.1%)	0.13	0.37	0.11	0.7427	1.13	0.54 to 2.35
Breast cancer	723 (4.8%)	125 (5.1%)	0.28	0.15	3.71	0.0539*	1.33	1.00 to 1.77
Lung cancer	160 (1.1%)	48 (2.0%)	0.75	0.27	7.79	0.0053**	2.12	1.25 to 3.60
Oesophagus cancer	12 (0.1%)	4 (0.2%)	1.67	0.69	5.87	0.0154**	5.32	1.37 to 20.55
Stomach cancer	46 (0.3%)	13 (0.5%)	0.96	0.43	5.03	0.0249**	2.60	1.13 to 6.00
Pancreas cancer	51 (0.3%)	8 (0.3%)	-0.43	0.73	0.35	0.5551	0.65	0.16 to 2.72
Liver and bile ducts cancer	25 (0.2%)	3 (0.1%)	0.10	0.75	0.02	0.8942	1.10	0.26 to 4.78
Colorectal cancer	287 (1.9%)	47 (1.9%)	0.03	0.26	0.01	0.9137	1.03	0.62 to 1.70
Kidney cancer	78 (0.5%)	8 (0.3%)	0.15	0.43	0.12	0.7338	1.16	0.50 to 2.70
Bladder cancer	116 (0.8%)	19 (0.8%)	0.27	0.36	0.55	0.4570	1.31	0.65 to 2.64
Prostate cancer	84 (0.6%)	13 (0.5%)	-0.02	0.54	0.00	0.9708	0.98	0.34 to 2.82
Uterus cancer	113 (0.7%)	21 (0.8%)	0.49	0.34	2.02	0.1550	1.62	0.83 to 3.17
Cervical cancer of the uterus	46 (0.3%)	11 (0.5%)	0.42	0.54	0.63	0.4273	1.53	0.54 to 4.37
Ovary cancer	72 (0.5%)	11 (0.5%)	0.33	0.43	0.58	0.4449	1.39	0.59 to 3.26
Vagina and vulva cancers	37 (0.2%)	21 (0.9%)	2.29	0.40	33.00	<0.0001**	9.85	4.51 to 21.50
Bone cancer	13 (0.1%)	1 (0.0%)	-18.24	6,264.52	0.00	0.9977	0.00	
Sarcoma	44 (0.3%)	14 (0.6%)	0.72	0.49	2.17	0.1405	2.06	0.79 to 5.40
Melanoma	114 (0.8%)	25 (1.0%)	-0.36	0.52	0.48	0.4897	0.70	0.25 to 1.93
Acute leukaemia	75 (0.5%)	15 (0.6%)	0.38	0.41	0.87	0.3502	1.46	0.66 to 3.24
Chronic leukaemia	41 (0.3%)	12 (0.5%)	0.96	0.50	3.74	0.0530*	2.62	0.99 to 6.96
Hodgkin's lymphoma	35 (0.2%)	10 (0.4%)	0.75	0.55	1,86	0.1730	2.11	0.72 to 6.20
Non-Hodgkin’s lymphoma	159 (1.1%)	48 (2.0%)	1.01	0.25	16.91	<0.0001**	2.75	1.70 to 4.45

Myelodysplastic syndrome	11 (0.1%)	6 (0.2%)	2.09	0.69	9.30	0.0023**	8.10	2.11 to 31.08
Multiple myeloma	44 (0.3%)	13 (0.5%)	1.11	0.43	6.68	0.0097**	3.03	1.31 to 7.03
Malignancy of unknown primary	297 (2.0%)	120 (4.9%)	1.46	0.16	83.73	<0.0001**	4.32	3.16 to 5.91
Other neoplasms	111 (0.7%)	28 (1.2%)	-0.38	0.26	2.12	0.1450	1.34	0.66 to 2.70

*****Borderline association, **significant association.

Table 3. Cox multivariate survival analysis assessing the impact of different cancer subtypes on mortality of systemic sclerosis patients.

Cancer	HR	95%CI	p-value
CNS cancer	2.86	1.62 to 5.05	0.0003
Oropharynx cancer	0.93	0.38 to 2.24	0.8665
Thyroid cancer	1.14	0.70 to 1.87	0.5979
Larynx cancer	2.39	1.28 to 4.47	0.0064
Sarcoma	1.43	0.81 to 2.52	0.2223
Melanoma	1.18	0.77 to 1.79	0.4502
Breast cancer	1.75	1.48 to 2.06	<0.0001
Lung cancer	4.59	3.66 to 5.77	<0.0001
Oesophagus cancer	3.62	1.88 to 6.99	0.0001
Stomach cancer	3.42	2.30 to 5.08	<0.0001
Liver cancer	5.30	3.37 to 8.34	<0.0001
Pancreas cancer	5.87	4.23 to 8.14	<0.0001
Colorectal cancer	1.63	1.31 to 2.02	<0.0001
Kidney cancer	1.97	1.35 to 2.87	0.0004
bladder cancer	2.27	1.71 to 3.03	<0.0001
Prostate cancer	0.92	0.58 to 1.46	0.7272
Ovarian cancer	2.67	1.92 to 3.80	<0.0001
Uterus cancer	1.61	1.13 to 2.28	0.0081
Cancer of the cervix uteri	1.85	1.05 to 3.26	0.0346
Vagina and vulva cancer	3.23	1.97 to 5.31	<0.0001
Acute leukemia	1.53	0.97 to 2.40	0.0667
Chronic leukemia	2.09	1.21 to 3.61	0.0083
Hodgkin’s lymphoma	3.72	2.23 to 6.21	<0.0001
Non-Hodgkin’s lymphoma	2.20	1.64 to 2.96	<0.0001
Multiple myeloma	3.56	2.31 to 5.48	<0.0001
myelodysplastic syndrome	2.48	0.93 to 6.63	0.0709
Cancer of unknown primary	1.65	1.27 to 2.13	0.0001
Other neoplasms	3.14	2.33 to 4.23	<0.0001

Table 4. Multivariate Cox proportional-hazard regression analysis for assessing the risk of cancer and of death related to different SSc-autoantibodies

*****HR was computed adjusting for age, gender, BMI, SES, and smoking status.

Autoantibody		Risk of cancer development in SSc-Patients			Risk of death in SSc-patients with cancer		
		HR*	**95%CI**	**p-value**	**HR***	**95%CI**	**p-value**
ANA							
	Overall risk	0.84	0.66 to 1.08	0.1666	0.64	0.50 to 0.83	**0.0007**
	Risk after SSc diagnosis	0.83	0.59 to 1.17	0.2894			
	Risk in ±36 months of SSc diagnosis	0.81	0.57 to 1.16	0.2565			
	High titre vs low	0.90	0.63 to 1.27	0.5385			
RNAPIII							
	Overall risk	1.94	1.00 to 3.73	**0.0488**	1.53	0.60 to 3.88	0.3763
	Risk after SSc diagnosis	1.96	0.70 to 5.52	0.2022			
	Risk in ±36 months of SSc diagnosis	1.97	0.67 to 5.79	0.2160			
Scl-70							
	Overall risk	1.13	0.90 to 1.43	0.2872	1.39	1.08 to 1.80	**0.0106**
	Risk after SSc diagnosis	1.41	1.05 to 1.90	**0.0224**			
	Risk in ±36 months of SSc diagnosis	1.23	0.89 to 1.72	0.2113			
Centromere							
	Overall risk	1.28	0.94 to 1.74	0.1116	1.42	0.99 to 2.03	0.0545
	Risk after SSc diagnosis	0.95	0.59 to 1.53	0.8324			
	Risk in ±36 months of SSc diagnosis	1.10	0.67 to 1.81	0.7192			
RNP							
	Overall risk	0.97	0.64 to 1.45	0.8734	0.50	0.23 to 1.09	0.0796
	Risk after SSc diagnosis	1.26	0.77 to 2.07	0.3620			
	Risk in ±36 months of SSc diagnosis	0.90	0.48 to 1.70	0.7414			

Legends for Figures.

Figure 1. (A) Cumulative frequency showing mean age at diagnosis of malignancy in systemic sclerosis in comparison to controls. Between age 30 to 70, cancers present at a younger age in SSc subjects (green line). (B) Kaplan-Meyer survival curve for systemic sclerosis patients and controls with and without cancer.

Figure 2. Kaplan-Meyer survival curve analysis for systemic sclerosis with cancer stratified according to positivity/negativity for a panel of autoantibodies (ANA, anti-centromere, RNA polymerase III, anti-RNP, anti-Scl-70. SSc-patients with cancer and positive for a SSc-related autoantibody were compared to overall SSc cohort with cancer but negative for the same antibody in terms of survival.

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