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ORIGINAL RESEARCH

Autoantibodies, cutaneous subset and immunosuppressants contribute to the cancer risk in systemic sclerosis

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ABSTRACT

Objective Systemic sclerosis (SSc) is associated with an increased risk of cancer. We aimed to assess the prevalence of cancer in our cohort and to explore possible associations with clinical, immunological and treatment characteristics.

Methods Our retrospective monocentric cohort study of patients with SSc recorded prevalent and incident cases of malignancy, including those diagnosed within 3 years of the SSc onset (defined as cancer-associated scleroderma) and sought associations with the clinical characteristics and the serum autoantibody profiling performed using RNA and protein immunoprecipitation, Western-blot, immunoblot and ELISA at the time of SSc diagnosis, prior to any specific treatment.

Results Among 290 patients with SSc, the overall prevalence of cancer was 20%, with 8% of cases being cancer-associated scleroderma. Both conditions were more frequent in elderly patients and in patients with positive anti-Ro52 or anti-U3-RNP. Cancer-associated scleroderma was significantly more prevalent among patients negative for both anti-centromere (ACA) and anti-topoisomerase-1 (TOPO1) antibodies, especially in the case of diffuse SSc. Immunosuppressants were not significantly associated with cancer. Patients triple negative for ACA, TOPO1 and anti-RNA polymerase III antibodies had a significantly higher risk of breast cancer.

Conclusions Cancer surveillance should be particularly careful in patients with diffuse SSc, increased age at disease onset and without classical SSc-related autoantibodies.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The prevalence and risk of malignancy are higher in patients with systemic sclerosis (SSc), compared with the general population. While anti-RNA polymerase III (POLR3) antibodies have represented a major stigma for cancer risk, recent evidence has suggested a more complex link between the serum autoantibody profile and malignancy.

WHAT THIS STUDY ADDS

⇒ This study presents a new report on the occurrence of cancer in a cohort of patients with SSc. It also highlights the complexity of the association between SSc and malignancy, possibly suggesting the emerging role of serum autoantibodies, including 'unconventional specificities' (eg, anti-Ro52), and their interaction with clinical characteristics, especially the cutaneous phenotype. This intricate interplay adds a fascinating layer to our understanding of SSc pathogenesis and emphasises the complexity of autoimmune processes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study confirms the importance of thoroughly assessing the presence of neoplasms in patients with SSc, also during follow-up. It enhances the understanding of the association between malignancy and SSc and emphasises the necessity to further elucidate such underlying factors in larger cohorts.

INTRODUCTION

Systemic sclerosis (SSc) is characterised by aberrant inflammation, fibrosis and small vessel vasculopathy[1 2] which are responsible for a wide spectrum of clinical manifestations.[3 4] Despite such heterogeneity, there are clusters of patients with similar SSc phenotypes and trajectories. Serum autoantibodies significantly contribute to patient stratification into clinically actionable subsets,[5] similarly to what is better established for idiopathic inflammatory myositis.[6 7]

The risk of cancer is increased in patients with SSc compared with the general population and identifying patients at risk represents an unmet need in disease management.[8–12] An increased risk of lung, breast and haematological malignancies has been reported in SSc, as to hypothesise the existence of a link between the autoimmune and oncological diathesis.[13] Urothelial and non-melanoma skin cancers have been associated with cyclophosphamide,

while the risk is elusive for other immunosuppressive therapies, namely mycophenolate mofetil (MMF), for which caution is however advised by the regulatory authorities.[13 14] It has also been proposed that SSc manifestations occurring within 3 years from cancer diagnosis represent a paraneoplastic phenomenon defined as cancer-associated scleroderma. Serum autoantibodies represent a powerful instrument to predict the risk of neoplasms in patients with SSc, with conflicting reports for anti-RNA polymerase III (POLR3)[15] and anti-RNPC-3 autoantibodies.[16] It has recently been proposed that the total number of autoantibody specificities and the presence of selected antigen associations may impact the risk of cancer,[17 18] similar to inflammatory myositis.[19 20]

We retrospectively analysed a cohort of phenotyped patients with SSc with the primary aim to evaluate the prevalence of cancer and cancer-associated SSc. As secondary objectives, we explored the potential impact of clinical, immunological and treatment characteristics that could be related to different profiles of cancer risk. Subgroup analyses were performed to evaluate for cancers diagnosed at different time points and to explore the features possibly associated to the most prevalent malignancies.

PATIENTS AND METHODS

Study population

We included SSc cases with an ongoing follow-up or a follow-up ≥5 years at the Scleroderma Unit of the Rheumatology and Clinical Immunology, Humanitas Research Hospital (Rozzano, Italy), starting from the 1 January 2012 to 30 September 2023. Patients were included if they fulfilled the 2013 American College of Rheumatology/European Alliance of the Associations for Rheumatology classification criteria for SSc.[21] Patients deceased for other causes than cancer before achieving at least 5 years of follow-up were excluded. Demographic and clinical data were comprehensively collected from the electronic medical records, including age at onset, smoking status, disease and follow-up duration, and clinical features. These included skin involvement; interstitial lung disease (ILD) detected at high-resolution CT as per guidelines[22]; primary heart involvement confirmed at cardiac magnetic resonance[23 24]; signs of pulmonary hypertension at echocardiography or pulmonary arterial hypertension (PAH) confirmed by right heart catheterisation when available; gastrointestinal involvement defined as the presence of symptoms (oesophageal reflux, dyspepsia, diarrhoea and constipation) and/or functional (oesophageal or anorectal manometry) and/or radiological (barium-contrasted oesophageal X-ray) signs of dysmotility.

In our centre, cancer screening is periodically prescribed in all patients, according to standard operating procedures including annual mammary ultrasound (if age ≥30) and mammography (if age ≥40), annual dosage of serum prostate-specific antigen for men ≥50 years, faecal occult blood testing every other year for patients aged ≥40 and annual gynaecological evaluation. Complete blood cell count and chest HRCT are performed according to the clinical needs during the follow-up of SSc. We recorded all cases of malignancy, with the date of the diagnosis, but excluded monoclonal gammopathies of uncertain significance and non-melanoma skin cancers as these two conditions have an unclear natural history and are generally excluded in studies of the neoplastic risk.[25 26] Cancer-associated scleroderma was diagnosed when cancer occurred less than 3 years before or after the onset of the first non-Raynaud's symptom (or the finding of scleroderma-like pattern at nailfold capillaroscopy)[18 27]; in such cases, cancer was considered synchronous to SSc. For patients with a diagnosis of cancer preceding at least 3 years, the clinical onset of SSc was considered as having previous cancer, while if cancer was diagnosed at least 3 years after SSc, it was considered as subsequent. Data were gathered at the latest follow-up visit for patients without cancer or those with previous and synchronous cancer. Conversely, the time of cancer diagnosis was selected as the reference point for assessing the follow-up of patients with subsequent cancer. In the following text, the 'cancer' group refers to all patients diagnosed with cancer at any timepoint (ie, previous, synchronous—cancer-associated SSc, or subsequent); otherwise, the respective adjectives will be used to specify the different clusters.

Autoantibody detection

Serum samples were collected from patients at the time of SSc diagnosis before starting any immunosuppressive treatment. RNA and protein immunoprecipitation were performed for the detection of specific autoantibodies. Western-blot confirmation analysis was performed in case of immunoprecipitation positivity.[28–30] ELISA was used for autoantibodies not forming precipitins, such as Ro52/TRIM21.[31] Results of immunoblotting assays (Euroline) for the detection of SSc-associated autoantibodies were considered in patients for whom IP was not available.

In the final analysis, we considered autoantibodies directed towards different specific antigens: centromere (ACA), topoisomerase I (TOPO1), POLR3, Ro52, Ro60, La, U1-RNP, Th/To, NOR90, fibrillarin (U3-RNP), RNPC-3, Ku, PM/Scl. The presence of rheumatoid factors (RF), antibodies directed towards citrullinated peptides (ACPA) and double-stranded DNA (anti-dsDNA, on *C. luciliae* immunofluorescence) was also registered. Patients testing positive in two separate occasions for lupus anticoagulant, anticardiolipin IgG or anti-beta-2 glycoprotein I IgG antibodies were considered to have antiphospholipid antibodies (aPL), as per current recommendations.[32] According to previous definition, patients testing triple negative for ACA, anti-TOPO1 and anti-POLR3 were defined as 'CTP-negative'.[33] To further investigate whether the absence of the two most common autoantibodies (ie, TOPO1 and ACA) is correlated with the rate of malignancy, we defined 'double negative' those patients without both anti-TOPO1 and ACA.

Statistical analysis

Patient characteristics and clinical outcomes are presented as mean (SD) or median (IQR) for continuous variables, depending on whether they were normally or non-normally distributed, respectively. For variables with missing data (ie, teleangiectasis, calcinosis, anti-Ro52 and anti-Ro60), statistical analysis was performed only for patients with complete data. Overall, the prevalence of missing data was very low and is reflected by the denominators in the tables.

Statistical analysis was performed using Stata V.16 software (Stata Corp.). Continuous variables were compared using the two-tailed t-test if they followed a normal distribution, or the Mann-Whitney U test otherwise. Similarly, multiple-group comparisons of continuous variables were performed with analysis of variance for normally distributed variables, or the Kruskal-Wallis test for variables non-normally distributed. Categorical variables were analysed with the χ^2 or Fisher's exact test, as appropriate. Values of $p < 0.05$ were considered statistically significant.

Multivariable logistic regression was performed to assess the association between explanatory variables and the overall cancer rate. Variables were selected based on their clinical relevance and statistical significance in univariable analysis ($p \leq 0.10$). To fit the multivariable model, a stepwise selection process was used to eliminate variables that were not significant in the multivariable framework; a statistical criterion of $p \leq 0.05$ was used to determine which variables to eliminate.

RESULTS

Patients' characteristics

Our analysis included 290 patients with SSc with a cumulative observation of 2591 patient years, a median age at diagnosis of 56.5 years (IQR 43–65) and a median disease duration of 6 years (IQR 3–13). Clinical features are reported in table 1, with diffuse cutaneous SSc (dcSSc) in 42 (15%), ILD in 97 (33%), primary heart involvement in 41 (14%) and increased pulmonary arterial pressure in 34 (12%; of whom in 17 (6%) PAH was diagnosed through right heart catheterisation). Profiling of SSc-associated and related autoantibodies is reported in table 1. Furthermore, no patient tested positive for anti-RNPC-3, RF was positive in 18 (6%) patients, ACPA in 2 (1%) and anti-dsDNA in 6 (2%). Eleven (4%) patients were positive for aPL antibodies.

At the time of our database lock on 30 September 2023, 262 (90%) patients were alive and 59/290 (20%) had been diagnosed with cancer. The median follow-up period was 6 (IQR 2–13) years for patients diagnosed with cancer, while was 6 (IQR 3–13) years for the cancer-free counterpart. Cancer-associated scleroderma was diagnosed in 23 (8%) patients, while 13/290 (4%) were identified as previous cancer cases and 23/290 (8%) as subsequent.

Overall cancer prevalence

Patients ever diagnosed with cancer were significantly older at the time of SSc onset, with a median age of 61 (IQR 48–70) years versus 54.5 (IQR 41–63) years in patients without cancer (p=0.005; table 1). We observed no difference in terms of mortality, sex ratio, smoking history or clinical features, except for a tendency towards a higher prevalence of dcSSc in the cancer group.

Considering serum autoantibodies, the overall cancer occurrence was significantly higher in the presence of anti-Ro52 (14% vs 4%; p=0.009) and U3-RNP (3% vs 0%; p=0.041; table 1). While cancer prevalence did not differ with or without anti-POLR3 autoantibodies, among the 27 patients with anti-POLR3 autoantibodies, cancer was diagnosed in 6 cases. Notably, all 6 of these cancer cases (100%) had isolated anti-POLR3 autoantibodies. In contrast, among the remaining 21 patients with anti-POLR3 autoantibodies but without cancer, only 11 (52%) had isolated anti-POLR3 autoantibodies, while the other 10 had concurrent autoantibodies directed towards other antigens (p=0.033; not shown in tables).

Diffuse cutaneous disease impacted on overall cancers in 'double negative' patients (12% vs 4%; p=0.025) and in those with isolated anti-POLR3 autoantibodies (9% vs 2%; p=0.033) (table 1). Also, when focusing on anti-TOPO1-negative cases, cancer prevalence was significantly higher in patients with dcSSc (8/19; 42%) compared with the other cutaneous subsets (42/203; 21%) (p=0.044; not shown in tables).

Multivariable analysis confirmed the overall cancer prevalence was increased with elderly age at the onset of SSc (OR 1.03; 95% CI 1.01 to 1.06; p=0.006) and in the presence of anti-Ro52 antibodies (OR 3.86; 95% CI 1.19 to 12.5; p=0.025).

Cancer-associated scleroderma

Compared with patients without cancer, cancer-associated scleroderma was observed in elder patients, with a median age of 65 (IQR 53–71) years (p=0.006), with shorter disease duration, and with a lower prevalence of gastrointestinal manifestations (17% vs 42%; p=0.024; table 1).

Considering autoantibodies, anti-Ro52 were more frequently observed with cancer-associated scleroderma (24% vs 4%; p=0.004), even when detected as the sole serum specificity (10% vs 1%; p=0.039; table 1). Cancer-associated scleroderma was more often diagnosed in patients without common autoantibodies, including both the 'double negative' (39% vs 20%; p=0.028) and CTP-negative cases (26% vs 11%; p=0.052). Remarkably, these associations were further strengthened in the presence of the diffuse cutaneous subset (table 1).

Cancer risk and MMF therapy

At the time of our analysis, 81/290 (28%) patients had ever been on immunosuppressive therapy during the disease course, while 76/290 (26%) were still on treatment: 52/290 (18%) on MMF 2–3 g/d; 9/290 (3%) on

Table 1 Comparison of demographic, clinical and serological features of patients with SSc without cancer, patients with SSc and overall cancer occurrence, and patients with cancer-associated scleroderma

	Whole cohort (n=290)	No cancer (n=231)	Overall cancers (n=59)	P value*	Cancer-associated SSc (n=23)	P value†
Demographic features						
Age at SSc onset	56.5 (43–65)	54.5 (41–63)	61 (48–70)	0.005‡	65 (53–71)	0.006§
Alive at 31 July 2023	262/290 (90.3)	211/231 (91.3)	51/59 (86.4)	0.255	21/23 (91.3)	0.995
Smoke (ever)	71/290 (24.5)	55/231 (23.8)	16/59 (27.1)	0.598	9/23 (39.1)	0.107
Sex (male)	20/290 (6.9)	14/231 (6.1)	6/59 (10.2)	0.259	3/23 (13.0)	0.190
Disease duration (years)	6 (3–13)	6 (3–13)	6 (2–13)	0.578	4 (1–5)	0.004§
Clinical features						
dcSSc	42/290 (14.5)	29/231 (12.6)	13/59 (22.0)	0.065	5/23 (21.7)	0.208
dcSSc versus lcSSc	42/203 (20.7)	29/161 (18.0)	13/42 (31.0)	0.065	5/14 (35.7)	0.151
mRSS	2 (0–6)	2 (0–6)	2 (0–6)	0.457	1 (0–4)	0.148
ILD	97/290 (33.5)	76/231 (32.9)	21/59 (35.6)	0.696	4/23 (17.4)	0.160
pHI	41/290 (14.1)	32/231 (13.9)	9/59 (15.3)	0.834	1/23 (4.3)	0.328
PAH	34/290 (11.7)	27/231 (11.7)	7/59 (11.9)	1.000	2/23 (8.7)	1.000
GI involvement	118/290 (40.7)	98/231 (42.4)	20/59 (33.9)	0.234	4/23 (17.4)	0.024§
Digital ulcers	57/290 (19.7)	45/231 (19.5)	12/59 (20.3)	0.882	3/23 (13.0)	0.584
Telangiectasis	110/289 (38.1)	86/230 (37.4)	24/59 (40.7)	0.643	4/23 (17.4)	0.068
Calcinosis	28/289 (9.7)	24/230 (10.4)	4/59 (6.8)	0.470	0/23 (0)	0.142
Autoantibodies						
ACA	161/290 (55.5)	128/231 (55.4)	33/59 (55.9)	0.943	12/23 (52.2)	0.828
TOPO1	68/290 (23.5)	59/231 (25.5)	9/59 (15.3)	0.121	2/23 (8.7)	0.078
dcSSc	23/290 (7.9)	18/231 (7.8)	5/59 (8.5)	0.792	0/23 (0)	0.385
TOPO1 isolated	48/290 (16.6)	43/231 (18.6)	5/59 (8.5)	0.076	1/23 (4.3)	0.143
dcSSc	17/290 (5.9)	15/231 (6.5)	2/59 (3.4)	0.539	0/23 (0)	0.374
Double negative	62/290 (21.4)	45/231 (19.5)	17/59 (28.8)	0.119	9/23 (39.1)	0.028§
dcSSc	16/290 (5.5)	9/231 (3.9)	7/59 (11.9)	0.025‡	4/23 (17.4)	0.021§
POLR3	27/290 (9.3)	21/231 (9.1)	6/59 (10.2)	0.803	3/23 (13.0)	0.464
dcSSc	13/290 (4.5)	8/231 (3.5)	5/59 (8.5)	0.149	2/23 (8.7)	0.226
POLR3 isolated	17/290 (5.9)	11/231 (4.8)	6/59 (10.2)	0.125	3/23 (13.0)	0.122
dcSSc	10/290 (3.5)	5/231 (2.2)	5/59 (8.5)	0.033‡	2/23 (8.7)	0.125
CTP negative	36/290 (12.4)	26/231 (11.3)	10/59 (16.9)	0.237	6/23 (26.1)	0.052§
dcSSc	3/290 (1.0)	1/231 (0.4)	2/59 (3.4)	0.106	2/23 (8.7)	0.022§
lcSSc	21/290 (7.2)	17/231 (7.4)	4/59 (6.8)	1.000	2/23 (8.7)	0.685
Ro52	17/277 (6.1)	9/221 (3.9)	8/56 (14.3)	0.009‡	5/21 (23.8)	0.004§
Ro52 isolated	4/277 (1.4)	2/221 (0.9)	2/56 (3.6)	0.183	2/21 (9.5)	0.039§
Ro60	10/278 (3.6)	8/222 (3.6)	2/56 (3.6)	1.000	0/21 (0)	1.000
SSB	4/290 (1.4)	3/231 (1.3)	1/59 (1.7)	1.000	0/23 (0)	1.000
U1-RNP	2/290 (0.7)	2/231 (0.9)	0/59 (0)	1.000	0/23 (0)	1.000
Ku	4/290 (1.4)	3/231 (1.3)	1/59 (1.7)	1.000	0/23 (0)	1.000
PM/Scl	5/290 (1.7)	4/231 (1.7)	1/59 (1.7)	1.000	0/23 (0)	1.000
NOR90	8/290 (2.8)	5/231 (2.2)	3/59 (5.1)	0.208	2/23 (8.7)	0.125
Th/To	6/290 (2.1)	4/231 (1.7)	2/59 (3.4)	0.605	1/23 (4.3)	0.380
U3-RNP	2/290 (0.7)	0/231 (0)	2/59 (3.4)	0.041‡	1/23 (4.3)	0.091
Overlapping autoimmune diseases						
Thyroiditis	33/290 (11.4)	28/231 (12.1)	5/59 (8.5)	0.500	0/23 (0)	0.087

Continued

Table 1 Continued

	Whole cohort (n=290)	No cancer (n=231)	Overall cancers (n=59)	P value*	Cancer-associated SSc (n=23)	P value†
PBC	28/290 (9.7)	21/231 (9.1)	7/59 (11.9)	0.471	2/23 (8.7)	1.000
SjS	19/290 (6.6)	13/231 (5.6)	6/59 (10.2)	0.237	3/23 (13.0)	0.166

*Comparison between patients with SSc with overall cancer versus patients with SSc without cancer.
†Comparison between patients with cancer-related scleroderma versus patients with SSc without cancer.
‡P <0.05 comparing patients with SSc with overall cancer versus patients with SSc without cancer.
§P< 0.05 comparing patients with cancer-related scleroderma and patients with SSc without cancer.
ACA, anticentromere antibodies; CTP-negative, triple ACA-, TOPO1-, POLR3-negative; dcSSc, diffuse cutaneous SSc; GI, gastrointestinal; ILD, interstitial lung disease; lcSSc, limited cutaneous SSc; MMF, mycophenolate mofetil; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; PBC, primary biliary cholangitis; PHI, primary heart involvement associated to SSc; POLR3, RNA polymerase III; SjS, Sjogren's syndrome.

methotrexate 10–15 mg/week; 7/290 (2%) on cyclophosphamide 6–9 g; 5/290 (2%) on azathioprine 2 mg/kg/d; 6/290 (2%) on rituximab 2 g every 6 months (4/6 on concomitant MMF; 1/6 on concomitant methotrexate; 1/6 on concomitant cyclophosphamide); 6/290 (2%) on tocilizumab 162 mg/week (2/6 on concomitant MMF; 1/6 on methotrexate; 3/6 monotherapy); 4/290 (1%) on nintedanib 150 mg twice daily (3/4 on concomitant MMF, 1/4 on concomitant cyclophosphamide).

To analyse the impact of immunosuppressive drugs on cancer, we excluded all patients who were diagnosed with cancer before the onset of SSc and obtained that the rate of cancers was 37/268 (14%). Exposure to immunosuppressive drugs at any timepoint was not associated with cancer occurrence, which was observed in 7/77 (9%) patients ever exposed and in 30/191 (16%; p=0.176) patients never exposed to immunosuppressants.

We then focused on MMF, being the most adopted drug. Of 268 patients, 49 were actively on MMF, with a median daily dosage of 2 g (IQR 2–3) and a treatment duration of 36 months (IQR 15–57). Of 219 patients not on active MMF, 6 had previously discontinued the drug due to disease progression (4/6), recurrent infections (1/6) or pregnancy desire (1/6); these subjects were excluded from subsequent analysis. A cancer diagnosis was formulated in 2/49 patients (4%) on MMF, while in 33/213 (15%) patients never receiving MMF (p=0.036).

Cancer onset timing and type

The features of patients with *previous*, *synchronous* (cancer-associated SSc) and *subsequent* cancers are compared in table 2. No difference was noted among patients with *previous* versus *synchronous* versus *subsequent* malignancy in terms of clinical features, serum autoantibodies or overlapping autoimmune diseases, with the exception of age at SSc onset and the frequency of telangiectasia. Remarkably, the disease duration was longer among patients with subsequent cancers. When considering the most common cancer subsets, breast cancers were significantly more observed in the *previous* group (p=0.030).

The most common cancer subtypes were breast, lung and haematological malignancy, accounting for 22/59 (37%), 8/59 (14%) and 7/59 (12%) cancer cases, respectively (table 3). Compared with patients without cancer, patients with breast cancer had significantly older age at SSc onset (62 (IQR 48–71) vs 54.3 (41–63) years, respectively; p=0.041), but no difference was noted in terms of clinical features. Considering autoantibodies, breast cancer was more frequently observed with anti-Ro52, particularly when detected as the only serum specificity (10% vs 1%; p=0.039), and in CTP-negative patients (p=0.043), especially with dcSSc phenotype (p=0.021) (table 3).

Compared with cancer-free cases, patients developing lung cancer were significantly older at the onset of SSc (69 (IQR 61–71) vs 54.3 (41–63) years; p=0.008) and more frequently males (50% vs 6%; p=0.001), while smoking was not a significant predictor. Lung malignancy was associated to high mortality, with only 5/8 (63%) patients being alive at the end of the follow-up period, compared with 211/231 (91%) patients without cancer (p=0.032). Despite ILD and pulmonary hypertension secondary to ILD were not associated with lung cancer, dcSSc and PAH tended to be more common compared with cancer-free patients. Lung malignancy was more frequent in anti-TOPO1+dcSSc (p=0.004) and with anti-U3-RNP/fibrillarin antibodies (13% vs 0%; p=0.033) (table 3).

Haematological malignancies included lymphoproliferative disorders (4/7; 57%), acute myeloid leukaemia (2/7; 29%) and myeloproliferative neoplasms (1/7; 14%) and were associated to a significantly lower chance of survival at the end of the follow-up period, compared with patients without cancer (57% vs 91%, respectively; p=0.021). The clinical profile of patients with haematological malignancies did not differ significantly from patients without cancer and so was for the distribution of serum autoantibodies, except for a trend towards higher prevalence of anti-U3-RNP (14% vs 0%; p=0.065) (table 3).

DISCUSSION

Our data on demographic, clinical and serological risk factors for cancer in SSc confirm previously reported and identify novel significant associations, particularly in

Table 2 Comparison of demographic, clinical and serological features of patients with SSc and cancer, according to the timing of cancer onset, that is: *previous* (occurring more than 3 years before the diagnosis of SSc), *subsequent* (occurring more than 3 years after the diagnosis of SSc), or *synchronous* (occurring within 3 years from the diagnosis of SSc)

	Previous (n=13)	Synchronous (n=23)	Subsequent (n=23)	P value
Demographic features				
Age at SSc onset	67 (36–83)	65 (29–86)	56 (23–71)	0.005*
Alive at 31 July 2023	12/13 (92.3)	21/23 (91.3)	18/23 (78.3)	0.433
Smoke (ever)	1/13 (7.7)	9/23 (39.1)	6/23 (26.1)	0.124
Sex (male)	0/13 (0)	3/23 (13.0)	3/23 (13.0)	0.389
Disease duration (years)	2 (2–11)	4 (1–5)	14 (13–22)	0.001*
Clinical features				
dcSSc	2/13 (15.4)	5/23 (21.7)	6/23 (26.1)	0.920
mRSS	0 (0–15)	0 (0–24)	3 (0–43)	0.092
ILD	6/13 (46.2)	4/23 (17.4)	11/23 (47.8)	0.064
pHI	2/13 (15.4)	1/23 (4.3)	6/23 (26.1)	0.135
PAH	0/13 (0)	2/23 (8.7)	5/23 (21.7)	0.139
Gut	6/13 (46.2)	4/23 (17.4)	10/23 (43.5)	0.114
Digital ulcers	1/13 (7.7)	3/23 (13.0)	8/23 (34.8)	0.137
Teleangiectasis	7/13 (53.8)	4/23 (17.4)	13/23 (56.5)	0.017*
Calcinosis	1/13 (7.7)	0/23 (0)	3/23 (13.0)	0.257
Autoantibodies				
ACA	8/13 (61.5)	12/23 (52.2)	13/23 (56.5)	0.943
TOPO1	2/13 (15.4)	2/23 (8.7)	5/23 (21.7)	0.529
POLR3	1/13 (7.7)	3/23 (13.0)	2/23 (8.7)	1.000
Ro52	0/12 (0)	5/21 (23.8)	3/23 (13.0)	0.201
Cancer type				
Breast cancer	9/13 (40.9)	7/23 (31.8)	6/23 (27.3)	0.030*
Lung cancer	0/13 (0)	4/23 (50)	4/23 (50)	0.333
Haematological cancers	0/13 (0)	3/23 (42.9)	4/23 (57.1)	0.358

P >0.05 for all the other tested autoantibodies, including anti-Ro60, -SSB, U1-RNP, -Ku, -PM/Scl, -NOR90, -Th/To, -U3-RNP.
*P <0.05 three-group comparison among patients with previous, synchronous and subsequent cancer.
ACA, anticentromere antibodies; CTP-negative, triple ACA-, TOPO1-, POLR3-negative; dcSSc, diffuse cutaneous SSc; GI, gastrointestinal; ILD, interstitial lung disease; lcSSc, limited cutaneous SSc; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; PBC, primary biliary cholangitis; pHI, primary heart involvement associated to SSc; POLR3, RNA polymerase III; SjS, Sjogren's syndrome.

terms of serum autoantibodies, cancer timing and cancer type.

Malignancy represents a significant cause of death in patients with SSc in the European Scleroderma Trials & Research Group database.[34] Our real-life cohort reports a high overall rate of malignancies, reaching 20%, while the prevalence of cancer-associated scleroderma is comparable to previous data.[33 35] In addition to confirming the association between cancer and older age at SSc onset,[36] our data demonstrate a link between elderly onset and cancer-associated scleroderma, and high suspicion for occult neoplasm is recommended in this scenario. However, these observations should seek confirmation with multivariable analysis in larger cohorts. Concerning clinical features, the overall cancer rate was higher with dcSSc compared with lcSSc, although the difference did not reach statistical significance. Similar findings were described in a large meta-analysis,[10] but conflicting evidence has also been reported.[33]

In contrast with previous evidence,[18 35–37] we did not observe an association between anti-POLR3 antibodies and the overall cancers or cancer-associated scleroderma. Our results may be affected by the sample size and low prevalence of anti-POLR3 antibodies, which is typical for a European cohort.[38] However, relevant considerations can still be made, considering the role of multiple autoantibody specificities. First, when focusing the analysis on patients with anti-POLR3, the overall cancer rate was significantly higher in those with isolated anti-POLR3 autoantibodies compared with subjects with other associated serum specificities.[17] Second, a higher rate of cancer-associated SSc was observed in 'double-negative'

Table 3 Demographic, clinical and serological features of patients with SSc according to different types of cancer (ie, breast, lung, haematological malignancies), and compared with the cancer-negative counterpart of the SSc cohort

	No cancer (n=231)	Breast (n=22)	Lung (n=8)	Haematologic (n=7)
Demographic features				
Age at SSc onset	54.5 (41–63)	62 (48–71)*	69 (61–71)†	53 (40–60)
Alive at 31 July 2023	211/231 (91.3)	20/22 (90.9)	5/8 (62.5)†	4/7 (57.1)‡
Smoke (ever)	55/231 (23.8)	4/22 (18.2)	4/8 (50.0)	3/7 (42.9)
Sex (male)	14/231 (6.1)	0/22 (0)	4/8 (50.0)†	1/7 (14.3)
Disease duration (years)	6 (3–13)	6 (2–13)	9 (6–13)	15 (1–15)
Clinical features				
dcSSc	29/231 (12.6)	5/22 (22.7)	3/8 (37.5) p = 0.077	1/7 (14.3)
dcSSc versus lcSSc			3/4 (75.0)†	
mRSS	2 (0–6)	2 (0–4)	0 (0–11)	2 (0–3)
ILD	76/231 (32.9)	9/22 (40.9)	3/8 (37.5)	3/7 (42.9)
pHI	32/231 (13.9)	4/22 (18.2)	0/8 (0)	1/7 (14.3)
PAH	27/231 (11.7)	0/22 (0)	3/8 (37.5) p = 0.065	0/7 (0)
Gut	98/231 (42.4)	7/22 (31.8)	3/8 (37.5)	2/7 (28.6)
Digital ulcers	45/231 (19.5)	4/22 (18.2)	3/8 (37.5)	2/7 (28.6)
Teleangiectasis	86/230 (37.4)	11/22 (50.0)	2/8 (25.0)	4/7 (57.1)
Calcinosis	24/230 (10.4)	2/22 (9.1)	1/8 (12.5)	0/7 (0)
Autoantibodies				
ACA	128/231 (55.4)	10/22 (45.5)	3/8 (37.5)	5/7 (71.4)
TOPO1	59/231 (25.5)	4/22 (18.2)	3/8 (37.5)	1/7 (14.3)
dcSSc	18/231 (7.8)	2/22 (9.1)	3/8 (37.5)†	0/7 (0)
TOPO1 isolated	43/231 (18.6)	2/22 (9.1)	2/8 (25.0)	1/7 (14.3)
dcSSc	15/231 (6.5)	1/22 (4.5)	2/8 (25.0)	0/7 (0)
Double negative	45/231 (19.5)	8/22 (36.4)	2/8 (25.0)	1/7 (14.3)
dcSSc	9/231 (3.9)	3/22 (13.6) p = 0.075	0/8 (0)	0/7 (0)
POLR3	21/231 (9.1)	1/22 (4.5)	0/8 (0)	0/7 (0)
CTP-negative	26/231 (11.3)	6/22 (27.3)*	2/8 (25.0)	1/7 (14.3)
dcSSc	1/231 (0.4)	2/22 (9.1)*	0/8 (0)	0/7 (0)
Ro52	9/221 (3.9)	3/21 (14.3) p = 0.074	1/7 (14.3)	1/7 (14.3)
Ro52 isolated	2/221 (0.9)	2/21 (9.5)*	0/7 (0)	0/7 (0)
U3-RNP	0/231 (0)	0/22 (0)	1/8 (12.5)†	1/7 (14.3) p = 0.065
Overlapping autoimmune diseases				
Thyroiditis	28/231 (12.1)	3/22 (13.6)	0/8 (0)	0/7 (0)
PBC	21/231 (9.1)	2/22 (9.1)	2/8 (25.0)	0/7 (0)
SjS	13/231 (5.6)	1/22 (4.5)	1/8 (12.5)	2/7 (28.6)‡

p>0.05 for anti-Ro60, -SSB, U1-RNP, -Ku, -NOR90, -Th/To, -PM/Scl antibodies.
*P <0.05 comparing cases of breast cancer with patients with SSc without cancer.
†P <0.05 comparing cases of lung cancer with patients with SSc without cancer.
‡P <0.05 comparing cases of haematological neoplasms with patients with SSc without cancer.
ACA, anticentromere antibodies; CTP-negative, triple ACA-, TOPO1-, POLR3-negative; dcSSc, diffuse cutaneous SSc; GI, gastrointestinal; ILD, interstitial lung disease; lcSSc, limited cutaneous SSc; mRSS, modified Rodnan skin score; PAH, pulmonary arterial hypertension; PBC, primary biliary cholangitis; pHI, primary heart involvement associated to SSc; POLR3, RNA polymerase III; SjS, Sjogren's syndrome.

and CTP-negative patients, corroborating previous evidence.[33] Akin to what elegantly postulated in other connective tissue diseases,[6 19 20 39] lower cancer risk has been reported in SSc with multiple antibody positivity.[40] Thus, the expansion of the autoantibody repertoire may be protective against cancer occurrence in patients

with SSc, especially at time points close to disease onset. However, this observation does not appear to be universally valid and might be antigen dependent, as we found opposite results with anti-Ro52.

The association between malignancy and the absence of 'conventional' autoantibodies raises concern for the role of rarer specificities. While no positivity for anti-RNPC-3 was observed in our cohort,[16 18] we report for the first time that, despite their rarity, antibodies towards U3-RNP/fibrillarin are significantly associated with both overall cancer and cancer-associated SSc. Also, even though we did not confirm data concerning enhanced cancer risk with anti-PM/Scl autoantibodies,[11 41] our observations on anti-U3-RNP further support the association between malignancy and autoantibodies directed to nucleolar antigens.[42] Further on the subject of unconventional antibodies, anti-SSA positivity was recently found to predict cancer occurrence in a French cohort of patients with SSc.[27] Since anti-SSA antibodies target either Ro52 or Ro60 antigens,[43] we separately detected antibodies to Ro52 and Ro60 in our cohort of patients with SSc and observed a significantly higher frequency of anti-Ro52 but not anti-Ro60 antibodies in patients with overall cancer and cancer-associated scleroderma, implementing recent observations made in a large case-control study.[18]

The dcSSc phenotype acts as a modifier of the autoantibody-related risk of cancer. Similar to findings from Igusa *et al*,[33] overall cancers and cancer-associated SSc tended to occur more frequently with anti-POLR3+dcSSc, particularly in patients with isolated antibodies. Also, an enhanced rate of malignancies was observed with dcSSc in patients without conventional autoantibody specificities, with 'double-negative patients' showing a higher prevalence of overall cancers and CTP-negative patients being more often diagnosed with synchronous cancers.

In line with the literature,[9 44–47] breast, lung and haematological neoplasms were the most frequently observed in our cohort of patients with SSc. Lung and haematopoietic malignancies strongly impacted on the overall survival. Breast cancer was nearly three times more common in CTP-negative subjects and was associated to anti-Ro52 autoantibodies, particularly when isolated. Of note, anti-Ro52 was the most common autoantibody detected in patients with breast cancer and no sign of systemic autoimmune diseases.[48] Since hyperexpression of Ro52 has been associated with favourable outcomes,[49] further research could explore the prognostic significance of anti-Ro52 in the progression of breast malignancy and pathophysiology of the anti-cancer response in patients with SSc.

In patients with SSc, lung cancer has been variably associated with traditional risk factors, including smoking, older age and male sex, along with longer disease duration of SSc and the presence of ILD.[13 46 50] However, conflicting evidence has been reported[10] and, similarly, we did not describe any association between ILD and lung cancer, which occurred more frequently with dcSSc and PAH. Concerning antibodies, we observed lung malignancy to be more frequent in patients with anti-TOPO1+diffuse SSc, along with anti-U3-RNP/fibrillarin. Due to the low sample size, these results should be interpreted with extreme caution.

A high prevalence of haematological malignancies has been reported in patients with SSc, in particular B-cell non-Hodgkin's lymphomas,[47] but the role of overlap SjS is unclear.[51] We report that the concurrence of SjS and SSc, although in a small number of patients, is associated with an increased risk of lymphoma: in our cohort, both patients with SSc/SjS overlap developing lymphoma had ACA, one of them displaying a high SjS serological risk profile, represented by concurrent positivity for anti-Ro52 and RFs.[51]

We did not observe any increase in the rate of malignancies with immunosuppressive therapies, particularly MMF. In transplanted patients, a slightly reduced risk of cancer was reported with MMF compared with azathioprine, including skin cancers.[52 53] From a mechanistic perspective, our group previously demonstrated that MMF exerts an inhibitory effect on B-cells and humoral immunity, without impairing the cell-mediated response,[54] which is crucial for anti-cancer immune surveillance.[55] Also, increased risk of cancer has been linked to subclinical chronic inflammation,[56] which is potentially present in patients with SSc not receiving immunosuppressants. Since our study was not designed to evaluate cancer occurrence in patients treated with immunosuppressants, we advocate for this crucial research question to be addressed in larger case-control studies.

Our study has several strengths. The cohort of patients was extensively profiled from both clinical and immunological perspective. Several strategies were adopted to minimise bias and enhance the validity and reliability of our findings. Comprehensive data recording was ensured for all patients, with a very small percentage of missing data, only a minority of patients. Standardised inclusion criteria were defined to maintain consistency, and reference methods were applied for the detection of serum autoantibodies. When feasible, multivariable logistic regression was employed to adjust for potential confounders.

The most important limitations of our study stem from its retrospective design and the explorative nature of the secondary aims, which led to multiple testing. Thus, caution is warranted when interpreting some results, for example, rarer autoantibodies and subgroup analyses, due to a possible increase in the rate of false positives. Multivariable analysis was performed only for overall cancers to avoid overfitting. Disease duration and follow-up varied across different clusters, with shorter durations observed in cancer-associated SSc and longer durations, for instance, in cases of subsequent cancers. The heterogeneity of follow-up duration, reaching decades for certain participants, and the regular prescription of cancer screening as per Rheumatologists' practice might have partially contributed to increase the overall cancer

rate. Considering clinical features, a high prevalence of SSc *sine* scleroderma was observed, possibly reflecting the high proportion of ACA and, therefore, the percentage of patients whose diagnosis relies on the combination of Raynaud's phenomenon, SSc antibodies, capillaroscopy alterations and telangiectasis.[57 58] However, no difference in the rate of cancers or cancer-associated SSc was observed comparing patients with SSc *sine* scleroderma w/o internal organ involvement (ie, ILD and PAH; data not shown). PAH confirmation through right-heart catheterisation was not available for all patients with echocardiographic suspicion. Similarly, we lack data on different nailfold capillaroscopy patterns. As shown in the online supplemental figure, immunoprecipitation was not available for the whole patient cohort; thus, a proportion of data concerning serum autoantibodies relies on results obtained from routine laboratories, possibly leading to bias.[59] On the other hand, we included in the analysis only patients with complete data on the autoantibodies we intended to investigate. We did not evaluate for changes in the autoantibody profile of patients at the time of cancer diagnosis as a prospective study could have allowed. Considering immunosuppressive therapies, we were not able to detect associations with other drugs but MMF, probably due to the sample size. Most importantly, as discussed, data on MMF are just meant to be speculative in nature, since a case-control study is needed to assess the role of immunosuppressants in determining the risk of malignancy in SSc.

To conclude, peculiar suspicion for cancer should arise in patients with SSc who are double and CTP negative, along with anti-Ro52 antibodies. There is a tendency for malignancy to develop more often in dcSSc, while no other clinical aspect seems to predispose to cancer. The most frequent cancers were breast, especially preceding the onset of SSc, lung and haematological neoplasms, for which surveillance programmes are warranted. Such an initiative should embrace a precision medicine approach, aiming to comprehensively investigate patients with varying degrees of malignancy risk.

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Contributors Conceptualisation: AT and MDS. Data collection: AT, FM, NI, RR and EN. Statistical analysis: AT, AC, SB and FM. Writing – original draft: AT and MDS. Writing – review and editing: AT, MDS, SB and CS. Guarantor: MDS. All the authors have read and approved the final version of the manuscript.

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