Anti-Th/To antibodies in systemic sclerosis: analysis of long-term follow-up of pulmonary involvement, organ damage accrual and mortality in an Italian cohort with a case-control study

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Abstract

Objective

In systemic sclerosis (SSc) American patients, anti-Th/To antibodies were reported to be associated with interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH). Few data in European patients are available, so we aimed at describing the clinical associations of anti-Th/To antibodies, focusing on ILD outcome, organ damage and mortality in an Italian single-centre cohort.

Methods

Case-control study: anti-Th/To+ SSc patients vs. anti-topoisomerase (anti-topo)1+, anticentromere (ACA)+ and quadruple-negative (anti-topo 1-, ACA-, anti-RNAP3-, anti-Th/To-) SSc patients (1:3; matched for sex and age at SSc onset). Organ damage was assessed with the SCTC-Damage Index.

Results

Thirteen anti-Th/To+ patients were evaluated: 100% had limited cutaneous involvement; 46% digital ulcers; none had PAH, synovitis, joint contractures. As compared to anti-topo 1+ and quadruple-negative patients, anti-Th/To+ patients developed less frequently ILD (40% vs. 85% and 84%), that required less immunosuppression (8% vs. 41% and 44%), and rarely had functional worsening (15.4% at 5 years), without development of long-term complications (no need for O_2, pulmonary hypertension, death). In anti-Th/To+ patients, the Damage Index was lower than in anti-topo 1+ and quadruple-negative patients at various timepoints, and remained low during the long-term follow-up (median: 16 years). The 5- and 10-year survival of anti-Th/To+ patients was 92% and 72%, respectively, and did not differ from those of the SSc matched patients; none of the anti-Th/To+ patients died due to SSc, while mortality was mainly related to cancer.

Conclusion

In this study, anti-Th/To+ patients showed a mild SSc phenotype, characterised by low organ damage, favourable ILD outcome and good survival.

Key words

anti-Th/To antibodies, systemic sclerosis, interstitial lung disease, organ damage accrual, mortality

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Introduction

Antinuclear autoantibodies have been suggested to be very useful markers in identifying distinct disease subsets in systemic sclerosis (SSc), a rare disorder with substantial clinical heterogeneity (1, 2). Although the clinical significance of the more common SSc-specific antibodies [anti-centromere (ACA), anti-topoisomerase (anti-topo), anti-RNA-polymerase (anti-RNAP)] has already been defined, further research is still needed to clarify the clinical associations of rarer autoantibodies (3). Among them, anti-Th/To autoantibodies (directed towards protein components of 2 endonucleases: the human RNase MRP and RNase P ribonucleoprotein complexes) are among the most common and have been shown to be fairly specific for SSc (3-5).

In SSc, anti-Th/To antibodies have been consistently associated with limited cutaneous involvement (lcSSc). In comparison with ACA+ patients, the most frequent subgroup among those with lcSSc, anti-Th/To+ SSc patients are younger and more frequently male (5). Moreover, they show shorter time between the onset of Raynaud's phenomenon and the onset of SSc, and earlier development of nailfold capillary microscopy abnormalities (5, 6), but less severe vascular and gastrointestinal involvement (7). On the other hand, pulmonary involvement is significantly more common (7, 8).

Some open questions regarding other clinical features related to anti-Th/To+ SSc subset still need to be addressed.

First, SSc patients with anti-Th/To antibodies were reported by several studies describing the large single-centre American cohort from Pittsburgh to have the worst survival among different subsets of patients with lcSSc, and it was suggested that this could be explained by the higher frequency of interstitial lung disease (ILD) and pulmonary hypertension (PH) (7-9). However, these data were not adjusted for the presence of concomitant factors that might explain a lower survival in SSc patients, such as male gender, non-Caucasian ethnicity, longer disease duration, and smoking habit, which are reported to be more prevalent among anti-Th/To+ patients (7-9). Furthermore, few data on long-term outcome of ILD in these patients are available, particularly regarding pulmonary function tests (PFT) evolution, need for immunosuppressive therapy or O_2 supply, onset of PH secondary to chronic hypoxia (9), and ILD-related mortality. Secondly, irreversible organ damage, associated with morbidity, mortality and reduction of patients' quality of life, usually accrues very early in the course of SSc (10), but data concerning this issue in anti-Th/To+ patients are lacking.

Thirdly, in an American cohort, anti-Th/To antibodies were associated with a reduced risk of cancer diagnosis within the first 3 years after SSc onset, as compared with other SSc subsets (11). On the contrary, others reported an overall high incidence of cancer history in Japanese patients with anti-Th/To antibodies and raised the concern that it could explain higher mortality in this SSc subset (12).

Therefore, the present study was focused on the analysis of long-term outcome of ILD, organ damage accrual, association with cancer, and mortality in Italian SSc patients with anti-Th/To autoantibodies, through a case-control study.

Materials and methods

Study design

A single-centre case-control retrospective study was conducted in an Italian Rheumatology and Clinical Immunology Unit.

Inclusion criteria and selection of control patients

Adult (≥18 years) patients with SSc, according to 2013 ACR/EULAR classification criteria, attending the Unit between 2000 and 2021 were considered. Among them, only those with a single SSc-specific autoantibody positivity were included. Cases were represented by patients positive for anti-Th/To antibodies. Notably, these antibodies were tested in patients with SSc clinical features who resulted negative for ACA, anti-topo 1 and anti-RNAP3 antibodies, through RNA immunoprecipitation (IP) test or immunoblotting (IB) assay (Systemic Sclerosis Pro-

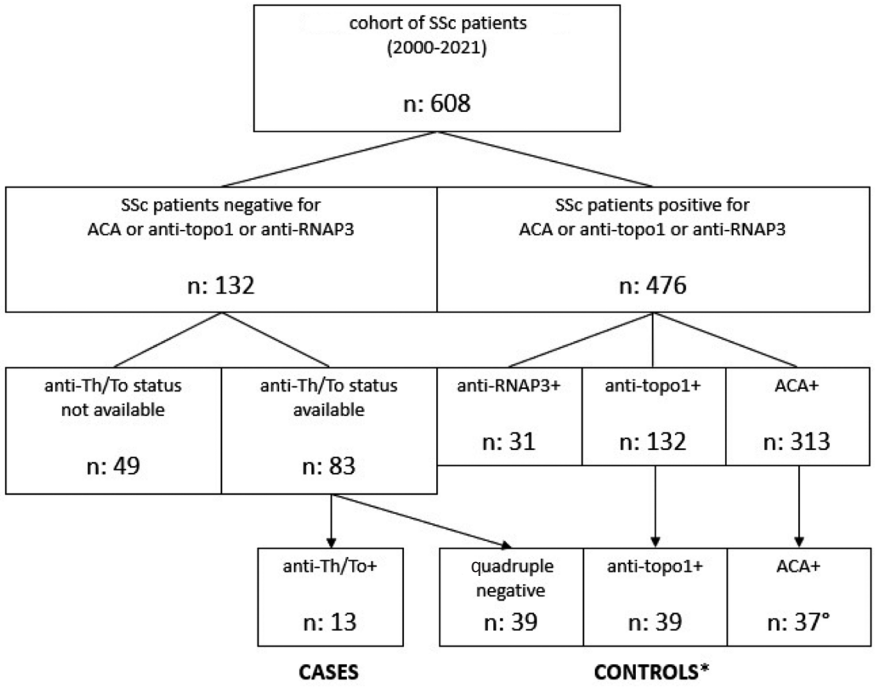


Fig. 1. Flow chart: identification of anti-Th/To+ patients (cases) and ACA+, anti-topo 1+ and quadruple-negative sex and age at disease onset-matched SSc patients (controls).
*Controls were matched for sex and age at disease onset (± 5 years).
°Among 313 ACA+ patients, 7 male patients were identified.

file (Nucleoli) 13 antigens; Euroimmun, Lübeck, Germany). Anti-Th/To antibodies tested through IB were considered positive only if present at medium-high titre (> positive ++) on a semi-quantitative scale. The cut-off values for antibody positivity, as indicated by the manufacturer are: negative (-) (0-5 AU), borderline (+) (6-10 AU), positive + (11-25 AU), positive ++ (26-50 AU) and positive +++ (51-256 AU). For each case (anti-Th/To+), three anti-topo 1+, three ACA+, and three negative for ACA, anti-topo 1, anti-RNAP3 and anti-Th/To antibodies (quadruple-negative) SSc control patients, matched for sex and age at disease onset (± 5 years), were selected. Clinical and laboratory data were retrieved from clinical charts and defined according the minimal essential datasets of the EUSTAR registry, as previously described (13).

Evaluated outcomes

The presence of ILD was defined by evidence of alveolitis and/or fibrosis on chest high-resolution computed tomography (HRCT). ILD course was assessed through PFTs. A relevant functional worsening was defined as a relative decline in the percent of predicted forced vital capacity (%pFVC) ≥10% or a relative decline in the %pFVC of 5% to 10% together with a relative decline in the percent of predicted diffuse capacity of carbon monoxide (%pDLCO) of ≥15% (14). PFTs values, including %pFVC and %pDLCO, were evaluated at least yearly.

Organ damage accrual was evaluated through the Scleroderma Clinical Trials Consortium Damage Index (SCTC-DI), with a score ranging from 0 to 55 and distinguishing three levels of organ damage: 0–5: low, 6–12: moderate, ≥13: severe (10). The SCTC-DI score was evaluated at T0 and at T1, T5 and T10.

SSc progression was defined as death, worsening ILD, onset of pulmonary arterial hypertension (PAH) or of scleroderma renal crisis (SRC), whichever occurred first.

Subgroup analysis

Several sub-analysis were performed comparing anti-Th/To+ patients with SSc controls: 1) in patients with lcSSc, to evaluate clinical features, including ILD, organ damage accrual and survival; 2a) in patients with ILD, to evaluate the ILD outcome (PFTs evolution, need for immunosuppressive treatments or O_2 supply, onset of secondary PH secondary, and ILD-related mortality); 2b) in patients matched for the estimated probability to have ILD, calculated by propensity score technique, to evaluate the ILD outcome; 3) comparing separately patients who received the SSc diagnosis within 2002 (the median year of diagnosis in the anti-Th/To+ group), and afterwards, to account for the wide period of patients selection.

Statistical analysis

Continuous variables were presented as median and interquartile range [IQR]. Categorical variables were presented as number/percentages. Continuous variables were compared using the non-parametric Mann-Whitney test. Categorical variables were compared using contingency tables, and *p*-value was calculated with Chi-Square, or Fisher exact test, when appropriated. Odds ratios (ORs) were calculated to assess the risk of the presence of each variable, with Haldane's correction, when needed. Survival and SSc progression were measured from the time of onset of SSc and were determined using Kaplan-Meier survival curves and log-rank analysis. To estimate the propensity score for having ILD, a logistic regression model was fitted to the variables identified as confounders (gender and disease subtype). We used a nearest neighbour 1:3 propensity score matching (PSM) algorithm. PSM was performed using IBM SPSS Statistics 25. Other statistical analyses were performed with GraphPad Prism 9.

The study was approved by the Ethical Committee of the Center (ASST Spedali Civili of Brescia) and performed according to the principles of the Declaration of Helsinki.

Results

Among 608 SSc patients, 132 (21.7%) resulted negative for ACA, anti-topo 1 and anti-RNAP3. Anti-Th/To status was available in 83/132 (62.8%) patients and anti-Th/To antibodies were positive in 13 out of 83 (15.7%) patients, (13/608, 2.1% of the entire cohort). The identifi-

Table I. Demographic and clinical features of anti-Th/To+ patients compared with anti-topo 1+, ACA+ and quadruple-negative sex- and age at disease onset-matched SSc controls.

	Anti-Th/To+ n: 13	Anti-topo 1+ n: 39	*p* value OR [CI 95%]	ACA+ n: 37	*p* value OR [CI 95%]	Quadruple-negative n: 39	*p* value OR [CI 95%]
Demographic features							
Males	3/13 (23.1)	9/39 (23.1)	*1.000*	7/37 (18.9)	*0.707*	9/39 (23.1)	*1.000*
Caucasians	13/13 (100.0)	37/39 (94.9)	*1.000*	36/37 (97.3)	*1.000*	37/39 (94.9)	*1.000*
Age at SSc onset°, years	50.0 [37.0-67.0]	52.0 [37.5-69.0]	*0.841*	52.0 [39.0-68.0]	*0.748*	51.0 [37.5-69.0]	*0.797*
Smoking habit, current or previous	7/13 (53.8)	12/39 (30.8)	*0.187*	14/37 (37.8)	*0.346*	14/39 (35.9)	*0.253*
Clinical features (ever recorded)							
Raynaud phenomenon (RP)	13/13 (100.0)	39/39 (100.0)	*1.000*	37/37 (100.0)	*1.000*	39/39 (100.0)	*1.000*
Duration of RP before SSc onset, years	1.0 [0.0 -11.0]	1.0 [0.0-3.0]	*0.556*	2.0 [0.0-8.0]	*0.955*	1.0 [0.0-2.0]	*0.104*
Limited cutaneous subtype	13/13 (100.0)	20/39 (51.3)	***0.003***	35/37 (94.6)	1.000	30/39 (76.8)	*0.091*
			25.68 [1.43-462.10]				
mRSS at first evaluation	3.0 [2.0-4.0]	12.2 [5.5-19.5]	***<0.001***	4.0 [2.0-6.0]	0.444	5.0 [3.0-11.5]	**0.046**
mRSS at last evaluation	2.0 [0.0-4.0]	8.5 [4.0-13.0]	***<0.001***	4.0 [2.0-4.0]	***0.045***	2.0 [2.0-6.0]	*0.234*
Digital ulcers	6/13 (46.2)	30/39 (76.9)	*0.079*	24/37 (64.9)	*0.236*	25/39 (64.1)	*0.253*
Pitting scars	6/13 (46.2)	30/39 (76.9)	*0.079*	19/37 (51.4)	*0.747*	24/39 (61.5)	*0.331*
Telangiectasias	5/13 (38.5)	19/39 (48.7)	*0.521*	26/37 (70.3)	***0.042***	17/39 (43.6)	*0.746*
					0.26 [0.07-0.99]		
Calcinosis	1/13 (7.7)	6/39 (15.4)	*0.664*	8/37 (21.6)	*0.414*	8/39 (20.5)	*0.290*
Myositis	2/13 (15.4)	0/39 (0.0)	*0.061*	0/37 (0.0)	*0.064*	10/39 (25.6)	*0.447*
Synovitis	0/13 (0.0)	11/39 (28.2)	***0.024***	1/37 (2.7)	*1.000*	7/39 (17.9)	*0.101*
			0.03 [0.01-1.68]				
Joint contractures	0/13 (0.0)	15/39 (38.5)	***0.005***	3/37 (8.1)	*0.558*	9/39 (23.1)	*0.091*
			0.06 [0.01-1.06]				
Esophageal symptoms	10/13 (76.9)	31/39 (79.5)	*1.000*	29/37 (78.4)	*1.000*	19/39 (48.7)	*0.076*
Gastro-intestinal involvement	2/13 (15.4)	5/39 (12.8)	*1.000*	6/37 (16.2)	*1.000*	6/39 (15.4)	*1.000*
Interstitial lung disease (HRCT)	4/10 (40.0)	28/33 (84.9)	***0.010***	3/20 (15.0)	*0.181*	27/32 (84.4)	***0.011***
			0.12 [0.02-0.58]				**0.12 [0.03-0.60]**
Cardiac involvement	5/13 (30.1)	8/39 (20.5)	*0.269*	7/37 (18.9)	*0.256*	7/39 (17.9)	*0.147*
Pulmonary arterial hypertension (RHC)	0/13 (0.0)	0/39 (0.0)	*1.000*	7/37 (18.9)	*0.0.166*	4/39 (10.3)	*0.561*
Scleroderma renal crisis	0/13 (0.0)	1/39 (2.6)	*1.000*	0/37 (0.0)	*1.000*	2/39 (5.1)	*1.000*
Synchronous* malignancies	1/13 (7.7)	2/39 (5.1)	*1.000*	4/37 (10.8)	*1.000*	4/39 (10.3)	*1.000*
Disease duration, years	16.0 [5.0-20.0]	11.0 [5.5-18.0]	*0.703*	10.0 [4.0-18.0]	*0.674*	9.0 [4.5-18.5]	*0.538*
Follow-up duration, years	16.0 [6.0-20.0]	9.0 [4.0-11.5]	*0.208*	7.0 [3.0-13.0]	*0.111*	6.0 [3.0-12.0]	*0.082*
Lost to follow-up	0/13 (0.0)	1/39 (2.6)	*1.000*	7/37 (18.9)	*0.168*	5/39 (12.8)	*0.174*
Deaths	4/13 (30.7)	22/39 (56.4)	*0.109*	12/37 (32.4)	*1.000*	21/39 (53.8)	*0.149*

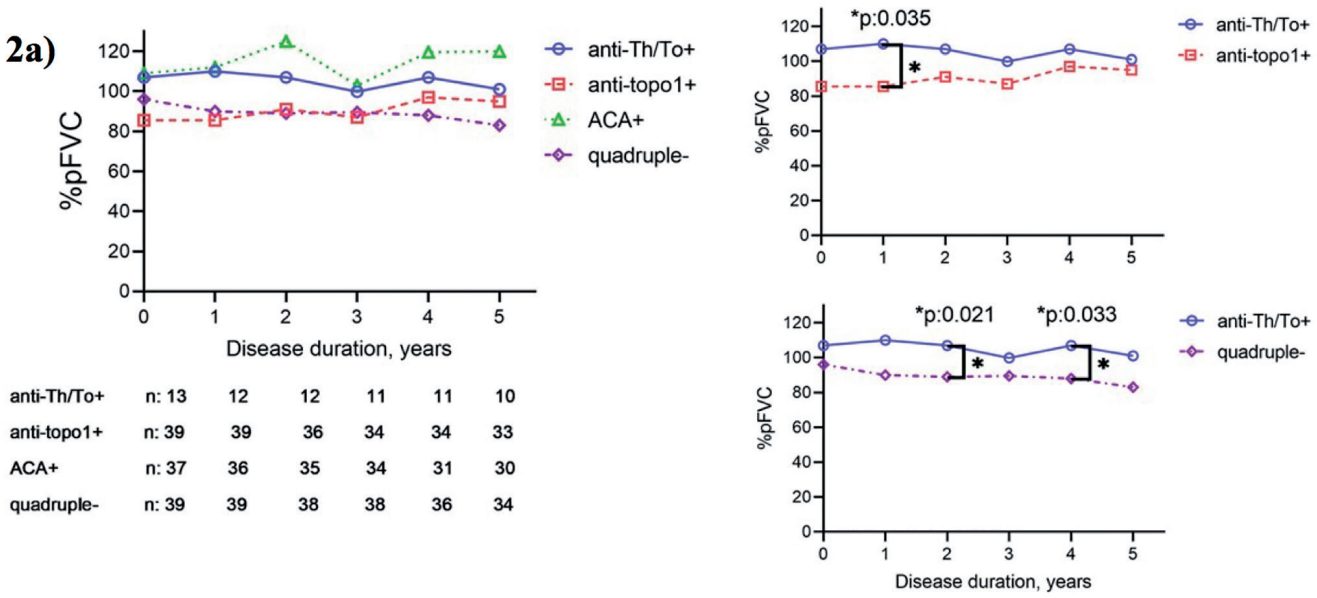
Continuous variables are presented as median [1st-3rd quartile] and compared with Mann-Whitney test; categorical variables are presented as number/number available data (%) and compared with Chi-square test/Fisher's exact test. Odds ratios were calculated, Haldane's correction was applied when needed.
°SSc onset: onset of first non-Raynaud manifestation. *Synchronous malignancy: ±3 years from SSc onset.
CI: confidence interval; HRCT: high resolution computed tomography; mRSS: modified Rodnan skin score; OR: odds ratio; RHC: right heart catheterisation; SSc: systemic sclerosis.

cation of the anti-Th/To+ SSc cases and of the ACA+, anti-topo 1+ and quadruple-negative matched SSc controls is showed in the flowchart in Figure 1; for one anti-Th/To+ male patient aged 28 years at disease onset, only one ACA+ male patient was found as control. Anti-Th/To antibodies were detected by RNA IP in 8 cases, as already described (5), and by IB in 5 cases. Among the latter, anti-Ro52 antibodies were also observed in 3 cases, and anti-signal recognition particle (SRP) antibodies in an additional case. In the control group of quadruple-negative SSc patients, the following antibodies were found: 1 anti-Nor90+, 1 anti-fibrillarin+, 3 anti-U1RNP+, 5 anti-Ku+, 6 anti-Pm/Scl+, 7 anti-Ro/SSA+ (2/7 anti-Ro52+) isolated and 16 positives for antinuclear antibodies without anti-ENA specificity. Anti-Ro/SSA+ were additionally found in association in 4/39 (2/4 anti-Ro52+) cases: 2/3 anti-U1RNP+, 1/6 anti-Pm/Scl+ and 1/1 anti-fibrillarin+ patients.

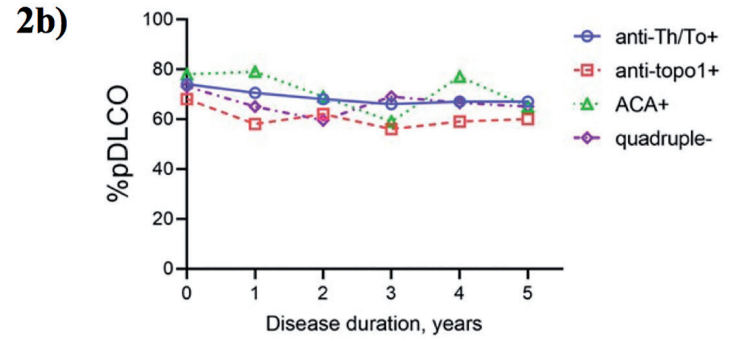
Clinical and demographic data of anti-Th/To+ patients and anti-topo 1+ (n: 39), ACA+ (n: 37) and quadruple-negative (n: 39) sex- and age at disease onset-matched SSc patients are reported in Table I.

Two out of 13 anti-Th/To+ patients (15.4%) had SSc/myositis overlap syndrome (one case associated with anti-SRP, the other without myositis-specific antibodies, associated to pancreatic cancer diagnosed 2 years after SSc onset). All anti-Th/To+ patients presented lcSSc and three quarter of them had oesophageal symptoms. Presence of digital ulcers, ILD and cardiac involvement were also frequently detected. In particular, heart involvement was observed in 5/13 (38.5%) anti-Th/To+ patients (3 pericardial effusion, 1 atrial fibrillation, and 1 myocarditis in the patient who additionally presented anti-SRP+ and myositis). None had synovitis, joint contractures, PAH diagnosed by right heart catheterisation (RHC), nor history of SRC.

As compared to anti-Th/To+ patients, anti-topo 1+ matched controls presented diffuse cutaneous involvement in half cases with a higher modified Rodnan Skin Score (mRSS) both at the first ($p<0.001$) and at the last ($p<0.001$) evaluation available and had a higher rate of synovitis ($p=0.047$) and joint contractures ($p=0.011$). ACA+ matched controls showed a higher prevalence of telangiectasias ($p=0.042$). In the quadruple-negative group, 8/39 (20.5%) patients had SSc/myositis overlap syndrome and 7/39 (17.9%) had heart involvement: 3 myocarditis, 2 pericar-



anti-Th/To+	n: 13	12	12	11	11	10
anti-topo1+	n: 39	39	36	34	34	33
ACA+	n: 37	36	35	34	31	30
quadruple-	n: 39	39	38	38	36	34



anti-Th/To+	n: 13	12	12	11	11	10
anti-topo1+	n: 39	39	36	34	34	33
ACA+	n: 37	36	35	34	31	30
quadruple-	n: 39	39	38	38	36	34

Fig. 2. Longitudinal evaluation of **a)** median %pFVC and **b)** median %pDLCO values in anti-Th/To+, anti-topo 1+, ACA+ and quadruple-negative sex- and age at disease onset-matched SSc patients.
All the control groups (anti-topo1+, ACA+, quadruple-negative) were compared with the anti-Th/To+ group (Mann-Whitney test). Statistically significant comparisons are shown in the figures, while all the other comparisons resulted not statistically significant: **a)** anti-Th/To+ *vs*. anti-topo 1+: T0: p=0.103, T2: p=0.200, T3: p=0.093, T4: p=0.327, T5: p=0.489; anti-Th/to+ *vs*. ACA+: T0: p=0.452, T1: p=0.279, T2: p=0.062, T3: p=0.755, T4: p=0.349, T5: p=0.489; anti-Th/to+ *vs*. quadruple-negative: T0: p=0.079, T1: p=0.094, T3: p=0.075, T5: p=0.133. **b)** anti-Th/To+ *vs*. anti-topo 1+: T0: p=0.089, T1: p=0.079, T2: p=0.252, T3: p=0.116, T4: p=0.171, T5: p=0.298; anti-Th/to+ *vs*. ACA+: T0: p=0.532, T1: p=0.249, T2: p=0.921, T3: p=0.529, T4: p=0.925, T5: p=0.987; anti-Th/to+ *vs*. quadruple-negative: T0: p=0.736, T1: p=0.476, T2: p=0.125, T3: p=0.823, T4: p=0.638, T5: p=0.969.

dial effusion, 2 arrhythmic disorders (1 atrial fibrillation and 1 ventricular ectopic beat requiring pacemaker and defibrillator implantation, respectively). SSc patients in the quadruple-negative group had a higher mRSS score at baseline as compared to anti-Th/To+ patients (p=0.046).
In a separate analysis which considered only lcSSc patients (13/13 anti-Th/To+, 20/39 anti-topo 1+, 35/37 ACA+ and 30/39 quadruple-negative), no significant differences in the clinical features between the four groups were observed except for ILD prevalence (see below).

ILD outcome

HRCT was performed in 10/13 (76.9%) anti-Th/To+ patients, detecting ILD in 4/10 (40.0%) cases after a median disease duration of 1.0 [1.0–6.3] years. In 1/13 (7.7%) case, treatment with cyclophosphamide followed by azathioprine as maintenance therapy was required, while no patients required oxygen therapy.
All patients (both ILD+ and ILD- patients) were regularly monitored with PFTs, with a median follow-up of 7.0 [2.8–14.0] years. In Figure 2a and Figure 2b the longitudinal courses of %pFVC and %pDLCO are presented. During the follow-up, %pFVC remained stable or improved (as compared to T0) in most anti-Th/To+ patients: 12/12 (100.0%) at T1, 8/10 (80.0%) at T5. None of the anti-Th/To+ patients developed secondary PH nor died because of ILD.
As compared to anti-Th/To+, in anti-topo 1+ and quadruple-negative patients, ILD at HRCT was more frequently detected (p:0.010 and p:0.011) (Table II). Furthermore, as compared to anti-topo 1+ and quadruple-negative, anti-Th/To+ patients less frequently required immunosuppression (p=0.039 and p=0.018) and never required O_2 therapy. In addition, while a relevant functional worsening on PTFs was observed in only 2/13 (15.4%) anti-Th/To+ patients after 4 years of disease duration, this occurred in 9/39 (23.1%) anti-topo 1+ patients and in 14/39 (35.9%) quadruple-negative patients, after a median interval of 1 and 2 years, respectively (Table II).
Notably, even when analysing separately only lcSSc patients, ILD was less

Table II. Case-control study on ILD course and outcome, comparing anti-Th/To+ patients with anti-topo 1+, ACA+ and quadruple-negative sex- and age at disease onset-matched SSc patients.

	Anti-Th/To+ n: 13	Anti-topo 1+ n: 39	*p* value OR [CI 95%]	ACA+ n: 37	*p* value OR [CI 95%]	Quadruple-negative n: 39	*p* value OR [CI 95%]
ILD on HRTC	4/10 (40.0)	28/33 (84.9)	***0.010*** **0.12 [0.02-0.58]**	3/20 (15.0)	*0.181*	27/32 (84.4)	***0.011*** **0.12 [0.03-0.60]**
Immunosuppressive therapy for ILD	1/13 (7.7)	16/39 (41.0)	***0.039*** **0.12 [0.01-1.02]**	0/37 (0.0)	*0.260*	17/39 (43.6)	***0.018*** **0.12 [0.01-0.91]**
Antifibrotic therapy for ILD	0/13 (0.0)	2/39 (5.1)	*1.000*	0/37 (0.0)	*1.000*	0/39 (0.0)	*1.000*
O_2 therapy required	0/13 (0.0)	7/39 (17.9)	*0.171*	6/37 (16.2)	*0.319*	5/39 (12.8)	*0.314*
PH secondary to ILD	0/13 (0.0)	6/39 (15.4)	*0.317*	0/37 (0.0)	*1.000*	5/39 (12.8)	*0.314*
SSc-ILD related death	0/13 (0.0)	7/39 (17.9)	*0.171*	0/37 (0.0)	*1.000*	5/39 (12.8)	*0.314*
%pFVC <70% T0	0/13 (0.0)	3/39 (7.7)	*0.564*	0/37 (0.0)	*1.000*	1/39 (2.5)	*1.000*
%pFVC <70% T1	0/12 (0.0)	2/39 (5.1)	*1.000*	0/36 (0.0)	*1.000*	3/39 (7.7)	*1.000*
%pFVC <70% T2	0/11 (0.0)	3/36 (8.3)	*1.000*	0/35 (0.0)	*1.000*	1/38 (2.6)	*1.000*
%pFVC <70% T3	0/11 (0.0)	3/34 (8.8)	*0.565*	1/34 (2.9)	*1.000*	2/38 (5.3)	*1.000*
%pFVC <70% T4	0/11 (0.0)	3/34 (8.8)	*0.565*	0/31 (0.0)	*1.000*	2/36 (5.6)	*1.000*
%pFVC <70% T5	0/10 (0.0)	1/33 (3.0)	*1.000*	0/30 (0.0)	*1.000*	2/34 (6.7)	*1.000*
%pDLCO <50% T0	0/13 (0.0)	2/39 (5.1)	*1.000*	1/37 (2.7)	*1.000*	3/39 (7.7)	*1.000*
%pDLCO <50% T1	1/12 (8.3)	6/39 (15.4)	*1.000*	2/36 (5.6)	*1.000*	5/39 (12.8)	*1.000*
%pDLCO <50% T2	0/11 (0.0)	5/36 (13.9)	*0.322*	1/35 (2.9)	*1.000*	7/38 (18.4)	*0.325*
%pDLCO <50% T3	0/11 (0.0)	5/34 (14.7)	*0.313*	5/34 (14.7)	*1.000*	5/38 (13.2)	*0.574*
%pDLCO <50% T4	1/11 (9.1)	5/34 (14.7)	*1.000*	4/31 (12.9)	*1.000*	4/36 (11.1)	*1.000*
%pDLCO <50% T5	1/10 (10.0)	5/33 (15.2)	*1.000*	2/30 (6.7)	*1.000*	2/34 (6.7)	*0.548*
Functional worsening* [T1-T0]	0/12 (0.0)	5/39 (12.8)	*0.323*	0/36 (0.0)	*1.000*	5/39 (12.8)	*0.323*
Functional worsening [T2-T1]	0/12 (0.0)	2/36 (5.6)	*1.000*	0/35 (0.0)	*1.000*	4/39 (10.5)	*0.561*
Functional worsening [T3-T2]	1/11 (9.1)	4/34 (11.8)	*1.000*	4/34 (11.8)	*1.000*	3/38 (7.9)	*1.000*
Functional worsening [T4-T3]	0/11 (0.0)	1/34 (2.9)	*1.00*	1/31 (3.2)	*1.000*	3/36 (8.3)	*1.000*
Functional worsening [T5-T4]	2/10 (20.0)	2/33 (6.1)	*0.226*	1/30 (3.3)	*1.000*	4/34 (11.8)	*0.606*
Functional worsening (ever)	2/13 (15.4)	9/39 (23.1)	*0.709*	5/37 (13.5)	*1.000*	14/39 (35.9)	*0.298*
Time to functional worsening, years	4.0 [3.5-4.5]	1.0 [1.0-3.0]	*0.211*	3.0 [3.0-3.0]	*0.469*	2.0 [1.0-3.8]	*0.222*

Continuous data are presented as median [1st-3rd quartile] and compared with Mann-Whitney test; categorical data are presented as number/number available data (%) and compared with Chi-square test/Fisher's exact test. Odds ratios were calculated; Haldane's correction was applied when needed.
*Functional worsening: decline in the %pFVC ≥10% or a decline in the %pFVC of 5% to 10% along with a decline in the %pDLCO of ≥15%.
CI: confidence interval; HRCT: high resolution computed tomography; ILD: interstitial lung disease; mRSS: modified Rodnan skin score; O_2: oxygen; OR: odds ratio; PH: pulmonary hypertension; pDLCO: predicted diffusion capacity of carbon monoxide; PF: progressive fibrosis; PFTs: pulmonary function tests; pFVC: predicted forced vital capacity; RHC: right heart catheterisation; SSc: systemic sclerosis.

frequent in anti-Th/To+ patients than in anti-topo 1+ and quadruple-negative patients: 40.0% *vs*. 81.3% (*p*=0.046) and *vs*. 80.8% (*p*:0.039), respectively.

In a separate analysis, which considered only ILD+ patients, no differences between SSc groups in PFTs evolution, need for immunosuppressive treatments or O_2 supply, onset of secondary PH secondary, and ILD-related mortality were found (data not shown). Finally, comparing anti-Th/To+ patients with SSc patients matched by propensity score analysis for the probability to have ILD (see Methods), no differences in PFTs evolution were found (data not shown).

Damage accrual (SCTC-DI)

Among anti-Th/To+ patients, the SCTC-DI score progressively increased over time: 0.0 [0.0–1.5] at T0; 0.5 [0.0–2-5] at T1; 2.5 [1.5–4.3] at T5; 2.5 [1.8–5.0] at T10 (Fig. 3a), mainly due to the onset of oesophageal involvement (76.9%), digital ulcers (46.2%), sicca symptoms (38.5%), ILD (30.8%) and pericardial effusion (23.1%). However, after a median follow-up of 16.0 [6.0–20.0] years, all the anti-Th/To+ patients presented low damage scores (SCTC-DI ≤5).

Organ damage accrual was significantly lower in anti-Th/To+ patients as compared to anti-topo 1+ matched controls at T1 (*p*=0.019), T5 (*p*=0.018) and T10 (*p*=0.010), and, as compared to quadruple-negative matched controls, at T0 (*p*=0.005) and T1 (*p*=0.013). DI determinants mostly contributing to the observed difference were, besides ILD, joint contractures and digital ulcers, that were more frequent in the anti-topo 1+ group (38.5% *vs*. 0.0%, *p*=0.005 and 76.9% *vs*. 46.2%, *p*=0.079, respectively) and significant weight loss, that was more frequent in the quadruple-negative group (25.6% *vs*. 0.0%, *p*=0.048). Organ damage accrual during the follow-up in anti-Th/To+ patients tended to be lower also when compared to ACA+ controls (Fig. 3a). In the ACA+ group the major DI determinants were: oesophageal involvement (64.9%), digital ulcers (64.9%), sicca symptoms (29.7%) and development of PAH (18.9%).

Even when comparing only lcSSc patients, DI values were higher at T0 (*p*=0.002) and T1 (*p*=0.014) in quadruple-negative patients, and later in disease course, at T10 (*p*=0.049), in anti-topo 1+ patients than in anti-Th/To+ patients (Fig. 3b).

However, when we evaluated DI changes over time, no differences between the four groups were found (data not shown).

Mortality

After a median disease duration of 9.8 [7.8–12.5] years, 4/13 (30.7%) anti-Th/To+ patients died at a median age of 80.8 [75.3–86.2] years. Causes of mortality were malignancies in 3 cases (2 pancreatic cancers and 1 lung cancer, diagnosed 2, 9 and 20 years after SSc onset, respectively) and diabetes mellitus complications in 1 patient. None of the anti-Th/To+ patients died for causes directly related to

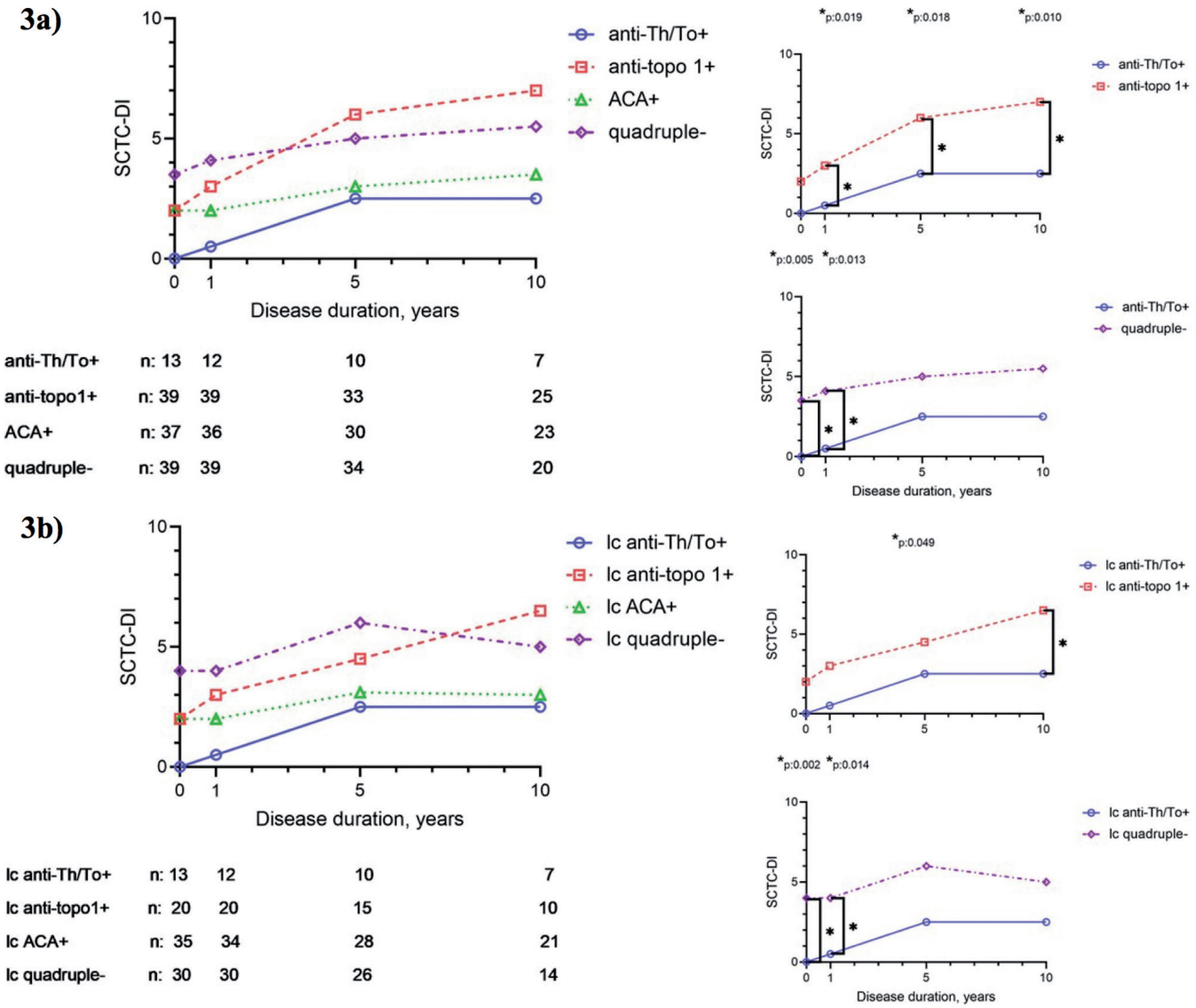


Fig. 3. a) Case-control study on organ damage accrual, comparing anti-Th/To+ SSc patients with anti-topo 1+, ACA+ and quadruple-negative matched SSc controls. **b)** Sub-analysis: case–control study on organ damage accrual, comparing lcSSc anti-Th/To+ SSc patients with lcSSc anti-topo 1+, lcSSc ACA+ and lcSSc quadruple-negative matched SSc controls.
All the control groups (anti-topo1+, ACA+, quadruple-negative) were compared with the anti-Th/To+ group (Mann-Whitney test). Statistically significant comparisons are shown in the figures, while all the other comparisons resulted not statistically significant: **a)** anti-Th/To+ *vs*. anti-topo 1+: T0: p=0.081; anti-Th/to+ *vs*. ACA+: T0: p=0.084, T1: p=0.251, T5: p=0.735, T10: p=644; anti-Th/to+ *vs*. quadruple-negative: T5: p=0.069, T10: p=0.086. **b)** lc anti-Th/To+ *vs*. lc anti-topo 1+: T0: p=0.187, T1: p=0.079, T5: p=0.121; lc anti-Th/to+ *vs*. lc ACA+: T0: p=0.071, T1: p=0.151, T5: p=0.629, T10: p=768; lc anti-Th/to+ *vs*. lc quadruple-negative: T5: p=0.085, T10: p=0.134.
SCTC-DI: Scleroderma Clinical Trials Consortium Damage Index.

SSc. In the anti-topo 1+ group, 22/39 (56.4%) patients died at a median age of 75.5 [65.3–82.8] years, after a disease duration of 16.0 [6.8–18.8] years. Among them, 10/39 (25.6%) patients died for causes related to SSc (25.6% *vs*. 0.0%, p=0.050), including 7 due to SSc-ILD. In the ACA+ group, 12/37 (32.4%) patients died at a median age of 75.6 [69.2–84.8] years, after a disease duration of 8.5 [6.0–11.5] years. Among them, 6/37 (16.2%) patients died for causes related to SSc, including 5 due to SSc-PAH. In the quadruple-negative group, 21/39 (53.8%) patients died at a median age of 73.2 [58.1–79.9] years, after a disease duration of 9.0 [5.0–24.0] years. Among them, 8/39 (20.5) patients died for causes related to SSc, including 5 due to SSc-ILD and 2 due to SSc-GI complications.

Survival curves of anti-Th/To+, anti-topo 1+, ACA+ and quadruple-negative patients are showed in Figure 4a and curves describing the time free from "SSc disease progression" (defined as death, worsening ILD, onset of PAH or of SRC) in Figure 4b: no differences between anti-Th/To+ patients and anti-topo 1+, ACA+ and quadruple-negative sex- and age at disease onset-matched controls were observed. In addition, no differences in survival between the 4 SSc groups were observed when comparing separately patients who received the SSc diagnosis within 2002 or afterwards (data not shown).

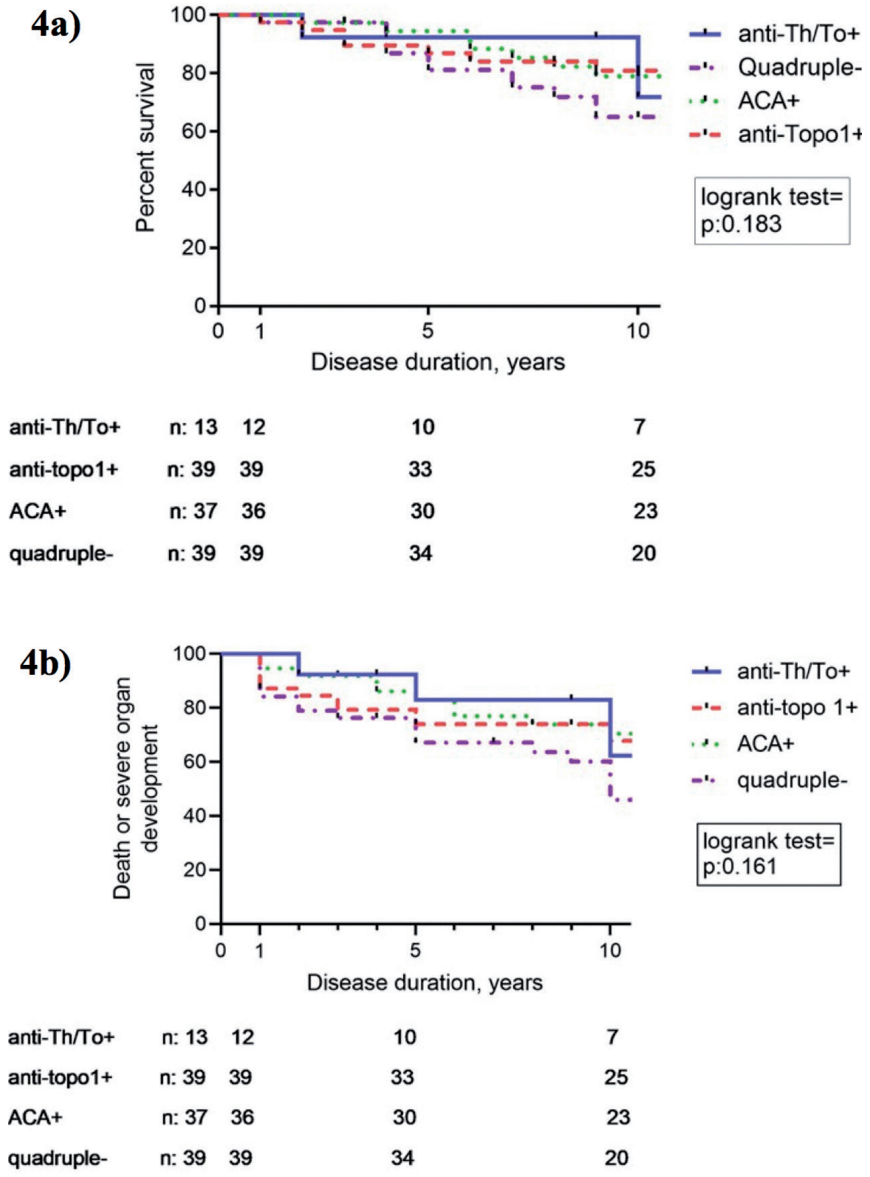


Fig. 4. a) Survival curves of anti-Th/To+ patients compared with anti-topo 1+, ACA+ and quadruple- matched SSc controls. **b)** Curves describing the time free from "SSc disease progression" (death, worsening ILD, PAH or SRC development).

Discussion

Anti-Th/To positivity was found in 2.1% of our entire cohort of SSc patients (this figure might be slightly underestimated by the fact that a small number of patients were never tested for anti-Th/To, even if negative for other SSc-specific antibodies). This figure is very similar to the frequency of anti-Th/To among SSc patients reported by a recent review of the literature who collected data from 25 articles (15); notably, these antibodies seemed to be less frequent in Europe (1.5%) than in other areas of the world (15).

We confirmed the main known clinical associations of anti-Th/To antibodies: all our patients were affected by limited cutaneous involvement, accordingly with previous reports (median percentage: 89%) (15), and ILD was detected by HRCT in almost half of the patients (median percentage in previous reports: 42.5%) (15).

Importantly, the long-term follow-up of PFTs (median: 16.5 years) demonstrated that a relevant functional worsening occurred rarely (15.4%) within the first 5 years of SSc course in our anti-Th/To+ patients, with only one patient requiring immunosuppressive treatment. Accordingly, none of these patients required O_2 supply, developed secondary PH or died because of ILD. Indeed, the very recent re-evaluation of 204 anti-Th/To+ SSc patients from the Pittsburgh cohort showed that only 5% of them developed group 3 PH related to ILD during the follow-up, whereas this complication was already present in 7% at the time of their first evaluation, that took place after a median of 8 years from SSc onset, as reported (9). This observation might lead to the hypothesis of a referral bias with selection of more severe cases in the Pittsburgh centre. In line with this hypothesis, a surprisingly high number of anti-Th/To+ SSc patients suffered from group 1 PAH at the time of their first visit in Pittsburgh (17%), but only 6% additionally developed PAH during the follow-up (9).

On the contrary, in the present cohort and in a Japanese case series of anti-Th/To+ patients (16), no cases of PAH were reported, even if it should be remarked that the number of patients included in these studies was much smaller than in the Pittsburgh one.

The difference recorded in clinical manifestations at the baseline is probably one of the main explanations of the discrepancy observed in the survival curves for anti-Th/To+ patients. Specifically, survival rate in our cohort at 5 and 10 years was 92.3% (SD: 7.4) and 71.8% (SD: 14.0) respectively, while in Pittsburgh the reported 5- and 10-year cumulative survival rates were 61% and 49% for the 1985-2000 period (7), only slightly improved in the latter report concerning patients firstly evaluated between 1980 and 2015 (5-year survival: 68%), but still significantly lower than in anti-Th/To-negative SSc controls (9). Noteworthy, several factors potentially associated with lower survival in SSc patients, including male gender, non-Caucasian ethnicity, longer disease duration, and smoking habit (17-22), were reported to be more prevalent among anti-Th/To+ patients followed in Pittsburgh (7, 9). The higher frequency of smokers among anti-Th/To+ patients might be particularly interesting, considering the potential link with the high frequency of ILD in this SSc subset, and was also reported by

others (11) and confirmed in our study. To account for some of these confounding factors, we designed a case-control study, matching patients for sex and age at SSc onset and, at variance with data originated from Pittsburgh, we did not observe differences in survival between anti Th/To+ and ACA+ or anti-topo 1+ matched controls.

Notably, none of our anti-Th/To+ patients died due to SSc-related causes, while cancer was the most frequent cause of death. An overall increased long-term incidence of cancer history in patients with anti-Th/To antibodies was reported by a small Japanese study (12), while anti-Th/To antibodies were associated with a reduced risk of cancer diagnosis within the first 3 years after SSc onset as compared with other SSc subsets in a larger American study (11). These results confirm that both time and autoantibodies are critical factors to consider when stratifying the risk of malignancies in SSc patients (11).

Finally, the favourable course of SSc in our cohort of anti-Th/To+ patients was confirmed by the mild organ damage observed in all the patients. This observation could be explained with the lower prevalence of life-threatening cardiopulmonary involvement (PAH or severe ILD), but also of other disease manifestations, such as joint contractures and digital ulcers, importantly affecting patients' quality of life (10). It is worth to note that even when considering separately only lcSSc patients, anti-Th/To+ patients suffered from less damage and less frequent ILD than anti-topo 1+ or quadruple-negative controls.

In conclusion, in this cohort, anti-Th/To+ patients followed during a median time of 16 years, showed a mild SSc phenotype, characterised by limited cutaneous involvement, low organ damage accrual over time, a favourable functional outcome of ILD, even if detected by HRCT in almost half of the patients and high survival rates. None of our anti-Th/To+ patients died due to SSc and the most frequent cause of death in them was cancer.

The main limitations of the present study are the small number of patients enrolled and the retrospective design. However, to our knowledge, this is the largest cohort of anti-Th/To+ patients evaluated in Europe until now (15). Another limitation of our study is the lack of information on the extent and the pattern of ILD on HRCT, but our patients were regularly assessed through PFTs during a median of 16.5 years. In addition, anti-Th/To antibodies were tested only in SSc patients negative for SSc-specific autoantibodies, such as ACA, anti-topo 1, or anti-RNAP3, so it is not possible to rule out the co-expression of anti-Th/To and SSc-specific antibodies. However, it should be noted that such co-expression is very rare, even when assays evaluating simultaneously multiple autoantibodies, like IB, are used (23). Therefore, we considered it unlikely that the results of our study were significantly affected by this limitation. Several open issues concerning anti-Th/To antibodies still need to be clarified; in particular, the relationship with cancer.

Considering the low prevalence of anti-Th/To antibodies in SSc, only the analysis of a large, international, multicenter cohort of SSc patients, and the comparison between anti-Th/To+ and anti-Th/To- patients well matched for confounding factors might provide further information on anti-Th/To clinical associations.

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