Assessment of myositis specific antibodies in primary chronic arthritis: single centre prospective study

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Abstract

Objective

Among the myositis specific antibodies (MSAs), the antisynthetase (anti-ARS) and the anti-MDA5 antibodies are those more frequently characterised by the occurrence of joint involvement. We aim to define the prevalence of MSAs in patients with established rheumatoid arthritis (RA), psoriatic arthritis (PsA), or undifferentiated polyarthritis (UPA).

Methods

From January 2021 to December 2024, all RA, PsA and UPA patients prospectively followed in our Early Arthritis Clinic (EAC), were evaluated. Changes in diagnosis, clinical/laboratory signs of muscle/lung/skin involvement at onset or during the follow-up, overlap syndromes, anti-ENA or cytoplasmic ANA positivity, Raynaud's phenomenon, and less than 24 months of follow-up were exclusion criteria. Baseline serum samples were tested for MSAs (line-blot). Positivity was defined according to manufacturers' instructions.

Results

143 patients were enrolled (93 females, 65%; 67 AR, 47%; 50 UPA, 35%, 26 PsA, 18%). Line-blot resulted positive in 10 (7%), weak-positive in 12 (8%), and borderline in 26 cases (18%). The remaining 95 patients (67%) were negative. MSAs positivity was anti-cN1A in 3 cases and anti-ARS and anti-MDA in 4 cases each. Weak positivity was found for anti-ARS (4), and anti-PM-Scl75 (3). Borderline results showed a high number of anti-ARS and aMDA5 (12, 46%). No variables were associated with MSA positivity.

Conclusions

MSAs positivity may be observed in one third of patients with primary isolated arthritis. About half of these cases displayed full or weak positivity for MSAs, whereas the remaining half displayed borderline results. Clinicians should be aware that MSA should be assessed only in case of effective clinical need.

Key words
myositis, arthritis, antisynthetase, autoantibody

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Introduction

Joint involvement is emerging as a common manifestation of anti-synthetase (ASSD), and anti-MDA5 syndrome (aMDA5), clinically mimicking Rheumatoid Arthritis (RA), Psoriatic Arthritis (PsA) or undifferentiated polyarthritis (UPA) (1, 2). Although isolated arthritis can be the presenting feature of both ASSD and aMDA5 (2-4) it is generally considered a transitory status, with most patients developing myositis, and/or Interstitial lung disease (ILD) during the disease course (1, 2). However, this knowledge mostly derives from retrospective studies (3, 5, 6) due to the low incidence of these conditions and the lack of standardised definitions helping in their early identification. The detection of myositis specific antibodies (MSAs) could be considered as a potentially useful marker to identify patients at risk of developing myositis related manifestations, despite the fair likelihood of false positive and negative results (7). Therefore, in real-world settings, it could be speculated that the presence of these antibodies is not always associated with clinical manifestations of the myositis spectrum, even in populations exhibiting symptoms that may be consistent with it, such as arthritis. With this study, we aimed at defining the prevalence of MSAs in a cohort of patients referring to our Early Arthritis Clinic (EAC) with established diagnosis of RA, PsA, or undifferentiated polyarthritis (UPA), presenting with an isolated joint involvement and with a long-term follow-up, after excluding patients with clinical or laboratory evidence of muscle, lung, or skin involvement or the concomitant occurrence of a connective tissue disease (CTD). Moreover, we also analysed the demographic and clinical differences between patients with and without MSA positivity.

Methods

From January 2021 to December 2024, we evaluated for study inclusion all patients followed at our EAC after signing the informed consent (Institutional Review Board Approval number 20070001302). To be included in the study, patients should have been in active follow-up for at least 24 months, and classified as RA (8), PsA (9), or UPA, without any change in diagnosis overtime. UPA was defined as the occurrence of joint swelling at physical examination involving more than 4 joints with at least one small joint, not fulfilling other classification criteria. Exclusion criteria encompassed: (i) the presence at any time-point of clinical or laboratory signs indicative of muscle (muscle weakness and/or CPK elevation), lung (dyspnoea and/or crackles at lung auscultation, ILD-related changes at baseline chest X-ray), or skin involvement along with signs of connective tissue disease (*e.g.*, recurrent oral ulcers, sicca symptoms, Raynaud's phenomenon, etc); (ii) a diagnosis of overlap syndrome, or microcrystalline arthritis; (iii) the cytoplasmic positivity of antinuclear antibody (ANA) test, or the positivity for extractable nuclear antigen (anti-ENA) antibodies, anti-dsDNA positivity (by Indirect Immunofluorescence in Crithidia Luciliae, after ELISA screening), or antiphospholipid antibodies; (iv) corticosteroid therapy at any dosage at the time of first assessment, or in the 3 previous months. Baseline demographic and clinical information were retrieved through chart-review. Two serum samples were obtained at baseline from each patient and underwent separate testing for MSAs using a commercially available line-blot assay (EUROLINE, Autoimmune Inflammatory Myopathies 16 Ag et CN1A). The assay employed a panel of 16 antigens plus anti-CN1A assay, specifically associated with autoimmune inflammatory myopathies, providing a robust and targeted evaluation of MSAs profile. Testing was performed in accordance with the manufacturer's instructions to ensure accuracy and reproducibility. Results were classified into three categories: 1) positive, 2) weak positive, and 3) borderline. To ensure reliable results, discrepancies between two tests were resolved by classifying the sample based on the lower value to avoid overestimating positivity. Furthermore, a single positive result

Table I. General characteristics of the enclosed cohort.

	RA (67 patients)	PsA (26 patients)	UPA (50 patients)	*p*-value	Overall (143 patients)
Female gender (%)	42 (63)	17 (65)	41 (68)	0.490	100 (70)
Median age at the onset (IQR)	58 (50.5-69)	52 (38-51)	50 (41-64)	0.054	54 (43.5-66.5)
Median disease duration (IQR)	42 (26-53)	30 (24-49.3)	29 (24-47.8)	0.05	37 (24.5-51)
RF positive patients (%)	27 (40)	0 (0)	3 (6)	<0.001	30 (21)
Post-hoc analysis	**Reference**	**<0.001**	**<0.001**	-	-
ACPA positive patients (%)	32 (48)	1 (4)	0 (0)	<0.001	33 (23)
Post-hoc analysis	**Reference**	**<0.001**	**<0.001**	-	-
ANA positive patients (%)	40 (60)	17 (65)	23 (46)	0.157	80 (56)
Median follow-up (IQR)	42 (26-53)	30 (24-56)	29 (24-48)	0.106	-
Joint erosion at X-rays (%)	22 (33)	2 (8)	4 (8)	<0.001	28 (20)
Post-hoc analysis	**Reference**	**0.014**	**0.002**	-	-
MSAs positive any degree (%)	20 (30)	9 (35)	19 (38)	0.65	48 (34)
Positive MSAs (%)	3 (4)	2 (8)	5 (10)	0.5	10 (7)
Weak positive MSAs	7 (10)	2 (8)	3 (6)	0.76	12 (8)
Borderline MSAs	10 (15)	5 (19)	11 (22)	0.99	26 (18)

RA: rheumatoid arthritis; PsA: psoriatic arthritis; UPA: undifferentiated polyarthritis; IQR: interquartile range; RF: rheumatoid factor; ACPA: anti-cyclic citrullinated peptide antibodies.

with a negative counterpart led to a conservative negative classification. In addition to MSAs testing, every patient was also tested for rheumatoid factor IgM (RF), anti-citrullinated peptide antibodies (ACPA), ANA and anti-ENA. Complementary laboratory investigations were performed to assess markers of systemic inflammation, muscle damage, and autoimmune activity. These included measurements of creatine phosphokinase (CPK), aldolase, and C-reactive protein (CRP) levels, as well as erythrocyte sedimentation rate (ESR). The follow-up of the patients and the treatments prescribed followed our previously described protocols (10, 11).

Statistical analyses were conducted using the Software Jamovi (version 2.6.23). Descriptive statistics were calculated for demographic and clinical variables, including means, medians, standard deviations (SD), and interquartile ranges (IQR) for continuous data, as well as frequencies and percentages for categorical data. The normality of continuous variables was assessed using the Shapiro-Wilk test. Comparisons between groups were performed using independent t-tests or Mann-Whitney U tests for continuous variables, depending on data distribution. Categorical variables were compared using chi-square tests or Fisher's exact tests, as appropriate. A significance level of $p< 0.05$ was considered statistically significant.

Table II. Distribution of different positivity for the myositis specific antibodies in our cohort, according to patients' classification.

		RA (67 patients)	PsA (26 patients)	UPA (50 patients)	Overall (147 patients)
Anti-CN1A (%)		**3 (4%)**	**1 (4%)**	**5 (10%)**	**9 (6%)**
	Positive	1 (1%)	0 (0%)	3 (6%)	4 (3%)
	Weak positive	1 (1%)	0 (0%)	1 (2%)	2 (1%)
	Borderline	1 (1%)	1 (4%)	1 (2%)	3 (2%)
Anti-PL7 (%)		**3 (4%)**	**2 (8%)**	**2 (4%)**	**7 (5%)**
	Weak positive	1 (1%)	1 (4%)	0 (0%)	2 (1%)
	Borderline	2 (3%)	1 (4%)	2 (4%)	5 (3%)
Anti-PL12 (%)		**1 (1%)**	**0 (0%)**	**3 (6%)**	**3 (2%)**
	Positive	0 (0%)	0 (0%)	1 (2%)	1 (1%)
	Weak positive	0 (0%)	0 (0%)	1 (2%)	1 (1%)
	Borderline	1 (1%)	0 (0%)	1 (2%)	2 (1%)
Anti-EJ (%)		**2 (3%)**	**0 (0%)**	**0 (0%)**	**2 (1%)**
	Positive	1 (1%)	0 (0%)	0 (0%)	1 (1%)
	Weak positive	1 (1%)	0 (0%)	0 (0%)	1 (1%)
Anti-MDA5 (%)		**2 (3%)**	**2 (8%)**	**3 (6%)**	**7 (5%)**
	Positive	0 (0%)	1 (4%)	0 (0%)	1 (1%)
	Borderline	2 (3%)	1 (4%)	3 (6%)	6 (4%)
Anti-PM-Scl75/100 (%)		**2 (3%)**	**1 (4%)**	**3 (6%)**	**6 (4%)**
	Weak positive	1 (1%)	1 (4%)	1 (2%)	3 (2%)
	Borderline	1 (1%)	0 (0%)	2 (4%)	3 (2%)
Anti-Mi2A/B (%)		**5 (7%)**	**0 (0%)**	**1 (2%)**	**6 (4%)**
	Positive	1 (1%)	0 (0%)	0 (0%)	1 (1%)
	Weak positive	1 (1%)	0 (0%)	0 (0%)	1 (1%)
	Borderline	3 (4%)	1 (4%)	1 (2%)	5 (3%)
Anti-SRP (%)		**0 (0%)**	**2 (8%)**	**1 (2%)**	**3 (2%)**
	Positive	0 (0%)	1 (4%)	0 (0%)	1 (1%)
	Borderline	0 (0%)	1 (4%)	1 (2%)	2 (1%)
Anti-SAE (%)		**1 (1%)**	**0 (0%)**	**1 (2%)**	**2 (1%)**
	Positive	0 (0%)	0 (0%)	1 (2%)	1 (1%)
	Weak positive	1 (1%)	0 (0%)	0 (0%)	1 (1%)
Anti-Ku (%)		**1 (1%)**	**0 (0%)**	**0 (0%)**	**1 (1%)**
	Weak positive	1 (1%)	0 (0%)	0 (0%)	1 (1%)

RA: rheumatoid arthritis; PsA: psoriatic arthritis; UPA: undifferentiated polyarthritis.

Results

We included in the study 143 patients (93 females, 65%), classified as RA (67, 47%), UPA (50, 35%), and PsA (26, 18%). Compared to PsA and UPA, patients with RA were more commonly RF and ACPA positive ($p<0.001$ both, at the post-hoc analysis) and with a more erosive joint disease ($p=0.014$ and $p<0.002$ respectively at the *post-hoc* analysis).

Table III. Comparison between patients with negative *vs*. positive MSAs.

		Negative assay (95 patients)	Positive MSAs (10 patients)	Weak-positive MSAs (12 patients)	Borderline MSAs (26 patients)	Overall positive (48 patients)
Female sex (%)		61 (64)	8 (80)	6 (50)	8 (31)	32 (67)
	Reference	0.317	0.338	0.634	0.771	
ANA (%)		51 (54)	4 (40)	8 (67)	17 (65)	29 (60)
	Reference	0.410	0.541	0.287	0.444	
RF (%)		20 (21)	3 (30)	5 (42)	2 (8)	10 (21)
	Reference	0.515	0.146§	0.156§	0.976	
ACPA (%)		23 (24)	2 (20)	4 (33)	4 (15)	10 (21)
	Reference	0.766	0.493§	0.432§	0.651	
Erosions (%)		20 (71)	2 (20)	3 (25)	3 (11)	8 (29)
	Reference	*1.000*§	*0.718*§	*0.399*§	*0.533*	
Median Age at the onset, years (IQR)		54.4 (43.4-66.7)	65.9 (56.9-72.8)	48.3 (43.4-60.7)	57.8 (46-70)	55 (44.2-64.7)
	Reference	0.084	0.708	0.550	0.712	

MSA: myositis specific antibodies; RA: rheumatoid arthritis; PsA: psoriatic arthritis; UPA: undifferentiated polyarthritis.
§Fisher's exact test.

Full results are shown in Table I.

In the overall cohort, 48 patients showed some degree of positivity for MSAs (33.5%): 10 patients (7%) positive, 12 (8%) weak positive, and 26 (18%) borderlines. In patients with RA, MSAs were found in 20 cases (30%), whereas patients with PsA and UPA displayed MSAs in 9 (35%) and 19 (38%) cases, respectively. No significant differences were found in the prevalence of positive, weak positive and borderline MSAs results amongst patients with RA, PsA and UPA (Table I). Anti-synthetase antibodies (anti-ARS) were the most frequent overall (12 patients, 8%), followed by anti-CN1A (9 patients, 6%), anti-MDA5 (7 patients, 5%), anti Pm-Scl 75/100 (6 patients, 4%), anti-Mi2A/B (6 patients, 4%), anti-SRP (3 patients, 2%), anti-SAE (2 patients, 1%), and anti-Ku (1 patient, 1%). Anti-ARS were the most common MSAs even when considering each cohort separately (9% in RA, 8% in PsA and 10% in UPA), whereas anti-CN1A were more prevalent in UPA (10%). Anti-CN1A was the most prevalent positive MSA identified, observed in 4 patients (3%), followed by anti-ARS anti anti-MDA5 in 3 cases each (Table II).

According to the degree of positivity, we evaluated if age at arthritis onset, female sex, occurrence (ever) of joint erosions at hands and feet X-rays, positivity of ANA test, RF, ACPA, and the underlying diagnosis (*e.g.*, RA, PsA or UPA) were statistically associated with MSA results. MSA results were stratified in positive, weak positive, borderline positive, overall positive and overall negative (negative assay results). The analysis did not show variables statistically associated with MSA testing results (Table III).

Discussion

To the best of our knowledge, this is the first study assessing MSA prevalence in a cohort of patients with established primary isolated arthritis prospectively followed in an EAC setting. We excluded all autoimmune CTD features except ANA positivity, as it's not rare in RA, PsA (12-14) and healthy individuals (15).

Our findings show MSA positivity in varying degrees in about one-third of primary arthritis patients. No associations were found between MSA results and RF, ACPA, ANA status, erosive disease, or arthritis classification. Although mostly borderline, the number of patients with positive or weakly positive MSAs was substantial. These results raise intriguing questions about the clinical implications of MSAs in this context, especially without overt CTD features. We're unsure if we identified false positives (7) or incomplete diseases with partially expressed phenotypes. We aimed to reduce false positives by a stringent line blot interpretation protocol. The likelihood of missing future clinical manifestations is lessened by the relatively long follow-up, though not entirely ruled out.

Considering this, clinicians should be aware that MSA positivity detected using commercial assays doesn't always carry true clinical significance. Our study's strengths include its prospective design, stringent inclusion/exclusion criteria, systematic follow-up, and double MSA testing

Testing should be reserved for cases where a positive result can be appropriately contextualised, thereby avoiding diagnostic uncertainty, overinterpretation of borderline results, or unnecessary diagnostic procedures

Limitations should also be acknowledged. First, the relatively small sample size may limit the findings' generalisability. Second, while our cohort's clinical stability supports no evolving autoimmune disease, longer follow-up may be needed to definitively exclude delayed-onset CTD. Third, arthritis treatment could have prevented some patients from developing additional, possibly CTD-indicative, signs or symptoms.

In conclusion, our study suggests that in patients with isolated early arthritis, MSAs tested using commercial assays may yield positive results in approximately one-third of cases. Half of these results are borderline, while the other half are either positive or weakly positive. The most detected antibodies were anti-ARS and anti-MDA5, which are frequently associated with arthritis, as well as anti-CN1a, typically linked to inclusion body myositis (IBM), and not associated with arthritis. These findings emphasize the importance of clinicians maintaining a critical approach when interpreting the results of these assays.

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