RHEUMATOLOGY

Rheumatology 2017;56:999–1007
doi:10.1093/rheumatology/kex021
Advance Access publication 4 March 2017

Original article

A longitudinal cohort study of the anti-synthetase syndrome: increased severity of interstitial lung disease in black patients and patients with anti-PL7 and anti-PL12 autoantibodies

Iago Pinal-Fernandez[1,*], Maria Casal-Dominguez[2,*], Julio A. Huapaya[2,*], Jemima Albayda[2], Julie J. Paik[2], Cheilonda Johnson[2], Leann Silhan[2], Lisa Christopher-Stine[2,†], Andrew L. Mammen[1,†] and Sonye K. Danoff[2,†]

Abstract

Objective. The aim was to study the prevalence, rate of appearance and severity of clinical features in patients with different anti-synthetase syndrome (ASyS) autoantibodies.

Methods. All Johns Hopkins Myositis Longitudinal Cohort subjects positive for any ASyS autoantibodies were included. Clinical information, including symptoms, signs, strength, creatine kinase concentrations and pulmonary function tests, were prospectively collected. The standardized mortality and cancer rates and the rate of appearance and intensity of the different organ manifestations were assessed using univariate and multivariate analysis and compared between ASyS autoantibodies.

Results. One hundred and twenty-four (73.4%) patients were positive for anti-Jo1, 23 (13.6%) for anti-PL12, 16 for anti-PL7 (9.5%) and 3 (1.8%) for anti-EJ or anti-OJ, respectively. The mean length of follow-up was 4.1 years. Anti-PL12 was more frequent in black subjects. Anti-PL12 and anti-PL7 were associated with more prevalent and severe lung involvement, often without muscle involvement. Anti-Jo1 displayed more severe muscle involvement compared with anti-PL12 patients. Concurrent anti-Ro52 was more prevalent in anti-Jo1 patients and was associated with earlier development of mechanic's hands, DM-specific skin findings and arthritis. Independent of ASyS antibody status, black patients demonstrated more severe lung involvement than white patients. There was no significant increase in mortality or cancer risk in ASyS patients compared with the general US population.

Conclusion. Different ASyS autoantibodies are associated with phenotypically distinct subgroups within the ASyS spectrum. Anti-PL7 and anti-PL12 are characterized by more severe lung involvement, whereas anti-Jo1 is associated with more severe muscle involvement. Black race is a major prognostic factor associated with lung disease severity.

Key words: myositis, anti-synthetase syndrome, dermatomyositis, polymyositis, cohort study, prognostic factors, anti-Ro52, anti-synthetase antibodies

CLINICAL SCIENCE

[1]National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda and [2]Johns Hopkins University School of Medicine, Baltimore, MD, USA

Submitted 13 October 2016; revised version accepted 26 January 2017

*Iago Pinal-Fernandez, Maria Casal-Dominguez and Julio Huapaya contributed equally to this study

†Lisa Christopher-Stine, Andrew L. Mammen and Sonye K. Danoff contributed equally to this study

Correspondence to: Sonye K. Danoff, Johns Hopkins University School of Medicine, 1830 E. Monument St., 5th Floor, Baltimore, MD 21287, USA.
E-mail: sdanoff@jhmi.edu

Rheumatology key messages

- Different anti-synthetase autoantibodies are associated with phenotypically distinct subgroups within the anti-synthetase spectrum.
- Anti-PL7 and anti-PL12 syndromes are characterized by more severe interstitial lung disease.
- Black race is a major prognostic factor associated with lung disease severity.

Introduction

The anti-synthetase syndrome (ASyS) is a complex autoimmune disorder characterized by the presence of autoantibodies against one of the aminoacyl-transfer (t)RNA synthetases. It is a clinical syndrome including variable expression of myositis, interstitial lung disease (ILD), polyarthritis, mechanic's hands, RP and/or fever.

Anti-Jo1, directed against the histidyl-tRNA synthetase protein, is the most common of the anti-synthetase antibodies [1]. Other aminoacyl-tRNA synthetases targeted by autoantibodies and associated with the ASyS include anti-PL7 [2], anti-PL12 [3], anti-OJ [4], anti-EJ [4], anti-KS [5], anti-Zo [6] and anti-Ha [7].

The type of ASyS autoantibody and the co-positivity with anti-Ro52 have been proposed to be major prognostic indicators predicting the manifestations and severity of ASyS. Thus, it has been suggested that anti-Jo1 patients have more prevalent muscle involvement, whereas patients with anti-PL7 and anti-PL12 are more likely to have ILD and gastrointestinal complications [8–10]. In addition, anti-Ro52 has been associated with a higher cancer risk and more severe muscle and joint involvement [11].

Two large cohort studies [8, 12] have compared the clinical features of anti-Jo1 patients with other ASyS autoantibodies, finding evidence that non-anti-Jo1 patients, particularly anti-PL7 and anti-PL12, were more likely to have isolated lung involvement and increased mortality. Moreover, two recent large multicentre cohort studies have analysed the natural history of anti-Jo1 ASyS [13, 14], clarifying the dynamic nature of ASyS manifestations over time, the heterogeneity in clinical features of the syndrome and the tendency to chronicity of ASyS ILD.

In this large single-centre study using an equivalently sized but independent cohort of patients from the Johns Hopkins Myositis Center cohort, we compare the different ASyS autoantibodies in terms of survival, cancer rate, clinical features at the onset of the disease and their rate of appearance. Moreover, we assess the role of anti-Ro52 as a disease modifier and study the severity of the lung, muscle and joint involvement over time.

Methods

Study population and autoantibody testing

Patients enrolled in the Johns Hopkins Myositis Center Longitudinal Cohort study between 2003 and 2016 were included if tested positive for an anti-synthetase autoantibody.The initial sera from all patients was screened for anti-Jo1, anti-PL7, anti-PL12, anti-EJ, anti-OJ and anti-Ro52 autoantibodies by ELISA, line blotting (Euroline Myositis Profile 4; Euroimmun), by immunoprecipitation at the Oklahoma Medical Research Foundation and/or using Quest Diagnostics myositis panels.

At each visit, arm abduction and hip flexion strength were evaluated by the examining physician using the Medical Research Council scale. This scale was transformed to Kendall's 0–10 scale for analysis purposes as previously described [15]. Serial strength measurements for each patient were made by the same physician. For the purposes of analyses, right- and left-side measurements for arm and hip strength were combined and the average was used for the calculations (possible range 0–10). Serum creatine kinase (CK) concentrations were included for analysis if obtained within 6 weeks of the date when strength testing was performed. Myositis-specific skin involvement, symptoms of oesophageal involvement and ASyS-associated clinical features (e.g. mechanic's hands, RP, arthritis, fever) were documented both retrospectively at the onset of the disease and prospectively at each visit. ILD was defined through a multidisciplinary approach as suggested by the American Thoracic Society [16]. Pulmonary function testing included spirometry, lung volumes measured by helium dilution and diffusing capacity by single-breath carbon monoxide based on American Thoracic Society criteria [17]. This study was approved by the Johns Hopkins Institutional Review Board; written informed consent was obtained from each participant.

Statistical analysis

Dichotomous variables were expressed as percentages and absolute frequencies, and continuous features were reported as means and S.D. Pairwise comparisons for categorical variables between groups were made using the χ^2 test or Fisher's exact test, as appropriate. Student's *t*-test was used to compare continuous variables among groups. CK, a highly positively skewed variable, was expressed as the median, first and third quartile for descriptive purposes and transformed through a base-10 logarithm for regression analysis.

Indirect standardization was used to compare the number of cases of cancer that we observed in our sample during the 3 years before or after the onset of the disease [18] with the number one would expect in the general population with the same age and sex distribution. Cancer incidence by age and sex groups was taken from the 2008–2012 United States Cancer Statistics registry. The observed and expected numbers of cases were compared using the standardized incidence ratio (observed/expected cases of cancer) and its 95% CI. The number of years at risk for cancer was allocated to the correct age interval. In the event a patient had

cancer, died or had <3 years of follow-up after disease onset, the number of years at risk for that patient would end at the occurrence of the event.

Patients who died were identified using Johns Hopkins medical record system and the March 2014 version of the USA Death Master File. Mortality incidence by age and sex groups was also compared with the general population using indirect standardization based on the 1999–2014 Compressed Mortality File. The number of years at risk from the disease onset to the date of death or to March 2014 was allocated to the correct age interval. The observed and expected numbers of cases were compared using the standardized mortality ratio (observed/expected deaths) and its 95% CI.

To account for the different number of visits per patient, the evolution of the pulmonary function tests, the CK concentrations and the muscle strength were studied using multilevel linear regression models with random slopes and random intercepts. The mean of hip flexor and arm abductor strength (range 0–10) was used as the strength outcome for regression analysis.

Locally weighted regression was applied to analyse graphically the evolution of the strength, CK concentrations and the pulmonary function tests. The Kaplan–Meier estimator and Cox regression were used to study the hazard to develop each one of the different clinical features over time and to compare cancer and mortality among autoantibody groups.

The influence of non-modifiable risk factors (sex, race, length of illness and age at the onset of the first symptoms), the CS dose and the administration of IVIG, rituximab, MTX, AZA and MMF were used as adjusting covariates. Other treatments administered to < 10% of the cohort were not included in the analysis.

All statistical analyses were performed using Stata/MP 14.1. To account for the number of statistical tests performed, a two-sided P-value of $\leqslant 0.001$ was considered statistically significant for the univariate analysis, whereas 0.05 was considered significant for the multivariate analysis.

Results

Patients

From the 2042 patients enrolled in the Myositis Centre Longitudinal Cohort Study, 1198 (59%) were tested for anti-synthetase antibodies and 169 of these were positive (14.1%). One hundred and twenty-four (73.4%) individuals were positive for anti-Jo1, 23 (13.6%) for anti-PL12, 16 for anti-PL7 (9.5%) and 3 (1.8%) for anti-EJ and anti-OJ, respectively (supplementary Fig. S1, available at *Rheumatology* Online). We analysed a total of 1458 visits, with a mean (S.D.) of 8.6 (6.7) visits per patient and a mean (S.D.) follow-up time of 4.1 (3.4 years). Considering the small sample size of anti-EJ and anti-OJ, they were not included for further subgroup analysis.

Of the ASyS patients, 73% were females, 32% were black, and the mean (S.D.) age at onset was 47.4 (13.5 years). Anti-PL12 autoantibodies were found more frequently in black patients. Anti-Jo1 was more common in white patients and was more frequently associated with anti-Ro52 autoantibodies. No differences were detected in the age at onset or sex distribution among autoantibody groups. CSs and AZA were the two drugs most commonly used in these patients, followed by MTX, MMF and IVIG (Table 1).

Clinical features: univariate analysis

The clinical features of these patients both at the onset and during their follow-up are shown in Table 2. In brief, exclusive, clinically apparent lung involvement at the onset of the disease was common in anti-PL12 and anti-PL7 patients (anti-PL12 65% and anti-PL7 56% *vs* anti-Jo1 26%), whereas sole muscle involvement was more common in anti-Jo1 patients (anti-Jo1 26% *vs* anti-PL12 0% and anti-PL7 12%). During the course of follow-up, most patients experienced both muscle and lung involvement (>60% in all groups). However, anti-PL12 and anti-PL7 patients trended towards having lung involvement without ever experiencing muscle involvement (anti-PL12 30% and anti-PL7 19% *vs* anti-Jo1 10%), and anti-Jo1 patients trended towards exclusive muscle involvement (anti-Jo1 26% *vs* anti-PL12 4% and anti-PL7 0%; Table 2).

The severity of weakness was not significantly different depending on sex, race, age at onset or anti-Ro52 status (Table 3). However, at the first visit, patients with anti-Jo1 showed a trend towards being weaker (mean hip flexor strength 8.3) than anti-PL12 and anti-PL7 patients (both mean hip flexor strength >9.4). The CK concentrations of anti-PL12 patients were significantly lower than the rest (median CK concentration 78 IU/L, $P < 0.001$).

Autoantibody status and race seemed to be the most important prognostic factors associated with ILD severity. Thus, anti-PL12 patients showed the most severe ILD phenotype [percentage forced vital capacity (%FVC) = 57%, percentage diffusing capacity for carbon monoxide (%DLCO) = 55%] and anti-Jo1 patients the mildest (%FVC = 71%, %DLCO = 67%). Anti-PL7 patients presented intermediate ILD severity (%FVC = 61%, %DLCO = 53%). Black patients showed strikingly more severe ILD (>15% lower FVC and DLCO, all $P < 0.001$) than the rest of the patients (Fig. 1A and B; supplementary Fig. S2, available at *Rheumatology* Online). The severity of ILD was not significantly different depending on sex, age at onset or anti-Ro52 status (Table 3).

Strength and ILD evolution: multivariate analysis

We performed a mixed effect regression analysis to assess the effect of the race and autoantibody status on the severity of weakness and ILD independent of possible confounding variables (age at onset, sex, time from the onset and immunosuppressant treatments). This analysis confirmed that patients with anti-PL12 autoantibodies showed lower CK concentrations and more severe ILD (lower FVC and DLCO) compared with patients with anti-Jo1 ASyS (all $P < 0.05$). Moreover, patients with anti-PL7 showed lower DLCO than anti-Jo1 patients

Table 1 General features of anti-synthetase patients

	Anti-Jo1 (n = 124)	Anti-PL12 (n = 23)	Anti-PL7 (n = 16)	Anti-EJ (n = 3)	Anti-OJ (n = 3)	Total (n = 169)
Age of onset, mean (S.D.), years	46.6 (13.5)	47.5 (14.9)	49.2 (12.1)	54.1 (7.6)	63.6 (6.7)*	47.4 (13.5)
Number of visits, mean (S.D.)	8.0 (6.6)*	12.3 (6.2)**	9.1 (6.3)	10.3 (4.5)	5.7 (4.0)	8.7 (6.6)
Length of follow-up, mean (S.D.), years	4.1 (3.3)	4.8 (3.9)	3.3 (3.3)	4.1 (1.7)	2.1 (1.2)	4.1 (3.4)
Sex, female, % (n)	73 (91)	83 (19)	69 (11)	33 (1)	33 (1)	73 (123)
Race, % (n)						
White	**71 (88)*****	**17 (4)*****	44 (7)	33 (1)	67 (2)	60 (102)
Black	**23 (28)*****	**70 (16)*****	50 (8)	33 (1)	33 (1)	32 (54)
Other races	6 (8)	13 (3)	6 (1)	33 (1)	0 (0)	8 (13)
Cancer-associated myositis, % (n)	2 (2)	9 (2)	6 (1)	0 (0)	0 (0)	3 (5)
Mortality, % (n)	6 (7)	13 (3)	19 (3)	0 (0)	0 (0)	8 (13)
Anti-Ro52, % (n)	**74 (92)*****	43 (10)*	44 (7)	67 (2)	0 (0)*	66 (111)
Treatments, % (n)						
CSs	92 (114)	100 (23)	100 (16)	100 (3)	33 (1)*	93 (157)
AZA	52 (64)	61 (14)	50 (8)	67 (2)	33 (1)	53 (89)
MTX	37 (46)**	17 (4)	12 (2)	0 (0)	0 (0)	31 (52)
MMF	34 (42)*	48 (11)	62 (10)*	67 (2)	33 (1)	39 (66)
IVIG	33 (41)	35 (8)	19 (3)	33 (1)	33 (1)	32 (54)
Rituximab	22 (27)	22 (5)	25 (4)	0 (0)	0 (0)	21 (36)

Dichotomous variables are expressed as a percentage (number of patients), whereas the age at onset is expressed as the mean (S.D.). Bivariate comparisons of continuous variables were made using Student's *t*-test, whereas bivariate comparisons of dichotomous variables were made using either the χ^2 test or Fisher's exact test, as appropriate. Bold values are statistically significant. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

(−14%, $P < 0.05$) and a trend towards lower FVC (−4%, $P > 0.05$). Consistent with the univariate study, black patients showed strikingly more severe ILD (18% lower FVC, $P < 0.001$; 12% lower DLCO, $P < 0.01$) than white patients, with no interaction detected between the autoantibody status and the race (all $P > 0.05$).

This analysis also showed that independent of the autoantibody status (and the rest of the confounding variables), CK concentrations tended to decrease over time ($\beta = 0.04$ IU/l/year, $P < 0.001$) and the %FVC tended to increase in a barely clinically relevant manner ($\beta = 0.89$%/year, $P < 0.01$), whereas the strength and DLCO remained stable over time (Table 4).

Independent of autoantibody status, race, sex, time from the onset, age at onset or treatments, CK concentrations were highly associated with the strength ($\beta = -0.66$, 95% CI: −0.87, −0.45; $P < 0.001$). Thus, a 10-fold decrease of the CK was estimated to be associated with a corresponding increase of 0.7 points of strength. The logarithm of CK concentrations continued to decrease linearly even after patients had reached maximal proximal muscle strength, which happened at ∼1 year of follow-up (Fig. 1C). Finally, anti-Ro52 autoantibodies were not associated with the severity of the weakness or ILD (all $P > 0.05$).

Rate of development of clinical manifestations

Independent of the race, sex and age at onset, anti-Ro52 was associated with earlier development of arthritis [hazard ratio (HR) = 2.0; 95% CI: 1.1, 3.8; P = 0.03], mechanic's hands (HR = 2.0; 95% CI: 1.1, 3.7; P = 0.03) and DM-specific skin signs (HR = 2.0; 95% CI: 1.1, 3.6; P = 0.02; supplementary Fig. S3, available at *Rheumatology* Online).

Both anti-PL12 (HR = 4.0, 95% CI: 1.5, 10.4) and anti-PL7 patients (HR = 4.2, 95% CI: 1.8, 11.1) developed symptoms of gastro-oesophageal reflux disease at a higher rate than anti-Jo1 patients (both $P < 0.004$; supplementary Fig. S4, available at *Rheumatology* Online).

Additional signs and symptoms associated with the ASyS showed no difference in the rate of development over time among the different autoantibody groups.

Mortality and cancer risk

Thirteen patients with ASyS died during the follow-up (seven anti-Jo1 and three anti-PL12 and anti-PL7, respectively). Five patients (two anti-Jo1 and one anti-PL12 and anti-PL7) were diagnosed with cancer (all adenocarcinomas: two colon, two breast and one lung, respectively) within 3 years of the onset of the disease. Compared with the general population, no increase in mortality or cancer was observed either in the whole cohort or when analysing each autoantibody group separately (Fig. 2). Also, independent of age, sex, race and age at onset, no autoantibody group showed a significant increase in mortality or cancer compared with the rest (all $P < 0.05$). Anti-Ro52 was not significantly associated with increased cancer (standardized incidence ratio 0.7, 95% CI: 0.09, 2.6) or mortality (standardized mortality ratio 1.4, 95% CI: 0.5, 3.0).

Table 2 Symptoms and signs of the patients with anti-synthetase syndrome

	Anti-Jo1 (n = 124)		Anti-PL12 (n = 23)		Anti-PL7 (n = 16)		Total (n = 169)	
	Onset	Cumulative	Onset	Cumulative	Onset	Cumulative	Onset	Cumulative
Simplified clinical groups[a]								
Lung and muscle involvement	35 (43)*	60 (74)	9 (2)*	65 (15)	31 (5)	81 (13)	30 (51)	62 (105)
Muscle involvement	26 (32)*	26 (32)**	0 (0)**	4 (1)*	12 (2)	0 (0)*	21 (36)	20 (34)
Lung involvement	**26 (32)*****	10 (13)*	**65 (15)*****	30 (7)*	56 (9)*	19 (3)	34 (57)	14 (24)
Exclusive joint involvement	10 (13)	3 (4)	22 (5)	0 (0)	0 (0)	0 (0)	11 (18)	2 (4)
No muscle, joint or lung involvement	3 (4)	1 (1)	4 (1)	0 (0)	0 (0)	0 (0)	4 (7)	1 (2)
Signs and symptoms								
Dyspnoea	52 (65)	63 (78)**	65 (15)	87 (20)*	75 (12)	94 (15)*	56 (94)	69 (117)
Cough	23 (29)	**38 (47)*****	39 (9)	70 (16)*	44 (7)	69 (11)	27 (46)	46 (78)
Muscle weakness	**60 (75)*****	85 (106)	**9 (2)*****	70 (16)	44 (7)	81 (13)	51 (87)	82 (139)
Arthritis	21 (26)	55 (68)*	17 (4)	52 (12)	12 (2)	25 (4)*	19 (32)	50 (84)
DM-specific skin involvement[b]	14 (17)	58 (72)	17 (4)	70 (16)	19 (3)	56 (9)	15 (25)	59 (100)
Raynaud's phenomenon	15 (19)	40 (49)*	4 (1)	17 (4)	19 (3)	31 (5)	14 (23)	35 (59)
Mechanic's hands	10 (13)	51 (63)	0 (0)	57 (13)	25 (4)	56 (9)	11 (18)	53 (89)
Dysphagia	10 (12)	21 (26)	9 (2)	30 (7)	12 (2)	19 (3)	9 (16)	22 (37)
Gastro-oesophageal reflux disease	11 (14)	**24 (30)*****	30 (7)*	61 (14)**	6 (1)	56 (9)*	14 (23)	33 (55)
Fever	10 (13)	19 (23)	17 (4)	39 (9)*	0 (0)	6 (1)	10 (17)	20 (33)
Calcinosis	3 (4)	11 (14)*	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	8 (14)

Values are given as a percentage (number of patients). Bold values are statistically significant. Bivariate comparisons of dichotomous variables were made between each category and the rest of the sample using either the χ^2 test or Fisher's exact test, as appropriate. [a]Muscle and lung involvement groups were categorized disregarding the status of joint involvement, whereas joint involvement excluded lung or muscle involvement. [b]DM-specific skin involvement includes heliotrope rash, Gottron sign or papules. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Discussion

Our findings confirm the hypothesis that different ASyS autoantibodies have clinically distinct phenotypes. Both anti-PL7 and anti-PL12 are associated with more prevalent and severe ILD, whereas anti-Jo1 was associated with more intense muscle involvement than anti-PL12. Moreover, independent of the autoantibody status, black race was a major prognostic factor for ILD severity. Anti-Ro52 was detected more frequently with anti-Jo1 and was associated with earlier development of arthritis, mechanic's hands and DM-specific skin findings.

Muscle involvement is one of the most characteristic features of the ASyS. However, its severity is milder compared with other types of myositis (e.g. anti-SRP- or anti-HMGCR-associated myositis) [19, 20]. After 1 year of follow-up, most of the patients recovered near-full proximal muscle strength no matter what combination of treatments was used (Fig. 1). This would suggest a conservative immunosuppressant schedule (e.g. CSs plus MTX or AZA) as the treatment of choice in ASyS myositis when not accompanied by lung disease. The CK continued to decrease after the strength had reached near-full strength (ceiling effect), suggesting that CK has a broader dynamic range as a marker of muscle injury than the strength as measured using the standard Medical Research Council scale. By using this surrogate marker, we detected milder muscle involvement in anti-PL12 myositis compared with anti-Jo1. Considering the low price, high availability and objectivity of the CK determination, our data suggest that the logarithm of the CK could be a good surrogate marker for muscle disease activity in ASyS patients.

We show that patients with anti-PL7 and, to an even greater degree, anti-PL12 have more severe lung involvement than those with anti-Jo-1. Nonetheless, regardless of the autoantibody status, the tendency in ASyS is towards stability in lung function with treatment over time, as previously suggested [13]. This course indicates that lung inflammation with irreversible damage may occur very early after the onset of the disease. If this were to be confirmed, it would suggest that early aggressive therapy in new-onset ILD might improve the long-term outcome.

The increase in the rate of gastro-oesophageal reflux disease detected in anti-PL7 and anti-PL12 patients is concordant with the increase in gastrointestinal manifestations suggested by Marie *et al*. [9]. However, this could be either secondary to an increase in the intrathoracic pressure swings attributable to ILD or to lower oesophageal involvement, which was suggested to be common in myositis patients but never demonstrated in a definite manner [21, 22].

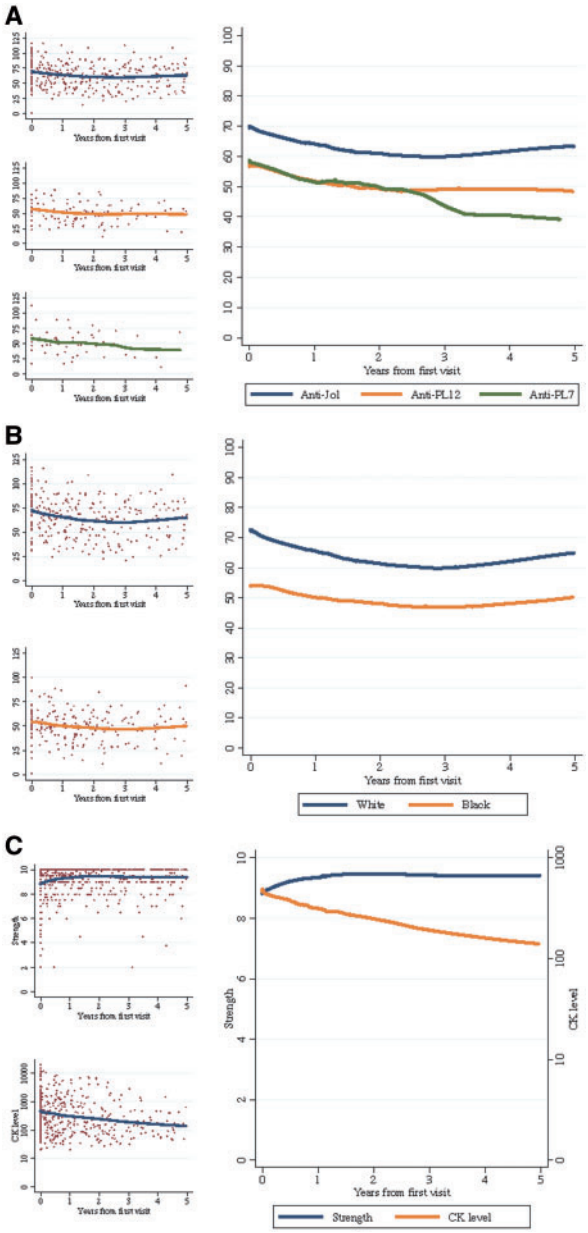
The mortality in our cohort was strikingly lower compared with previous studies. For example, Trallero-Araguás *et al.*

Table 3 Univariate analysis of muscle and lung involvement in patients with the anti-synthetase syndrome

	Anti-Jo1 Mean (S.D.)	Anti-PL12 Mean (S.D.)	Anti-PL7 Mean (S.D.)	Sex			Race			Age of onset		Anti-Ro52			Total (95% CI)
				Female	P-value	Male	White	P-value	Black	Corr. coeff	P-value	No	P-value	Yes	
Hip flexors															
Hip flexor strength at first visit	8.3 (2.2)*	9.4 (0.9)	9.7 (0.7)	8.6	0.5	8.3	8.3	0.07	9.1	0.0	1.0	8.8	0.3	8.4	8.5 (8.1, 8.9)
Follow-up hip flexor strength	9.0 (1.4)	9.3 (0.8)	9.5 (0.6)	9.0	0.02	9.6	9.1	0.8	9.2	0.0	0.8	9.2	0.7	9.1	9.1 (8.9, 9.3)
Hip flexor strength at last visit	9.1 (1.5)	9.3 (1.1)	9.6 (0.7)	9.0	0.05	9.6	9.0	0.6	9.2	−0.0	0.8	9.1	0.9	9.2	9.1 (8.9, 9.4)
Arm abductors															
Arm abductor strength at first visit	8.9 (1.7)	9.5 (0.9)	9.6 (0.7)	9.1	0.8	9.0	9.0	0.7	9.2	−0.1	0.1	9.1	0.8	9.0	9.0 (8.7, 9.3)
Follow-up arm abductor strength	9.4 (1.1)	9.4 (0.9)	9.7 (0.4)	9.3	0.02	9.8	9.5	0.8	9.4	0.1	0.5	9.5	0.4	9.4	9.4 (9.2, 9.6)
Arm abductor strength at last visit	9.4 (1.0)	9.5 (1.0)	9.8 (0.4)	9.3	0.08	9.7	9.4	1.0	9.4	0.1	0.6	9.7	0.2	9.3	9.4 (9.2, 9.6)
CK concentration [median (Q1–Q3)]															
CK at first visit	498 (150–1606)	**78 (46–242)*****	508 (424–762)	377	0.1	498	410	0.7	448	−0.2	0.06	404	0.7	461	428 (274, 634)
Follow-up CK	205 (99–863)	90 (54–204)*	285 (79–938)	149	0.06	254	192	0.4	176	−0.2	0.03	176	0.5	189	190 (138, 238)
Maximal CK	647 (178–2330)*	123 (50–531)**	634 (264–1597)	474	0.1	809	469	0.2	857	−0.2	0.02	430	0.1	647	518 (396, 778)
%FVC															
FVC at first visit	68.1 (19.1)*	51.2 (16.9)**	60.7 (17.2)	62.9	0.2	69.3	**71.4**	**<0.001**	**54.3**	0.2	0.09	65.1	0.9	64.3	64.6 (60.6, 68.6)
Follow-up FVC	71.4 (19.5)**	57.2 (14.3)**	61.3 (22.8)	66.7	0.4	70.3	**77.0**	**<0.001**	**55.2**	0.2	0.05	67.7	1.0	67.6	67.7 (63.8, 71.5)
FVC at last visit	76.6 (17.1)**	58.1 (17.1)**	66.1 (13.6)	69.9	0.3	75.5	**80.6**	**<0.001**	**59.4**	0.3	0.05	69.2	0.4	73.8	72.0 (66.8, 77.1)
%DLCO															
DLCO at first visit	70.0 (24.3)**	54.8 (16.6)*	56.5 (25.5)	65.1	0.7	67.5	**71.9**	**<0.001**	**54.3**	0.0	0.7	61.4	0.2	68.6	65.7 (60.8, 70.7)
Follow-up DLCO	**67.4 (23.7)****	54.5 (17.5)	53.1 (19.1)	62.5	0.9	62.9	**68.8**	**<0.001**	**53.2**	0.1	0.5	59.2	0.2	65.0	62.6 (58.2, 67.1)
DLCO at last visit	62.6 (20.2)	55.1 (12.7)	47.4 (19.3)	61.0	0.5	56.9	61.9	0.3	56.3	0.1	0.6	56.4	0.4	61.4	59.4 (54.1, 64.7)

Strength and FVC values are expressed as the mean (S.D.) and CK as the median [quartile 1–quartile 3 (Q1–Q3)]. Bivariate comparisons were made using Student's *t*-test for the strength and the Wilcoxon rank-sum test for CK. Pearson's *r* was used to calculate the correlation coefficient for strength and Spearman's ρ for the CK. Follow-up strength was defined as the mean strength of all the visits, excluding the first one. Bold values are statistically significant. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$. CK: creatine kinase; DLCO: diffusing capacity for carbon monoxide; FVC: forced vital capacity.

FIG. 1 Longitudinal evolution of percentage predicted diffusing capacity for carbon monoxide, strength and creatine kinase concentrations



Trends were calculated using locally weighted scatterplot smoothing. (**A**) Percentage predicted DLCO by autoantibody status. (**B**) Percentage predicted DLCO by race. (**C**) Strength and CK concentrations. CK: creatine kinase; DLCO: diffusing capacity for carbon monoxide.

[13] reported 5 and 10 year survival rates of 88 and 75% in anti-Jo1 patients, whereas ours were 97 and 89%. The definition of the onset of the disease (at first symptoms instead of at diagnosis), a younger population of patients and the different origin of the patients (Rheumatology [14], Internal Medicine [13] or a Multidisciplinary Clinic in our study) might explain these differences. Non-Jo1 anti-synthetase patients [12] and myositis patients with ILD [23] have been associated with higher mortality rates. We did not find significant differences in mortality between autoantibody groups, possibly because of our limited sample size or the high survival rates.

The rate of cancer was not significantly higher in this cohort than in the general population. Although some reports have associated ASyS with cancer [9, 11], those studies were not adjusted for age and sex. Further studies have refuted this observation, suggesting that ASyS is not associated with cancer [24]. Our data support the absence of association for anti-Jo1 and might suggest that cancer screening could be more limited in these patients.

Anti-Ro52 is associated with many autoimmune diseases, but it is especially prevalent in anti-Jo1 patients [11, 25]. In the present study, we confirm that the association is stronger for anti-Jo1 (74%) than for anti-PL12 (43%) or anti-PL7 (44%). Anti-Ro52 does not appear to portend worse lung involvement in ASyS but is associated with earlier development of arthritis, mechanic's hands and DM-specific skin findings.

Finally, we found a striking increase in the severity of the ILD in black compared with white patients and confirmed an association between the race and the type of ASyS autoantibody [26]. The severity of the lung involvement in black patients does not seem to be attributable to the higher prevalence of anti-PL12 in this group of patients, because both factors were independent predictors of the ILD severity without any detectable interaction between them. Thus, this would suggest that: (i) there is a mechanistic link between race and anti-PL12 autoantibodies; and (ii) both the race and the autoantibody status act as independent modifiers of the disease severity.

The present study has several limitations. First, most of the conclusions of this study are based on signs and symptoms that were recorded prospectively from the start of the study in 2003. Consequently, we could not include activity and damage tools or comprehensive arthritis scoring systems that were not available when the study started. Second, as this is a reference centre for myositis, it is possible that the most severe patients of the spectrum were selected; however, comparing our data with similar cohort studies it seems that the severity of our patients was similar or even lower than in previous studies [13, 14]. Finally, the increased ILD severity that we detected in black patients might be partly explained by socio-economic factors that we could not take into account. However, these socio-economic factors would presumably also have led to increased muscle weakness, and we could not detect this.

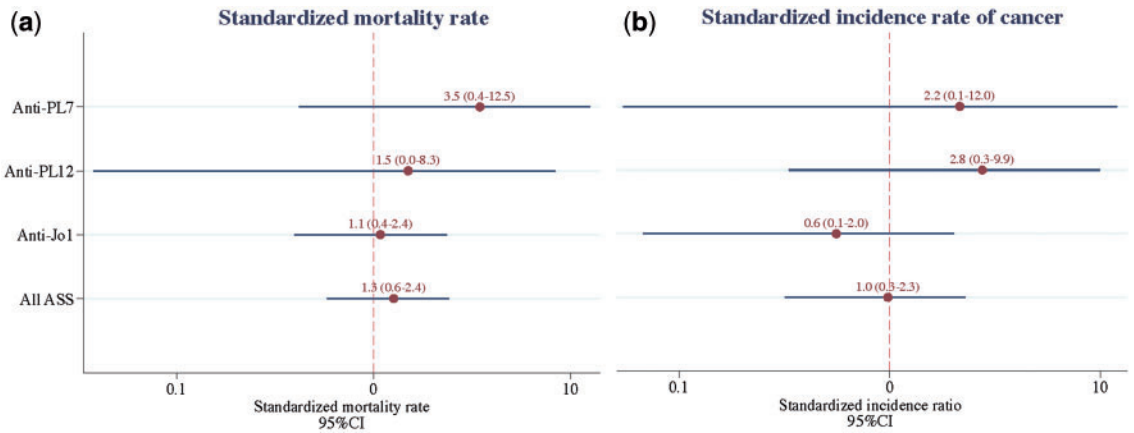
These limitations notwithstanding, this is the largest ASyS cohort study conducted longitudinally in a single centre and provides valuable information suggesting that the different anti-synthetase autoantibodies define different diseases within the ASyS spectrum. Thus, anti-PL7 and anti-PL12 syndromes are characterized by more severe ILD, whereas anti-Jo1 patients show more severe muscle involvement. Black race was identified as a major

Table 4 Multivariate longitudinal analysis of muscle and lung involvement in patients with the anti-synthetase syndrome

	Mean strength Coefficient (95% CI)	CK concentration (logarithmic scale) Coefficient (95% CI)	%FVC Coefficient (95% CI)	%DLCO Coefficient (95%CI)
Autoantibody (compared with anti-Jo1)				
Anti-PL12	0.28 (−0.27, 0.82)	**−0.42 (−0.71, −0.14)****	**−9.70 (−17.56, −1.84)***	**−10.47 (−19.71, −1.24)***
Anti-PL7	0.32 (−0.25, 0.89)	0.03 (−0.27, 0.33)	−3.92 (−13.63, 5.80)	**−13.95 (−25.42, −2.48)***
Time from onset to visit (1 year)	0.02 (−0.01, 0.06)	**−0.04 (−0.07, −0.02)*****	**0.89 (0.28,1.51)****	−0.09 (−0.49, 0.31)
Age of onset (each 10 years)	−0.05 (−0.18, 0.09)	**−0.09 (−0.17, −0.02)***	0.59 (−1.61, 2.80)	−2.15 (−4.76, 0.46)
Female *vs* male	−0.12 (−0.51, 0.28)	−0.12 (−0.33, 0.10)	−4.73 (−11.00, 1.54)	2.04 (−5.18, 9.26)
Black *vs* white	0.04 (−0.36, 0.45)	0.08 (−0.13, 0.29)	**−17.53 (−23.87, −11.18)*****	**−11.73 (−19.13, −4.32)****

Multilevel regression with random slopes and random intercepts. Multivariate analysis was adjusted by time from onset, age at onset, sex, race and immunosuppressant drugs (CS dose and treatment with IVIGs, rituximab, MMF, MTX or AZA). Bold values are statistically significant. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Fig. 2 Standard mortality and cancer incidence rates



prognostic factor associated with the severity of the ASyS ILD.

Acknowledgements

Thanks to William Kelly for his technical support in maintaining the Johns Hopkins Myositis Centre Cohort Database. S.K.D., L.C.-S. and the Myositis Research Database are supported by the The Huayi and Siuling Zhang Discovery Fund. I.P.-F.'s research is supported by a Fellowship from the Myositis Association.

Funding: This research was supported in part by the Intramural Research Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health.

Disclosure statement: L.C.-S. reports consultant fees from advisory board partipations for Novartis, Mallinckrodt, Octopharma, Idera, Option Care and MedImmune and receives royalties from Inova Diagnostics for intellectual property and grant support from CSL Behring. All other authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

References

1 Nishikai M, Reichlin M. Heterogeneity of precipitating antibodies in polymyositis and dermatomyositis. Characterization of the Jo-1 antibody system. Arthritis Rheum 1980;23:881–8.

2 Mathews MB, Reichlin M, Hughes GR, Bernstein RM. Anti-threonyl-tRNA synthetase, a second myositis-related autoantibody. J Exp Med 1984;160:420–34.

3 Bunn CC, Bernstein RM, Mathews MB. Autoantibodies against alanyl-tRNA synthetase and tRNAAla coexist and are associated with myositis. J Exp Med 1986;163:1281–91.

4 Targoff IN. Autoantibodies to aminoacyl-transfer RNA synthetases for isoleucine and glycine. Two additional synthetases are antigenic in myositis. J Immunol 1990;144:1737–43.

5 Hirakata M, Suwa A, Nagai S *et al*. Anti-KS: identification of autoantibodies to asparaginyl-transfer RNA synthetase associated with interstitial lung disease. J Immunol 1999;162:2315–20.

6 Betteridge Z, Gunawardena H, North J, Slinn J, McHugh N. Anti-synthetase syndrome: a new autoantibody to phenylalanyl transfer RNA synthetase (anti-Zo) associated with polymyositis and interstitial pneumonia. Rheumatology 2007;46:1005–8.

7 Hashish L, Trieu EP, Sadanandan P, Targoff IN. Identification of autoantibodies to tyrosyl-tRNA synthetase in dermatomyositis with features consistent with anti-synthetase syndrome. Arthritis Rheum 2005;52:S312.

8 Hervier B, Devilliers H, Stanciu R *et al*. Hierarchical cluster and survival analyses of antisynthetase syndrome: phenotype and outcome are correlated with anti-tRNA synthetase antibody specificity. Autoimmun Rev 2012;12:210–7.

9 Marie I, Josse S, Decaux O *et al*. Comparison of long-term outcome between anti-Jo1- and anti-PL7/PL12 positive patients with antisynthetase syndrome. Autoimmun Rev 2012;11:739–45.

10 Kalluri M, Sahn SA, Oddis CV *et al*. Clinical profile of anti-PL-12 autoantibody. Cohort study and review of the literature. Chest 2009;135:1550–6.

11 Marie I, Hatron PY, Dominique S *et al*. Short-term and long-term outcome of anti-Jo1-positive patients with anti-Ro52 antibody. Semin Arthritis Rheum 2012;41:890–9.

12 Aggarwal R, Cassidy E, Fertig N *et al*. Patients with non-Jo-1 anti-tRNA-synthetase autoantibodies have worse survival than Jo-1 positive patients. Ann Rheum Dis 2014;73:227–32.

13 Trallero-Araguás E, Grau-Junyent JM, Labirua-Iturburu A *et al*. Clinical manifestations and long-term outcome of anti-Jo1 antisynthetase patients in a large cohort of Spanish patients from the GEAS-IIM group. Semin Arthritis Rheum 2016;46:225–31.

14 Cavagna L, Nuño L, Scirè CA *et al*. Clinical spectrum time course in Anti Jo-1 positive antisynthetase syndrome: results from an International Retrospective Multicenter Study. Medicine 2015;94:e1144.

15 Rider LG, Werth VP, Huber AM *et al*. Measures of adult and juvenile dermatomyositis, polymyositis, and inclusion body myositis: Physician and Patient/Parent Global Activity, Manual Muscle Testing (MMT), Health Assessment Questionnaire (HAQ)/Childhood Health Assessment Questionnaire (C-HAQ), Childhood Myositis Assessment Scale (CMAS), Myositis Disease Activity Assessment Tool (MDAAT), Disease Activity Score (DAS), Short Form 36 (SF-36), Child Health Questionnaire (CHQ), physician global damage, Myositis Damage Index (MDI), Quantitative Muscle Testing (QMT), Myositis Functional Index-2 (FI-2), Myositis Activities Profile (MAP), Inclusion Body Myositis Functional Rating Scale (IBMFRS), Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI), Cutaneous Assessment Tool (CAT), Dermatomyositis Skin Severity Index (DSSI), Skindex, and Dermatology Life Quality Index (DLQI). Arthritis Care Res 2011;63 (Suppl 11):S118–57.

16 Travis WD, Costabel U, Hansell DM *et al*. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013;188:733–48.

17 MacIntyre N, Crapo RO, Viegi G *et al*. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. Eur Respir J 2005;26:720–35.

18 Troyanov Y, Targoff IN, Tremblay JL *et al*. Novel classification of idiopathic inflammatory myopathies based on overlap syndrome features and autoantibodies: analysis of 100 French Canadian patients. Medicine 2005;84:231–49.

19 Werner JL, Christopher-Stine L, Ghazarian SR *et al*. Antibody levels correlate with creatine kinase levels and strength in anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase-associated autoimmune myopathy. Arthritis Rheum 2012;64:4087–93.

20 Pinal-Fernandez I, Parks C, Werner JL *et al*. Longitudinal course of disease in a large cohort of myositis patients with autoantibodies recognizing the signal recognition particle. Arthritis Care Res 2017;69:263–70.

21 Mulcahy KP, Langdon PC, Mastaglia F. Dysphagia in inflammatory myopathy: self-report, incidence, and prevalence. Dysphagia 2012;27:64–9.

22 Oh TH, Brumfield KA, Hoskin TL *et al*. Dysphagia in inflammatory myopathy: clinical characteristics, treatment strategies, and outcome in 62 patients. Mayo Clin Proc 2007;82:441–7.

23 Johnson C, Pinal-Fernandez I, Parikh R *et al*. Assessment of mortality in autoimmune myositis with and without associated interstitial lung disease. Lung 2016;194:733–7.

24 Chinoy H, Fertig N, Oddis CV, Ollier WE, Cooper RG. The diagnostic utility of myositis autoantibody testing for predicting the risk of cancer-associated myositis. Ann Rheum Dis 2007;66:1345–9.

25 Rutjes SA, Vree Egberts WT, Jongen P *et al*. Anti-Ro52 antibodies frequently co-occur with anti-Jo-1 antibodies in sera from patients with idiopathic inflammatory myopathy. Clin Exp Immunol 1997;109:32–40.

26 Johnson C, Connors GR, Oaks J *et al*. Clinical and pathologic differences in interstitial lung disease based on antisynthetase antibody type. Respir Med 2014;108:1542–8.