Malignancies in Patients with Anti-RNA Polymerase III Antibodies and Systemic Sclerosis: Analysis of the EULAR Scleroderma Trials and Research Cohort and Possible Recommendations for Screening

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ABSTRACT. ***Objective.*** To analyze the characteristics of anti-RNA polymerase III antibodies (anti-RNAP3)–positive patients with systemic sclerosis (SSc) in the European League Against Rheumatism Scleroderma Trials and Research group (EUSTAR) registry with a focus on the risk of cancer and the characteristics of malignancies, and the aim to provide guidelines about potential cancer screening in these patients.

Methods. (1) Analysis of the EUSTAR database: 4986 patients with information on their anti-RNAP3 status were included. (2) Case-control study: additional retrospective data, including malignancy history, were queried in 13 participating EUSTAR centers; 158 anti-RNAP3+ cases were compared with 199 local anti-RNAP3– controls, matched for sex, cutaneous subset, disease duration, and age at SSc onset. (3) A Delphi exercise was performed by 82 experts to reach consensus for cancer screening in anti-RNAP3+ patients.

Results. In the EUSTAR registry, anti-RNAP3 were associated in multivariable analysis with renal crisis and diffuse cutaneous involvement. In the case-control study, anti-RNAP3 were associated with gastric antral vascular ectasia, rapid progression of skin involvement, and malignancies concomitant to SSc onset (OR 7.38, 95% CI 1.61–33.8). When compared with other anti-RNAP3+ patients, those with concomitant malignancies had older age ($p < 0.001$) and more frequent diffuse cutaneous involvement ($p = 0.008$). The Delphi exercise highlighted the need for malignancy screening at the time of diagnosis for anti-RNAP3+ patients and tight followup in the following years.

Conclusion. Anti-RNAP3+ patients with SSc have a high risk of concomitant malignancy. These results have implications for clinical practice and suggest regular screening for cancer in anti-RNAP3+ patients. (First Release January 15 2017; J Rheumatol 2017;44:639–47; doi:10.3899/jrheum.160817)

Key Indexing Terms:
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Systemic sclerosis (SSc) is characterized by considerable heterogeneity of clinical manifestations in affected individuals[1,2,3]. Autoantibodies are seen in almost all patients and might have a pathogenic role[4]. They are currently considered the best available markers to stratify patient's heterogeneity: different autoantibodies are associated with clinically and genetically distinct disease subsets[2,4], identifying patients at highest risk of particular clinical manifestations and providing longterm prognostic information[5].

In particular, anti-RNA polymerase III antibodies (anti-RNAP3) have specificity for SSc and association with diffuse cutaneous involvement and renal crisis[6,7]. The development of ELISA, more suited to routine use[8], allowed the identification of novel clinical correlates of anti-RNAP3, such as gastric antral vascular ectasia (GAVE)[9,10], and increased risk of malignancy diagnosis[11,12,13,14,15,16].

However, the prevalence of anti-RNAP3 in SSc is very heterogeneous among studies, probably because of geographic/ethnic factors[17]. Their prognostic value still needs to be better focused and the previous studies, mostly based on monocentric series and/or with low to moderate sample size, precluded firm conclusions. This is even more important because of data showing a link between *RNAP3* gene mutations, the emergence of tumor cells, and immune response leading to anti-RNAP3 production[18].

We took advantage of the worldwide registry of the European League Against Rheumatism (EULAR) Scleroderma Trials and Research group (EUSTAR). We first queried the EUSTAR database, and then asked centers to participate in a retrospective case-control study, providing additional data not included in the database. In particular, we sought data on the history of malignancies, with the aim of defining the time relationship and the characteristics of anti-RNAP3+ SSc and malignancies. Finally, a Delphi exercise was performed to obtain expert-based conclusions for clinical practice.

MATERIALS AND METHODS

Analysis of the EUSTAR database. This database records the minimal essential dataset (MEDS) of a longitudinally followed cohort of patients with SSc[2,3]. Data were extracted from the registry in March 31, 2014, when 11,399 patients from 118 centers fulfilling either the 1980 American College of Rheumatology (ACR) or the 2013 ACR/EULAR classification criteria for SSc centers were recorded[19,20]. Data on malignancy were not recorded in the EUSTAR database at that time. Data on anti-RNAP3 were recorded since December 2008. MEDS variables were defined as previously reported[2,3].

Patients were included in our study when anti-RNAP3 status was available in at least 1 visit, whereas patients with no information or unknown status in the registry were excluded. Patients were considered positive for anti-RNAP3 when the test was positive in at least 1 determination at the baseline or during the followup. Patients positive both for anti-RNAP3 and for other SSc-specific antibodies [anticentromere antibodies (ACA) or antitopoisomerase I (anti-topo I)] were excluded from comparisons between anti-RNAP3+ and anti-RNAP3– patients. To evaluate the association between anti-RNAP3 and other demographic, clinical, and laboratory variables, the MEDS data from the last available visit were used. The method of anti-RNAP3 detection was at the discretion of the participating center according to local practice and was not recorded in the database.

Ethics approval was obtained from each site [Brescia, Spedali Civili di Brescia, n.1072; Zurich, Ethic committee Canton of Zurich, number PB_2016-01515; Lund, Regionala Etikprövningsnämnden, Lund (regional ethics board) n.590/2008; Pecs, Hungarian National Ethics Committee, n.430/PI/20I2, 426/2013; Gent, Universitair Ziekenhuis Gent, n.385/2008; Verona, Azienda Ospedaliera e Istituti Ospitalieri Verona progetto n.1570, n.24934; Roma, Azienda Policlinico Umberto I, n.1469; Lille, Ethical Committee Ile de France III n.2561; Haifa, Rambam Helsinki committee, n.2440; Strasbourg, Ethical Committee Ile de France III n.2561; Lisboa, Centro Hospitalar de Lisboa Central, n.160/2014; Paris, Ethical Committee Ile de France III n.2561].

Case-control study. EUSTAR centers were invited to participate in a specifically designed case-control study. Retrospective data for all the anti-RNAP3+ patient of the participating centers (cases) were collected through a dedicated form. Anti-RNAP3+ patients also positive for other SSc-specific antibodies were excluded. Centers were asked to provide 1 or, if possible, 2 local controls (anti-RNAP3–) for each case, matched for sex, disease duration, cutaneous subset as defined by Leroy, *et al*[21], and age at disease onset (by 5-yrs class of age). All patients included in our analysis were tested for anti-RNAP3. Data on anti-RNA polymerase I were not collected. The method of anti-RNAP3 detection, as well as the panel of SSc-specific autoantibody tests, was at the discretion of the participating center and was recorded in a dedicated form, also collecting the time of SSc onset (defined as the onset of the first non-Raynaud symptom; T0), progression of skin involvement evaluated by the modified Rodnan Skin Score (mRSS) at T0 and at 6, 12, and 24 months after T0, peak of mRSS and its time interval from T0, renal crisis, gastrointestinal involvement, malignancies and their histologic type, and date of diagnosis. Malignancies were classified as "synchronous" with SSc when the diagnosis was made between 6 months before and 12 months after SSc onset[12,15], or in separate analyses in a larger interval including 2 years before and after SSc onset[16].

Delphi exercise on possible screening for malignancies in anti-RNAP+ patients with SSc. A Web-based approach was used to address the questions concerning possible screening for malignancies in anti-RNAP3+ patients with SSc. First, the results of the EUSTAR cohort analysis were presented to all EUSTAR centers. Centers were then asked to participate in a Delphi exercise, voting on the opportunity of screening for malignancies in anti-RNAP3+ patients and its modality. In the second stage of this exercise, questions that remained unsolved at the first step and new suggestions from experts were re-proposed. Finally, possible recommendations were drafted and participants were asked to vote on them in stage 3 of the Delphi exercise. The level of agreement for each statement was voted using a 10-point visual analog scale (10 = fully agree).

Statistical analysis. Frequencies and percentages of categorical variables were compared using the chi-square test with Pearson correction or Fisher's exact test, and continuous variables using the Student t test, Mann-Whitney U test, or ANOVA, as appropriate.

A multivariate logistic regression analysis (adjusted for sex, age, and disease duration) was performed with calculation of OR estimates and 95%

CI. Besides *a priori* potential confounders, variables associated with $p < 0.05$ in univariable analysis were considered. Bonferroni correction for multiple comparison was applied.

The Kaplan-Meier method and the log-rank test were applied to analyze the progression of mRSS and survival.

RESULTS

Clinical associations of anti-RNAP3 based on the analysis of the EUSTAR database. Data on anti-RNAP3 were available in 4986 patients with SSc recorded in the EUSTAR database (Figure 1). No difference was observed between them and those for whom information on anti-RNAP3 status was not available.

Among evaluable patients, 223 (4.5%) were reported as anti-RNAP3+ in at least 1 visit, while 4763 were always reported as anti-RNAP3–. Among anti-RNAP3+ patients, 47 were also positive for other SSc-specific antibodies in at least 1 visit (15 for ACA, 30 for anti-topo I, and 2 for both) and were excluded from further analysis, which finally compared 176 anti-RNAP3+ and 4763 anti-RNAP3– patients with SSc.

In univariable analysis (Table 1), positivity for anti-RNAP3 was associated with male sex ($p < 0.0001$), arterial hypertension ($p = 0.03$), diffuse cutaneous involvement ($p < 0.0001$), renal crisis ($p < 0.0001$), and joint contractures ($p < 0.0001$).

In the multivariable model (adjusted for sex, age at disease onset, and disease duration; Table 1), anti-RNAP3 positivity was independently associated with renal crisis ($p < 0.0001$) and diffuse cutaneous involvement ($p < 0.0001$).

Association of anti-RNAP3 with cancer based on the case-control study. Thirteen EUSTAR centers participated in the case-control study, collecting retrospective data from 158 anti-RNAP3+ SSc cases (95.9% of which were included in the EUSTAR database) and 199 anti-RNAP3– local SSc controls, matched for sex, disease duration, cutaneous subset, and age at disease onset. Among controls, 48% were anti-topo I+ and 22% ACA+. The interval between diagnosis and the last visit available was shorter in anti-RNAP3+ cases than in controls [median (interquartile range; IQR) 77 (38–132) mos vs 100 (52–155), $p = 0.008$]. There was no difference in the number of deaths and their causes between cases (n = 25: 13 due to SSc, 10 to cancer, 2 to other reasons) and controls (n = 31: 15 due to SSc, 6 to cancer, 8 to other reasons, and 2 unknown). Cumulative survival was not different between the 2 groups [at 5 yrs after SSc diagnosis, anti-RNAP3+ 91.6% (SE 2.3) vs anti-RNAP3– 94.4% (SE 1.7); at 10 years, anti-RNAP3+ 87.0% (SE 3.2) vs anti-RNAP3– 84.0% (SE 3.2), log-rank test $p = 0.72$].

In multivariable analyses, anti-RNAP3 positivity was negatively associated with gastroesophageal reflux disease ($p = 0.003$), but was positively associated with renal crisis ($p = 0.0005$) and GAVE ($p = 0.0009$; Table 2). No difference was observed between the 2 groups for peak of mRSS. However, Kaplan-Meier analysis showed that the time to reach the peak of mRSS was shorter in anti-RNAP3+ patients than in matched controls ($p = 0.013$). In particular, the peak of mRSS was reached within 1 or 2 years in 71% and 87%

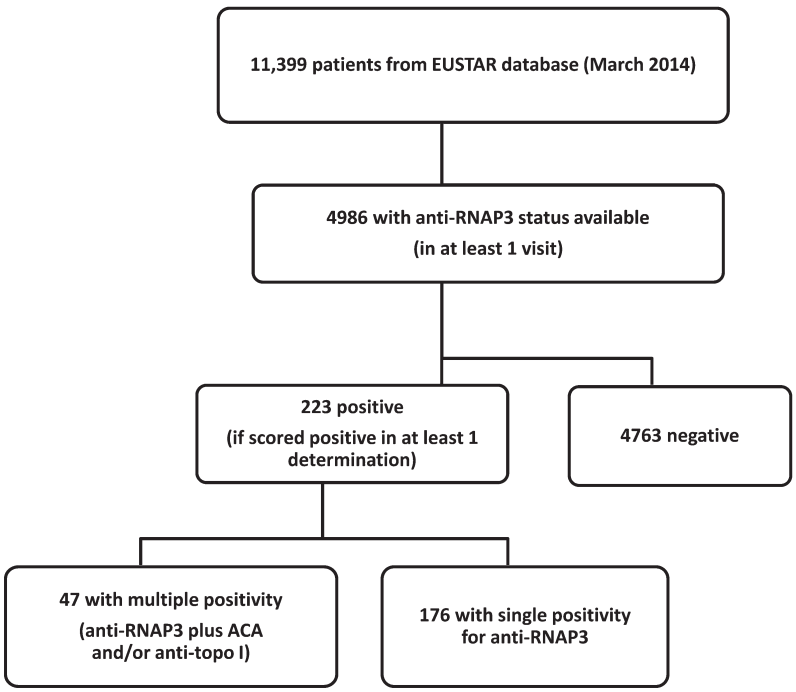

Figure 1. Patient selection process for inclusion in the study. EUSTAR: European League Against Rheumatism Scleroderma Trials and Research group; anti-topo I: antitopoisomerase I; anti-RNAP3: anti-RNA polymerase III antibodies; ACA: anticentromere antibodies.

Table 1. Results of the univariable and multivariable analysis (adjusted on sex, age at disease onset, and disease duration) comparing anti-RNAP3– and anti-RNAP3+ patients at the last visit (n = 4939). Values are no./no. available data (%) unless otherwise specified.

Characteristics	Univariable Analysis			Multivariable Analysis		
	Anti-RNAP3–	Anti-RNAP3+	p	Available Data, n (%)	OR (95% CI)	p*
Age at disease onset, yrs, mean (SD), (n available)	46.5 (14.2) (3946)	46.4 (13.1) (140)	0.981	4086 (82.7)		0.461
Disease duration, mos, mean (SD), (n available)	138.6 (102.6) (3943)	131.0 (125.0) (140)	0.391	4083 (82.7)		0.843
Male	684/4763 (14.4)	45/176 (25.6)	< 0.0001	4939 (100.0)		0.306
Ethnicity						
White	3425/3605 (95.0)	134/144 (93.1)	0.295	3749 (75.9)		
Asian	34/3605 (0.9)	3/144 (2.1)	0.175	3749 (75.9)		
Black	47/3605 (1.3)	1/144 (0.7)	0.524	3749 (75.9)		
Others	99/3605 (2.7)	6/144 (4.2)	0.311	3749 (75.9)		
Raynaud phenomenon	4242/4546 (93.3)	157/165 (95.2)	0.351	4711 (95.4)		
Esophageal symptoms	2721/4612 (59.0)	99/167 (59.3)	0.942	4779 (96.8)		
Stomach symptoms	1026/4536 (22.6)	42/163 (25.8)	0.346	4699 (95.1)		
Intestinal symptoms	1132/4603 (24.6)	50/167 (29.9)	0.116	4770 (96.6)		
Arterial hypertension	1040/4600 (22.6)	50/168 (29.8)	0.030	4768 (96.5)		0.508
Scleroderma renal crisis	59/4608 (1.3)	21/169 (12.4)	< 0.0001	4777 (96.7)	7.06 (3.77–12.2)	< 0.0001
Dyspnea, significant	481/3999 (12.0)	18/150 (12.0)	0.992	4149 (84.0)		
Diffuse cutaneous subtype	1289/4573 (28.2)	98/169 (58.0)	< 0.0001	4742 (96.0)	2.35 (1.58–3.49)	< 0.0001
Scleroderma/puffy fingers	1759/4144 (42.4)	27/101 (26.7)	0.739	4245 (85.9)		
Active digital ulcers	583/4523 (12.9)	17/166 (10.2)	0.316	4689 (94.9)		
Joint synovitis	563/4560 (12.3)	13/167 (7.8)	0.077	4727 (95.7)		
Joint contractures	1346/4489 (30.0)	74/160 (46.3)	< 0.0001	4659 (94.3)		0.104
Tendon friction rubs	259/4479 (5.8)	13/158 (8.2)	0.199	4637 (93.9)		
Muscle weakness	802/4499 (17.8)	30/160 (18.8)	0.764	4659 (94.3)		
Muscle atrophy	417/4494 (9.3)	18/159 (11.3)	0.385	4653 (94.2)		
Conduction blocks	462/3810 (12.1)	14/118 (11.9)	0.932	3928 (79.5)		
Elevated sPAP, ECHO	628/3882 (16.2)	18/133 (13.5)	0.415	4015 (81.3)		
Lung fibrosis on plain radiograph	1046/3346 (31.3)	42/112 (37.5)	0.162	3458 (70.0)		
Lung fibrosis on HRCT	1175/2520 (46.6)	41/74 (55.4)	0.136	2594 (52.5)		

* p value after Bonferroni correction was obtained for p values < 0.007. sPAP: systolic pulmonary arterial pressure; ECHO: echocardiogram; HRCT: high-resolution computed tomography; anti-RNAP3: anti-RNA polymerase III antibodies.

of anti-RNAP3+ patients, respectively, as compared with 62% and 74% of controls.

The overall rate of malignancies was higher in anti-RNAP3+ patients than in controls (17.7% vs 9.0%, p = 0.015; Table 2). In particular, cancers synchronous with SSc were more frequent, considering those diagnosed either between 6 months before and 12 months after SSc onset (7.0% vs 1.0%, p = 0.004), or within a larger time interval extended to 2 years before and after SSc onset (9.0% vs 2.5%, p = 0.007). In particular, cancer was diagnosed after the SSc diagnosis within the first 2 years in 9 patients (with a median delay of 4 mos).

On the other hand, the risk of cancer was not increased beyond 2 years since SSc diagnosis (Table 2).

Looking at the malignancy type, the frequency of solid tumors in anti-RNAP3+ patients was higher than in controls (p = 0.012), particularly for breast cancers (p = 0.03). Notably, the diagnosis of breast cancer was synchronous (± 2 yrs) to the onset of SSc in 7 out of 155 evaluable anti-RNAP3+ patients and in none of 199 anti-RNAP3– matched SSc controls (p = 0.003). The percentage of women with breast cancer diagnosis synchronous to the onset of SSc was 6.0% (95% CI 3.0–12.0).

When the 14 anti-RNAP3+ patients with malignancies synchronous to SSc onset were compared with the other 144 anti-RNAP3+ patients (Table 3), they had an older mean age at SSc onset (p < 0.001) and an increased proportion of diffuse cutaneous involvement (p = 0.008). In fact, in patients with diffuse cutaneous involvement, the prevalence of synchronous malignancies was higher among anti-RNAP3+ than anti-RNAP3– patients (p = 0.001), but no differences were observed comparing anti-RNAP3+ and anti-RNAP3– patients with limited cutaneous SSc. Conversely, among anti-RNAP3+ patients, the prevalence of synchronous malignancies was higher in those with diffuse than with limited cutaneous involvement (p = 0.009). A trend for increased proportion of men was also observed comparing patients with malignancies synchronous to SSc onset with other anti-RNAP+ patients (p = 0.058; Table 3). In particular, the risk of non-breast cancer synchronous with SSc was much

Table 2. Results of the univariable analysis comparing anti-RNAP3+ patients with SSc (cases) and anti-RNAP3– SSc controls, matched for sex, disease duration, cutaneous subset, and age at disease onset. Anti-RNAP3 positivity was identified by ELISA in 91 patients, by line immunoassay in 38, and by immunoprecipitation in 29. Anti-RNAP3– patients were evaluated by ELISA, line immunoassay, and immunoprecipitation in 132, 30, and 37 cases, respectively. Values are no./no. available data (%) unless otherwise specified.

Characteristics	Anti-RNAP3+	Anti-RNAP3–	p	OR (95% CI)	Anti-RNAP3– Anti-Topo I+	p§	Anti-RNAP3– ACA+	p§
Age at disease onset, yrs, mean (SD), (n available)	50.7 (13.9) (144)	48.5 (13.2) (192)	0.145		48.3 (12.6) (61)	0.255	38.4 (13.0) (30)	0.400
Male	34/150 (22.7)	47/195 (24.1)	0.755		19/65 (29)	0.307	1/30 (3)	0.011
White	36/39 (92.3)	80/84 (95.2)	0.678		44/47 (94)	1.000	19/19 (100)	0.544
Country of origin								
Italy	43/158	54/199	0.115					
Swiss	22	38						
Sweden	21	34						
Hungary	21	21						
France	27	28						
Others	24	24						
Diffuse cutaneous subtype	74/121 (61.2)	114/168 (67.9)	0.239		55/64 (86)	< 0.0001	9/29 (31)	0.006
Peak mRSS (0/51 to 51/51), (n available)	21.3 (12.0) (95)	18.6 (10.6) (157)	0.131		22.3 (8.7) (52)	0.594	9.9 (7.0) (21)	< 0.0001
Gastroesophageal reflux disease	100/157 (63.7)	155/199 (77.9)	0.003	0.50 (0.30–0.82)	52/65 (80)	0.018	24/30 (80)	0.095
Anorectal incontinence	4/158 (2.5)	5/199 (2.5)	1.000		0/65 (0)	0.325	2/30 (7)	0.245
SIBO requiring therapy	6/157 (3.8)	13/199 (6.5)	0.344		2/65 (3)	1.000	3/30 (10)	0.159
Primary biliary cirrhosis	3/154 (1.9)	2/198 (1.0)	0.657		0/65 (0)	0.557	0/30 (0)	1.000
GAVE	13/157 (8.3)	2/197 (1.0)	0.0009	8.80 (1.85–57.4)	0/65 (0)	0.012	0/30 (0)	0.133
Scleroderma renal crisis	19/158 (12.0)	5/199 (2.5)	0.0005	5.30 (1.81–16.6)	2/65 (3)	0.043	0/30 (0)	0.047
Death	25/158 (15.8)	31/198 (15.7)	0.966		13/65 (20)	0.441	1/30 (3)	0.085
Malignancies	28/158 (17.7)	18/199 (9.0)	0.015	2.17 (1.15–4.08)	4/65 (6)	0.034	2/30 (7)	0.176
Malignancies synchronous, –6/+12 mos	11/158 (7.0)	2/199 (1.0)	0.004	7.38 (1.61–33.8)	0/65 (0)	0.004	0/30 (0)	0.079
Malignancies nonsynchronous, < –6/> +12 mos	17/158 (10.7)	16/199 (8.0)	0.486		4/65 (6)	0.327	2/30 (7)	0.745
Malignancies synchronous, ± 2 yrs*	14/155 (9.0)	5/199 (2.5)	0.007	3.85 (1.36–10.9)	1/61 (2)	0.073	0/30 (0)	0.131
Malignancies nonsynchronous, < –2/> +2 yrs*	14/155 (9.0)	13/199 (6.5)	0.423		3/61 (5)	0.120	2/30 (7)	1.000
Solid tumors	22/158 (13.9)	12/199 (6.0)	0.012	2.52 (1.21–5.27)	4/65 (6)	0.113	1/30 (3)	0.133
Solid tumors synchronous, ± 2 yrs*	13/155 (8.4)	4/199 (2.0)	0.010	4.46 (1.32–16.6)	1/61 (2)	0.120	0/30 (0)	0.132
Breast cancers	11/158 (7.0)	4/199 (2.0)	0.030	3.65 (1.14–11.7)	1/65 (2)	0.187	1/30 (3)	0.694
Breast cancers synchronous, ± 2 yrs*	7/155 (4.5)	0/199 (0)	0.003	20.2 (1.41–355)	0/61 (0)	0.195	0/30 (0)	0.600
Solid tumors other than breast cancer synchronous, ± 2 yrs*	6/155 (3.9)	4/199 (2.0)	0.344		1/61 (2)	0.676	0/30 (0)	0.591
Hematologic malignancies, including leukemia	1/158 (0.6)	2/199 (1.0)	0.702		0/65 (0)	1.000	0/30 (0)	1.000
Nonmelanoma skin cancer	3/158 (1.9)	4/199 (2)	0.940		0/65 (0)	0.558	1/30 (3)	0.504
Melanoma	2/158 (1.3)	0/199 (0)	0.195		0/65 (0)	1.000	0/30 (0)	1.000

* Patients with < 2 years of followup were excluded. § Compared with anti-RNAP3+ patients. SSc: systemic sclerosis; mRSS: modified Rodnan skin score; SIBO: small intestinal bacterial overgrowth; GAVE: gastric antral vascular ectasia; anti-RNAP3: anti-RNA polymerase III antibodies; anti-topo I: antitopoisomerase I; ACA: anticentromere antibodies.

higher in male than in female patients (43% vs 0.8%, p < 0.001, OR 95.2, 95% CI 10.2–890).

Based on our results, the number of anti-RNAP3+ patients needed to screen to find 1 synchronous malignancy was 17.

Delphi exercise on possible screening for malignancies in anti-RNAP3+ patients with SSc. To derive a possible list of recommendations guiding clinicians in everyday practice to screen anti-RNAP3+ patients with SSc for cancer, a Web-based Delphi approach was used. Eighty-two experts from EUSTAR centers participated in the third stage of the Delphi exercise by voting on possible recommendations. The results are shown in Table 4.

Table 3. Results of the univariable analysis comparing anti-RNAP3+ patients with or without synchronous cancer. Values are no./available data no. (%) unless otherwise specified.

Characteristics	Anti-RNAP3+ with Synchronous Cancer	Anti-RNAP3+ without Synchronous Cancer	p	OR (95% CI)
Age at disease onset, yrs, mean (SD), (n available)	65.3 (10.0) (12)	49.3 (13.3) (128)	< 0.001	
Male	6/14 (42.9)	28/136 (20.6)	0.058	
Diffuse cutaneous subtype	13/14 (92.9)	67/121 (55.4)	0.008	10.5 (1.33–82.6)
Peak mRSS, 0/51 to 51/51, mean (SD), (n available)	23.3 (13.7) (12)	21.0 (11.8) (83)	0.537	
Time to peak of mRSS, mos, mean (SD), (n available)	12.0 (14.7) (12)	14.5 (17.8) (83)	0.587*	
Gastroesophageal reflux disease	8/14 (57.1)	92/143 (64.3)	0.593	
GAVE	3/14 (21.4)	10/143 (7.0)	0.061	
Scleroderma renal crisis	2/14 (14.3)	17/144 (11.8)	0.785	

* Log rank test, Kaplan-Meier analysis. mRSS: modified Rodnan skin score; GAVE: gastric antral vascular ectasia; anti-RNAP3: anti-RNA polymerase III antibodies.

A high level of agreement was achieved on the indication to screen for malignancies at the time of diagnosis in these patients.

Possible recommendations included:

(1) screening for breast cancer in women;

(2) screening for other malignancies, guided by clinical suspicion and patient age and sex, at least with noninvasive tests (e.g., fecal occult blood, gynecological evaluation, prostatic-specific antigen, ultrasound studies);

(3) tight surveillance in the first years after SSc diagnosis. The length of this period of tight control was one of the most-discussed points, even if we and others[14] did not demonstrate an increased risk of cancer diagnosis beyond 2 or 3 years after SSc diagnosis.

Experts recommended prospective studies to clarify some still debated issues such as the use of positron emission tomography (PET)/computed tomography (CT) and longterm followup.

DISCUSSION

In our study, we analyzed the clinical associations of anti-RNAP3 in SSc, taking advantage of the EUSTAR collaborative group, focusing particularly on the association with malignancies.

Previous reports described a more severe cutaneous involvement in anti-RNAP3+ than in anti-topo I+ patients[6,7,22,23] and a shorter interval between the appearance of Raynaud phenomenon and the first non-Raynaud SSc symptom[24]. Our study confirms that skin thickening progression is particularly rapid in anti-RNAP3+ patients[11]. Because organ complications, such as renal crisis or GAVE, usually develop early during the disease course[9,10,25], these features indicate that anti-RNAP3 identify a SSc subset characterized by particularly rapid onset and progression. This scenario might be in accordance with the hypothesis of a disease induced by some trigger, which in some cases might be identified in a concomitant malignancy.

The identification of anti-RNAP3 as a marker of a disease subset with concomitant onset of cancer and SSc was first made by a single-center American study in which anti-RNAP3 were evaluated both by immunoprecipitation

Table 4. Results of the Delphi exercise. Degree of agreement with the proposed recommendations about screening for synchronous cancer in anti-RNAP3+ patients with SSc among 82 EUSTAR experts (1–10, with 10 = fully agree).

Proposed Recommendations	Mean	SD
Screening for synchronous malignancies is recommended	8.73	1.70
Screening for synchronous breast cancer in female patients is recommended with mammography (or US/MRI when needed)	9.02	1.35
Screening for other malignancies should be guided by clinical suspicion and patient age. Noninvasive tests (e.g., fecal occult blood, gynecological evaluation, prostatic-specific antigen) may be considered in all patients	8.63	1.48
Serum tumor markers are NOT useful for screening in every patient	8.59	2.18
A period of 2–5 yrs of tight surveillance for cancer is recommended	8.26	2.01
If the screening tests for cancer performed at the diagnosis of scleroderma are negative, tests for breast cancers should be repeated (e.g., annually); other tests should be repeated in case of clinical suspicion	7.90	2.14
PET/CT may be considered when unspecific systemic signs suggest the possible presence of neoplasms	8.00	2.02
Further tight surveillance for cancer is not recommended after a period of 2–5 yrs	7.10	2.27
Prospective studies are needed to clarify how long the tight surveillance should last and which examinations are most indicated for screening of cancer in these patients	9.56	1.04

SSc: systemic sclerosis; EUSTAR: European League Against Rheumatism Scleroderma Trials and Research group; US: ultrasound; MRI: magnetic resonance imaging; PET: positron emission tomography; CT: computed tomography; anti-RNAP3: anti-RNA polymerase III antibodies.

and ELISA[11]. This was subsequently confirmed by several reports, whatever the geographic origin of the patients, despite some differences in research design[12,13,14,15,16]. The OR for diagnosis of cancer in anti-RNAP3+, as compared with other patients with SSc, was calculated at 5.08 (95% CI 1.60–16.1)[16] and 5.83 (95% CI 3.1–10.9)[14], in an interval of 2 or 3 years around the onset of SSc, respectively. We herein extend these findings using our large cohort, showing an OR of 7.38 (95% CI 1.61–33.8) within an interval between 6 months before and 12 months after SSc onset.

We also observed an increased overall prevalence of cancer in anti-RNAP3+ patients, with a low OR, similar to what is found in a previous study[14], but not in others including smaller numbers of cancer cases[13,15,16]. In years distant from the onset of SSc, we like others[14] did not observe any increased frequency of cancer in anti-RNAP3+ patients. Therefore, no available data thus far suggest that the risk of cancer is extended beyond an interval of a few years around SSc onset.

The large majority of malignancies associated with the onset of anti-RNAP3+ SSc were solid cancers; in particular, anti-RNAP3+ patients were more likely to develop breast cancer within 2 years as compared with matched SSc controls with an OR of 20.2. Although the CI were wide, it is noteworthy that a similar OR of 19.0 as compared with ACA+ patients within a 3-year interval was previously reported[14].

Many previously published case series described a higher incidence of breast cancer in patients with SSc compared with the general population, and close temporal relationship with SSc onset was frequently reported[26,27,28]. A metaanalysis demonstrated the association of SSc with lung and hematological malignancies, but did not confirm the association with breast cancer[29]. However, the analysis excluded breast cancer cases diagnosed before SSc, which are fairly common in the years close to SSc onset in anti-RNAP3+ patients[16]. Taken together, these data suggest that the mechanisms underlying the association of SSc with lung and hematological neoplasms, in which DNA oxidative damage and the use of alkylating agents may be relevant[29], are different from those explaining the association with breast cancer.

Indeed, genetic alterations in the *POLR3A* gene, encoding for RNAP3 polypeptide A, and humoral and cell-mediated immune response against this mutated antigen were demonstrated in anti-RNAP3+ patients, but not in patients with other SSc-specific antibodies and cancer[30]. These data suggest that an autoimmune response initiated by mutation in the autoantigen in the cancer cells may explain the onset of SSc as a paraneoplastic disease in these patients[18]. These genetic alterations were found not only in cases of breast cancer[30], and therefore this putative mechanism of cancer-induced autoimmune response as a trigger of SSc may be not limited to this type of malignancy.

From a practical point of view, in our series, the crude frequency of having a diagnosis of cancer within 2 years before or after SSc onset in anti-RNAP3+ patients was 11%. In our experience, as in other series[13,14,15,16], around half of these malignancies were breast cancers.

These results suggest the possible need of a cancer screening program for anti-RNAP3+ patients, similar to what is generally applied in dermatomyositis[18], considering that the number of patients needed to screen to find 1 synchronous malignancy would be relatively low.

We also herein defined clinical and demographic features characteristic of anti-RNAP3+ patients with SSc who have simultaneous malignancy. Patients with older age or diffuse cutaneous involvement were particularly at risk, and malignancies other than breast cancers were much more frequent in men. These risk factors may help in clinical practice to institute appropriate cancer screening at SSc diagnosis in these individuals.

EUSTAR experts agreed to recommend such screening in these patients. Minimal tests to be performed at diagnosis should include screening for breast cancer in women and noninvasive tests guided by clinical suspicion and patient age for other malignancies. Although most experts agreed on the proposed statements, some degree of discordance was found on the use of PET/CT and the program of longterm followup. In particular, lower agreement was found for the duration of tight surveillance and which examinations would be most indicated to be repeated, even if a first determination is negative. The experts strongly agreed that prospective studies are needed to clarify these issues.

Besides the association of anti-RNAP3 with malignancy, our analysis of the large EUSTAR registry confirmed the associations with renal crisis and diffuse cutaneous involvement[4,6,7,13,31]. The associations with arterial hypertension and joint contractures, already reported[13], were not confirmed by multivariable analysis, suggesting that they may be indirectly explained by covariates. The higher frequency of men among anti-RNAP3+ patients was probably due to the high prevalence of women among ACA+ patients[13,22,23,32], whereas we, like others, did not observe differences in the sex distribution between anti-RNAP3+ and anti-topo I+ patients[4,6,7,13,22,23]. We also confirmed the association of anti-RNAP3 with GAVE[9,10], a complication associated with renal crisis, hypertension, reduced DLCO/alveolar volume, and telangiectasia[9,33,34,35], thus reinforcing the characterization of anti-RNAP3 as markers of an SSc subset particularly prone to microangiopathic complications.

Accordingly, anti-RNAP3 were associated with increased risk of pulmonary hypertension (PH)[36]. This could not be confirmed, but the phenotyping of PH in the EUSTAR cohort (initially based on echocardiographic data) was only recently improved, and thus our analysis could not yet include enough patients with proven diagnosis of PH. Further studies are therefore needed to clarify this important issue.

Limitations are inherent in the design of a large, international, and multicenter study based, as far as the association between SSc and cancer, on retrospective data. However, because the awareness of increased risk of malignancy in anti-RNAP3+ patients has arisen among clinicians only recently, it is unlikely that the results of our retrospective analysis (which enrolled all historic patients among participating centers) were biased by more rigorous cancer screening in anti-RNAP3+ than in other patients with SSc. Although methods of anti-RNAP3 evaluation were at the discretion of the participating center, ELISA and line immunoassay tests for anti-RNAP3 have good sensitivity and a specificity higher than 90% when compared with the gold standard of the immunoprecipitation method[37,38,39,40]. Moreover, the association with malignancies was recognized by previous studies irrespective of the method of antibody detection[11,12,13,14,15,16]. Intrapatient variability in anti-RNAP3 levels was observed in longitudinal studies[41], and it is possible that positivity for anti-RNAP3 disappears during the course of the disease. Because the diffusion of anti-RNAP3 tests is relatively recent, we cannot exclude that an initial anti-RNAP3 positivity was not recognized. This might lead to a false-negative classification of patients. However, if false-negative data were included in our analysis, the clinical associations of anti-RNAP3 observed here would be weakened.

In fact, it is noteworthy that, when available in the literature, data derived from single-center observational studies on the relative risk of cancer simultaneous to the onset of SSc[14,16] were very similar to the results of our study, even for specific tumors such as breast cancers[14].

Anti-RNAP3 antibodies are associated with poor prognostic manifestations of SSc, including increased risk of malignancy diagnosis (in particular of breast cancer) close to the SSc onset. Despite the clear potential benefit of anti-RNAP3 evaluations, they are still not always routinely evaluated. Although anti-RNAP3 and other SSc-specific autoantibodies are in most cases mutually exclusive[11,37], a reasonable strategy might be to evaluate anti-RNAP3 not only in all patients with SSc negative for anti–topo I and ACA, but also in all patients with diffuse cutaneous involvement, particularly in older patients and in men, and in all conditions with discrepancies between antibodies status and clinical course. When anti-RNAP3+ SSc is diagnosed, the association with synchronous cancer requires careful screening and followup for malignancies.

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REFERENCES

1. Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. N Engl J Med 2009;360:1989-2003.
2. Walker UA, Tyndall A, Czirják L, Denton C, Farge-Bancel D, Kowal-Bielecka O, et al. Clinical risk assessment of organ manifestations in systemic sclerosis: a report from the EULAR Scleroderma Trials And Research group database. Ann Rheum Dis 2007;66:754-63.
3. Meier FM, Frommer KW, Dinser R, Walker UA, Czirják L, Denton CP, et al. Update on the profile of the EUSTAR cohort: an analysis of the EULAR Scleroderma Trials and Research group database. Ann Rheum Dis 2012;71:1355-60.
4. Steen VD. The many faces of scleroderma. Rheum Dis Clin North Am 2008;34:1-15.
5. Wirz EG, Jaeger VK, Allanore Y, Riemekasten G, Hachulla E, Distler O, et al; EUSTAR coauthors. Incidence and predictors of cutaneous manifestations during the early course of systemic sclerosis: a 10-year longitudinal study from the EUSTAR database. Ann Rheum Dis 2016;75:1285-92.
6. Okano Y, Steen VD, Medsger TA Jr. Autoantibody reactive with RNA polymerase III in systemic sclerosis. Ann Intern Med 1993;119:1005–13.
7. Bunn CC, Denton CP, Shi-wen X, Knight C, Black CM. Anti-RNA polymerases and other autoantibody specificities in systemic sclerosis. Br J Rheumatol 1998;37:15–20.
8. Kuwana M, Kimura K, Kawakami Y. Identification of an immunodominant epitope on RNA polymerase III recognized by systemic sclerosis sera: application to enzyme-linked immunosorbent assay. Arthritis Rheum 2002;46:2742-7.
9. Ceribelli A, Cavazzana I, Airò P, Franceschini F. Anti-RNA polymerase III antibodies as a risk marker for early gastric antral vascular ectasia (GAVE) in systemic sclerosis. J Rheumatol 2010;37:1544.
10. Ghrénassia E, Avouac J, Khanna D, Derk CT, Distler O, Suliman YA, et al. Prevalence, correlates and outcomes of gastric antral vascular ectasia in systemic sclerosis: a EUSTAR case-control

study. J Rheumatol 2014;41:99-105.
11. Shah AA, Rosen A, Hummers L, Wigley F, Casciola-Rosen L. Close temporal relationship between onset of cancer and scleroderma in patients with RNA polymerase I/III antibodies. Arthritis Rheum 2010;62:2787–95.
12. Airò P, Ceribelli A, Cavazzana I, Taraborelli M, Zingarelli S, Franceschini F. Malignancies in Italian patients with systemic sclerosis positive for anti-RNA polymerase III antibodies. J Rheumatol 2011;38:1329–34.
13. Nikpour M, Hissaria P, Byron J, Sahhar J, Micallef M, Paspaliaris W, et al. Prevalence, correlates and clinical usefulness of antibodies to RNA polymerase III in systemic sclerosis: a cross-sectional analysis of data from an Australian cohort. Arthritis Res Ther 2011;13:R211.
14. Moinzadeh P, Fonseca C, Hellmich M, Shah AA, Chighizola C, Denton CP, et al. Association of anti-RNA polymerase III autoantibodies and cancer in scleroderma. Arthritis Res Ther 2014;16:R53.
15. Saigusa R, Asano Y, Nakamura K, Miura S, Ichimura Y, Takahashi T, et al. Association of anti-RNA polymerase III antibody and malignancy in Japanese patients with systemic sclerosis. J Dermatol 2015;42:524-7.
16. Shah AA, Hummers LK, Casciola-Rosen L, Visvanathan K, Rosen A, Wigley FM. Examination of autoantibody status and clinical features associated with cancer risk and cancer-associated scleroderma. Arthritis Rheumatol 2015;67:1053-61.
17. Sobanski V, Dauchet L, Lefevre G, Lambert M, Morell-Dubois S, Sy T, et al. Prevalence of anti-RNA polymerase III antibodies in systemic sclerosis: new data from a French cohort and a systematic review and meta-analysis. Arthritis Rheumatol 2014;66:407-17.
18. Shah AA, Casciola-Rosen L, Rosen A. Cancer-induced autoimmunity in the rheumatic diseases. Arthritis Rheumatol 2015;67:317-26.
19. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum 1980;23:581–90.
20. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum 2013;65:2737–47.
21. Leroy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 1988;15:202-5.
22. Satoh T, Ishikawa O, Ihn H, Endo H, Kawaguchi Y, Sasaki T, et al. Clinical usefulness of anti-RNA polymerase III antibody measurement by enzyme-linked immunosorbent assay. Rheumatology 2009;48:1570-4.
23. Motegi SI, Toki S, Yamada K, Uchiyama A, Ishikawa O. Demographic and clinical features of systemic sclerosis patients with anti-RNA polymerase III antibodies. J Dermatol 2015; 42:189-92.
24. Cavazzana I, Ceribelli A, Airò P, Zingarelli S, Tincani A, Franceschini F. Anti-RNA polymerase III antibodies: a marker of systemic sclerosis with rapid onset and skin thickening progression. Autoimmun Rev 2009;8:580-4.
25. Codullo V, Cavazzana I, Bonino C, Alpini C, Cavagna L, Cozzi F, et al. Serologic profile and mortality rates of scleroderma renal crisis in Italy. J Rheumatol 2009;36:1464-9.
26. Colaci M, Giuggioli D, Vacchi C, Lumetti F, Iachetta F, Marcheselli L, et al. Breast cancer in systemic sclerosis: Results of a cross-linkage of an Italian rheumatologic center and a population-based cancer registry and review of the literature. Autoimmunity Rev 2014;13:132-7.
27. Abu-Shakra M, Guillemin F, Lee P. Cancer in systemic sclerosis. Arthritis Rheum 1993;36:460-4.
28. Launay D, Le Berre R, Hatron PY, Peyrat JP, Hachulla E, Devulder B, et al. Association between systemic sclerosis and breast cancer: eight new cases and review of the literature. Clin Rheumatol 2004;23:516-22.
29. Bonifazi M, Tramacere I, Pomponio G, Gabrielli B, Avvedimento EV, La Vecchia C, et al. Systemic sclerosis (scleroderma) and cancer risk: systematic review and meta-analysis of observational studies. Rheumatology 2013;52:143-54.
30. Joseph CG, Darrah E, Shah AA, Skora AD, Casciola-Rosen LA, Wigley FM, et al. Association of the autoimmune disease scleroderma with an immunologic response to cancer. Science 2014;343:152-7.
31. Kuwana M, Kaburaki J, Mimori T, Tojo T, Homma M. Autoantibody reactive with three classes of RNA polymerases in sera from patients with systemic sclerosis. J Clin Invest 1993;91:1399-404.
32. Elhai M, Avouac J, Walker UA, Matucci-Cerinic M, Riemekasten G, Airò P, et al; EUSTAR co-authors. A gender gap in primary and secondary heart dysfunctions in systemic sclerosis: a EUSTAR prospective study. Ann Rheum Dis 2016;75:163-9.
33. Hung EW, Mayes MD, Sharif R, Assassi S, Machicao VI, Hosing C, et al. Gastric antral vascular ectasia and its clinical correlates in patients with early diffuse systemic sclerosis in the SCOT trial. J Rheumatol 2013;40:455-60.
34. Marie I, Ducrotte P, Antonietti M, Herve S, Levesque H. Watermelon stomach in systemic sclerosis: its incidence and management. Aliment Pharmacol Ther 2008;28:412-21.
35. Ingraham KM, O'Brien MS, Shenin M, Derk CT, Steen VD. Gastric antral vascular ectasia in systemic sclerosis: demographics and disease predictors. J Rheumatol 2010;37:603-7.
36. Nihtyanova SI, Schreiber BE, Ong VH, Rosenberg D, Moinzadeh P, Coghlan JG, et al. Prediction of pulmonary complications and long-term survival in systemic sclerosis. Arthritis Rheumatol 2014;66:1625-35.
37. Parker JC, Burlingame RW, Webb TT, Bunn CC. Anti-RNA polymerase III antibodies in patients with systemic sclerosis detected by indirect immunofluorescence and ELISA. Rheumatology 2008;47:976-9.
38. Kuwana M, Okano Y, Pandey JP, Silver RM, Fertig N, Medsger TA Jr. Enzyme-linked immunosorbent assay for detection of anti-RNA polymerase III antibody: analytical accuracy and clinical associations in systemic sclerosis. Arthritis Rheum 2005; 52:2425–32.
39. Codullo V, Morozzi G, Bardoni A, Salvini R, Deleonardi G, De Pità O, et al; Forum Interdisciplinare per la Ricerca sulle Malattie Autoimmuni (F.I.R.M.A.) study group. Validation of a new immunoenzymatic method to detect antibodies to RNA polymerase III in systemic sclerosis. Clin Exp Rheumatol 2007;25:373–7.
40. Villalta D, Imbastaro T, Di Giovanni S, Lauriti C, Gabini M, Turi MC, et al. Diagnostic accuracy and predictive value of extended autoantibody profile in systemic sclerosis. Autoimmunity Rev 2012;12:114–20.
41. Nihtyanova SI, Parker JC, Black CM, Bunn CC, Denton CP. A longitudinal study of anti-RNA polymerase III antibody levels in systemic sclerosis. Rheumatology 2009;48:1218–21.