Clinical features of systemic sclerosis patients with anti-RNA polymerase III antibody in a single centre in Spain

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ABSTRACT

Objective. *To evaluate the clinical features and survival of patients with positive anti-RNA polymerase III (anti-RNAP III) in a Spanish single centre.*

Methods. *We analysed 221 patients with SSc according to LeRoy and Medsger criteria. Twenty-six patients with positivity for anti-RNAP III antibodies were compared with 195 negative patients. Epidemiological, clinical, immunological features and survival were analysed.*

Results. *In patients with anti-RNAP III positivity diffuse cutaneous SSc (dcSSc) subset was the most prevalent (20, 76.9% vs. 35, 17.9%, p<0.001), with shorter diagnosis delay (4.11±7.34 years vs. 6.77±9.22 years, p=0.005). Patients with anti-RNAP III antibodies had higher frequency of arterial hypertension (13, 50% vs. 55, 28.2%, p=0.024), scleroderma renal crisis (SRC) (3, 11.5% vs. 3, 1.5%, p=0.023), arthritis (9, 34.6% vs. 35, 17.9%, p=0.046), tendon friction rubs (4, 15.4% vs. 1, 0.5%, p=0.001) and contractures (5, 19.2% vs. 10, 5.1%, p=0.02). There were no differences found in the presence of cancer or in global survival. In the multivariate survival analysis, severe interstitial lung disease (ILD) (HR: 8.61, 95%CI 3.40–21.81), pulmonary arterial hypertension (PAH) (HR: 4.05, 95%CI 1.42–11.61) and SRC (HR: 17.27, 95%CI 3.36–88.97) were the only factors associated with poor prognosis.*

Conclusion. *In this cohort anti-RNAP III antibodies are related with dcSSc subset, shorter diagnostic delay and higher prevalence of musculoskeletal involvement, arterial hypertension and SRC. ILD, PAH and SRC were independent prognostic factors.*

Introduction

Systemic sclerosis (SSc) is a multiorgan connective tissue disease of autoimmune nature characterised by fibrosis of the skin and internal organs, activation of immune system and prominent vascular and microvascular damage and oxidative stress (1, 2). In addition, current investigations support the activation of the immune system plays a crucial role in the pathogenesis of SSc (3, 4). One relevant feature of the immune system activation is the presence of antinuclear antibodies (ANAs) in almost 90% of patients. Moreover, ANAs react against different intracellular components that play crucial roles in transcription, splicing and cell division, leading to the expression of a specific autoantibody (5). The most recent classification criteria developed by the American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) include anti-centromere antibodies (ACA), anti-topoisomerase I (ATA) antibodies and anti RNA polymerase III antibodies (anti-RNAP III) (6). ACA are associated with limited cutaneous involvement, pulmonary arterial hypertension (PAH) and more favourable prognosis than patients with other SSc-related ANA (7-9). Anti-topoisomerase I (anti-topo I) are related to diffuse skin involvement and interstitial lung disease (ILD) (8). Anti-RNAP III antibody have been linked to dcSSc subtype (8, 10-12), the development of scleroderma renal crisis (SRC) (10, 12, 13) and gastric antral vascular ectasia (GAVE) (14). In addition presence of anti-RNAP III was associated with the concomitant existence of cancer (14-16).

A recent meta-analysis established an overall prevalence of 11% in SSc patients with a high degree of heterogeneity due to demographic and geographic factors (17). However, few studies have been focused in analysing the characteristics of south European population with positivity for this antibody (15, 18-21).

This study was undertaken to examine the main epidemiological, clinical, immunological, vascular features and survival of SSc patients who were anti-RNAP III positive and compare with anti-RNAP negative in a SSc cohort of a single Spanish centre.

Methods

Patients

We selected 221 Caucasian patients (21 men and 192 women) diagnosed with SSc who were assessed in the Scleroderma Unit at Vall d'Hebron Hospital since 1980. The study was approved by the ethics Committee for Clinical Research (PG(AG)07/2015), and all patients provided written informed consent for their participants. All patients met the LeRoy and Medsger criteria (22) and 196 (88.6%) patients fulfilled the 2013 ACR/EULAR classification criteria of SSc (6).

Two hundred and twenty-one patients were analysed consecutively. Among them, 26 with anti-RNAP III positivity were compared with 195 patients who were negative for anti-RNAP III.

Age at onset of disease was defined as the presented at first clinical event including the Raynaud's phenomenon (RP), reported by the patient. We also include age at onset of first clinical feature other than RP. Diagnosis delay was defined as the time from the first symptom of the disease (first symptom attributable to the disease including RP or first symptom non-RP) to the time when the diagnosis was established.

Patients were classified according to Leroy and Medsger's subsets (22): limited cutaneous SSc (lcSSc) was defined if skin thickening was confined distally to the elbows and knees and may affect the face; diffuse cutaneous SSc (dcSSc) skin sclerosis also affected proximally to the elbows and knees or the trunk; *sine* scleroderma SSc (ssSSc) was defined by RP or equivalents (pitting scars, typical capillaroscopic alterations), antinuclear antibodies and scleroderma visceral involvement (PAH, ILD, SRC, oesophageal hypomotility or sclerodermic myocardiopathy) without skin sclerosis. Capillaroscopic study was classified in "early", "active" and "late" pattern according to Cutolo's classification (23). Gastrointestinal involvement was defined as the incompetence of the lower esophageal sphincter (pressure <15mmHg) and esophageal hypomotility, gastroparesis, the presence of gastric antral vascular ectasia (GAVE) (watermelon stomach), or bacterial overgrowth. Liver involvement was determined as elevated liver enzymes without another cause, presence of autoimmune hepatitis or concomitant primary biliary cholangitis (24). PAH was defined as mean pulmonary arterial pressure (mPAP) ≥25mmHg in right heart catheterisation (RHC) with pulmonary artery wedge pressure (PAWP) ≤15mmHg and pulmonary vascular resistance (PVR) >3 Wood units in RHC (25). Both groups were followed similarly in relation to PAH, based on annual screening through pulmonary function test (PFT), echocardiogram and clinical monitoring (26). ILD was considered if there was radiological evidence of interstitial disease on high resolution computer tomography (HRCT). Heart involvement was defined as a pericardial involvement demonstrated by echocardiogram, computed tomography (CT) or nuclear magnetic resonance (NMR); conduction abnormalities established by electrocardiogram; ischaemic heart disease in absence of classical cardiovascular risk factors (CVRF) made evident by left catheterisation, NMR or myocardial scintigraphy; scleroderma cardiomyopathy or cardiac fibrosis confirmed by NMR; mitral insufficiency without CVRF and diastolic dysfunction without CVRF proved with echocardiogram as well as left ventricular ejection fraction <50% and right ventricular ejection fraction <40% made evident by echocardiogram or NMR. SRC was defined by the presence of a rapid decline of renal function within an interval of less than one month or by the combination of sudden onset or aggravation of moderate or severe arterial hypertension (>160/90mmHg) in association with manifestations of malignant hypertension (27). Cancer data were obtained by pathology report.

Association with other autoantibodies, cancer prevalence, overall survival and survival from the disease onset were analysed.

Immunological test

Antinuclear autoantibodies (ANAs) were determined by indirect immunofluorescence on HEp-2 cells. The presence of anti-RNAP III antibodies was detected by commercial line blot assay to RP155 and RP11 proteins (EUROLINE Systemic Sclerosis Profile, Euroimmun, Germany), or enzyme-linked immunosorbent assay (ELISA) (Quanta Lite RNA Pol III, Inova Diagnostics). Using the immunoblot, positive results were considered as determined by the manufacturer; borderline results were taken into account as negative. ELISA test defined a positive result according to manufacturer MBL kit with a cut-off value > 28UI/mL.

Statistical analysis

Fisher's exact probability or chi-squared test was used for categorical variables. For continuous variables t-Student test were used. Survival analysis was performed using Kaplan-Meier curves and log-rank test. Independent risk factors from univariate analyses, at a significance level of 20% were included in the multivariate Cox's regression model.

In one analysis any symptom including RP was considered as de onset of disease, while in another was considered the first symptom-non RP, in both cases reported by patient or doctor. Statistical analysis was accomplished using SPSS Statistics 20.0 (Inc., Chicago, USA). Statistical significance was defined as a *p*-value lower than 0.05.

Results

Of 221 patients included in the study, 26 (11.7%) showed reactivity anti-RNAP III. Twenty-four patients were positive by immunoblot, 22 were RP155+/RP11+, one was RP155+/RP11- and another was RP155-/RP11+ (the latter was also positive by ELISA). Among these 24 patients diagnosed by immunoblot, a total of 4 patients were positive also by ELISA. In addition, 2 more patients were anti-RNAP III positive by ELISA without having been tested by the immunoblot. The main demographic characteristics, clinical manifestations, immunological features, survival and causes of death are represented in Table I. The

Table I. Demographic and clinical characteristics of 221 SSc patients, with and without anti-RNAP III reactivity.

Demographic and clinical characteristics, n (%)	Overall SSc 221 (100)	Anti-RNAP III positive 26 (11.8)	Anti-RNAP III negative 195 (88.2)	*p*
Female gender, n (%)	192 (86.9)	21 (80.8)	171 (87.7)	0.350
SSc subsets (N=221)				
Limited cutaneous SSc, n (%)	137 (62)	6 (23.1)	131 (67.2)	**<0.001**
Sine escleroderma SSc, n (%)	29 (13.1)	0 (0)	29 (14.9)	**0.030**
Diffuse cutaneous SSc, n (%)	55 (24.9)	20 (76.9)	35 (17.9)	**<0.001**
Age at disease onset, mean ± SD, y	40.5 ± 15.6	38.3 ± 14.0	40.8 ± 15.8	0.580
Age at onset of first non-RP symptom, mean ± SD, y	45.3 ± 14.8	41.0 ± 15.5	45.8 ± 14.7	0.223
Age at SSc diagnosis, mean ± SD, y	46.9 ± 15.6	42.4 ± 15.6	47.4 ± 15.5	0.170
Diagnosis delay since first symptom, mean ± SD, y	6.3 ± 9.0	4.11 ± 7.3	6.8 ± 9.2	**0.005**
Diagnosis delay since non RP symptom, mean ± SD, y	2.0 ± 5.1	1.4 ± 2.6	2.0 ± 5.4	0.205
Time of follow-up since first symptom, mean ± SD, y	18.7 ± 12.1	16.2 ± 10.5	19.1 ± 12.3	0.268
Time of follow-up since first non RP symptom, mean ± SD, y	13.9 ± 9.3	13.4 ± 9.2	14.0 ± 9.4	0.765
Patients who met 2013 ACR/EULAR criteria, n (%)	196 (88.69)	26 (100)	170 (87.1)	0.051
Cutolo late pattern, n (%)	55/209 (26.3)	6/23 (26.1)	49/186 (26.3)	0.979
Cardiovascular risk factors				
Arterial hypertension, n (%)	68/221 (30.8)	13/26 (50)	55/195 (28.2)	**0.024**
Diabetes Mellitus, n (%)	11/221 (5)	1/26 (3.8)	10/195 (5.1)	1.000
Dyslipidaemia, n (%)	47/221 (21.3)	6/26 (23.1)	41/195 (21)	0.810
Peripheral vascular manifestations, n (%)	220/221 (99.5)	26/26 (100)	194/195 (99.5)	0.710
Raynaud's phenomenon, n (%)	216/221 (97.7)	26/26 (100)	190/195 (97.4)	1.000
Digital ulcers, n (%)	115/221 (52)	16/26 (61.5)	99/195 (50.8)	0.302
Telangiectasies, n (%)	166/221 (75.1)	20/26 (76.9)	146/195 (74.9)	0.820
Gastrointestinal involvement, n (%)	192/221 (86.9)	23/26 (88.5)	169/195 (86.7)	1.000
Oesophagus involvement, n (%)	181/221 (81.9)	20/26 (76.9)	161/195 (82.6)	0.587
Stomach involvement, n (%)	41/221 (18.8)	8/26 (30.8)	33/195 (16.9)	0.106
GAVE, n (%)	15/112 (13.4)	2/15 (13.3)	13/97 (13.4)	1.000
Intestinal involvement, n (%)	37/221 (16.7)	6/26 (23.1)	31/195 (15.9)	0.400
Liver involvement, n (%)	17/221 (7.7)	2/26 (7.7)	15/195 (7.7)	0.679
Lung involvement (ILD and/or PAH)	109/221 (49.3)	15/26 (57.7)	94/195 (48.2)	0.363
Interstitial lung disease, n (%)	96/221 (43.4)	14/26 (53.8)	82/195 (42.1)	0.505
Pulmonary arterial hypertension, n (%)	26/221 (11.8)	3/26 (11.5)	23/195 (11.8)	1.000
FVC < 70%, n (%)	74/221 (33.8)	9/26 (36.0)	65/195 (33.5)	0.804
FVC < 50%, n (%)	22/221 (10.0)	2/26 (8.0)	20/195 (10.3)	1.000
Heart involvement, n (%)	169/221 (76.5)	19/26 (73.1)	150/195 (76.9)	0.664
LVEF < 50%, n (%)	7/221 (3.2)	0/26 (0)	7/195 (3.6)	1.000
Pericarditis, n (%)	23/221 (13.1)	5/26 (19.2)	24/195 (12.3)	0.352
Coronary heart disease, n (%)	4/221 (1.8)	0/26 (0)	4/195 (2.1)	1.000
Coronary microvascular disease, n (%)	17/221 (7.7)	3/26 (11.5)	14/195 (7.2)	0.431
LV diastolic dysfunction, n (%)	101/221 (47.4)	11/26 (44)	89/195 (47.9)	0.716
Mitral insufficiency, n (%)	127/221 (59.6)	14/26 (53.8)	112/195 (60.1)	0.694
Conduction abnormalities, n (%)	50/221 (34)	6/26 (35.3)	44/195 (33.8)	0.906
Myocardial fibrosis, n (%)	4/221 (1.8)	1/26 (3.8)	3/195 (1.5)	0.396
Renal involvement				
Scleroderma renal crisis, n (%)	6/221 (2.7)	3/26 (11.5)	3/195 (1.5)	**0.023**
Musculoskeletal involvement, n (%)	107/221 (48.4)	16/26 (61.5)	91/195 (46.7)	0.150
Arthritis, n (%)	44/221 (19.4)	9/26 (34.6)	35/195 (17.9)	**0.046**
Tendon friction rubs, n (%)	5/221 (2.3)	4/26 (15.4)	1/195 (0.5)	**0.001**
Contractures, n (%)	15/221 (6.8)	5/26 (19.2)	10/195 (5.1)	**0.020**
Myositis, n (%)	13/221 (5.9)	0/26 (0)	13/195 (6.7)	0.373
Non-inflammatory myopathy, n (%)	16/221 (7.2)	1/26 (3.8)	15/195 (7.7)	0.701
Calcinosis, n (%)	54/221 (24.4)	7/26 (26.9)	47/195 (24.1)	0.753
Cancer	25/221 (11.3)	4/26 (15.4)	21/195 (10.8)	0.508
Causes of death				
Total, n (%)	29/221 (13.1)	4/26 (15.4)	25/195 (12.8)	0.757
SSc related:				
ILD or PAH, n (%)	12/29 (41.3)	3/4 (75)	9/25 (36)	0.126
Cardiopathy, n (%)	3/29 (10.3)	0/4 (0)	3/25 (12)	1.000
Scleroderma renal crisis, n (%	1/29 (3.4)	0/4 (0)	1/25 (4)	1.000
Non SSc-related	13/29 (44.8)	1/4 (25)	12/25 (48)	0.900

SSc: systemic sclerosis; anti-RNAP III: anti-RNA polymerase III; y: years; RP: Raynaud's phenomenon; GAVE: gastric antral vascular ectasia; ILD: interstitial lung disease; PAH: pulmonary arterial hypertension; FVC: Forced Vital Capacity; LVEF: left ventricle ejection fraction; LV: left ventricle; bold *p*: $p<0.05$.

Table II. Immunological features of 221 SSc patients with and without anti-RNAP III reactivity.

	Overall SSc 221 (100)	Anti-RNAP III positive 26 (11.8)	Anti-RNAP III negative 195 (88.2)	*p*
Anti-nuclear, n (%)	217 (98.2)	26 (100)	191 (97.9)	1.000
Anti-centromere, n (%)	77 (34.8)	0 (0)	77 (39.5)	**<0.001**
Anti-topoisomerase, n (%)	49 (22.2)	3 (11.5)	46 (23.6)	0.165
Anti-PM/Scl, n (%)	17 (7.7)	0 (0)	17 (8.7)	0.230
Anti-RNP, n (%)	6 (2.7)	0 (0)	6 (3.1)	1.000
Anti-Ro52, n (%)	61 (27.6)	3 (11.5)	58 (29.7)	0.051
Anti-Ro60, n (%)	24 (10.9)	2 (7.7)	22 (11.3)	0.740
Anti-La, n (%)	6 (2.7)	1 (3.8)	5 (2.6)	0.530

Bold *p*: $p<0.05$.

female (21, 80.8% and 171, 87.7%) was the most frequent gender in both groups. Patients with anti-RNAP III antibody did not differ from seronegative group in terms of mean age at disease onset (32.2±13.9 years *vs*. 40.7±15.7 years) or mean age at diagnosis (42.4±15.6 years *vs*. 47.4±15.5 years). A shorter diagnosis delay in patients with anti-RNAP III was observed (4.1±7.3 years *vs*. 6.7±9.2 years, $p=0.005$). DcSSc was significantly more frequent in the anti-RNAP III group (20, 76.9% *vs*. 35, 17.9%, $p<0.001$). The patients anti-RNAP III fulfil more frequently ACR/EULAR 2013 classification criteria (6) (26, 100% *vs*. 170, 87.2%, $p=0.051$). With reference to traditional cardiovascular risk factors, patients anti-RNAP III presented higher prevalence of arterial hypertension (13, 50% *vs*. 55, 28.2%, $p=0.024$) with no differences in diabetes mellitus (1, 3.8% *vs*. 10, 5.1%) or dyslipidaemia (6, 23.1% *vs*. 41, 21%). RP was less frequently presented as the first manifestation of the disease in the anti-RNAP III group (14, 53.8% *vs*. 153, 78.5% $p=0.006$). Among peripheral vascular manifestations there were no differences in the presence of digital ulcers (16, 61.5% *vs*. 99, 50.8%), or in the prevalence RP (26, 100% *vs*. 190, 97.4%) or telangiectasias (20, 76.9% *vs*. 146, 74.9%). Neither were there significant differences in the global gastrointestinal involvement (23, 88.5% *vs*. 169, 86.7%), esophageal (20, 76.9 *vs*. 161, 82.6%), gastric (8, 30.8% *vs*. 33, 16.9%), the presence of GAVE (2, 7.7% *vs* 13, 6.7%), intestinal (6, 23.1% *vs*. 31, 15.9%) or liver involvement (2, 7.7% *vs*. 15, 7.7). No differences in lung involvement were found in the form of ILD (14, 53.8% *vs*. 82, 42.1%) or PAH (3, 11.5% *vs*. 23, 11.8%). Regarding PFT, there were no differences among patients with forced vital capacity (FVC) below 70% or 50% of predicted (9, 36% *vs*. 65, 33.5% and 2, 8% *vs*. 20, 10.3% respectively). No differences in global heart involvement were detected (19, 73.1% *vs*. 150, 76.9%). Scleroderma renal crisis (SRC) was significantly more frequent in patients with anti-RNAP III (3, 11.5% *vs*. 3, 1.5% $p=0.023$). Regarding musculoskeletal involvement, arthritis (9, 34.6% *vs*. 35, 17.9% $p=0.046$), tendon friction rubs (4, 15.4% *vs*. 1, 0.5% $p=0.001$) and contractures (5, 19.2% *vs*. 10, 5.1% $p=0.02$) were more frequent in anti-RNAP III group, whereas myositis (0, 0% *vs*. 13, 6.7%), non-inflammatory myopathy (1, 3.8% *vs*. 15, 7.7%) and calcinosis (7, 26.9% *vs*. 47, 24.1%) showed no differences between both groups.

No differences were detected concerning capillaroscopy pattern, late pattern by Cutolo was presented in 55/209 (26.3%) patients (6/23, 26.1 *vs*. 49/186, 26.3%) (23). In the study of other associated autoantibodies there was only found a not reactivity against anticentromere antibodies (0, 0% *vs*. 77, 35.9%, $p<0.001$) in the patients with anti-RNAP III (Table II).

There were no differences in the presence of cancer in the patients with RNAP III positivity (4, 15.4% *vs*. 25, 12.8%), although a tendency of higher development of synchronic neoplasms for 60 months prior and after SSc onset was observed (2, 7.7% vs. 7, 3.6%, $p=0.28$). Both groups were followed up for a

Table III. Univariate and multivariate Cox's regression survival analysis.

	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Diffuse cutaneous SSc	2.76	(1.25-6.13)	**0.012**[a]	1.36	(0.44-4.22)	0.593
Anti-RNA polymerase III positive	1.59	(0.55-4.60)	0.391			
Anti-centromere positive	0.51	(0.23-1.13)	0.095[a]	0.94	(0.33-2.66)	0.902
Anti-topoisomerase	1.73	(0.76-3.91)	0.192[a]	0.80	(0.27-2.66)	0.679
Male	1.45	(0.50-4.20)	0.489			
Age at diagnosis since first symptom	1.11	(1.07-1.14)	**<0.001**			
Age at diagnosis (since non-RP symptom)	1.05	(1.03-1.09)	**<0.001**	1.07	(1.04-1.11)	<0.001
ILD with Forced Vital Capacity < 50%	7.06	(3.32-15.01)	**<0.001**[a]	8.61	(3.40-21.81)	**<0.001**
Pulmonary arterial hypertension	2.12	(0.85-5.27)	0.106[a]	4.05	(1.42-11.61)	**0.001**
Scleroderma renal crisis	10.47	(3.09-35.43)	**<0.001**[a]	17.27	(3.36-88.97)	**0.001**
Heart involvement	1.12	(0.43-2.95)	0.816			
Cancer	1.40	(0.54-3.68)	0.490			

HR: hazard ratio; CI: confidence interval; bold *p*: $p<0.05$; RP: Raynaud's phenomenon; ILD: interstitial lung disease.
[a]Variables included in the multivariate analysis.

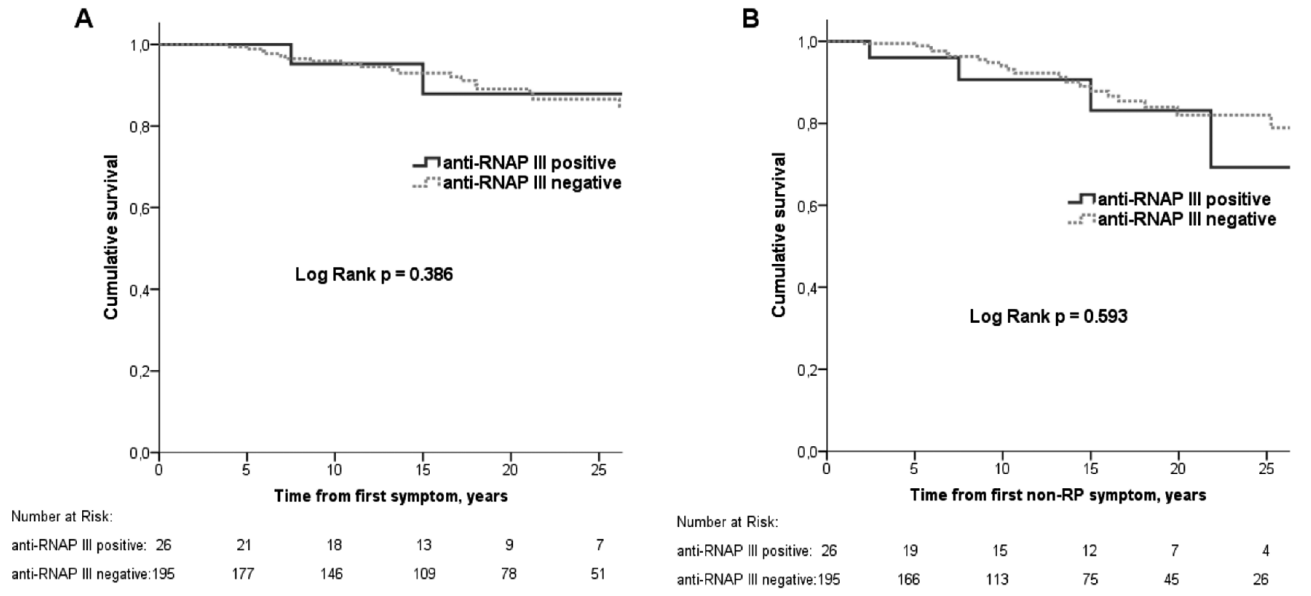

Fig. 1. Kaplan-Meier survival plots.
A: Cumulative survival since first SSc symptom. **B**: Cumulative survival since first SSc symptom excluding Raynaud's phenomenon.

mean of 17.8±12.0 years with no differences between them, nevertheless there were no differences found in mortality comparing the two groups (4, 15.4% *vs*. 25, 12.8%), nor in the causes of death. The main causes of death related to SSc were lung disease (ILD or PAH) (3/4, 75% *vs*. 9/25, 36%), heart disease (0/4, 0% *vs*. 1/4, 25%) and SRC (0/4, 0% *vs*. 1/4, 25%). The remaining deceased patient in anti-RNAP III group was because of breast cancer. Other causes in anti-RNAP III negative group were 3 cancer (gastric neoplasm, lung cancer and acute myeloblastic leukaemia), one infectious pneumonia, two cases of chronic obstructive pulmonary disease, one chronic pachypleuritis, one intestinal ischaemia, one liver cirrhosis, stroke in two patients and one sudden death. Death data were obtained from local and national records in both groups.

There were no statistical differences in the cumulative survival from the onset of the disease including the RP as first manifestation (log rank p=0.386, Fig. 1A). Cumulative survival rates from the RP at 5, 10, 15, 20 and 25 years in anti-RNAP III positive group were 100%, 95%, 87%, 87% and 87%, respectively and in seronegative group were 98%, 95%, 93%, 89% and 86%, respectively. Analysing the time from the first non-RP manifestation of SSc, neither were differences found in the survival between patients with anti-RNAP III antibodies compared with patients without this antibody (Fig. 1B). Cumulative survival rates from the first non-RP symptom at 5, 10, 15, 20 and 25 years in the anti-RNAP III positive group were 96%, 90%, 83%, 83% and 69%, respectively, and in the seronegative group were 98%, 93%, 87%, 82% and 82%, respectively.

Univariate Cox survival revealed that dcSSc was associated with a worse survival (hazard ratio (HR): 2.76, 95%CI 1.25–6.13), as well as ILD with a FVC lower than 50% (HR: 7.06, 95%CI 3.32–15.01) and SRC (HR: 10.47, 95%CI 3.09–35.43) (Table III). Variables with a p<0.20 were selected to perform the multivariate Cox's regression survival analysis. Multivariate study showed the presence of ILD with a FVC lower than 50% (HR: 8.61, 95%CI 3.40–21.81), PAH (HR: 4.05, 95%CI 1.42–11.61) and SRC (HR: 17.27, 95%CI 3.36–88.97) were the only factors associated with death during follow-up period (Table III). None of the autoantibodies was associated with a worse outcome.

Discussion

In our study, we analysed in a single centre the clinical associations and survival of anti-RNAP III in a SSc Spanish cohort. We found an association between anti-RNAP III antibody and shorter diagnosis delay, diffuse cutaneous subtype and more prevalence of arterial hypertension, SRC and some musculoskeletal manifestations such as arthritis, tendon friction rubs and contractures. In reference to overall survival no differences were found in the distinct subgroups analysed. ILD, PAH and SRC were independent prognostic factors.

This study provided new data of anti-RNAP III prevalence in Spanish population in a single centre. We found a prevalence of anti-RNAP III in our cohort of 11.7%, similar to the overall prevalence reported in the last meta-analysis (17). Moreover, it is comparable to that found in southern European countries where prevalence is among 6.6–12.1% (18-21) but lower than described in the United States (24.3%) (15), Canada (18.6%) (28), in northern European countries (21.7–22%) (29, 30) and in New Zealand (20%) (31). Whilst there are different studies that have established the prevalence of anti-RNAP III in Europe (15-19), according to prior literature, this is the first time that it has been described in Spain. The similarity of prevalence between geographically close regions may indicate that there are genetic factors implicated (32) and environmental factors in-

volved may play a role too. The latter is further supported by the comparable prevalence among countries with similar latitude.

Diffuse cutaneous subtype was present in 76.9% of patients, being the most frequent cutaneous subtype in the RNAP-III group, which agrees with other series (56.5-64.3%) (11, 33). With reference to the presence of GAVE there is a disparity between published data. We found a similar prevalence among two groups (13.3% *vs*. 13.4%) according to Hung *et al*. (35.7% *vs*. 28.9%) (34). However, EUSTAR study reported significant differences (63% *vs*. 27%, p=0.01) (35).

We observed a higher incidence of musculoskeletal manifestations such as arthritis in the anti-RNAP III group presumably due to the association between antibody expression and diffuse subset. Similarly, there are series in Europe in which this trend is already observed when compared to ACA subset (43.5% *vs*. 20.1%) (36). In addition, an association has been described between the presence of anti-RNAP III and other connective tissue disease with articular expression mainly as rheumatoid arthritis (11).

We detected that approximately half of patients with anti-RNAP III have ILD, percentage slightly higher than that published in other studies (37-39) but similar to results observed in a Norwegian study (51%) (40). Both studies HRCT was used for ILD diagnosis criteria. In our daily practice we perform a HRCT at the moment of the SSc diagnosis in all patients, then we can detect pulmonary fibrosis in subclinical stages. According to the results of HRCT and pulmonary function test, Hoffmann-Vold *et al*. (40) propose to classify patients with anti-RNAP III into two groups at the time of diagnosis. One subset with normal pulmonary findings and low risk to develop ILD and another subset with raised risk in which closer monitoring and early treatment is needed. Furthermore, the same group emphasise that anti-RNAP III was associated with a greater progression of pulmonary fibrosis than in the other subset antibodies. Hoffmann-Vold *et al*. (40) did not find differences in relation to FVC between anti-RNAP III and ATA positive patients. In this same line, we did not find significant differences between anti-RNAP III positive and negative groups regarding FVC variables, probably due to a low number of ATA in the negative group.

Regarding pulmonary vascular disease, in our cohort PAH rates are comparable to those previously reported without establishing association between the presence of the antibody and this complication (7, 15, 20, 38, 40). Most studies did not find any association between anti-RNAP III and PH but only in a few of them this complication was diagnosed by RHC (20, 41). In a multivariable analysis performed in a cohort of about 400 patients it was observed that the anti-RNAP III is an independent predictor of PH measured by RHC (42). Nonetheless, we found that the frequency of PAH was 11.8% in the anti-RNAP III patients, which is similar to the global cohorts, emphasising that PH surveillance is an important issue in these patients.

SRC is a well-described complication associated in many previous studies with the presence of anti-RNAP III antibodies (7, 43-46). Comparable to most of the previous published data, we found association between presence of anti-RNAP III and renal involvement in form of SRC and arterial hypertension, so it is essential to perform frequent blood pressure controls and blood analysis. It is important to note that, as mentioned before, anti-RNAP patients are a group with an especially high prevalence of tendon friction rubs, contractures and arthritis, which exposes them to a possible greater use of glucocorticoids, so care must be taken given the known relationship between the use of glucocorticoids and SRC (45).

The correlation between anti-RNAP III and cancer is widely known, which has been confirmed in an large study including more than 2100 patients (14), which has also demonstrated a temporal relationship between the onset of SSc and cancer diagnosis (16, 47). Mutations in the polymerase III polypeptide A *(POLR3A)* gene in tumours from SSc patients were found in six of eight patients with anti-RNAP III antibodies suggesting that antigenic trigger is encoded by mutated genes located in tumour cells (48). Moreover, a recent study by the EUSTAR group proposed a cancer screening program in these patients (49). We detected a similar prevalence of cancer in anti-RNAP III group than previously reported (14, 46) but consistent with other studies recently published, we found no differences compared to those who did not express the antibody (40, 50).

In our present cohort, despite the fact of the long follow-up period, we did not observe differences among overall survival since the first SSc symptom including the RP or from the first clinical feature excluding RP, agreeing with a previous cohort from the north of Europe (51). Multivariate Cox survival analysis confirmed that a worse prognosis was related to developing a SRC, ILD with a FVC lower than 50% of expected or PAH diagnosed by RHC, nevertheless neither dcSSc, anti-RNAP III, anti-Scl 70 nor anti-centromere antibodies were related to a different outcome. To the best of our knowledge this is the first study that defines independent risk factors associated with worse survival in a south European cohort with anti-RNAP III determined.

Limitations of our study were different method of anti-RNAP III evaluation since we used ELISA and immunoblot assay, as we have the latter technique available since 2015. However, both techniques have a high sensitivity and specificity when compared with radioimmunoprecipitation method which is considered the gold standard (39, 52, 53). Some of the strengths were the study population was extremely homogenous; the data were collected from a single cohort of patients minimising the inter-observer variability of the clinical findings, the performance of a complementary test or even giving a similar interpretation of results and that we defined the risk factors associated with worse survival.

Conclusions

The anti-RNAP III antibody is included in the last ACR/EULAR classification criteria and this positivity may have diagnostic and prognostic implications

and can lead the clinician to take decisions to prevent certain conditions. To our knowledge this is the first study focusing on the prevalence of anti-RNAP III in the Spanish population and their clinical, epidemiological and microvascular characteristics as well as survival analysis. We found that the group anti-RNAP III had more prevalence of dcSSc, shorter diagnosis delay, less frequency of RP as the first SSc manifestation, and increased prevalence of SRC and arterial hypertension.

The analysis of anti-RNAP III antibody in SSc patients is useful, since its association with potentially serious manifestations is clear.

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