Cardiopulmonary Disease Development in Anti-RNA Polymerase III-positive Systemic Sclerosis: Comparative Analyses from an Unselected, Prospective Patient Cohort

Anna-Maria Hoffmann-Vold, Øyvind Midtvedt, Anders H. Tennøe, Torhild Garen, May Brit Lund, Trond M. Aaløkken, Arne K. Andreassen, Fadi Elhage, Cathrine Brunborg, Eli Taraldsrud, and Øyvind Molberg

ABSTRACT. Objective. Extensive skin disease and renal crisis are hallmarks of anti-RNA polymerase III (RNAP)-positive systemic sclerosis (SSc), while lung and heart involvement data are conflicting. Here, the aims were to perform time-course analyses of interstitial lung disease (ILD) and pulmonary hypertension (PH) in the RNAP subset of a prospective unselected SSc cohort and to use the other autoantibody subsets as comparators.

Methods. The study cohort included 279 patients with SSc from the observational Oslo University Hospital cohort with complete data on (1) SSc-related autoantibodies, (2) paired, serial analyses of lung function and fibrosis by computed tomography, and (3) PH verified by right heart catheterization.

Results. RNAP was positive in 33 patients (12%), 79% of which had diffuse cutaneous SSc. Pulmonary findings were heterogeneous; 49% had no signs of fibrosis while 18% had > 20% fibrosis at followup. Forced vital capacity at followup was < 80% in 39% of the RNAP subset, comparable to the antitopoisomerase subset (ATA; 47%), but higher than anticentromere (ACA; 13%). Accumulated frequency of PH in the RNAP subset (12%) was lower than in ACA (18%). At 93% and 78%, the 5- and 10-year survival rates in RNAP were comparable to the ATA and ACA subsets.

Conclusion. In this cohort, the RNAP subset was marked by cardiopulmonary heterogeneity, ranging from mild ILD to development of severe ILD in 18%, and PH development in 12%. These data indicate that cardiopulmonary risk stratification early in the disease course is particularly important in RNAP-positive SSc. (J Rheumatol First Release January 15 2017; doi:10.3899/jrheum.160867)

Key Indexing Terms:
SYSTEMIC SCLEROSIS AUTOANTIBODIES PULMONARY FIBROSIS
PULMONARY HYPERTENSION OUTCOME RESEARCH

Systemic sclerosis (SSc) is a complex, heterogeneous, and serious systemic connective tissue disease (CTD) characterized by progressive fibrosis of skin and internal organs, vasculopathy, and a range of autoantibodies[1,2]. There is no disease-modifying therapy available. Mortality is high and mainly driven by 2 cardiopulmonary disease components: pulmonary hypertension (PH) causing right-sided heart failure and progressive interstitial lung disease (ILD)[3,4].

Antinuclear antibodies (ANA) are a hallmark of SSc. They were first described in the disease in 1957, and are present in

From the Department of Rheumatology, the Department of Respiratory Medicine, the Department of Radiology and Nuclear Medicine, and the Department of Cardiology, Oslo University Hospital – Rikshospitalet; Institute of Clinical Medicine, University of Oslo; Institutes of Immunology, Oslo University Hospital; Oslo Centre for Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital, Oslo, Norway.

Supported by grants from the Norwegian Women's Public Health Association and the Norwegian Rheumatology Foundation.

A.M. Hoffmann-Vold, MD, PhD, Department of Rheumatology, Oslo University Hospital – Rikshospitalet, and Institute of Clinical Medicine, University of Oslo; Ø. Midtvedt, MD, Department of Rheumatology, Oslo University Hospital – Rikshospitalet; A.H. Tennøe, MD, Department of Rheumatology, Oslo University Hospital – Rikshospitalet, and Institute of Clinical Medicine, University of Oslo; T. Garen, MS, Department of Rheumatology, Oslo University Hospital – Rikshospitalet; M.B. Lund, MD, PhD, Professor, Institute of Clinical Medicine, University of Oslo, and Department of Respiratory Medicine, Oslo University Hospital – Rikshospitalet; T.M. Aaløkken, PhD, Department of Radiology and Nuclear Medicine, Oslo University Hospital – Rikshospitalet; A.K. Andreassen, PhD, Department of Cardiology, Oslo University Hospital – Rikshospitalet; F. Elhage, MD, Institutes of Immunology, Oslo University Hospital; C. Brunborg, MS, Oslo Centre for Biostatistics and Epidemiology, Research Support Services, Oslo University Hospital; E. Taraldsrud, MD, Professor, Department of Respiratory Medicine, Oslo University Hospital – Rikshospitalet; Ø. Molberg, MD, PhD, Professor, Department of Rheumatology, Oslo University Hospital – Rikshospitalet, and Institute of Clinical Medicine, University of Oslo.

Address correspondence to Dr. A.M. Hoffmann-Vold, Oslo University Hospital – Rikshospitalet, Pb 4950 Nydalen, 0424 Oslo, Norway. E-mail: a.m.hoffmann-vold@medisin.uio.no

Accepted for publication November 24, 2016.

at least 90% of the SSc population worldwide[5,6]. There are 3 dominating ANA specificities in SSc: antitopoisomerase I antibodies (ATA), anticentromere antibodies (ACA), and anti-RNA polymerase III antibodies (RNAP), all highly specific for SSc[6,7,8,9,10]. They are generally mutually exclusive, although different combinations of ATA, ACA, and RNAP were identified in individual patients with SSc[11,12]. Additionally, less frequent autoantibodies have been described. Some, such as antifibrillarin and anti-Th/To, seem specific for SSc, while others such as anti-Nor90 are not.

In general, autoantibodies are among the strongest predictors for clinical outcome in SSc[6,13,14,15], and several studies have shown that the different antibodies are associated with distinct phenotypes. ATA is related to diffuse cutaneous SSc (dcSSc) and the development of ILD and PH-ILD, while ACA is associated with limited cutaneous SSc (lcSSc) and the late development of pulmonary arterial hypertension (PAH)[9,16,17]. Studies on RNAP are less extensive, but have consistently shown associations to extensive skin disease, scleroderma renal crisis, and malignancies[9,18,19,20,21]. Data on RNAP and cardiopulmonary involvement have been conflicting[13,14,19,22]. While some studies have reported a lower frequency of ILD and PH in RNAP-positive patients[9,14,16,22], Nihtyanova and Denton described the predictive value of RNAP for PH development in multivariate analyses[13]. To the best of our knowledge, the associations between RNAP and development of ILD and PH have not been analyzed in a prospective SSc cohort with longitudinal, paired data on cardiopulmonary disease components. Recently, we established a large prospective SSc cohort that covers the whole spectrum of disease severities and has complete paired, serial data on lung fibrosis extent by high-resolution computer tomography (HRCT) and pulmonary function, as well as longitudinal data on echocardiography and right heart catheterizations (RHC) in suspect PH cases[23,24,25]. The aim of our present study was to examine the association between RNAP and the extent of lung fibrosis, pulmonary function, PH development, and survival in this well-characterized SSc cohort.

MATERIALS AND METHODS

Study cohort and clinical variables. At the Oslo University Hospital (OUH), all patients with SSc were included in an ongoing, prospective, observational SSc cohort. All the patients are followed by rheumatologists at the OUH, and clinical, laboratory, and imaging variables were systematically recorded in the Norwegian systemic CTD and vasculitis registry (NOSVAR) at the hospital[23,25]. Our current study cohort included all the patients with SSc from this unselected cohort that (1) met the 2013 European League Against Rheumatism/American College of Rheumatology classification criteria for SSc, (2) had cardiopulmonary data available from baseline and followup examinations, and (3) had complete data available on SSc-related serum antibodies[23,25,26]. Data on patient demographics, clinical data, and SSc subset were obtained from NOSVAR[27]. SSc subsets were defined as lcSSc and dcSSc[28].

Disease onset was defined as the first non-Raynaud symptom: the time from disease onset to study end (June 2016) or time of death was defined as disease duration. Using the Norwegian personal identification numbers, we identified every death in the SSc cohort during the observation period. Malignancies were registered as ever present and no information was available about the time of diagnosis. The study was approved by the regional committee of health and medical research ethics in Southeast Norway (No. 2009/1035).

Serial assessment of SSc-ILD. Paired pulmonary function test (PFT) and HRCT lung images were obtained both at baseline and at the last available followup visit. Extent of fibrosis on baseline and followup lung HRCT was measured as previously described[23]. Briefly, CT images were reconstructed at 1.25-mm section thickness in 10-mm intervals and reviewed in a blinded fashion. Reticular pattern abnormalities and super-imposed ground-glass opacities were defined as equivalent to fibrosis[29] and measured precisely by freehand drawing of the region of interest. Fibrosis was expressed as percentage of total lung volumes at baseline and at followup. Annual fibrosis progression rate was defined as the difference in extent of fibrosis between the baseline and followup HRCT divided by the actual followup period in years. PFT were performed within 1 month of the corresponding HRCT and carried out according to the American Thoracic Society/European Respiratory Society guidelines using automated Vmax V6200 (SensorMedics)[30]. Recorded PFT variables were forced vital capacity (FVC) and forced expiratory volume in 1 s. Gas diffusing variables were the transfer factor for carbon monoxide (DLCO) and DLCO divided by alveolar volume. PFT values were expressed in absolute values and as percentage of predicted values[31].

Serial assessment of PH development. Paired N-terminal pro-brain natriuretic peptide (NT-proBNP) data and systolic pulmonary artery pressure (sPAP) measured by echocardiography (ECHO) were obtained both at baseline and at the last available followup visit, and were available for all patients. All cases with suspect PH underwent RHC. Suspect PH was defined as a systolic pressure of > 40 mmHg on ECHO, increasing or unexplained dyspnea, or significant decline in DLCO%, or 6-min walking distance. PH was diagnosed according to the updated European Society of Cardiology guidelines with a mean pulmonary arterial pressure ≥ 25 mmHg measured by RHC, further divided into pre- and postcapillary PH according to a pulmonary wedge pressure ≤ 15 mmHg or > 15 mmHg, respectively[32]. Diagnosis was made by an experienced cardiologist. PAH was defined as the presence of precapillary PH and < 20% fibrosis on HRCT and FVC% predicted > 70% at both baseline and followup, with other causes of precapillary PH ruled out[33]. PH-ILD consisted of patients with precapillary PH combined with findings of fibrosis > 20% and/or FVC < 70%. Angina pectoris and myocardial infarction was registered if ever present.

ANA screening. Sera samples were taken at baseline from all included patients with SSc. ANA were detected by indirect immunofluorescence (IIF) and ELISA. ACA and ATA were detected by automated ELISA (EliA, Phadia) or by immune blot (SSc blot, Euroimmun). RNAP, antifibrillarin, anti-Th/to, anti-RNP, and PmScl75/100 were detected by immune blot (SSc blot, Euroimmun). The immune blot was considered as positive when ++ and above. For descriptive purposes, we grouped the cases with fibrillarin, Th/To, PmScl75/100, RNP, and ANA (ANA IIF) without defined molecular specificity as "other antibodies" (AA). Autoantibody-negative patients (no antibodies) were negative for both ANA IIF and ANA ELISA.

Statistical analyses. Analyses were performed by IBM SPSS version 22 and STATA version 14. Cox regression analyses with HR with its 95% CI were applied to analyze cardiopulmonary outcome and survival analyses. Independent risk factors from univariate analyses, at a significance level of 20%, were included in the multivariate Cox proportional hazard regression analysis. A manual backward stepwise elimination procedure was performed to identify independent risk factors for outcome measures. Multivariate analyses were preceded by estimation of correlation between risk factors. Global tests (ANOVA) were conducted for a combined antibody variable before separate comparisons between single antibody subgroups were performed. Pearson chi-square test, Fisher's exact test, or independent sample Student t test were used as appropriate. Cumulative survival rates were computed by the Kaplan-Meier method and significance was tested

with the log-rank test. Items with significant effects on survival were entered into the Cox proportional hazards model.

RESULTS

RNAP and demographic and clinical variables. The SSc study cohort included 279 of 305 eligible patients with SSc. The 26 patients excluded because of missing data did not differ significantly from the included patients with respect to age, sex distribution, and clinical characteristics. ACA was the most frequent serum antibody detected, with 134 patients (48%) being positive, while ATA was detected in 46 (16%) and RNAP in 33 (12%; Table 1). The remaining 66 patients had either anti-RNP (n = 15), antifibrillarin antibodies (n = 6), anti-Th/To (n = 10), anti-PmScl75/100 (n = 4), ANA alone (n = 20), or no autoantibodies (n = 11). Six patients with SSc were positive for multiple antibodies, 1 for RNAP and ATA, 3 for RNAP and ACA, and 2 for ACA and ATA. These patients were grouped with the strongest positive reaction; RNAP and ATA, and RNAP and ACA in the RNAP subgroup and the patient with ATA and ACA in the ATA subgroup. In the total cohort, 95% were white. Mean age at disease onset was 49 years (SD 15.3), total observation period was 11.5 years (SD 7.6), and 59 patients (21%) were men (Table 1). The mean time from serum sampling to the clinical followup visit was 3 years (SD 2.3). Patients with RNAP were more frequently classified as dcSSc than the ACA and ATA subsets; they had higher modified Rodnan Skin Score (mRSS) at baseline and more frequently developed scleroderma renal crisis and gastric antral vascular ectasia (GAVE) throughout the followup period (Table 1). There were no significant associations between RNAP and malignancies, digital ulcers, or other gastrointestinal involvement. Notably, there were no data available about the date of diagnosis in malignancies.

RNAP and ILD, lung fibrosis, and lung function. The ILD findings in RNAP-positive patients were heterogeneous; 49% of the patients did not develop any signs of fibrosis across the observation period, while 33% had 1%–20% fibrosis and 18% showed > 20% fibrosis at followup (Figure 1; and Supplementary Table 1, available with the online version of this article). The relative frequency of patients progressing to > 20% fibrosis at followup from < 20% at baseline was higher in the RNAP subset than in the other autoantibody subsets (Figure 1). Separate analyses of exclusively patients with lung fibrosis present at baseline (n = 104) showed that the RNAP subset had a mean 8.9% (SD 8.5) extent of fibrosis at baseline and 14.9% (SD 10.2) fibrosis at followup. This was significantly less than patients with ATA (baseline fibrosis 22.3%, SD 20.2, p = 0.002, and followup fibrosis 25.5%, SD 20.0, p = 0.020), but comparable with patients with ACA at baseline (baseline fibrosis 7.5%, SD 6.7, p = 0.575) and significantly more than at followup (9.2%, SD 6.6, p = 0.05). The annual fibrosis progression rate was higher in RNAP (1.2%, SD 2.0) compared with ATA (0.5%, SD 5.0, p = 0.535) and ACA (0.7%, SD 1.4, p = 0.437), but did not reach statistical significance (Supplementary Table 1, available with the online version of this article).

There were no significant differences between RNAP- and ATA-positive patients regarding any FVC variables, neither at baseline nor at followup (Figure 2; and Supplementary Table 2, available with the online version of this article). FVC% predicted was significantly lower in RNAP-positive patients compared with ACA at baseline (88.2% and 98.4%,

Table 1. Comparison of ever present clinical characteristics between SSc patient groups stratified by antibody status. Values are n (%) unless otherwise specified.

Characteristics	Total Cohort	RNAP	ACA	ATA	Other Ab	No Ab	p[a]	p[b]
No. patients	279	33 (12)	134 (48)	46 (16)	55 (20)	11 (4)		
Age at disease onset, yrs, mean (SD)	49 (15.3)	46 (13.6)	52 (14.1)	46 (16.1)	45 (17.4)	52 (15.0)	0.909	0.050
Disease duration at baseline[c], yrs, mean (SD)	5.5 (6.8)	3.3 (3.4)	5.2 (6.7)	6.2 (7.4)	6.3 (7.3)	7.6 (8.8)	0.027	0.120
Disease duration at followup[c], yrs, mean (SD)	9.9 (7.5)	8.2 (5.1)	9.5 (7.3)	10.4 (8.8)	11.1 (8.4)	11.5 (10.4)	0.155	0.335
Disease duration < 3 yrs at baseline	147 (53)	20 (65)	70 (60)	24 (56)	28 (51)	6 (55)	0.470	0.617
Males	59 (21)	7 (21)	24 (18)	13 (28)	12 (22)	3 (27)	0.477	0.662
Dead	66 (24)	6 (18)	24 (18)	14 (30)	13 (24)	9 (82)	0.217	0.971
Diffuse cutaneous SSc	76 (21)	26 (79)	8 (16)	24 (52)	13 (24)	5 (46)	0.016	< 0.001
Modified Rodnan skin score, mean (SD)[d]	9.0 (8.8)	18.0 (11.9)	5.9 (6.8)	10.8 (7.7)	8.1 (6.1)	16.8 (12.3)	0.002	< 0.001
Renal crisis	10 (4)	9 (27)	0 (0)	0 (0)	1 (2)	0 (0)	< 0.001	< 0.001
Digital ulcers	123 (44)	15 (46)	58 (43)	22 (48)	25 (46)	3 (27)	0.096	0.650
GAVE	18 (7)	5 (16)	8 (6)	1 (2)	4 (7)	1 (9)	0.036	0.068
Esophagus dysmotility	202 (72)	27 (82)	96 (72)	30 (65)	42 (76)	7 (64)	0.069	0.624
Dysphagia	137 (49)	17 (52)	66 (49)	16 (35)	32 (58)	6 (55)	0.132	0.799
GERD	138 (50)	19 (58)	61 (46)	25 (54)	28 (51)	5 (46)	0.785	0.413
Malignancies	49 (18)	7 (23)	24 (19)	7 (16)	9 (16)	2 (18)	0.495	0.688

[a] p values between RNAP and ATA. [b] p values between RNAP and ACA. [c] Time from onset of first non-Raynaud symptom to baseline or followup consultation. [d] Estimated at baseline. SSc: systemic sclerosis; RNAP: RNA polymerase III; ACA: anticentromere antibody; ATA: antitopoisomerase I; Ab: antibody; GAVE: gastric antral vascular ectasia; GERD: gastroesophageal reflux disease.

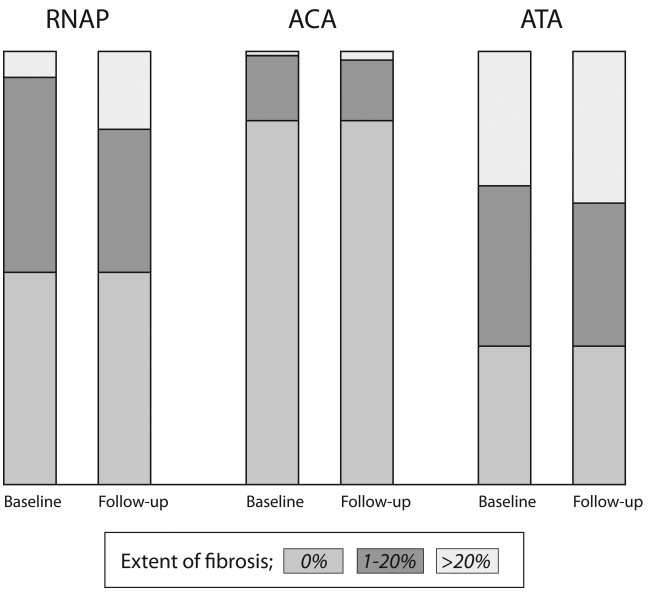

Figure 1. Extent of lung fibrosis by HRCT at baseline and followup in the RNAP-, ACA-, and ATA-positive patient subsets. The bars represent the frequency of patients in each subset with 0%, 1–20%, and > 20% of fibrosis at the baseline and followup HRCT examinations. HRCT: high-resolution computed tomography; RNAP: RNA polymerase III; ACA: anticentromere antibody; ATA: antitopoisomerase I.

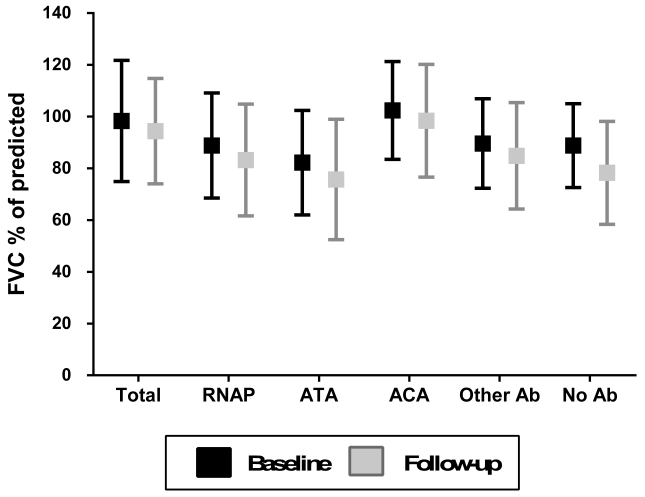

Figure 2. FVC% at baseline and followup segregated by antibody subsets. Values are presented as mean FVC% predicted with SD. FVC: forced vital capacity; RNAP: RNA polymerase III; ATA: antitopoisomerase I; ACA: anticentromere antibody; Ab: antibody.

p < 0.001) and at followup (83.2% and 102.4%, p < 0.001; Figure 2). There were no significant differences in any DLCO variables between the antibody subgroups (Supplementary Table 2, available with the online version of this article).

In univariate Cox analyses, RNAP was associated with fibrosis progression from < 20% at baseline to > 20% at followup, while ACA and ATA were negatively and positively associated with fibrosis > 20% and FVC < 70% at followup (Table 2). Because of low numbers, no multivariate analyses were performed.

RNAP and PH. In the OUH cohort, 51/279 patients (18%) developed PH during the observation period; 33 (12%) of these were defined as PAH and the remaining 18 had PH-ILD. Total frequency of PH in the RNAP subset was 4/33 (12%), with PAH in 1/33 (3%; Table 3). The PAH frequency in RNAP was lower than in ACA (18%, p = 0.025). The different antibody subgroups had comparable baseline and followup values of estimated sPAP by ECHO and serum NT-proBNP (Table 3). ACA was in univariate analyses significantly associated with PAH development (HR 2.8, 95% CI 1.24–6.36, p = 0.014), but there was no significant association in multivariate analyses. Neither ACA nor any of the other autoantibody specificities were associated with PH development in univariate Cox regression analyses. Factors associated with PH were in multivariate analyses baseline sPAP on ECHO (HR 1.03, 95% CI 1.02–1.04, p < 0.001), baseline DLCO (HR 0.95, 95% CI 0.93–0.97, p < 0.001), and age at SSc onset (HR 1.06, 95% CI 1.04–1.09, p < 0.001).

RNAP and mortality. Five-year and 10-year cumulative survival rates in the RNAP subset were 93% and 78%. These rates were comparable to the rates in the ACA subset (94% and 85%) and in the ATA subgroup (91% and 82%, p = 0.537; Supplementary Figure 1, available with the online version of this article).

In univariate and multivariate Cox proportional hazard analyses, none of the antibodies showed an association with mortality. In multivariate Cox proportional hazard analyses, these factors were associated with mortality: age at onset (HR 1.1, 95% CI 1.08–1.14, p < 0.001), mRSS (HR 1.04, 95% CI 1.02–1.07, p = 0.002), sPAP on ECHO at baseline (HR 1.01, 95% CI 1.01–1.02, p < 0.001), FVC% at baseline (HR 0.98, 95% CI 0.97–0.99, p = 0.024), and DLCO% at baseline (HR 0.97, 95% CI 0.96–0.99, p = 0.004).

DISCUSSION

Autoantibodies targeting RNAP are associated with the development of extensive skin disease and scleroderma renal crisis, but it is not clear whether these antibodies may predict specific cardiopulmonary outcomes. Here, we examined the frequency and progression rate of ILD and the development of PH in the RNAP-positive subset of a prospective SSc cohort. We found that the RNAP subset was heterogeneous, and could be divided into 3 ILD categories: (1) no signs of ILD (50% of the subset), (2) moderate and slowly progressive ILD (around 30%), and (3) severe and progressive ILD (nearly 20%). With 12%, the frequency of PH in RNAP was comparable to ATA, but lower than ACA.

Previous studies on RNAP and ILD were cross-sectional, partly based on chest radiographs and did not quantify lung fibrosis, and the results were inconsistent. Steen found that severe pulmonary fibrosis was rare in RNAP[9] while other studies have reported total frequencies of pulmonary fibrosis

Table 2. Univariate Cox analyses for pulmonary outcomes at followup with different autoantibodies in the systemic sclerosis cohort.

Antibodies	Fibrosis at Followup > 20%, HR (95% CI)	p	Fibrosis Progression from < 20% to > 20%, HR (95% CI)	p	FVC at Followup < 70%, HR (95% CI)	p
Antipolymerase III	1.7 (0.72–4.17)	0.223	4.4 (1.29–15.12)	0.018	1.9 (0.87–4.03)	0.109
Antitopoisomerase	3.5 (1.83–6.77)	< 0.001	1.1 (0.24–5.16)	0.889	2.3 (1.24–4.12)	0.008
Anticentromere	0.1 (0.02–0.27)	< 0.001	0.1 (0.01–0.85)	0.034	0.3 (0.13–0.51)	< 0.001

FVC: forced vital capacity.

Table 3. Comparison of PH subgroups, pulmonary artery pressures, and NT-proBNP in systemic sclerosis groups stratified by antibody status. sPAP and NT-proBNP levels were available for all patients. Values are n (%) unless otherwise specified.

No. Patients	Total Cohort, n = 279	RNAP, n = 33 (12%)	ACA, n = 134 (48%)	ATA, n = 46 (16%)	Other Ab, n = 55 (20%)	No Ab, n = 11 (4%)	p*	p**
Time from onset to PH, yrs, mean (SD)	8.5 (7.9)	7.6 (10.0)	8.3 (9.2)	11.8 (7.6)	7.4 (5.0)	8.3 (3.9)	0.413	0.886
PH	51 (18)	4 (12)	28 (21)	5 (11)	11 (20)	3 (27.3)	0.796	0.105
PAH	33 (12)	1 (3)	24 (18)	1 (2)	6 (11)	1 (9)	0.722	0.025
PH-ILD	18 (6)	3 (9)	4 (3)	4 (9)	5 (9)	2 (18)	0.858	0.025
mPAP at PH diagnosis, mmHg, mean (SD)	29.9 (11.9)	25.3 (10.1)	30.7 (12.4)	26.0 (7.5)	32.8 (12.7)	39.8 (10.8)	0.485	0.064
Baseline sPAP, mmHg, mean (SD)	27.7 (19.8)	25 (14.8)	28.8 (20.1)	24.0 (9.3)	28.5 (25.8)	37.2 (19.4)	0.725	0.333
Followup sPAP, mmHg, mean (SD)	34.9 (23.7)	30.4 (17.7)	32.1 (16.4)	36.6 (26.4)	34.5 (24)	46.4 (31.1)	0.564	0.684
Baseline NT-ProBNP, pmol/l, mean (SD)	78.2 (321.5)	25.7 (80.1)	59.9 (198.9)	31.6 (50.1)	58.9 (134.8)	53.7 (49.2)	0.134	0.191
Followup NT-ProBNP, pmol/l, mean (SD)	218.7 (606.8)	287.6 (780.1)	221.7 (701.0)	106.9 (606.1)	189.4 (412.9)	298.4 (485.9)	0.807	0.713
Myocardial infarction	24 (9)	1 (2)	12 (9)	3 (9)	5 (9)	3 (27)	0.222	0.636
Angina pectoris	23 (8)	0 (0)	14 (11)	3 (7)	5 (9)	1 (9)	0.183	0.038

* p values between RNAP and ATA. ** p values between RNAP and ACA. PH: pulmonary hypertension; NT-proBNP: N-terminal pro-brain natriuretic peptide; sPAP: systolic pulmonary artery pressure on echocardiogram; RNAP: RNA polymerase III; ACA: anticentromere antibody; ATA: antitopoisomerase I; Ab: antibody; PAH: pulmonary arterial hypertension; ILD: interstitial lung disease; mPAP: mean pulmonary artery pressure by right heart catheterization, data shown from PH patients.

in RNAP ranging from 17%–41.5%[10,14,16,22]. Consistent with our data, Airo, *et al* found significantly more ILD in RNAP-positive patients compared with ACA[19].

At followup, 18% of the RNAP subset had severe lung fibrosis > 20%. Interestingly, a subgroup of these patients had a very high annual fibrosis progression rate. A possible explanation might be that RNAP patients are evaluated very early in their disease course because of the aggressive skin involvement. Hence, we speculate that the RNAP patients with aggressive skin disease and lung involvement often are identified at a disease stage where they have very active and progressive lung inflammation. Overall, we have observed that the baseline HRCT and PFT evaluations divide the RNAP-positive patients into 2 subsets, 1 with normal pulmonary findings and a very low risk for ILD development, and 1 subset with substantial risk for progressive ILD. The latter subset should probably be followed by tight controls with early institution of targeted ILD therapies in the patients that seem to progress.

There are some studies assessing PH in RNAP. Most of these studies did not find any associations between RNAP and PH[9,14,16,19,22,34], but only few of them had RHC-verified PH[8,13,34]. However, Nihtyanova and Denton reported that RNAP was associated with increased risk of RHC-verified PH in multivariate Cox regression analyses[13]. In our cohort, we did not observe any significant associations between RNAP and PH development. Nonetheless, we found that the accumulated frequencies of PH (12%) and PAH (3%) in the RNAP subset were comparable to previous studies, emphasizing that PH surveillance is an important issue in these patients[8,14,18,19].

Studies regarding survival segregated by antibody status have given varying results[9,13,14,35,36]. In our present cohort, survival was decreased, but we did not find any significant differences between the antibody subgroups and none of the antibodies predicted survival. This is in line with a previous Swedish report from Hesselstrand, *et al*[16] and might support the concept of regional differences in SSc characteristics because of genetic backgrounds and environmental differences.

Previous studies have shown varying RNAP prevalence.

Sobanski, *et al*[37] published a metaanalysis showing a pooled RNAP prevalence of 11% with a large variation of 0–41% worldwide. Our results with an RNAP prevalence of 12% support these findings. We also support reported associations between RNAP and dcSSc, scleroderma renal crisis, and GAVE[9,18,38]. It is unclear why RNAP is significantly associated with vasculopathic changes such as scleroderma renal crisis and GAVE, but not with PAH. One might speculate that the pathogenesis of vascular complications differs between the different organ systems in SSc, but mechanistic studies are needed to address this issue. Malignancy is not associated with RNAP in our study. However, there is a lack of information regarding the timepoint of cancer diagnosis, making this result difficult to interpret.

Our current study has major strengths. First and probably most important is that it is based on longitudinal and complete data, including HRCT-verified extent of lung fibrosis, lung function, ECHO results, clinical data, and RHC-verified PH in a largely unselected SSc cohort. Second, the study population is extremely homogenous with 95% genetic Norwegian whites. Third, because of the unique identification numbers in Norway and the official mortality statistics, no patients with serial assessments were "lost to followup" at the end of study.

There are some limitations. Most important are the inter-individual variations in disease duration and the variation in observational length. This is because of the OUH SSc study design where patients are included consecutively and then followed annually. With our study design, survival bias is also an unavoidable limitation. Finally, since the study population is mainly white and genetically very homogenous, the results may not be transferable to other ethnic groups.

The results from our prospective SSc cohort demonstrate that the RNAP subset is marked by cardiopulmonary heterogeneity with progression to extensive ILD and PH development in a subgroup. Our data indicate that cardiopulmonary risk stratification early in the disease course is particularly important in RNAP-positive SSc.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

REFERENCES

1. Pattanaik D, Brown M, Postlethwaite BC, Postlethwaite AE. Pathogenesis of systemic sclerosis. Front Immunol 2015;6:272.
2. Allanore YA, Denton C, Matucci-Cerinic, M. Systemic sclerosis. In: Bijlsma JWJ, editor. EULAR textbook on rheumatic diseases, 1st Edition. London: BMJ Group; 2012:546-67.
3. Rubio-Rivas M, Royo C, Simeón CP, Corbella X, Fonollosa V. Mortality and survival in systemic sclerosis: systematic review and meta-analysis. Semin Arthritis Rheum 2014;44:208-19.
4. Hoffmann-Vold AM, Molberg Ø, Midtvedt Ø, Garen T, Gran JT. Survival and causes of death in an unselected and complete cohort of Norwegian patients with systemic sclerosis. J Rheumatol 2013;40:1127-33.
5. Salazar GA, Assassi S, Wigley F, Hummers L, Varga J, Hinchcliff M, et al. Antinuclear antibody-negative systemic sclerosis. Semin Arthritis Rheum 2015;44:680-6.
6. Domsic RT. Scleroderma: the role of serum autoantibodies in defining specific clinical phenotypes and organ system involvement. Curr Opin Rheumatol 2014;26:646-52.
7. Mierau R, Moinzadeh P, Riemekasten G, Melchers I, Meurer M, Reichenberger F, et al. Frequency of disease-associated and other nuclear autoantibodies in patients of the German Network for Systemic Scleroderma: correlation with characteristic clinical features. Arthritis Res Ther 2011;13:R172.
8. Patterson KA, Roberts-Thomson PJ, Lester S, Tan JA, Hakendorf P, Rischmueller M, et al. Interpretation of an extended autoantibody profile in a well-characterized Australian systemic sclerosis (scleroderma) cohort using principal components analysis. Arthritis Rheumatol 2015;67:3234-44.
9. Steen VD. Autoantibodies in systemic sclerosis. Semin Arthritis Rheum 2005;35:35-42.
10. Meyer OC, Fertig N, Lucas M, Somogyi N, Medsger TA Jr. Disease subsets, antinuclear antibody profile, and clinical features in 127 French and 247 US adult patients with systemic sclerosis. J Rheumatol 2007;34:104-9.
11. 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.
12. Kayser C, Fritzler MJ. Autoantibodies in systemic sclerosis: unanswered questions. Front Immunol 2015;6:167.
13. Nihtyanova SI, Denton CP. Autoantibodies as predictive tools in systemic sclerosis. Nat Rev Rheumatol 2010;6:112-6.
14. Hamaguchi Y, Hasegawa M, Fujimoto M, Matsushita T, Komura K, Kaji K, et al. The clinical relevance of serum antinuclear antibodies in Japanese patients with systemic sclerosis. Br J Dermatol 2008;158:487-95.
15. 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.
16. Hesselstrand R, Scheja A, Shen GQ, Wiik A, Akesson A. The association of antinuclear antibodies with organ involvement and survival in systemic sclerosis. Rheumatology 2003;42:534-40.
17. Jacobsen S, Halberg P, Ullman S, Van Venrooij WJ, Høier-Madsen M, Wiik A, et al. Clinical features and serum antinuclear antibodies in 230 Danish patients with systemic sclerosis. Br J Rheumatol 1998;37:39-45.
18. 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.
19. Airo 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.
20. 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.
21. 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.
22. 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.

23. Hoffmann-Vold AM, Aaløkken TM, Lund MB, Garen T, Midtvedt Ø, Brunborg C, et al. Predictive value of serial high-resolution computed tomography analyses and concurrent lung function tests in systemic sclerosis. Arthritis Rheumatol 2015;67:2205-12.
24. Hoffmann-Vold AM, Tennøe AH, Garen T, Midtvedt Ø, Abraityte A, Aaløkken TM, et al. High level of chemokine ccl18 is associated with pulmonary function deterioration, lung fibrosis progression, and reduced survival in systemic sclerosis. Chest 2016;150:299-306.
25. Hoffmann-Vold AM, Gunnarsson R, Garen T, Midtvedt Ø, Molberg Ø. Performance of the 2013 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Systemic Sclerosis (SSc) in large, well-defined cohorts of SSc and mixed connective tissue disease. J Rheumatol 2015;42:60-3.
26. 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.
27. Hoffmann-Vold AM, Midtvedt Ø, Molberg Ø, Garen T, Gran JT. Prevalence of systemic sclerosis in south-east Norway. Rheumatology 2012;51:1600-5.
28. 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.
29. Hansell DM, Bankier AA, MacMahon H, McLoud TC, Muller NL, Remy J. Fleischner Society: glossary of terms for thoracic imaging. Radiology 2008;246:697-722.
30. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:5-40.
31. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;40:1324-43.
32. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Heart J 2016;37:67-119.
33. Goh NS, Desai SR, Veeraraghavan S, Hansell DM, Copley SJ, Maher TM, et al. Interstitial lung disease in systemic sclerosis: a simple staging system. Am J Respir Crit Care Med 2008; 177:1248-54.
34. Meyer O, De Chaisemartin L, Nicaise-Roland P, Cabane J, Tubach F, Dieude P, et al. Anti-RNA polymerase III antibody prevalence and associated clinical manifestations in a large series of French patients with systemic sclerosis: a cross-sectional study. J Rheumatol 2010;37:125-30.
35. Strickland G, Pauling J, Cavill C, Shaddick G, McHugh N. Mortality in systemic sclerosis-a single centre study from the UK. Clin Rheumatol 2013;32:1533-9.
36. Jacobsen S, Ullman S, Shen GQ, Wiik A, Halberg P. Influence of clinical features, serum antinuclear antibodies, and lung function on survival of patients with systemic sclerosis. J Rheumatol 2001;28:2454-9.
37. 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.
38. Santiago M, Baron M, Hudson M, Burlingame RW, Fritzler MJ. Antibodies to RNA polymerase III in systemic sclerosis detected by ELISA. J Rheumatol 2007;34:1528-34.