Clinical impact of anti-SSA antigenic specificity on disease activity and patient-reported outcomes in primary Sjögren's disease: a real-life cohort study

S. Fonzetti[1], G. La Rocca[1], G. Fulvio[1], B. Dei[1], C. Porciani[2], E. Elefante[1], F. Ferro[1], P. Migliorini[2], M. Mosca[1], C. Baldini[1]

[1]Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Pisa; [2]Clinical Immunology Unit, Department of Internal Medicine, University of Pisa, Italy.

Abstract

Objective

Anti-Ro/SSA antibodies are a hallmark of Sjögren's disease (SjD), yet the clinical implications of distinct antigenic targets remain insufficiently defined. We aimed to determine whether specific anti-SSA antigenic profiles-isolated anti-Ro52, isolated anti-Ro60, double anti-Ro52/anti-Ro60, or triple anti-Ro52/anti-Ro60/anti-La/SSB positivity, are associated with distinct systemic, serological, and patient-reported phenotypes in real-life clinical practice.

Methods

We analysed 279 anti-SSA-positive SjD patients fulfilling 2016 ACR/EULAR criteria. Participants were stratified into the four serological subsets and compared for demographics, cumulative ESSDAI domains, systemic activity (ESSDAI), immunologic markers (IgG, C3, C4), and patient-reported outcomes (ESSPRI and subdomains) at study entry. Group comparisons used ANOVA/ANCOVA and Kruskal-Wallis tests, with modified Poisson regression for adjusted prevalence ratios and general linear models for adjusted means.

Results

Triple-positive patients displayed the highest systemic inflammatory activity (ESSDAI median 3 [IQR 1–6]) and IgG levels (1497 [1180–1790] mg/dL; $p<0.001$), enriched for haematologic, lymphoid, glandular and biological domains. In contrast, isolated anti-Ro60 patients showed the mildest systemic activity (ESSDAI 0 [0–0]) yet the highest patient-reported burden (ESSPRI 6.08 [0.52]; $p<0.01$), revealing a marked dissociation between objective inflammation and symptoms. Isolated anti-Ro52 displayed the broadest and most heterogeneous phenotype, and older age at diagnosis. In adjusted analyses, only triple-positive patients remained independently associated with increased systemic activity and B-cell hyperactivity (all $p<0.05$). Symptom measures showed the opposite gradient: isolated anti-Ro52 and anti-Ro60 subsets had the highest adjusted ESSPRI, pain, and fatigue scores, whereas double- and triple-positive patients reported substantially fewer symptoms. When stratified by combined ESSDAI/ESSPRI scores, low-activity/high-symptom cases predominated and were enriched among isolated anti-Ro52 and anti-Ro60 patients.

Conclusion

Anti-SSA antigenic specificity delineates biologically and clinically distinct phenotypes within SjD. These data support serology-informed, multidimensional disease stratification as a foundation for precision-targeted management in SjD.

Key words

Sjögren's disease, anti-SSA, Ro52, ESSDAI, ESSPRI

Silvia Fonzetti, MD
Gaetano La Rocca, MD
Giovanni Fulvio, MD
Beatrice Dei, MD
Caterina Porciani, MD
Elena Elefante, MD
Francesco Ferro, MD
Paola Migliorini, MD
Marta Mosca, MD, PhD
Chiara Baldini, MD, PhD

Please address correspondence to:
Chiara Baldini
U.O. di Reumatologia,
Dipartimento di Medicina
Clinica e Sperimentale,
Università di Pisa,
Via Roma 67,
56126 Pisa, Italy.
E-mail: chiara.baldini74@gmail.com

Received on November 4, 2025; accepted in revised form on December 11, 2025.

Competing interests: M. Mosca has served as advisor/speaker for Abbvie, AstraZeneca, Biogen, BMS, Chugai, GSK, Idorsia, LIlly, Miltenyi, Novartis, Roche and UCB.
C. Baldini has received consultancy fees from Amgen, Aurina, BMS, GSK, J&J, Novartis and Sanofi.
The other authors have declared no competing interests.

Introduction

Sjögren's disease (SjD) is a heterogeneous systemic autoimmune disorder that predominantly affects middle-aged women and is characterised by lymphocytic infiltration of exocrine glands, with clinical phenotypes ranging from isolated sicca symptoms to multi-organ involvement (1-4).

Anti-Ro/SSA antibodies are a serological hallmark of SjD and carry major diagnostic weight (three points) in the 2016 ACR/EULAR classification criteria (5). They can be present in up to 70% of SjD patients according to the detection method and are frequently accompanied by anti-La/SSB autoantibodies (1, 6).

The anti-Ro/SSA system comprises two distinct antigens: Ro52 (TRIM21) and Ro60 (TROVE2) that differ in molecular structure, biological function and clinical associations (7). Ro60 is a ribonucleoprotein involved in the quality control of misfolded RNA, whereas Ro52 is an E3 ubiquitin ligase implicated in cell-cycle regulation, innate immune responses, and interferon signalling (8-9). Although anti-Ro52 and anti-Ro60 antibodies are traditionally grouped together under the anti-SSA umbrella, accumulating evidence indicates that they reflect divergent biological pathways and are also associated with partially distinct clinical profiles. Anti-Ro60 is frequent in SjD and systemic lupus erythematosus (SLE), often accompanied by anti-Sm or anti-RNP, while anti-Ro52 occurs in SjD but also across inflammatory myopathies, systemic sclerosis, autoimmune liver disease, and other immune-mediated or even infectious/malignant conditions (7, 10). Because autoantibodies may target these proteins independently, separate detection of anti-Ro52 and anti-Ro60 has been strongly advocated (11).

Given the heterogeneity of SjD, anti-SSA antigenic specificity may contribute not only to the disease diagnosis but also to patients' stratification with clinically meaningful differences in disease expression, prognosis, and therapeutic needs. Namely, within SjD, double positivity for anti-Ro52 and anti-Ro60 has been consistently associated with higher disease activity, enhanced B-cell hyperactivity, and a stronger type I interferon signature, whereas the phenotypic implications of isolated anti-Ro52 reactivity remain more heterogeneous and less clearly defined (12-14). Deroo *et al*. (15) further demonstrated that patients triple-positive for anti-Ro52, anti-Ro60, and anti-La/SSB display more severe glandular and extra glandular involvement, suggesting that serological combinations may reflect underlying immunologic states.

Despite these insights, the clinical implications of isolated anti-Ro52, isolated anti-Ro60, double, or triple anti-Ro reactivity remain insufficiently understood, particularly in real-life, deeply phenotyped cohorts. Indeed, most available studies have been limited by incomplete phenotyping or by lack of patient-reported outcomes (PROs). Therefore, by integrating cumulative systemic involvement, current disease activity, and symptom burden, our study aims to clarify whether anti-SSA antigenic specificity delineates clinically meaningful subsets relevant for both prognosis and therapeutic stratification.

Material and methods

We conducted a monocentric, cross-sectional observational study nested within a longitudinal cohort of patients with primary SjD. All patients had been managed and monitored at the same Rheumatology Unit by a dedicated multidisciplinary team, according to shared diagnostic and follow-up procedures. This setting allowed for homogeneous, longitudinal phenotyping from diagnosis through subsequent visits.

A total of 352 consecutive patients fulfilling the 2016 ACR/EULAR classification criteria for SjD (5) were enrolled from November 2024 onwards. Out of them, for the present analysis, we included the 279 patients who tested positive for anti-SSA antibodies.

Current disease activity and PROs were assessed cross-sectionally at the time of study inclusion using the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) (16) and the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI), respectively (17). Accordingly, systemic activity was

categorised as low (ESSDAI <5) and moderate-high (ESSDAI ≥5) (16). An acceptable symptom burden was defined as an ESSPRI <5 (17). At study entry, laboratory biomarkers including IgG levels, C3, C4 were also recorded.
In parallel, cumulative organ involvement was retrospectively derived from the entire longitudinal follow-up at our centre. For each patient, lifetime ESSDAI-defined systemic domains were retrieved through a detailed review of medical records from diagnosis onwards. Age at diagnosis and disease duration were also retrieved.
Autoantibodies were detected using a commercially available line immunoblot assay (EUROIMMUN, Lübeck, Germany). Antigen-coated immunoblot strips were automatically processed and digitally analysed using the EUROLineScan software (EUROIMMUN). Anti-SSA-positive patients were stratified into four serological subsets according to antigenic specificity: isolated anti-Ro52 reactivity, isolated anti-Ro60 reactivity, double anti-Ro52/anti-Ro60 positivity, and triple anti-Ro52/anti-Ro60/anti-La/SSB positivity.
This study was approved by the local Ethics Committee (ID 26517, protocol "SJOGREN PERSPECT", 29 October 2024) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Statistical analysis
Descriptive statistics were used to summarise demographic, serological, histological, and clinical features across the four anti-SSA serological subsets. Group comparisons were performed using ANOVA or ANCOVA (with adjustment for age at diagnosis or disease duration when appropriate) for continuous variables, and χ^2 test for categorical variables. A two-tailed p-value <0.05 was considered statistically significant.
For cumulative ESSDAI domains (binary outcomes), we fitted generalised linear models to estimate adjusted associations between serological subset and organ involvement. Given the prevalence of most outcomes, modified Poisson regression with robust variance was used to report adjusted prevalence ratios (PRs) and 95% confidence intervals, including sex and disease duration as covariates (isolated anti-Ro60 as reference group). SPSS v. 19 (IBM Corp., Armonk, NY, USA) was used for all analyses.

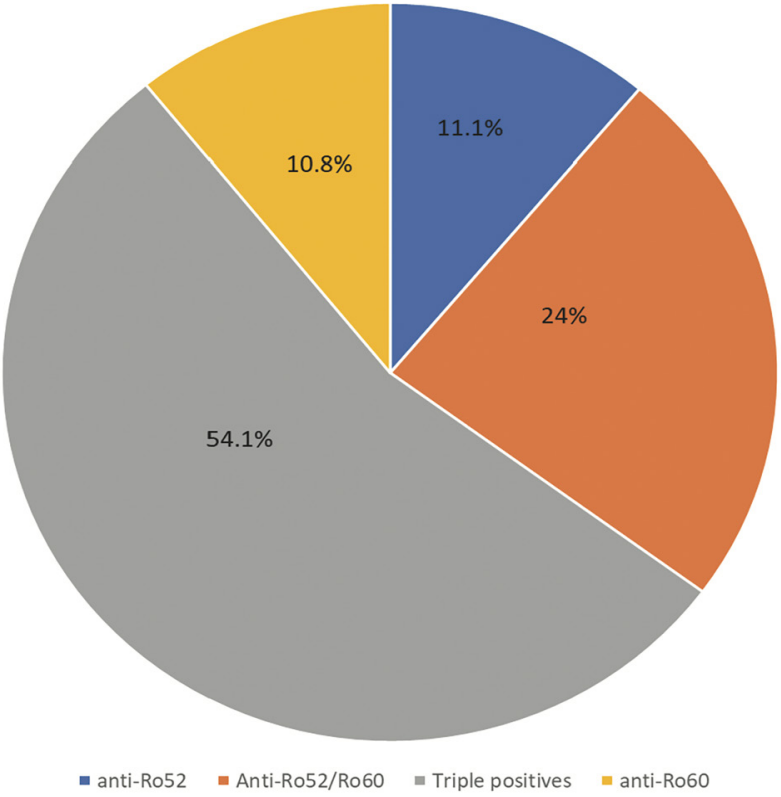

Fig. 1. Distribution of anti-SSA serological subsets in the study cohort.
Proportion of 279 anti-SSA-positive patients were stratified according to antigenic specificity. Triple anti-Ro52/Ro60/La (SSB) positivity represented the majority of the cohort (54.1%), followed by double anti-Ro52/Ro60 reactivity (24.0%), isolated anti-Ro52 (11.1%), and isolated anti-Ro60 (10.8%).

Results

Study population
A total of 279 anti-SSA-positive patients were included in the analysis, of whom 264 (94.6%) were female. Based on anti-SSA antigenic specificity, 31 (11.1%) presented isolated anti-Ro52 antibodies, 67 (24.0%) had double anti-Ro52/anti-Ro60 positivity, 151 (54.1%) were triple-positive for anti-Ro52/anti-Ro60 and anti-La/SSB, and 30 (10.8%) displayed isolated anti-Ro60 antibodies (Fig 1).
Sex distribution was comparable across the four serological subsets, with a consistent female predominance. Notably, isolated anti-Ro52 patients included the highest proportion of males, although this difference was not statistically significant. Current age at study inclusion was similar across groups. In contrast, clear differences emerged in age at diagnosis and disease duration: isolated anti-Ro52 patients were diagnosed at a substantially older age and had shorter disease duration than double- and triple-positive individuals (Table I).

Cumulative extra-glandular involvement
Cumulative extra-glandular involvement showed a consistent serology-dependent gradient (Table II). Triple-positive patients displayed the broadest and most pronounced systemic involvement, with the highest frequencies of haematologic, lymphoid, glandular, articular and biological abnormalities. Double anti-Ro52/Ro60 patients showed an intermediate pattern, whereas isolated anti-Ro52 and especially isolated anti-Ro60 patients represented the lower end of the inflammatory spectrum. Notably, isolated anti-Ro60 patients consistently exhibited the lowest prevalence of systemic domains and the highest frequency of fibromyalgia,

Table I. Demographic features of the study population stratified by serological subset.

	Isolated anti-Ro60 (n=30)	Isolated anti-Ro52 (n=31)	Double anti-Ro52/Ro60 (n=67)	Triple Ro52/Ro60/SSB (n=151)	*p*-value
Gender: female, n (%)	29 (96.7)	27 (87.1)	66 (98.5)	142 (94)	0.122
Age at diagnosis, mean (SD)	51.47 (8.96)	58.13 (13.75)	48.15 (13.12)	48.71 (15.11)	**0.005**
Disease duration, mean (SD)	7.73 (6.01)	5.84 (5.13)	10.81 (9.92)	11.18 (8.93)	**0.006**

Statistically significant differences were observed for age at diagnosis and disease duration, with isolated anti-Ro52 patients being older and double/triple-positive patients having longer disease duration.

Table II. Frequency of cumulative extra glandular involvement across serological subsets.

Domain (cumulative)	Isolated anti-Ro60 (n=30)	Isolated anti-Ro52 (n=31)	Double anti-Ro52/Ro60 (n=67)	Triple Ro52/Ro60/SSB (n=151)	p (χ^2)
Cutaneous	3 (10.0%)	5 (16.1%)	9 (13.4%)	35 (23.2%)	0.183
Haematologic	6 (20.0%)	8 (25.8%)	22 (32.8%)	68 (45.0%)	**0.018**
Renal	0 (0.0%)	2 (6.5%)	2 (3.0%)	12 (7.9%)	0.245
Articular	7 (23.3%)	12 (38.7%)	25 (37.3%)	75 (49.7%)	**0.035**
CNS	0 (0.0%)	0 (0.0%)	2 (3.0%)	1 (0.7%)	0.361
PNS	1 (3.3%)	0 (0.0%)	3 (4.5%)	6 (4.0%)	0.711
Muscle domain	0 (0.0%)	0 (0.0%)	1 (1.5%)	4 (2.6%)	0.620
Lung (non-ILD)	0 (0.0%)	3 (9.7%)	11 (16.4%)	16 (10.6%)	0.117
ILD	0 (0.0%)	2 (6.5%)	5 (7.5%)	9 (6.0%)	0.524
Constitutional	0 (0.0%)	2 (6.5%)	4 (6.0%)	12 (7.9%)	0.448
Lymphadenopathy	6 (20.0%)	5 (16.1%)	22 (32.8%)	70 (46.4%)	**0.001**
Salivary gland enlargement	5 (16.7%)	6 (19.4%)	19 (28.4%)	64 (42.4%)	**0.005**
Biological	11 (36.7%)	14 (45.2%)	37 (55.2%)	115 (76.2%)	**<0.001**
MALT	0 (0.0%)	1 (3.2%)	2 (3.0%)	8 (5.3%)	0.538
Fibromyalgia	16 (53.3%)	9 (29.0%)	25 (39.1%)	37 (25.9%)	**0.017**
Thyroid involvement	9 (34.6%)	11 (39.3%)	18 (28.6%)	35 (25.2%)	0.419
PBC	0 (0.0%)	1 (3.2%)	3 (4.5%)	1 (0.7%)	0.191

Frequencies represent lifetime occurrence of ESSDAI-defined organ involvement across serological subsets. Significant differences (Pearson's χ^2 test) were observed for haematologic, articular, lymphadenopathy, salivary gland enlargement, and biological domains ($p<0.05$), with triple-positive patients consistently showing the highest cumulative burden. Fibromyalgia, although not an ESSDAI domain, was included due to its clinical relevance and showed an inverse association with systemic inflammatory activity, being most prevalent in isolated anti-Ro60 and least prevalent in triple-positive patients.
Bold values indicate statistically significant results.

supporting a predominantly symptom-driven phenotype.

Interstitial lung disease occurred exclusively in anti-Ro52-positive groups (isolated Ro-52, double-positive, and triple-positive), although numbers were small and differences did not reach statistical significance. No substantial between-group differences were observed for renal, cutaneous, constitutional, muscular, neurologic, or thyroid involvement. MALT lymphoma was rare across all subsets (3–5%) and absent in isolated anti-Ro60 patients, without statistically significant differences.

After adjustment for disease duration and sex (Table III), triple-positive patients remained significantly more likely to present haematologic, lymphoid, salivary-gland and biological involvement compared with isolated anti-Ro60. In contrast, fibromyalgia was significantly less frequent among triple-positive individuals, further rein-

Table III. Adjusted prevalence ratios (PRs) for cumulative ESSDAI domain involvement by anti-SSA serological subset.

Domain	Subset	Adjusted PR (95% CI)	*p*-value
Haematologic	Isolated Ro52	1.35 (0.54–3.43)	0.522
	Double Ro52/Ro60	1.57 (0.71–3.47)	0.264
	Triple Ro52/Ro60/SSB	**2.17 (1.04–4.54)**	**0.039**
Lymphadenopathy	Isolated Ro52	0.76 (0.26–2.23)	0.621
	Double Ro52/Ro60	1.68 (0.76–3.72)	0.201
	Triple Ro52/Ro60/SSB	**2.32 (1.11–4.85)**	**0.025**
SGE	Isolated Ro52	1.16 (0.40–3.38)	0.786
	Double Ro52/Ro60	1.64 (0.67–3.99)	0.277
	Triple Ro52/Ro60/SSB	**2.42 (1.06–5.52)**	**0.037**
Biological domain	Isolated Ro52	1.23 (0.67–2.26)	0.511
	Double Ro52/Ro60	1.50 (0.90–2.52)	0.123
	Triple Ro52/Ro60/SSB	**2.07 (1.28–3.34)**	**0.003**
Fibromyalgia	Isolated Ro52	0.59 (0.31–1.13)	0.110
	Double Ro52/Ro60	0.68 (0.43–1.09)	0.108
	Triple Ro52/Ro60/SSB	**0.46 (0.30–0.72)**	**0.001**

Adjusted prevalence ratios (PRs) and 95% confidence intervals were derived from modified Poisson regression models with robust variance estimation (GENLIN procedure, Poisson distribution, log link, COVB=ROBUST), adjusting for sex and disease duration. The isolated anti-Ro60 subset served as the reference category (PR=1.00), presenting the lowest level of activity and the highest prevalence of fibromyalgia within the cohort. Triple anti-Ro52/Ro60/SSB positivity remained independently associated with haematologic, lymphadenopathy, salivary-gland enlargement (SGE), and biological domains, whereas fibromyalgia showed the lowest adjusted prevalence in triple-positive patients.

Table IV. Adjusted estimated marginal means of current systemic activity (ESSDAI) and serum IgG levels by serological subset.

Serological subset	Adjusted ESSDAI mean (95% CI)	Adjusted IgG mean, mg/dL (95% CI)
Isolated anti-Ro60	2.27 (0.26–4.28)	1276 (1003–1550)
Isolated anti-Ro52	3.64 (0.72–5.53)	1179 (927–1432)
Double Ro52/Ro60	4.05 (2.44–5.66)	1395 (1170–1620)
Triple Ro52/Ro60/SSB	4.51 (3.22–5.80)	1616 (1427–1806)

General linear models adjusted for disease duration and sex estimated means with 95% CIs.
Adjusted estimated marginal means and 95% confidence intervals were derived from general linear models controlling for sex and disease duration (Type III SS). The overall effect of serological subset was not statistically significant for ESSDAI (p=0.108), although a biological gradient was apparent, with higher systemic activity in double- and triple-positive patients. In contrast, IgG levels differed significantly across subsets (p<0.001), showing a progressive increase from isolated anti-Ro60 and anti-Ro52 to double- and triple-positive patients.

forcing the divergence between disease activity-dominant and symptom-dominant serological subsets.

Current disease activity: ESSDAI domains at study inclusion

At study entry, overall systemic activity was low across all serological subsets (median 2 [IQR 0–5]). Nonetheless, a clear gradient emerged: triple-positive patients displayed the highest current ESSDAI scores (median 3 [1–6]), followed by double-positive (2 [0–4]) and isolated anti-Ro52 patients (1 [0–3]), whereas isolated anti-Ro60 individuals consistently showed minimal activity (0 [0–0]) (p=0.002). Most organ-specific domains were inactive in the majority of patients; the main exception was the biological domain, which showed the greatest between-group variability with median values of 1 (IQR 0–1) in triple-positive and double Ro52/Ro60 groups, compared with 0 (IQR 0–1) in isolated anti-Ro52 and 0 (IQR 0–0) in isolated anti-Ro60 patients (p<0.001).

Immunological features followed similar patterns. Triple-positive patients exhibited the highest IgG levels (median 1497 mg/dL [IQR 1180–1790]), with progressively lower values in double-positive (1363 mg/dL [1080–1690]), isolated anti-Ro52(1081 mg/dL [920–1530]), and isolated anti-Ro60 subsets (median 1181 mg/dL [1050–1390]) (p<0.001), reflecting a continuum from activity-dominant to low-activity phenotypes. Complement levels (C3 and C4) did not differ significantly across groups.

These trends remained evident in adjusted analyses. Although differences in ESSDAI did not reach statistical significance after adjusting for disease duration and sex, the estimated marginal means preserved the same gradient observed in the unadjusted data: triple- and double-positive patients showed the highest adjusted ESSDAI values, while isolated anti-Ro60 individuals consistently demonstrated the lowest activity. Adjusted IgG levels displayed an analogous pattern, reinforcing the biological distinction between higher-activity (double/triple positive) and lower-activity subsets (isolated anti-Ro52 and anti-Ro60) (Table IV).

PROs across subgroups at study inclusion

PROs at study entry differed substantially across serological subsets, revealing a pattern broadly opposite to that observed for systemic activity (Table V). Isolated anti-Ro52 and isolated anti-Ro60 patients consistently reported the highest symptom burden, with markedly elevated ESSPRI, pain and fatigue scores, whereas double-positive and especially triple-positive patients exhibited lower levels of self-reported symptoms. Dryness scores showed weaker discrimination among groups and did not follow a clear serology-driven pattern.

Adjusted ESSPRI, pain, and fatigue scores were highest in isolated anti-Ro52 and isolated anti-Ro60 subsets, intermediate in double-positive patients, and lowest in triple-positive individuals (Table VI, Fig. 2).

Composite clinical phenotypes integrating ESSDAI and ESSPRI

Integrating ESSDAI and ESSPRI into composite clinical phenotypes further highlighted these contrasts. Triple-positive patients, who displayed the highest levels of systemic activity, reported the lowest symptom burden, consistent with an inflammation-dominant phenotype. Conversely, isolated anti-Ro52 and isolated anti-Ro60 patients showed the opposite configuration: markedly elevated ESSPRI scores despite minimal ESSDAI activity, thus defining a symptom-dominant, low-active phenotype. Double anti-Ro52/Ro60 patients occupied an intermediate position, suggesting a biological and clinical continuum between the two extremes (Fig. 3).

Overall, when stratifying patients into composite phenotypes according to systemic inflammatory activity (ESSDAI <5 *vs.* ≥5) and patient-reported symptom burden (ESSPRI <5 *vs.* ≥5), clear serology-specific distribution patterns emerged (p<0.001). The dominant phenotype low inflammatory activity but high symptom burden (ESSDAI<5/ESSPRI≥5) was strikingly

Table V. Patient-reported outcomes (non-adjusted) across serological subsets (median [IQR]).

	Isolated anti-Ro60 (n=30)	Isolated anti-Ro52 (n=31)	Double anti-Ro52/Ro60 (n=67)	Triple anti-Ro52/Ro60/SSB (n=151)	p (Kruskal-Wallis)
ESSPRI	7.7 [6.1–7.9]	6.8 [6.0–7.6]	6.3 [5.5–6.7]	5.2 [4.9–5.7]	**<0.001**
VAS-dryness	7.0 [5.9–8.1]	6.5 [5.7–7.6]	7.0 [5.7–7.1]	7.0 [5.7–6.6]	0.25
VAS-ocular dryness	8.0 [5.7–8.1]	8.0 [5.8–8.1]	7.0 [5.7–7.1]	7.0 [5.4–6.4]	0.18
VAS-oral dryness	7.0 [5.5–7.7]	6.0 [4.7–7.0]	7.0 [5.2–6.8]	6.5 [5.6–6.6]	0.63
VAS-fatigue	8.0 [6.0–8.0]	7.0 [5.9–7.6]	7.0 [5.4–6.8]	5.0 [4.8–5.8]	**0.004**
VAS-pain	8.0 [5.9–6.9]	7.0 [6.0–7.9]	6.0 [5.0–6.5]	5.0 [3.9–5.0]	**<0.001**

Table VI. Adjusted estimated marginal means of current patient-reported outcomes (PROs) by serological subset.

	ESSPRI (95% CI)	VAS pain (95% CI)	VAS fatigue (95% CI)	VAS ocular dryness (95% CI)	VAS oral dryness (95% CI)	VAS dryness (95% CI)
Isolated anti-Ro60	6.08 (5.05–7.10)	6.11 (4.74–7.48)	6.30 (5.06–7.54)	6.83 (5.55–8.11)	6.27 (4.96–7.59)	5.92 (4.91–6.93)
Isolated anti-Ro52	6.00 (5.04–6.96)	6.18 (4.88–7.49)	6.05 (4.90–7.21)	6.75 (5.55–7.95)	5.62 (4.40–6.83)	5.15 (4.26–6.04)
Double Ro52/Ro60	4.89 (4.04–5.74)	4.66 (3.52–5.81)	5.07 (4.05–6.09)	5.89 (4.84–6.94)	5.35 (4.28–6.42)	5.08 (4.37–5.79)
Triple Ro52/Ro60/SSB	4.19 (3.50–4.87)	3.48 (2.53–4.44)	4.41 (3.60–5.22)	5.65 (4.81–6.49)	5.60 (4.75–6.45)	6.12 (5.04–7.20)

General linear models adjusted for disease duration and sex (Type III SS); estimated means with 95% CIs.
Estimated marginal means and 95% confidence intervals were obtained from general linear models adjusted for sex and disease duration (Type III SS). Serological subsets differed significantly in global symptom burden (ESSPRI, $p<0.001$), pain ($p<0.001$) and fatigue ($p=0.001$), with double- and triple-positive patients reporting markedly lower adjusted symptom levels. In contrast, isolated anti-Ro60 and anti-Ro52 subsets showed consistently higher ESSPRI, pain and fatigue scores. Among dryness measures, ocular dryness showed only a non-significant trend ($p=0.097$), oral dryness did not differ across subsets ($p=0.588$), while global dryness VAS demonstrated a borderline, non-significant difference ($p=0.092$).

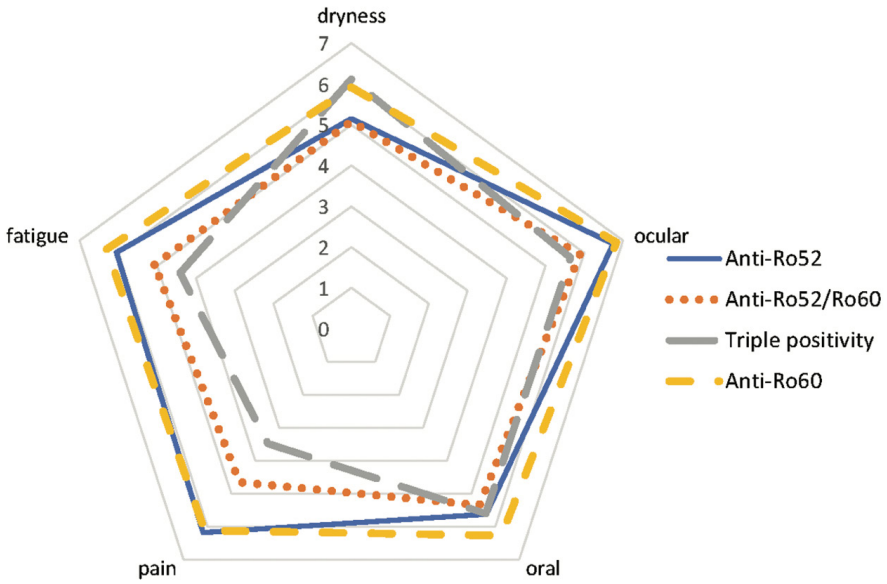

Fig. 2. Patient-reported outcomes (PROs) across serological subsets.
Radar plot illustrating adjusted mean scores of PROs: pain, fatigue, ocular dryness, oral dryness, and global dryness-across the four serological subsets. Values represent estimated marginal means derived from general linear models adjusted for sex and disease duration (Type III SS). Double- and triple-positive patients report lower symptom burden, whereas isolated anti-Ro60 and anti-Ro52 show higher PRO scores.

over-represented among isolated anti-Ro52 (76%) and isolated anti-Ro60 (72%) patients, while accounting for only 38% of triple-positive cases. Conversely, patients with low symptom burden were relatively enriched in triple-positive individuals and least frequent among isolated Ro52. The fully high-activity/high-symptom phenotype (~16% overall) remained numerically limited and did not show a strong serology-dependent pattern.

Discussion

In this deeply phenotyped, real-life cohort of 279 patients, anti-SSA antigenic specificity delineated distinct clinical and biological profiles. Triple-positive individuals showed the highest systemic activity, elevated IgG levels, and broader cumulative organ involvement. At the opposite end, isolated anti-Ro52 and isolated anti-Ro60 patients exhibited minimal systemic activity but a disproportionate symptom burden. Interstitial lung disease occurred exclusively in anti-Ro52-positive subsets, whereas isolated anti-Ro60 defined a distinctly low-activity, ILD-sparing phenotype. Double-positive patients showed intermediate levels of systemic activity, biological markers, and patient-reported symptoms, bridging the activity-dominant and symptom-dominant profiles observed at the two extremes of the serological spectrum.

Our findings refine and extend previous serology-based stratification studies. Robbins *et al*. (7), Armagan *et al*. (12) and Deroo *et al*. (15) had already suggested that triple positivity identifies a strongly B-cell-driven, interferon-high state. Consistent with these observations, triple-positive patients in our cohort showed the most pronounced systemic activity, and the lowest prevalence of fibromyalgia. Conversely, isolated anti-Ro60 emerged as the lowest active phenotype with very limited systemic involvement and near-absence of ILD. Nevertheless, these patients reported the greatest fatigue, pain, and ESSPRI burden. Taken together, these observations support an evolving paradigm in which Ro60 marks a predominantly glandular autoimmune profile with limited systemic extension, whereas Ro52 subsets reflect a distinct interferon-driven systemic biology with a particular predilection for lung involvement (8, 14, 18-22). Notably, the isolated anti-Ro52 subset displayed marked internal heterogeneity. While many patients aligned with the symptom-dominant, low-inflammatory phenotype, a subset exhibited features suggestive of a more complex immunological background. Older age at diagnosis and a less markedly female distribution further support the view that isolated anti-Ro52 represents a composite rather than a uniform serological entity (21-23).

Integration of ESSDAI and ESSPRI further highlighted this divergence. The low-ESSDAI/high-ESSPRI phenotype was markedly enriched in isolated anti-Ro52 and anti-Ro60 patients, whereas the reciprocal high-ESSDAI/low-ESSPRI profile occurred almost exclusively in the double- and triple-positive subsets. This dissociation between sys-

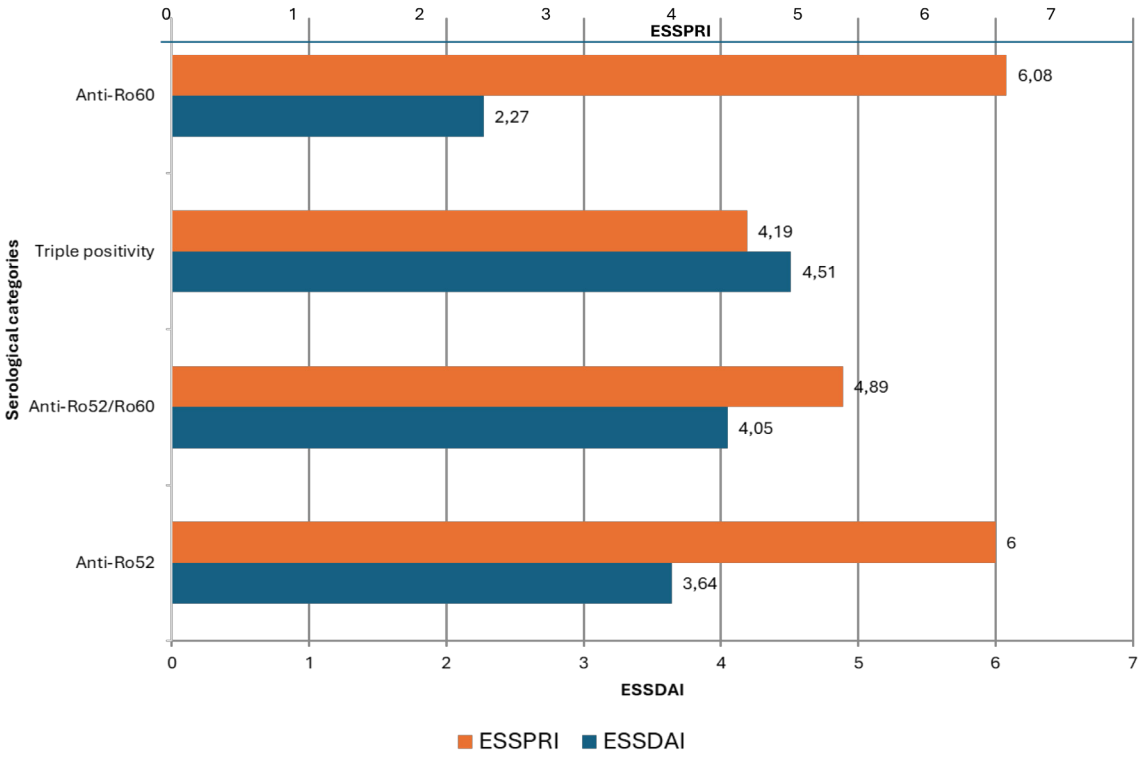

Fig. 3. Divergent patterns of systemic activity and patient-reported burden across anti-SSA serological subsets.
Adjusted mean ESSDAI (blue) and ESSPRI (orange) scores across the four anti-SSA serological subsets. Values represent estimated marginal means from general linear models adjusted for sex and disease duration (Tables III and IV). Triple-positive patients exhibited the highest systemic inflammatory activity (ESSDAI) and the lowest symptom burden (ESSPRI), defining an inflammation-dominant phenotype. In contrast, isolated anti-Ro52 and anti-Ro60 subsets showed disproportionately high ESSPRI despite low ESSDAI scores, consistent with symptom-dominant, low-active profiles. Double-positive patients displayed intermediate values between these extremes.

temic inflammation and patient-reported burden has not been described in previous serology-focused cohorts, which seldom incorporated PROs. Integrating the evaluation of clinical disease activity and symptom burden at study entry with biological markers and cumulative organ involvement provides a multidimensional stratification model that advances current frameworks and aligns with precision-medicine objectives in SjD.

A major strength of our study is its monocentric design, which ensured uniform clinical assessment and laboratory evaluation based on harmonised procedures. All serological and immunological tests were performed in the same laboratory using standardised methods, thereby eliminating the inter-laboratory variability that may affect multicentre studies (24). This homogeneous approach minimises interobserver differences and reduces methodological noise, allowing true biological differences between serological subsets to emerge more clearly. Furthermore, to our knowledge, this is the first study to integrate PROs with detailed serological stratification within a monocentric SjD cohort assessed using fully standardised clinical and laboratory procedures.

This study has limitations. The cross-sectional assessment of ESSDAI and PROs does not allow inference on longitudinal trajectories. Nevertheless, differences in cumulative ESSDAI support the stability of these serological phenotypes. Although one of the largest real-life anti-Ro52/anti-Ro60 cohorts to date, sample size remains limited for rare manifestations such as ILD or lymphoma. In addition, the absence of quantitative antibody titres and interferon signatures restricts further biological interpretation. Finally, anti-Ro52 antibodies may target diverse epitopes (25), but epitope-level stratification was not performed, precluding more detailed phenotypic resolution.

Overall, however, our findings support the presence of two distinct clinical-immunological patterns. Double- and triple-positive patients show a phenotype characterised by higher systemic activity and stronger B-cell-driven inflammation, whereas isolated anti-Ro52 and isolated anti-Ro60 subsets present with low inflammatory activity but a disproportionately high symptom burden. From a clinical standpoint, triple-positive patients appear to be the most suitable candidates for immune-modifying and B-cell-directed therapies. In contrast, in isolated anti-Ro52 or anti-Ro60 patients, the dissociation between systemic activity and symptom severity indicates that treatment strategies focused on symptom management-such as approaches targeting pain and fatigue-may be more appropriate than intensifying immunosuppression.

In conclusion, anti-SSA antigenic specificity identifies distinct clinical phenotypes within primary SjD, that are not captured by disease activity alone. These findings support serology-informed, multidimensional profiling as a key step toward precision medicine, as activity-driven and symptom-dominant subsets clearly require different therapeutic approaches.

References

1. BRITO-ZERÓN P, BALDINI C, BOOTSMA H *et al*.: Sjögren syndrome. *Nat Rev Dis Primers* 2016; 2:16047. https://doi.org/10.1038/nrdp.2016.47
2. TROMBY F, MANFRÈ V, CHATZIS LG *et al*.: Clinical manifestations, imaging and treatment of Sjögren's disease: one year in review 2024. *Clin Exp Rheumatol* 2024; 42(12): 2322-35. https://doi.org/10.55563/clinexprheumatol/5xq3fb
3. BALDINI C, CHATZIS LG, FULVIO G, LA ROCCA G, PONTARINI E, BOMBARDIERI M: Pathogenesis of Sjögren's disease: one year in review 2024. *Clin Exp Rheumatol* 2024; 42(12): 2336-43. https://doi.org/10.55563/clinexprheumatol/i8iszc
4. LONGHINO S, CHATZIS LG, DAL POZZOLO R *et al*.: Sjögren's syndrome: one year in review 2023. *Clin Exp Rheumatol* 2023; 41(12): 2343-56. https://doi.org/10.55563/clinexprheumatol/255qsx
5. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al*.: 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: a consensus and data-driven methodology involving three international patient cohorts. *Arthritis Rheumatol* 2017; 69(1): 35-45. https://doi.org/10.1002/art.39859
6. BALDINI C, FERRO F, ELEFANTE E, BOMBARDIERI S: Biomarkers for Sjögren's syndrome. *Biomark Med* 2018; 12(3): 275-86. https://doi.org/10.2217/bmm-2017-0297
7. ROBBINS A, HENTZIEN M, TOQUET S *et al*.: Diagnostic utility of separate anti-Ro60 and Anti-Ro52/TRIM21 antibody detection in autoimmune diseases. *Front Immunol* 2019; 10: 444. https://doi.org/10.3389/fimmu.2019.00444
8. ZAMPELI E, MAVROMMATI M, MOUTSOPOULOS HM, SKOPOULI FN: Anti-Ro52 and/or anti-Ro60 immune reactivity: autoantibody and disease associations. *Clin Exp Rheumatol* 2020; 38 (Suppl. 126): S134-41.
9. JONES EL, LAIDLAW SM, DUSTIN LB: TRIM21/Ro52 - roles in innate immunity and autoimmune disease. *Front Immunol* 2021; 12: 738473. https://doi.org/10.3389/fimmu.2021.738473
10. DIDIER K, BOLKO L, GIUSTI D *et al*.: Autoantibodies associated with connective tissue diseases: what meaning for clinicians? *Front Immunol* 2018; 9: 541. https://doi.org/10.3389/fimmu.2018.00541
11. PEENE I, MEHEUS L, DE KEYSER S, HUMBEL R, VEYS EM, DE KEYSER F: Anti-Ro52 reactivity is an independent and additional serum marker in connective tissue disease. *Ann Rheum Dis* 2002; 61(10): 929-33. https://doi.org/10.1136/ard.61.10.929
12. ARMAĞAN B, ROBINSON SA, BAZOBERRY A *et al*.: Antibodies to both Ro52 and Ro60 for identifying Sjögren's syndrome patients best suited for clinical trials of disease-modifying therapies. *Arthritis Care Res* (Hoboken) 2022; 74(9): 1559-65. https://doi.org/10.1002/acr.24597
13. NAKAMURA H, MORIMOTO S, SHIMIZU T, TAKATANI A, NISHIHATA SY, KAWAKAMI A: Clinical manifestations in anti-Ro52/SS-A antibody-seropositive patients with Sjögren's syndrome. *Immunol Med* 2021; 44(4): 252-62. https://doi.org/10.1080/25785826.2021.1919342
14. BETTACCHIOLI E, SARAUX A, TISON A *et al*.: Association of combined anti-Ro52/TRIM21 and anti-Ro60/SSA antibodies with increased Sjögren disease severity through interferon pathway activation. *Arthritis Rheumatol* 2024; 76(5): 751-62. https://doi.org/10.1002/art.42789
15. DEROO L, ACHTEN H, DE BOECK K *et al*.: The value of separate detection of anti-Ro52, anti-Ro60 and anti-SSB/La reactivities in relation to diagnosis and phenotypes in primary Sjögren's syndrome. *Clin Exp Rheumatol* 2022; 40(12): 2310-17. https://doi.org/10.55563/clinexprheumatol/l70874
16. SEROR R, RAVAUD P, BOWMAN SJ *et al*.: EULAR Sjögren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjögren's syndrome. *Ann Rheum Dis* 2010; 69(6): 1103-9. https://doi.org/10.1136/ard.2009.110619 Erratum in: *Ann Rheum Dis* 2011; 70(5): 880.
17. SEROR R, RAVAUD P, MARIETTE X *et al*.: EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI): development of a consensus patient index for primary Sjögren's syndrome. *Ann Rheum Dis* 2011; 70(6): 968-72. https://doi.org/10.1136/ard.2010.143743
18. QIU Y, LV C, WANG Y *et al*.: Clinical and molecular characterization of ILD in patients with overlapping ASyS and SjD: a retrospective observational study. *Rheumatology* (Oxford) 2025 Oct 29. https://doi.org/10.1093/rheumatology/keaf566
19. NAYEBIRAD S, MOHAMADI A, YOUSEFI-KOMA H *et al*.: Association of anti-Ro52 autoantibody with interstitial lung disease in autoimmune diseases: a systematic review and meta-analysis. *BMJ Open Respir Res* 2023; 10(1): e002076. https://doi.org/10.1136/bmjresp-2023-002076
20. LEE AYS, PUTTY T, LIN MW *et al*.: Isolated anti-Ro52 identifies a severe subset of Sjögren's syndrome patients. *Front Immunol* 2023; 14: 1115548. https://doi.org/10.3389/fimmu.2023.1115548
21. LEE AYS, LIN MW, REED JH: Anti-Ro52/TRIM21 serological subsets identify differential clinical and laboratory parameters. *Clin Rheumatol* 2022; 41(11): 3495-501. https://doi.org/10.1007/s10067-022-06299-5
22. BUVRY C, CASSAGNES L, TEKATH M *et al*.: Anti-Ro52 antibodies are a risk factor for interstitial lung disease in primary Sjögren syndrome. *Respir Med* 2020; 163: 105895. https://doi.org/10.1016/j.rmed.2020.105895
23. RETAMOZO S, AKASBI M, BRITO-ZERÓN P *et al*.: Anti-Ro52 antibody testing influences the classification and clinical characterisation of primary Sjögren's syndrome. *Clin Exp Rheumatol* 2012; 30(5): 686-92.
24. INFANTINO M, CARBONE T, BRUSCA I *et al*.: Current technologies for anti-ENA antibody detection: State-of-the-art of diagnostic immunoassays. *J Immunol Methods* 2022; 507: 113297. https://doi.org/10.1016/j.jim.2022.113297
25. INFANTINO M, MEACCI F, GROSSI V *et al*.: Serological epitope profile of anti-Ro52-positive patients with systemic autoimmune rheumatic diseases. *Arthritis Res Ther* 2015; 17: 365. https://doi.org/10.1186/s13075-015-0871-3