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ORIGINAL RESEARCH

Impact of autoantibody status on stratifying the risk of organ involvement and mortality in SSc: experience from a multicentre French cohort of 1605 patients

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ABSTRACT

Introduction Systemic sclerosis (SSc) is a rare autoimmune disease currently classified into two subgroups based on skin extension. The aim of this study was to determine in a large cohort whether the determination of autoantibody (AAb) profile among a full antinuclear AAbs panel including nine specificities had a higher impact than skin phenotype on stratifying the risk of organ involvement and mortality in SSc.

Methods Data for patients with SSc followed in seven French university hospitals were retrospectively analysed in terms of skin phenotype, AAbs (anti-topoisomerase I (ATA), anticentromere (ACA), anti-RNA polymerase III (anti-RNAPIII), anti-U1RNP, anti-U3RNP, anti-Pm/Scl, anti-Ku, anti-Th/To, anti-NOR90), organ involvement and mortality. Multivariate analyses were performed to identify independent factors associated with organ involvement and mortality.

Results We included 1605 patients with SSc (367 with diffuse cutaneous SSc). On multivariate analysis, ATAs were associated with interstitial lung disease and mortality (OR=3.27 (95% CI 2.42 to 4.42); HR=1.9 (95% CI 1.01 to 3.58)), anti-RNAPIII with scleroderma renal crisis and mortality (OR=7.05 (95% CI 2.98 to 16.72); HR=2.35 (95% CI 1.12 to 4.93)), anti-U1RNP with arthritis (OR=3.79 (95% CI 2.16 to 6.67)), anti-Pm/Scl and anti-Ku with myositis (OR=7.09 (95% CI 3.87 to 12.98) and 7.99 (95% CI 2.41 to 26.46)). The skin phenotype was not associated with survival or organ involvement on multivariate analysis without stepwise selection.

Conclusion This study unravels, by contrast with skin phenotype, a strong association between AAbs specificities, organ involvement and outcome in SSc and suggests that patients' classification based on only skin extension is not sufficient for defining prognosis and phenotype.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Historically, classification of systemic sclerosis (SSc), based on skin phenotype (diffuse or limited cutaneous SSc), has been used to predict prognosis but with a lack of precision, leading to growing interest in the weight of autoantibodies (AAbs) status.

WHAT THIS STUDY ADDS

⇒ Our study highlighted strong independent associations between each AAb and internal organ involvement in this disease. By contrast, the skin phenotype was not an independent factor associated with organ involvement and mortality in SSc.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our results suggests that patients' classification based on only skin extension is not sufficient for defining prognosis and phenotype. Consequently, the systematic and accurate determination of antinuclear AAb specificities at diagnosis could help clinicians to better stratify the individual risk of developing SSc complications and personalise monitoring.

INTRODUCTION

Systemic sclerosis (SSc) is a rare connective tissue disease characterised by skin and internal organ fibrosis, vasculopathy and autoimmunity. Despite recent improvements in understanding its pathogenesis, which offers new therapeutic opportunities, the mortality rate remains high in a lot of patients, with no existing curative treatment.[1 2] Several organs can be damaged during the disease course and lead to life-threatening involvements such as

interstitial lung disease (ILD), pulmonary arterial hypertension (PAH) and scleroderma renal crisis (SRC).[2 3] The frequency of such complications varies highly among patients with SSc and is difficult to predict individually. Patients are classified as having diffuse cutaneous SSc (dcSSc) or limited cutaneous SSc (lcSSc) depending on the extent of skin fibrosis.[4] Usually, patients with dcSSc are more likely to develop ILD and SRC, whereas PAH is commonly associated with lcSSc.[5] The mortality rate differs depending on the skin phenotype, with a higher rate in dcSSc than lcSSc.[2 3] Nevertheless, prediction of organ involvement and prognosis based on only skin phenotype is insufficient because ILD is also observed in patients with lcSSc and PAH can occur in patients with dcSSc.[6–9] Moreover, skin fibrosis does not develop in some patients. Consequently, additional and early markers are needed to better stratify the individual risk of severe complications and to personalise the screening of organ involvement and disease management.

A main feature that characterises the SSc pathogenesis is the breach of self-tolerance towards various nuclear antigens.[5] Antinuclear antibodies (ANA) represent a heterogeneous family of autoantibodies (AAbs) whose antigen targets are not yet fully determined. SSc AAbs display some remarkable features including early appearance (generally before clinical symptoms[10]), and mutual exclusion (only a single specific nuclear AAb is found in the serum of each patient with SSc, except in rare cases[11]). Besides the three well-known AAbs—antitopoisomerase I antibody (ATA), also called anti-Scl70, anticentromere antibody (ACA) and anti-RNA polymerase III AAbs (anti-RNAPIII)—a dozen of identified AAbs are associated with SSc.[12]

Most previous studies examining only three AAbs (ATA, ACA, anti-RNAPIII) in large cohorts reported associations between AAb subtype, SSc phenotype and prognosis.[5 6] Thus, ATA was found associated with dcSSc and ILD, ACA with lcSSc and anti-RNAPIII antibody with dcSSc and SRC.[13] Three years ago, an innovative study focused on the effect of five AAbs on the timing of organ complication development and disease prognosis in a large but single-centre cohort of patients with SSc and highlighted significant associations between AAb subtype and cumulative incidence of organ complications.[14] Taken together, the five analysed AAbs (ATA, ACA, anti-RNAPIII, anti-U3RNP, anti-Pm/Scl AAbs) are found in 50%–80% of patients with SSc. Consequently, studies including a larger panel of SSc-associated AAbs are needed.

Techniques to test patients' sera for nine SSc-associated AAbs are now available in several centres. This development raises opportunities for clinicians to more accurately determine AAb specificities in patients with SSc. Thus, we conducted a multicentre study to analyse the associations between organ complication development, outcome and nine SSc-associated AAbs (ATA, ACA, anti-RNAPIII, anti-U1RNP, anti-U3RNP, anti-Pm/Scl, anti-Ku, anti-Th/To, anti-NOR90 antibodies). We hypothesised that a precise and early AAb identification in each patient could help to better and early stratify the individual risk of organ involvement and mortality.

PATIENTS AND METHODS

Study design and population

We included patients with SSc over age 18, at the time of the inclusion, who were followed up to 2019 in seven French university hospital centres and fulfilled the inclusion criteria and no exclusion criteria as detailed below.[15] SSc diagnosis has been made clinically, but for inclusion, patients had to fulfil the ACR/EULAR classification criteria. Patients with antisynthetase syndrome, or other diseases that may mimic SSc complications were excluded. Patients who were lost to follow-up for more than 3 years were included only if they were screened again for internal organ involvement after resumption of medical follow-up. Eligible patients had to have heart sonography, a pulmonary function test and at least one high-resolution CT (HRCT) scan during follow-up, either annually or more frequently. We excluded all patients without any follow-up within the last 3 years before the beginning of the study. In each of the selected centres, a patient register was available with filling procedures and consensual definitions of organ complications. A clinical report form (CRF) has been set up for this study. The CRF was completed using centre registers and patient medical files by the same person (KD) in all centres. The following data were collected: age at disease onset (defined as the age at which the first non-Raynaud's phenomenon symptom appeared), disease duration, sex, skin phenotype, organ involvement during follow-up (PAH, ILD, SRC, digital ulcer (DU), arthritis, myositis, intestinal pseudo-obstruction (IPO)), presence (or absence) of ANA and the specific AAb (ATA, ACA, anti-RNAPIII, anti-U1RNP, anti-U3RNP, anti-Pm/Scl, anti-Ku, anti-Th/To, anti-NOR90) or none of these nine AAbs.

Patients were classified as having dcSSc or lcSSc according to the LeRoy and Medsger classification.[4] Patients with SSc *sine scleroderma* were classified as having lcSSc. ILD was defined as the presence of at least one usual sign of SSc-associated ILD on HRCT, defined by the identification of fibrotic features on chest HRCT generally most pronounced in the lung bases (commonly non-specific interstitial pneumonia[16]). PAH was diagnosed based on right-heart catheterisation according to diagnostic criteria at the time of inclusion (mean pulmonary arterial pressure ≥25 mm Hg and pulmonary capillary wedge pressure ≤15 mm Hg in a patient with no ILD or ILD with forced vital capacity % predicted ≥70% and extent of ILD on HRCT ≤20%). SRC was defined as the abrupt onset of severe hypertension and/or decline in renal function, with proteinuria but without an alternative aetiology and/or microangiopathy visualised on a renal biopsy. Myositis was diagnosed with muscle weakness associated with increased creatine phosphokinase level or abnormal findings on electromyography, muscle

MRI or muscle biopsy. Arthritis was defined as inflammatory pain and/or the presence of synovitis. IPO was defined as a clinical and/or radiological appearance of intestinal obstruction without a clearly defined ischaemic, mechanical or postsurgical cause. All deaths (related or not to SSc) during follow-up were collected.

Ethical considerations

The database was constituted in accordance with the reference methodology MR004 of the Commission Nationale de l'Informatique et des Libertés (no. 2206749, 13 September 2018). As such, non-opposition was obtained from each patient included in the study for the use of their deidentified medical record data, and data management complied with current French legislation governing nominative personal data, the General Data Protection Regulation of the European Union and the French legislation pertaining to informatics and liberties of 6 January 1978 and its modification in 2018.

Laboratory testing

At least one serum sample from each patient was analysed for AAbs in the referral centre for the patient. A first screening test was performed to detect ANA on HEp-2 cells by indirect immunofluorescence: titers of at least 1:160 or 1:200 dilution (according to the laboratory) were considered positive. The search for AAb targets was driven by the ANA's fluorescence pattern: ATA as homogeneous and nucleolar or speckled, ACA as discrete-coarse speckled, anti-RNAPIII as speckled and/or nucleolar, anti-U1RNP and anti-Ku as speckled, anti-U3RNP, anti-Pm/Scl, anti-Th/To and anti-NOR90 as nucleolar.[17] ATA, ACA, anti-RNAPIII, anti-U1RNP, anti-U3RNP, anti-Pm/Scl and anti-Ku were tested in all serum samples in all centres. Anti-Th/To and anti-NOR90 were tested in all serum samples in four and three centres, respectively.

The following methods were used to detect the AAb specificity, according to the laboratory and the AAb subtype: chemiluminescent method (Theradiag-Fidis, BioFlash apparatus or BioPlex 2200 ANA Screen), ELIA (Elia symphony on Phadia250), line-dot immunoassay (SCL10DIV-24, D-Tek or SCL12D-24, D-Tek or ANA10D-IV-24, D-Tek or DL 1532–1601 G, EuroImmun or DL 1530-1601-4 G, EuroImmun or AD-SPS12D, Eurobio) or ELISA (704555 QUANTA Lite RNA Pol III).

Statistical analysis

Categorical variables are described with number (percentage), and quantitative variables with mean and SD. Factors associated with dcSSc and lcSSc were studied by univariate analysis (Student's t-test, Wilcoxon test, χ^2 test or Fisher exact test, as appropriate). Factors associated with the presence of each defined complication were studied by univariate analysis (Student's t-test, Wilcoxon test, χ^2 test or Fisher exact test, as appropriate) and multivariate analysis (logistic regression without stepwise selection, with all factors significant at p≤0.10 included). Survival curves were established with the Kaplan-Meier method with data censored after 20 years of follow-up. Patients producing several specific AAbs were excluded from analysis to avoid some bias. Variables associated with global survival were identified by univariate analysis (Log-rank test) and multivariate analysis (Cox proportional-hazard model without stepwise selection, with all factors significant at p≤0.10 included). ORs, HRs and 95% CIs were estimated. P<0.05 was considered statistically significant. All analyses were performed with SAS V.9.4 (SAS Institute Inc, Cary, North Carolina, USA).

RESULTS

Clinical and immunological features of the SSc cohort

The clinical and biological characteristics of the 1605 patients with SSc included are summarised in table 1: 1299 (80.9%) were female and the mean (SD) age at diagnosis was 52 (15.2) years. Twenty (1.2%) patients were diagnosed before 18-years-old. Patients were followed for a mean of 10 (8.2) years. Among the 1605 patients, 275 (17.1%) patients presented with overlap syndrome (table 1).

During follow-up, 553 (34.5%) patients developed ILD, 142 (8.9%) patients PAH, 60 (3.7%) patients experienced SRC, 574 (35.8%) DU, 349 (21.7%) arthritis, 129 (8.0%) myositis and 14 (0.9%) IPO (table 1). Death occurred in 110 patients (6.9%, the overall survival curve of the population is available in online supplemental figure 1).

A total of 1572 (97.9%) sera were positive for ANA. Among them, 1403 (89.2%) contained at least one from the nine AAbs tested. Most patients' sera contained only one SSc-related AAb subtype, but 32 (1.9%) had AAbs directed against several tested antigens (figure 1). The description of the patients displaying more than one specific AAb is summarised in online supplemental table 1).

Among this cohort, 367 (22.9%) patients had dcSSc and 1238 (77.1%) lcSSc. The patients with lcSSc were more often women (85.0% vs 67.3% for dcSSc, p<0.0001) and older at diagnosis (mean age 52.7 (15.0) and 49.7 (15.5) years old, p=0.0009) than patients with dcSSc (table 1). More patients with dcSSc than lcSSc experienced ILD (56.4% vs 28%, p<0.0001), SRC (10.6% vs 1.7%, p<0.0001), DU (49.3% vs 31.8%, p<0.0001), arthritis (26.7% vs 20.3%, p=0.009) and IPO (1.9% vs 0.6%, p=0.02). The two groups did not differ in the occurrence of PAH (p=0.47) or myositis (p=0.33). Overall, the mortality was higher in patients with dcSSc with 9.3% of patients with dcSSc dying (from any causes) during follow-up as compared with 6.1% patients with lcSSc (p=0.001) (figure 2A). More patients with dcSSc than lcSSc had ATA (51.2% vs 14.3%, p<0.0001), anti-RNAPIII (17.4% vs 2.8%, p<0.0001) or anti-U3RNP (4.9% vs 1.9%, p=0.001). More patients with lcSSc than dcSSc had ACAs (61.6% vs 7.4%, p<0.0001) or anti-U1RNP (4.6% vs 2.2%, p=0.04). The two groups did not differ in terms of the prevalence of anti-Pm/Scl (p=0.15), anti-Ku (p=0.32), anti-Th/To (p=0.22) or anti-NOR90 (p=0.49) AAbs.

Table 1 Main features of the SSc population

	Total (n=1605)	dcSSc (n=367)	lcSSc (n=1238)	P value
General features				
Female, n (%)	1299 (80.9)	247 (67.3)	1052 (85.0)	<0.0001
Age at diagnosis, years, mean (SD)	52.0 (15.2)	49.7 (15.5)	52.7 (15.0)	0.0009
≤16-years-old at diagnosis, n (%)	14 (0.9)	3 (0.8)	11 (0.9)	0.9999
Disease duration, years, mean (SD)	10.0 (8.2)	8.2 (7.2)	10.5 (8.4)	<0.0001
Death, n (%)	110 (6.9)	34 (9.3)	76 (6.1)	0.0001
Organ involvement, n (%)				
PAH	142 (8.9)	29 (7.9)	113 (9.1)	0.47
ILD	553 (34.5)	207 (56.4)	346 (28.0)	<0.0001
SRC	60 (3.7)	39 (10.6)	21 (1.7)	<0.0001
Digital ulcer	574 (35.8)	181 (49.3)	393 (31.7)	<0.0001
Arthritis	349 (21.7)	98 (26.7)	251 (20.3)	0.009
Myositis	129 (8.0)	34 (9.3)	95 (7.7)	0.33
IPO	14 (0.9)	7 (1.9)	7 (0.6)	0.02
Overlap diseases, n (%)				
All overlap diseases	275 (17.1)	33 (9.0)	242 (19.6)	<0.0001
Systemic lupus	36 (2.2)	3 (0.8)	33 (2.7)	0.04
Sjögren syndrome	136 (8.5)	19 (5.2)	117 (9.5)	0.01
Rheumatoid arthritis	38 (2.4)	7 (1.9)	31 (2.5)	0.70
Autoimmune myositis	18 (1.1)	3 (0.8)	15 (1.2)	0.78
Primary biliary cholangitis	38 (2.4)	0 (0)	38 (3.1)	<0.0001
ANCA-vasculitis	4 (0.3)	0 (0)	4 (0.3)	0.58
SSc-related AAbs, n (%)				
AAN	1572 (97.9)	361 (98.4)	1211 (97.8)	0.46
At least one AAb specificity identified	1403 (89.2)	309 (85.6)	1094 (90.3)	0.02
ATA	365 (22.7)	188 (51.2)	177 (14.3)	<0.0001
ACA	789 (49.2)	27 (7.4)	762 (61.6)	<0.0001
Anti-RNAPIII	98 (6.1)	64 (17.4)	34 (2.8)	<0.0001
Anti-U1RNP	65 (4.1)	8 (2.2)	57 (4.6)	0.04
Anti-U3RNP	41 (2.6)	18 (4.9)	23 (1.9)	0.001
Anti-Pm/Scl	55 (3.4)	17 (4.6)	38 (3.1)	0.15
Anti-Ku	12 (0.8)	1 (0.3)	11 (0.9)	0.32
Anti-Th/To*	9 (0.9)	0 (0.0)	9 (1.1)	0.22
Anti-NOR90*	3 (0.4)	1 (0.6)	2 (0.3)	0.49

*Data concerning anti-Th/To AAb and anti-NOR90 AAbs were available for 1014 and 864 patients respectively.
AAb, autoantibody; AAN, antinuclear antibody; ACA, anticentromere antibody; Anti-RNAPIII, anti RNA polymerase III antibody; ATA, anti-topoisomerase I antibody; dcSSc, diffuse cutaneous systemic sclerosis; ILD, interstitial lung disease; IPO, intestinal pseudo-obstruction; lcSSc, limited systemic sclerosis; PAH, pulmonary arterial hypertension; SRC, scleroderma renal crisis; SSc, systemic sclerosis.

Among the 1238 patients with lcSSc, 74 were classified as *sine scleroderma* with median disease duration of 6.8 years (SD 3.6 years). None of them have been previously classified as dcSSc. We found no statistical difference between patients sine scleroderma compared with lcSSc concerning complications, except for the occurrence of DUs (respectively 20.3% and 32.5%, p=0.03). Among these 74 patients, 8 had ATA, 49 had ACA. The other AAb detected were anti-RNAPIII (n=1), anti-U1RNP (n=1), anti-U3RNP (n=1), anti-Pm/Scl (n=2) or anti-Th/To (n=1).

Factors associated with the occurrence of each type of organ complication according to multivariate analysis in SSc

Because of the high phenotypical heterogeneity occurring in SSc, multivariate analyses were performed to

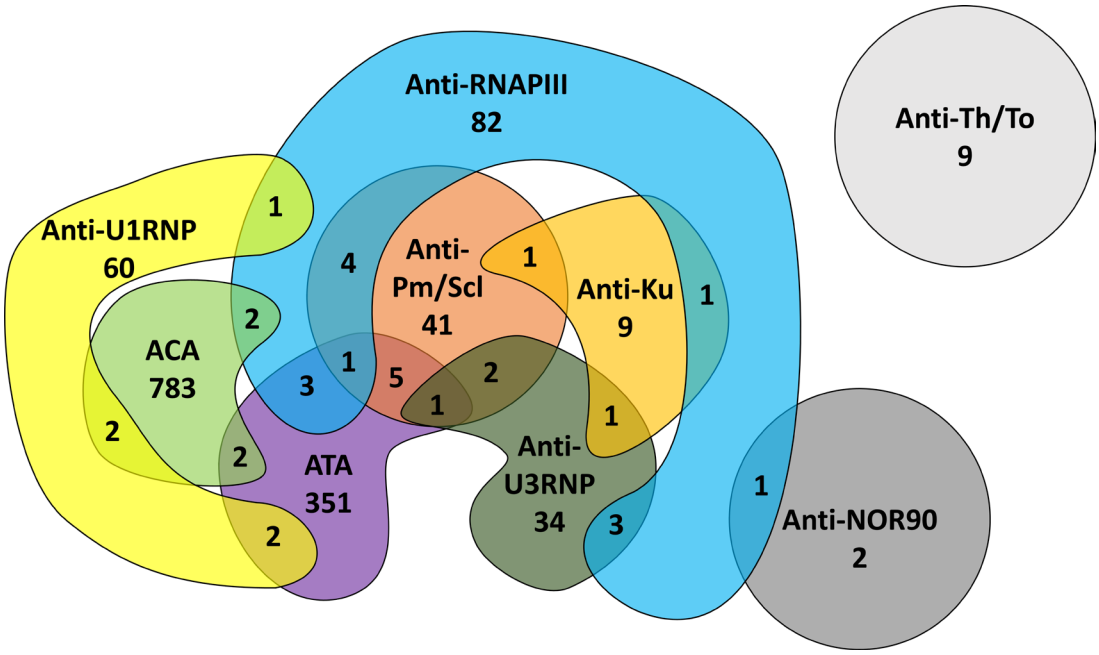

Figure 1 Schematic prevalence of nine SSc-related AAbs in the population: 365 patients had ATA, 789 ACA, 98 anti-RNAPIII, 65 anti-U1RNP, 41 anti-U3RNP, 55 anti-Pm/Scl, 12 anti-Ku, 9 anti-Th/To and 2 anti-NOR90. Thirty-two patients (1.9%) had several AAbs: 14 patients had ATA, 6 ACA, 16 anti-RNAPIII, 5 anti-U1RNP, 7 anti-U3RNP, 14 anti-Pm/Scl, 3 anti-Ku and 1 anti-NOR90. AAbs, autoantibodies; ATA, antitopoisomerase I antibody; ACA; anticentromere antibody; Anti-RNAPIII, anti RNA polymerase III antibody.

identify risk factors associated with the main complications observed in patients with SSc .

ILD was associated with male sex (OR=1.4 (95% CI=1.05 to 1.9), p=0.02), presence of ATA (OR=3.27 (95% CI=2.42 to 4.42), p<0.0001), whereas ILD was negatively associated with ACA (OR=0.18 (95% CI=0.13 to 0.24), p<0.0001). ILD was not associated with skin phenotype subtype or presence of another AAb (table 2 and online supplemental table 2).

PAH was associated with only age at diagnosis (per one additional year, OR=1.04 (95% CI=1.03 to 1.05), p<0.0001) (table 2 and online supplemental table 3). PAH was not associated with the skin phenotype subtype or presence of a specific AAb.

SRC was found associated with anti-RNAPIII (OR=7.05 (95% CI=2.98 to 16.72), p<0.0001) as well as male sex although not statistically significant (OR=2.05 (95% CI=0.91 to 4.66), p=0.09). SRC was negatively associated with the presence of ACA (OR=0.17 (95% CI=0.04 to 0.78), p=0.02). SRC was not associated with skin phenotype subtype or presence of another AAb (table 2 and online supplemental table 4).

DU was associated with age at diagnosis (per 1 additional year, OR=0.97 (95% CI=0.96 to 0.98), p<0.0001), dcSSc subtype (OR=1.53 (95% CI=1.14 to 2.05), p=0.005) or presence of ATA (OR=2.42 (95% CI=1.76 to 3.31), p<0.0001). No other association was found (table 2 and online supplemental table 5).

Arthritis was associated with ATA (OR=1.49 (95% CI=1.03 to 2.15), p=0.04), anti-RNAPIII (OR=2.01 (95% CI=1.21 to 3.34], p=0.007), anti-U1RNP AAb (OR=3.79 (95% CI=2.16 to 6.67), p<0.0001) or anti-U3RNP AAb (OR=2.28 (95% CI=1.15 to 4.52) p=0.02) and negatively associated with the presence of ACA (OR=0.62 (95% CI=0.44 to 0.89), p=0.009). Arthritis was not associated with skin phenotype subtype or another AAb (table 2 and online supplemental table 6).

Myositis was associated with the presence of anti-U1RNP AAb (OR=2.56 (95% CI=1.3 to 5.03), p=0.006), anti-U3RNP AAb (OR=3.32 (95% CI=1.58 to 7.0), p=0.002), anti-Pm/Scl AAb (OR=7.09 (95% CI=3.87 to 12.98), p<0.0001) or anti-Ku AAb (OR=7.99 (95% CI=2.41 to 26.46), p=0.0007), whereas this inflammatory muscle disease was negatively associated with ACA (OR=0.24 (95% CI=0.14 to 0.42), p<0.0001). Myositis was not associated with the skin phenotype subtype (table 2 and online supplemental table 7).

Survival and factors associated with mortality

Survival analyses were first performed to assess the effect of the AAbs on death. Patients with ACA or anti-U1RNP AAb exhibited a better survival (p=0.0003 and 0.05), whereas a worse outcome was observed in patients with ATA (p=0.007), anti-RNAPIII (p<0.0001) or anti-U3RNP AAb (p=0.004) (figure 2, panels B–F).

Anti-ATA and anti-RNAPIII were also identified as independent factors associated with increased mortality by multivariate analysis (anti-ATA: HR=1.9 (95% CI=1.01 to 3.58), p=0.05 and anti-RNAPIII: HR=2.35 (95% CI=1.12 to 4.93), p=0.02). Age at diagnosis, presence of PAH or occurrence of SRC were three other independent factors found associated with increased mortality. On the

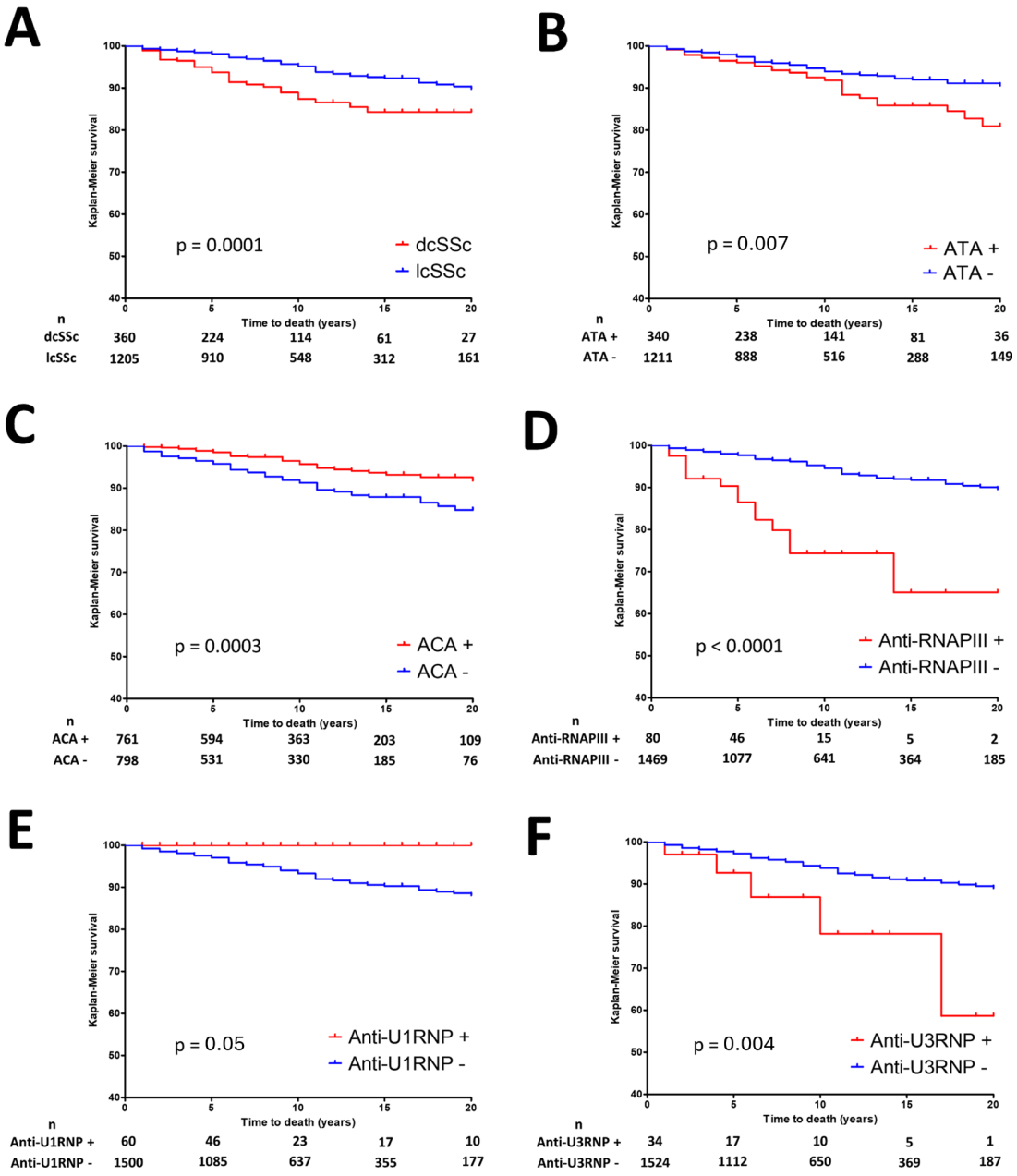

Figure 2 Survival curves of the SSc population by subgroups. Analyses were according to the skin phenotype (A) and the presence or absence of selected AAbs (B–F). AAb, autoantibody; ACA, anticentromere antibody; ATA, antitopoisomerase I antibody; Anti-RNAPIII, anti RNA polymerase III antibody; dcSSc, diffuse cutaneous systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis.

contrary, outcome was not linked with the skin phenotype (table 3 and online supplemental table 8).

DISCUSSION

In this work, we report data from a large multicentric cohort of patients with SSc focusing on the association between a wide range of AAb specificities, organ complications and disease prognosis. Our observations highlighted the importance of an accurate AAb analysis to guide patient personalised monitoring, as the association between solid organ involvement was much stronger with AAb profile than with skin phenotype in SSc.

We enrolled seven French university hospital centres set up in distinct geographical areas, all belonging to the rare autoimmune and autoinflammatory diseases national network (FAI2R). These centres were chosen because they all applied national guidelines regarding SSc management, including regular screening of organ complications. Moreover, these seven centres were able to detect the AAb specificity against many antigens recently found associated with SSc. Within each centre, all patients with a diagnosis of SSc according to the ACR/EULAR criteria were included. Consequently, with this project, we report data for one of the largest SSc cohorts,

Table 2 Multivariate analysis of factors associated with organ complications in SSc

	PAH (n=1605)		ILD (n=1605)		SRC (n=864)		Digital ulcer (n=1603)		Arthritis (n=1605)		Myositis (n=1603)	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
General features												
Age at diagnosis (per 1 year)	**1.04 (1.03 to 1.05)**	<0.0001	–	–	–	–	**0.97 (0.96 to 0.98)**	<0.0001	0.99 (0.97 to 1.0)	0.15	0.99 (0.99 to 1.01)	0.91
Male sex	–	–	**1.41 (1.05 to 1.9)**	0.02	2.05 (0.91 to 4.66)	0.09	1.14 (0.86 to 1.51)	0.35	–	–	0.89 (0.56 to 1.4)	0.61
dcSSc subtype	–	–	1.01 (0.75 to 1.34)	0.98	1.63 (0.71 to 3.76)	0.25	**1.53 (1.14 to 2.05)**	0.005	0.89 (0.65 to 1.23)	0.48	–	–
Antibody												
ATA	0.93 (0.54 to 1.62)	0.80	**3.27 (2.42 to 4.42)**	<0.0001	–	–	**2.42 (1.76 to 3.31)**	<0.0001	**1.49 (1.03 to 2.15)**	0.04	–	–
ACA	1.43 (0.93 to 2.2)	0.10	**0.18 (0.13 to 0.24)**	<0.0001	**0.17 (0.04 to 0.78)**	0.02	1.08 (0.81 to 1.44)	0.61	**0.62 (0.44 to 0.89)**	0.009	**0.24 (0.14 to 0.42)**	<0.0001
Anti-RNAPIII	–	–	–	–	**7.05 (2.98 to 16.72)**	<0.0001	0.69 (0.41 to 1.17)	0.17	**2.01 (1.21 to 3.34)**	0.007	–	–
Anti-U1RNP	–	–	–	–	–	–	–	–	**3.79 (2.16 to 6.67)**	<0.0001	**2.56 (1.3 to 5.03)**	0.006
Anti-U3RNP	–	–	–	–	–	–	–	–	**2.28 (1.15 to 4.52)**	0.02	**3.32 (1.58 to 7.0)**	0.002
Anti-Pm/Scl	–	–	1.05 (0.59 to 1.86)	0.87	–	–	–	–	–	–	**7.09 (3.87 to 12.98)**	<0.0001
Anti-Ku	–	–	–	–	–	–	–	–	–	–	**7.99 (2.41 to 26.46)**	0.0007
Anti-Th/To	–	–	–	–	–	–	–	–	–	–	–	–
Anti-NOR90	–	–	–	–	6.15 (0.46 to 81.72)	0.17	<0.001 (<0.001 to >999)	0.97	–	–	–	–

Representation of multivariate analysis of factors associated with organ complications in SSc using a heatmap representation (OR = 1 or non-significant; OR = 0; highest OR).
Statistically significant values are put in bold.
ACA, anti-centromere antibody; anti-RNAPIII, anti-RNA polymerase III antibody.; ATA, anti-topoisomerase I antibody; dcSSc, diffuse cutaneous systemic sclerosis; ILD, interstitial lung disease; PAH, pulmonary arterial hypertension; SRC, scleroderma renal crisis.

Table 3 Cox proportional-hazards analysis of factors associated with mortality in SSc

	HR (95% CI)	P
General features		
Age at diagnosis (per 1 year)	**1.07 (1.06 to 1.09)**	<0.0001
Male sex	1.49 (0.93 to 2.4)	0.10
Clinical features		
dcSSc subtype	1.2 (0.73 to 1.97)	0.48
PAH	**4.46 (2.99 to 6.67)**	<0.0001
ILD	0.87 (0.56 to 1.37)	0.56
SRC	**3.56 (1.78 to 7.1)**	0.0003
Antibody		
ATA	**1.9 (1.01 to 3.58)**	0.05
ACA	0.77 (0.4 to 1.48)	0.43
Anti-RNAPIII	**2.35 (1.12 to 4.93)**	0.02
Anti-U1RNP	0.44 (0.06 to 3.39)	0.43
Anti-U3RNP	2.63 (0.95 to 7.28)	0.06
Anti-Pm/Scl	–	–
Anti-Ku	–	–
Anti-Th/To	–	–
Anti-NOR90	–	–

Statistically significant values are put in bold.
ACA, anti-centromere antibody; anti-RNAPIII, anti RNA polymerase III antibody; ATA, anti-topoisomerase I antibody; dcSSc, diffuse systemic sclerosis; PAH, pulmonary arterial hypertension; SRC, scleroderma renal crisis; SSc, systemic sclerosis.

including more than 1600 non-selected patients with SSc with a mean disease duration of 10 (8.2) years, a full phenotypic description, and an extensive immunological exploration. This cohort displayed the classical features of SSc concerning organ involvement and overlap-syndrome frequencies and we assume that this large cohort is representative of SSc populations.[13 14 18–21] Regarding immunological markers, a total of 1572 (97.9%) patients were positive for ANA, and a SSc-related nuclear target was identified in 89.2% of the cohort. Our study gives a full description of the frequency and associated phenotype regarding nine AAbs and confirmed that double SSc-specific AAb positivity is extremely rare (32 patients representing less than 2% of the cohort produced more than one AAb).

Given the new therapeutic opportunities in SSc, we need to identify factors associated with the most frequent and severe complications related to SSc to personalise patient management and propose the best therapeutic strategy.[22] For this purpose, we logically focused on clinical and biological variables available early in the course of the disease. Currently, the skin phenotype subtype is the main factor used to classify patients with SSc and predict prognosis and organ involvement.[5 23] In our cohort, univariate analyses highlighted that patients with dcSSc had a higher risk than those with lcSSc to experience ILD, SRC, DU, arthritis and IPO, whereas there was no difference concerning the risk of PAH or myositis, as previously described in other cohorts.[6 7 14] Interestingly, there was no statistical difference between patients *sine scleroderma* compared with lcSSc concerning complications, except for the occurrence of DUs. To further assess the association between cutaneous phenotype and outcome in another way, we performed multivariate analyses without stepwise selection and evidenced that the cutaneous phenotype subtype did not predict any organ outcome in our model, except the development of DU. The association between the dcSSc subtype and DU has been previously reported and may be due to the higher frequency of mechanical ulcers on the back of these patients' hands.[24] Overall, our data demonstrate that the skin phenotype is not a sufficient and reliable factor to formally predict organ damage and disease complications for clinicians.

By contrast to the skin phenotype, we evidenced a strong association between several AAb specificities and organ damages on multivariate analyses without stepwise selection. Indeed, except PAH, each type of organ involvement was found associated with a single or some AAb(s). ILD was found related to the presence of ATA, SRC with anti-RNAPIII, myositis with anti-U1RNP, anti-U3RNP AAb, anti-Pm/Scl or anti-Ku AAbs. Regarding arthritis, this other musculoskeletal complication was observed in patients with ATA, anti-RNAPIII, anti-U1RNP and anti-U3RNP. Patients with ACA were identified as a subgroup at decreased risk for developing musculoskeletal complications (arthritis, myositis), SRC and ILD by our analyses. Previous studies have reported similar associations between the three most frequently identified AAbs (ATA, ACA, anti-RNAPIII) and SSc phenotype in many cohorts.[5 6] ATA was found associated with dcSSc and ILD, ACA with lcSSc and with PAH in some but not all studies, and anti-RNAPIII with dcSSc and SRC.[13 14 18 25] Regarding the other AAbs, data appear more scarce. Anti-U1RNP AAb was found associated with ILD and myositis, and also with PAH in a Japanese and an Australian cohort; anti-U3RNP AAb with PAH and myositis; anti-Pm/Scl and anti-Ku AAbs were both associated with myositis and ILD, and anti-Th/To AAb with PAH.[13 18 26–29] Regarding PAH, this severe complication was found associated with age at diagnosis but without any ANA specificity in our population. Previous studies suggested an association between ACA and PAH and this notion is commonly reported in reviews on the topic.[25] Nevertheless the definition of PAH may be objectionable in a part of these previous studies. Two important analyses on large populations observed no association between ACA and PAH, as reported here.[14 18] Anti-U3RNP and anti-Th/To AAb have been found associated with PAH in some studies focusing on these subgroups of patients producing these rare AAbs.[14 27 29] We may have missed these associations because of lack of patients with these two AAbs. Whatever, the majority of patients with SSc developing PAH do

not produce anti-U3RNP or anti-Th/To AAb, suggesting that AAbs are not key tools to help clinicians in PAH screening. Some teams are currently endeavouring to identify serum biomarkers for PAH based on pathophysiological considerations or without any a priori tools like multiplexed approaches.[30 31]

The mortality rate was about 10% at 10 years in our study, similar to that reported in an Italian cohort and lower than that estimated in another French cohort (about 30%), but this one was smaller and reported by specific centres who probably care for the most severe patients because of their leading role in the disease.[2 23] Logically, older age at diagnosis, PAH and SRC were found associated with reduced survival, as reported in other cohorts.[2 3] Surprisingly, ILD was not associated with a worsen prognosis in the present cohort. Because all patients performed a CT-scan at least at diagnosis, whatever their symptom or respiratory function test result, is it possible that there was an over-representation of 'non-clinically significant ILD" in this cohort by contrast with other cohort performing CT-scan only in case of altered respiratory function test. The skin phenotype was not found associated with mortality in our study, according to both the analyses in subgroups based on immunological profile and to multivariate analyses without stepwise selection in the entire population. This result is supported by 8 of the 10 previously published studies based on multivariate analyses, as shown in online supplemental table 9)[23 32–39] . Of note, Nihtyanova *et al* reported lower survival for dcSSc than patients with lcSSc mainly because of a higher mortality of patients with dcSSc in the first 5 years after disease onset in a well-characterised United Kingdom's cohort.[14] As highlighted by this team, survival rates were the same for lcSSc and dcSSc patients after 5 years of follow-up when survival analyses were performed according to presence or absence of organ involvement. They also analysed in the same cohort the associations between distinct immunological profiles (ACA, ATA, anti-RNAPIII, anti-U3RNP, anti-Pm/Scl, other identified ANA, unidentified ANA, no ANA) and organ complications and survival.[14] Accordingly, the patients who carried ACA had the highest survival, while the patients with unidentified ANA had the lowest survival. In our cohort with a larger panel of ANA specificities determination multivariate analyses without stepwise selection identified two among the nine AAb specificities, ATA and anti-RNAPIII, as factors associated with a higher mortality, whereas patients with ACA or anti-U1RNP AAb exhibited a better survival. These associations are in accordance with older studies on survival focusing only on patients with ATA, ACA and anti-RNAPIII.[40]

ANAs represent relevant biomarkers to improve predictive value of a new patient's outcome-based classification in SSc. Three arguments advocate for this recommendation: (1) these AAbs are produced before any symptoms and complications, (2) they are generally mutually exclusive as observed by all studies including ours[11] and (3) each ANA subtype is found significantly associated with a separate disease profile and outcome as demonstrated here by multivariate analyses without any a priori assumption. Other teams have previously published convincing studies regarding the weight of AAbs for patient classifications by using cluster analysis methods. In a study based on a cohort of 407 patients, adding five SSc-specific AAbs in the cluster analysis allowed to identify a group of patients at high risk of organ involvement with high sensitivity.[41] A cluster analysis within the European Scleroderma Trials and Research (EUSTAR) database distinguished six homogeneous groups of patients with SSc according to skin phenotype subtype, AAb profile, organ involvement and mortality.[8] All these elements raise old questions about the pathogenic role of this family of AAbs in SSc. It is believed that the nuclear nature of their target prevents any accessibility, precluding any direct pathophysiological considerations. Nevertheless, recent data suggest a pathogenic role of these AAbs in the disease. Different potential mechanisms of pathogenicity have been described, notably concerning ATA leading to endothelial cell apoptosis and/or fibroblast and immune cell activation, but many aspects remain to be elucidated.[42]

We consider that the systematic determination of the high number of ANA specificities is a major strength and factor of originality of our study since an important part of SSc population display ANA specificities that were not considered in most previous published studies. In the present cohort, ANA specificities were determined for up to 89% of the patients with SSc, whereas they were usually available in 50%–70% in other studies. The other strengths of our study include the lack of any a priori assumptions in the analyses, the real-life nature of the patient series, the number of included patients from several centres with a well-defined and long-term follow-up and very few lacking data. All centres were officially qualified to manage patients with SSc, but most of them were not recognised as referral centres. This is an important point to avoid an overrepresentation of the most severe cases.

Our study has limitations mainly due to the retrospective collection of the data and the observational nature of our cohort. To limit some bias, we excluded each patient with a lack of follow-up within the last 3 years before the beginning of the study. Furthermore, important factors that could influence organ involvement and mortality, such as other comorbidities, especially cancer and medication data were not available for analysis. Considering immunologic profile and despite the inclusion of more than 1600 patients, we may have missed some associations with AAb specificities because of the low prevalence of some AAbs. We also assume that the methods of detection of the nine AAbs could be different among the different centres. Nevertheless, all methods used assessed high to very high sensitivity and specificity and displayed a high concordance with a Cohen's κ ranged from 0.67 to 0.99.[43–49] Moreover, all centres applied the same algorithm for AAb detection. Capillaroscopic patterns at

diagnosis and during follow-up represent key factors to be considered in patients with SSc evaluation since the importance of nailfold microangiopathy and its evolution have been found to be associated with a higher prevalence of internal organ involvement.[50] Unfortunately, data on nailfold capillaroscopic patterns were missing for a large part of the patients of our multicentre cohort. Furthermore, among the 1605 patients, 240 (14.9%) were diagnosed within the last 3 years. Thus, we could have missed some association because of a short disease duration. Nevertheless, there was no statistical difference between patients with a disease duration inferior to 3 years than patients with a disease duration more than 3 years concerning the prevalence of PAH, ILD, myositis and arthritis (data not shown). Patients with a disease duration inferior to 3 years were more prompted to develop SRC (p=0.009), whereas patients with a disease lasting for more than 3 years suffered more from DUs (p=0.03). SRC is described as an early complication in SSc, whereas DUs occur later in the course of the disease.[14 51] Finally, most of our patients were Caucasian, and therefore extrapolation of our results to other SSc populations with different genetic backgrounds is hazardous.[52]

In conclusion, this study unravelled the strong association among nine ANA specificities, organ involvement and outcome in Caucasian patients with SSc. We provide new evidence for the value of a systematic determination of ANA specificities at diagnosis in patients with SSc since this information could help clinicians to better stratify the individual risk of complication and personalise monitoring. However, a classification based only on ANA would probably be insufficient to fully evaluate patient phenotype and other disease attributes including dynamic biomarkers, nailfold capillary patterns and tissue gene expression signatures have been proposed as innovative means of SSc subsetting and deserve further research.

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