Agreement between local and central anti-synthetase antibodies detection: results from the Classification Criteria of Anti-Synthetase Syndrome project biobank

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Abstract

Objective

The CLASS (Classification Criteria of Anti-Synthetase Syndrome) project is a large international multicentre study that aims to create the first data-driven anti-synthetase syndrome (ASSD) classification criteria. Identifying anti-aminoacyl tRNA synthetase antibodies (anti-ARS) is crucial for diagnosis, and several commercial immunoassays are now available for this purpose. However, using these assays risks yielding false-positive or false-negative results, potentially leading to misdiagnosis. The established reference standard for detecting anti-ARS is immunoprecipitation (IP), typically employed in research rather than routine autoantibody testing. We gathered samples from participating centers and results from local anti-ARS testing. As an "ad-interim" study within the CLASS project, we aimed to assess how local immunoassays perform in real-world settings compared to our central definition of anti-ARS positivity.

Methods

We collected 787 serum samples from participating centres for the CLASS project and their local anti-ARS test results. These samples underwent initial central testing using RNA-IP. Following this, the specificity of ARS was reconfirmed centrally through ELISA, line-blot assay (LIA), and, in cases of conflicting results, protein-IP. The sensitivity, specificity, positive likelihood ratio and positive and negative predictive values were evaluated. We also calculated the inter-rater agreement between central and local results using a weighted κ co-efficient.

Results

Our analysis demonstrates that local, real-world detection of anti-Jo1 is reliable with high sensitivity and specificity with a very good level of agreement with our central definition of anti-Jo1 antibody positivity. However, the agreement between local immunoassay and central determination of anti-non-Jo1 antibodies varied, especially among results obtained using local LIA, ELISA and "other" methods.

Conclusion

Our study evaluates the performance of real-world identification of anti-synthetase antibodies in a large cohort of multi-national patients with ASSD and controls. Our analysis reinforces the reliability of real-world anti-Jo1 detection methods. In contrast, challenges persist for anti-non-Jo1 identification, particularly anti-PL7 and rarer antibodies such as anti-OJ/KS. Clinicians should exercise caution when interpreting anti-synthetase antibodies, especially when commercial immunoassays test positive for non-anti-Jo1 antibodies.

Key words

immunoprecipitation, anti-synthetase syndrome, classification criteria, myositis-specific antibodies, real-world, line immunoblot, ELISA, idiopathic inflammatory myositis, myositis-associated antibodies

Affiliations: see page 285.

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Competing interests: see page 286.

Introduction

Myositis-specific (MSAs) and myositis-associated antibodies (MAAs) are present in up to 60% of adult and juvenile-onset idiopathic inflammatory myopathy (IIM) patients (1-3). These antibodies significantly changed the clinical approach to IIMs, identifying new disease subsets and facilitating earlier recognition of these conditions in the real world (4-7). MSAs and MAAs may influence the prognosis and guide subsequent investigations and treatment decisions (8, 9).

Anti-synthetase syndrome (ASSD) is a rare and heterogeneous disease encompassing various clinical manifestations, such as interstitial lung disease, myopathy, arthritis, Raynaud's phenomenon, fever, and mechanics hands (10). Detection of serum anti-aminoacyl tRNA synthetase autoantibodies (anti-ARS) such as anti-Jo-1, anti-PL7, anti-PL12, anti-EJ, anti-OJ, anti-KS, anti-Zo and anti-YRS/HA can suggest the presence of an underlying ASSD (3, 10).

Protein and RNA immunoprecipitation (IP) is the gold standard for MSA identification, with high clinical sensitivity and specificity (8). It is commonly used for research, confined to specialised centres, as it can help identify known and unknown antibodies. IP is not widely used in clinical practices and commercial labs due to being labor-intensive and the requirement of a high level of expertise (8, 11). The reading of precipitation bands is subjective, given that it requires expert interpretation as many MSAs migrate in the same narrow area of gel electrophoresis (12). Using commercial enzyme-linked immunosorbent assays (ELISA) or line blot immunoassays (LIA) allows for the rapid assessment of the presence of MSAs and MAAs at a lower cost and in a more accessible way for different laboratories (8). While LIA allows simultaneous testing for several MSAs with a single assay, it is associated with an increased likelihood of false positive and false negative results (1). The reliability of commercial immunoassays varies based on the individual MSA/MAA being assessed, and up to 16% of the healthy population can demonstrate positivity for an MSA on LIA (8, 13, 14). Often, false positives are associated with an incongruent ANA pattern or multiple positive MSAs (1, 8). More than 80% of clinicians believed MSA testing influenced their diagnostic confidence of IIM and prognostic information relayed to their patients, with 73% reporting that the presence of antibodies influenced their treatment decisions (15).

The Classification Criteria of Anti-Synthetase Syndrome (CLASS) project is a collaborative study involving multiple centers to develop and validate classification criteria for ASSD. This project was coordinated by the University of Pavia (Co-PI: LC) and the University of Pittsburgh (Co-PI: RA) with support from the American College of Rheumatology (ACR) and the European Alliance of Associations for Rheumatology (EULAR). The CLASS database has a total of 4179 patients enrolled, which provided a unique opportunity to evaluate sera samples to assess the real-world performance of local detection of anti-ARS using different antibody detection techniques.

Methods

Objectives

In this sub-study of the CLASS project, we aimed to evaluate the real-world performance of anti-ARS detection by comparing the results obtained in local laboratories of participating centres to those obtained from central laboratories in Italy (University and IRCCS Policlinico San Matteo Foundation Hospital of Pavia and Humanitas Clinical and Research Center, Milan) and UK (University of Bath). With multiple publications, these centres are recognised as international reference hubs for autoantibody testing (1, 16, 17). We also evaluated whether the performance of local testing was affected by anti-ARS antibody specificity and the technique used for detection.

Study population

All patients aged 18 years or older, diagnosed by the local expert physician as a case (ASSD) or as a control (systemic autoimmune rheumatic diseases, lung or skin diseases mimicking ASSD), who signed the informed con-

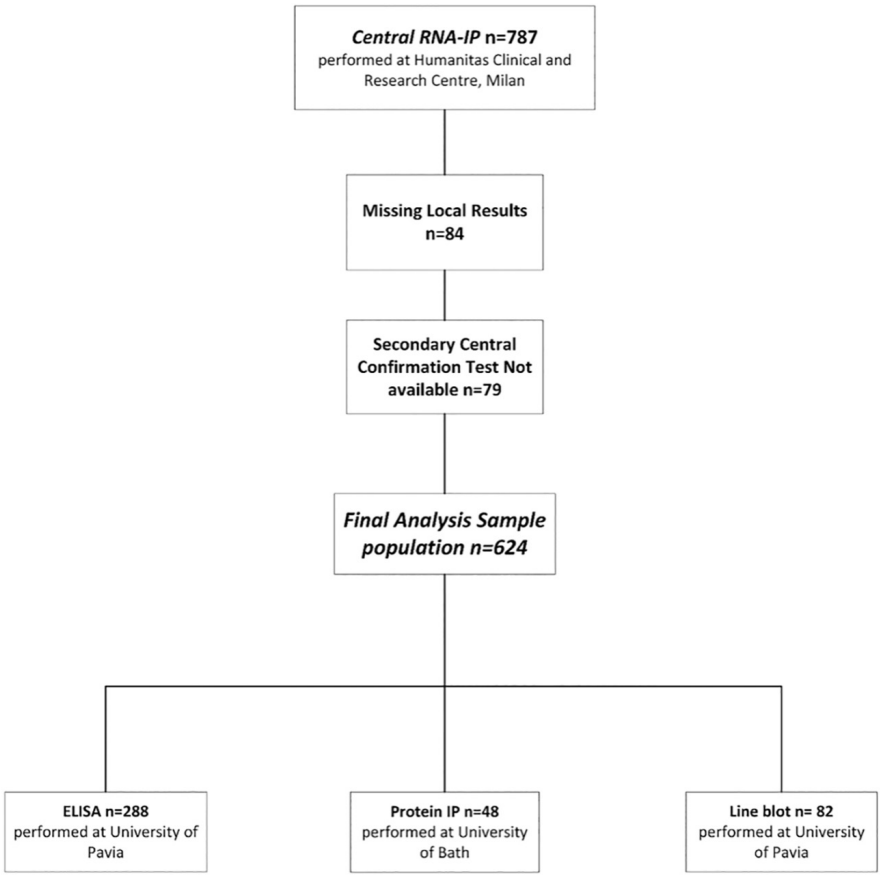

Fig. 1. Flow diagram describing the process determining samples included in the final cohort for analysis to validate the performance of local immunoassays compared to central testing and our gold-standard definition. 787 RNA-IP samples were performed by the University of Pavia, with confirmatory testing performed as listed below, including 288 ELISA samples and 82 Lineblot samples. 48 samples were retested with protein-IP at the University of Bath, UK.

sent for data and serum collection, were included in the study. The diagnosis of the patients enrolled as controls included rheumatoid arthritis (RA), systemic sclerosis (SSc), dermatomyositis (DM), polymyositis (PM), immune-mediated necrotising myopathy (IMNM), cancer-associated myositis, inclusion body myositis (IBM), scleromyositis, primary Sjögren's disease (p-SjD), ANCA-associated vasculitis (AAV), polymyalgia rheumatica (PMR), systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), psoriatic arthritis (PsA), psoriasis or eczema, interstitial pneumonia with autoimmune features (IPAF), connective tissue disease-associated ILD (CTD-ILD), idiopathic pulmonary fibrosis (IPF), undifferentiated connective tissue disease (UCTD) and overlap syndromes. Investigators collected diagnoses and clinical information through a secured online database (RedCap).

Study design

Serum or plasma samples, preserved in a frozen state, were dispatched from participating centres to the central laboratory located at the University of Pavia, Italy, following approved collection, storage, and shipment guidelines. Local anti-ARS assessment was conducted per the prevailing clinical protocols of each centre. This encompassed methodologies such as ELISA/ELiA, LIA and IP. All patients with local and central anti-ARS testing were included in this sub-study of the CLASS project, whereas patients without local results for anti-ARS were excluded.

Central RNA-IP was performed in all the available samples. According to the local result, samples that were negative for central RNA-IP and consistent with the local results on testing did not undergo further investigation and were considered negative. Samples that resulted negative for central RNA-IP but displayed a local positivity for anti-ARS were further tested through ELISA for anti-Jo1 and, if negative, underwent protein-IP. Samples that resulted positive for central RNA-IP underwent further testing through ELISA for anti-Jo1, anti-PL7, anti-PL12, anti-EJ and anti-KS. If no anti-ARS specificity was found, central LIA was performed. A flow diagram describing this decision-making scheme is available for review in Supplementary Fig. S1.

We used a stringent gold standard to define positive anti-ARS results, as detailed below:

1. For anti-Jo1 antibody positivity, the *gold standard* was defined as the positivity of central RNA IP and (or isolated) ELISA/protein IP.
2. For non-anti-Jo1 anti-synthetase antibody positivity, the *gold standard* was defined as the positivity of central RNA IP and confirmed by at least one other central technique (ELISA/EliA/LIA/protein IP).

The samples that did not meet this definition were either excluded or considered negative according to the following statements:

1. Samples were considered negative for anti-ARS when other autoantibodies could be detected by other central tests.
2. Samples were excluded from the analysis when the autoantibodies' specificity could not be identified due to the negativity of the other central tests or if a second central test was not performed.

IP procedures were performed by laboratories with well-recognised expertise in this technique, according to previously published protocols (1, 12). ELIA and LIA were conducted in Pavia, Italy, according to the manufacturer's instructions using EliA Symphony Well, Thermo Fisher Scientific Inc, and EUROLINE Autoimmune Inflammatory Myopathies 16 Ag, Euroimmun, Lübeck, respectively. Furthermore, locally developed ELISA assays for central testing for the different anti-ARS antibodies, including anti-Jo1, anti-PL7, anti-PL12, anti-EJ, and anti-KS antibodies, were developed and employed. Investigators performing the central determination of anti-ARS were blinded to the diagnosis

and results from local laboratories, ensuring an impartial and rigorous assessment process.

Sample selection

We received 1254 serum samples, including cases and controls, from various participating centres. 787 samples were randomly selected based on cost and logistics to perform central IP testing. Selected samples underwent central testing, encompassing IP, ELISA/EliA and LIA. However, samples missing local antibody results and those without confirmatory central testing were excluded from the final analysis for the study. For the main CLASS project, a secondary confirmatory test was not required. Therefore, not all samples that underwent central immunoprecipitation necessarily had a second confirmatory test using another testing method. After excluding missing results (Fig. 1), 624 samples were included in the final analysis.

Statistical analysis

We assessed the performance of the immunoassays in the real world by calculating the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) compared to our gold-standard definition. We also determined the positive likelihood ratios (PLR) using the calculated sensitivity and specificity. Inter-rater agreement was calculated between central and local results using a weighted ϰ co-efficient. The strength of agreement of the weighted κ co-efficient was assessed using the following values: poor (<0.20), fair (0.20–0.40), moderate (0.41–0.60), good (0.61–0.80) or very good (0.81-1.00) (18). A negative κ value suggested disagreement or agreement worse than expected or a random chance associated with the result (19).

Ethics

The CLASS project was approved by the Ethical Committee of the IRCCS Policlinico S. Matteo Foundation (P-201190088730, Prot. 20190094533) on October 23rd, 2019. Centers that sent samples obtained approval from their local ethics committee. All participants' clinical information was stored on an online database: RedCap (https://redcap.ospfe.it).

Results

Study population

A total of 787 serum samples were randomly selected from 12 distinct countries, namely Italy (n=392), Spain (n=90), Japan (n=65), USA (n=51), Germany (n=50), India (n=47), Chile (n=35), Sweden (n=19), France (n=18), Portugal (n=8), Israel (n=7) and Canada (n=5). From these samples, 84 were excluded as no local data were recorded regarding anti-ARS testing (Fig. 1) and 79 samples were excluded due to the absence of a secondary confirmatory central test. Characteristics regarding the excluded sera samples are described in Supplementary Table S1. 624 samples were included in the final analysis.

Based on geographic location, the LIA was the most commonly recorded method in anti-ARS assessment in Europe (76.4%), South America (86.2%) and India (100%), ELISA was most prevalent in North America (45.8%) and IP in Japan (70.8%). The performance of the different testing methods based on geographic location is available for review in Supplementary Table S2.

Local testing results

The majority of the 624 available samples were initially tested locally by LIA (419, 67%), ELISA (92, 15%), IP (N=56, 9%), and other assays (57, 9%). The overall positivity for anti-ARS was 47% (n=294), with the higher prevalence of anti-Jo1 (n=186, 30%), followed by anti-PL12 (n=44, 7%), anti-PL7 (n=32, 5%), anti-EJ (n=29, 5%), anti-OJ (n=4, 0.5%), and anti-KS (n=5, 0.5%) (Fig. 2). Six patients (0.5%) were included with a double anti-ARS positivity from local testing, described in Supplementary Table S3. The overall results of the local testing, including all local methods of assessment for the presence of anti-ARS, are reported in Table I.

Central testing results

According to the central anti-ARS positivity definition, 270 patients (43%) were anti-ARS positive. Among these, 191 (31%) were anti-Jo1, of which 37/191 were ELISA positive/IP negative, 33 (12.5%) anti-PL12, 24 (38%) anti-EJ, 21 (8%) anti-PL7, 2 (0.5%) anti-KS, and 0 (0%) anti-OJ positive (Fig. 2). Only one patient had double positivity for anti-ARS (anti-PL7 and anti-PL12), as reported in Supplementary Table S3.

The final analysis included 314 ARS cases determined by local clinicians. Among them, 57 samples (18.2%) did not meet our criteria for anti-ARS positivity. Out of these 57 samples, local testing detected an anti-ARS in 32 cases (56.1%). Furthermore, 13/310 control cases (4.2%) met the criteria for ARS positivity, of which three enrolled individuals were also reported to have anti-Jo1 positivity detected through local testing methods.

Comparison of local determination of ARS antibodies vs. central definition

- *All local immunoassay vs. central definition*

Local and central testing identified anti-ARS positivity in 294 samples (47%) and 270 samples (43%), respectively (Table I). This yielded a sensitivity of 95.9% and a specificity of 90.1%, with a PPV of 0.88 and an NPV of 0.97. Anti-Jo1 demonstrated the highest PLR of 484.5. In comparison, the non-Jo1 antibody with the highest PLR was anti-EJ, with a PLR of 55.5.

When combining the local assessment methods for all anti-ARS antibodies, the weighted κ coefficient was 0.85. Anti-Jo1 antibodies exhibited the highest κ coefficient at 0.97, while anti-PL7 antibodies had the lowest at 0.55, and anti-KS showed a negligible κ coefficient of -0.004. Unfortunately, we could not calculate the κ coefficient for anti-OJ antibodies since central confirmation was not obtained for any serum samples.

Specifically, central testing did not confirm local anti-ARS positivity in 35/624 samples (5.6%), including all cases of local anti-OJ positivity. Additionally, 11 patients who were negative in local testing (1.8%, 11/624) were found to be centrally positive for anti-ARS, of which 9/11 (81.2%) were thought to be control cases, and 2/11 (18.8%) were diagnosed by their local

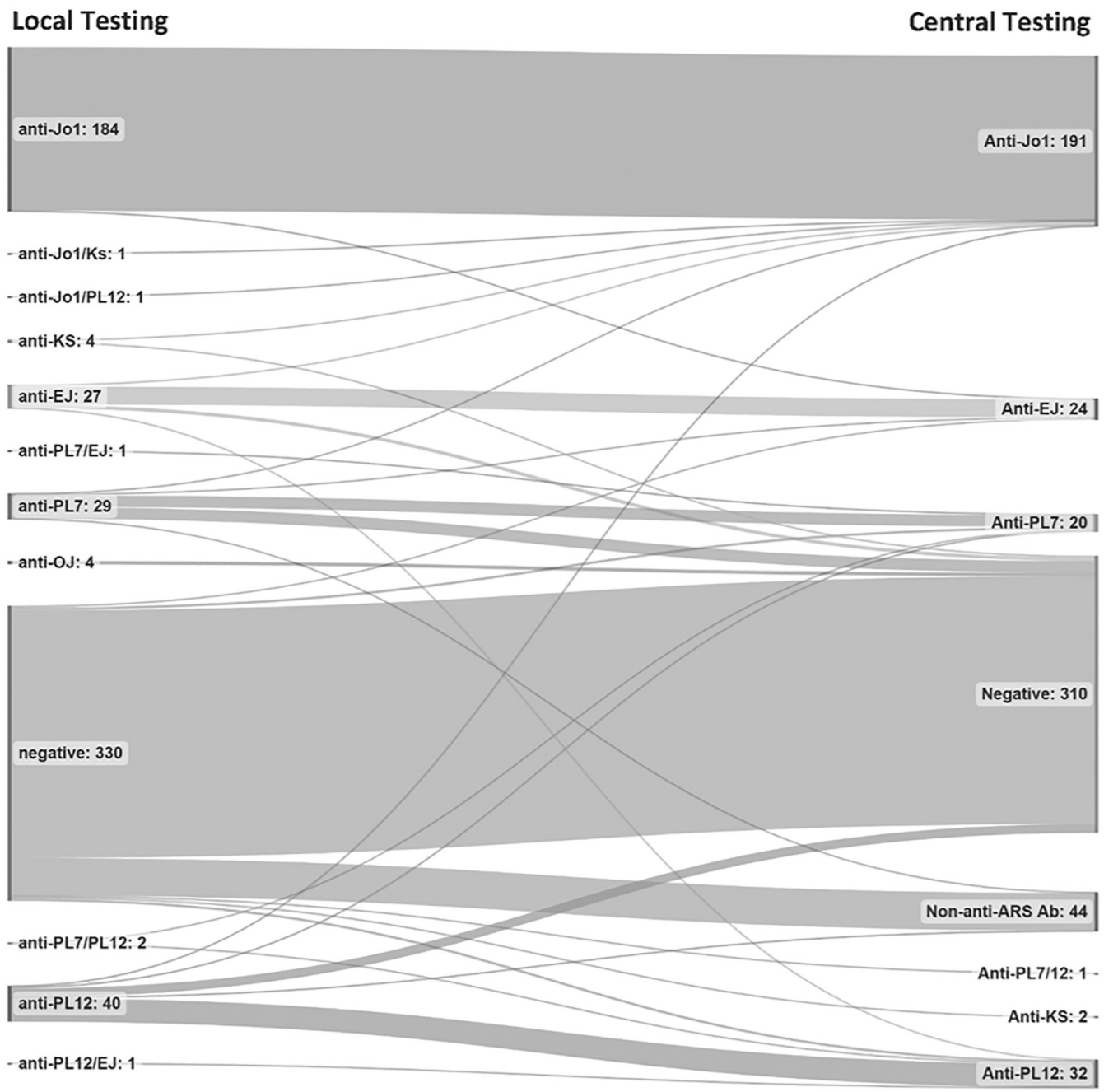

Fig. 2. Sanke plot illustrating the variation in results obtained through local testing compared to the result obtained on central testing.

clinician with ASSD. The specificity of anti-ARS changed with respect to local results in eleven cases (1.8%), as demonstrated in Figure 2.

Central confirmation was not obtained among the six local cases with double positivity observed. Central testing results demonstrated two sera samples with anti-Jo1, two with anti-PL7, and two with anti-PL12 antibodies (Suppl. Table S3). The only sample that tested positive for multiple anti-ARS antibodies through central testing (anti-PL7 and anti-PL12) was negative for anti-ARS on the local testing.

- *Local line blot vs. central definition*

Among patients who underwent local testing using LIA, 184 (43.9%) were determined to be positive for anti-ARS locally, while 166 (39.6%) tested positive in central testing (Table II). This resulted in a sensitivity of 95.2% and a specificity of 89.7%, with a PPV of 0.86 and an NPV of 0.97. Anti-Jo1 demonstrated the highest PLR of 324.3. In comparison, the non-Jo1 antibody with the highest PLR was anti-EJ, which had a PLR of 57.7. The best overall performance, in terms of sensitivity, specificity, PPV, and NPV, was observed with anti-Jo1 antibodies.

The analysis of the combined anti-ARS results obtained through LIA yielded a weighted κ coefficient of 0.83. Notably, anti-Jo1 antibodies had the highest κ coefficient at 0.98, while anti-PL7 antibodies had the lowest at 0.57, and anti-KS exhibited a coefficient of -0.002.

Table I. Calculated sensitivity, specificity, PPV, NPV and weighted κ comparing the evaluation of all local testing methods in comparison to our gold-standard definition for anti-ARS positivity. Some patients (six local and one central determination) were double positive, and they are described in further detail in the Supplementary files.

Antibodies	Local detection (%)	Central detection (%)	Sensitivity (%)	Specificity (%)	PPV	NPV	Positive likelihood ratio	Inter-rater agreement Weighted κ
Number of patients: 624 (all local immunoassays)								
ARS (combined)	294 (47)	270 (43)	95.9	90.1	0.88	0.97	9.7	0.85 (95CI% 0.81 – 0.89)
Jo1	186 (30)	191 (31)	96.9	99.8	0.99	0.99	484.5	0.97 (95%CI 0.95 – 0.99)
Non-Jo1	110 (18)	79 (13)	84.8	92.1	0.61	0.98	10.7	0.66 (95%CI 0.58 – 0.74)
PL7	32 (5)	21 (3.5)	71.4	97.2	0.47	0.99	25.5	0.55 (95% CI 0.38 – 0.71)
PL12	44 (7)	33 (5)	84.8	97.3	0.64	0.99	31.4	0.71 (95% CI 0.59 – 0.83)
EJ	29 (5)	24 (4)	83.3	98.5	0.69	0.99	55.5	0.74 (95% CI 0.61 – 0.88)
OJ	4 (0.5)	0	-	99.4	-	1	n/a	-
KS	5 (0.5)	2 (0.5)	0	99.2	0	99.7	0	-0.004 (95%CI -0.01 – 0)

Table II. Calculated sensitivity, specificity, PPV, NPV and weighted κ comparing the evaluation of LIA local testing methods *versus* our gold-standard definition for anti-ARS positivity.

Antibodies	Local detection (%)	Central detection (%)	Sensitivity (%)	Specificity (%)	PPV	NPV	Positive likelihood ratio	Inter-rater agreement Weighted κ
Local Line blot Immunoassay n=41**9**								
ARS (combined)	184 (43.9)	166 (39.6)	95.2	89.7	0.86	0.97	9.2	0.83 (95%CI 0.78 – 0.89)
Jo1	110 (26)	112 (26.7)	97.3	99.7	0.99	0.99	324.3	0.97 (95%CI 0.95 – 0.99)
Non-Jo1	75 (17.9)	54 (12.9)	83.3	91.7	0.6	0.97	10	0.64 (95% CI 0.54 – 0.75)
PL7	27 (6.4)	20 (4.8)	70	96.7	0.52	0.98	21.2	0.57 (95% CI 0.4 – 0.74)
PL12	33 (7.9)	21 (5)	90.5	96.5	0.58	0.99	25.9	0.68 (95% CI 0.54 – 0.83)
EJ	15 (3.6)	13 (3.1)	69.2	98.8	0.64	0.99	57.7	0.63 (95% CI 0.42 – 0.84)
OJ	2 (0.5)	0	-	99.5	-	1	n/a	-
KS	1 (0.2)	1 (0.2)	0	99.8	0	0.99	0	-0.002 (95%CI -0.006 – 0)

- *Enzyme-linked immunosorbent assay vs. central definition*

Among patients who underwent local ELISA testing, 56 (61%) tested positive for anti-ARS locally, and 55 (60%) tested positive in central testing (Table III). This resulted in a sensitivity of 96.4% and a specificity of 91.9%, with a PPV of 0.95 and an NPV of 0.94. Anti-Jo1 PLR could not be determined due to the 100% specificity. In comparison, the non-Jo1 antibody with the highest PLR was anti-EJ, which had a PLR of 90.9. The best overall performance, in terms of sensitivity, specificity, PPV, and NPV, was achieved with anti-Jo1 antibodies.

Analysing the combined anti-ARS results obtained via ELISA yielded a weighted κ coefficient of 0.89. Notably, anti-Jo1 antibodies had the highest κ coefficient at 0.89, while anti-PL12 antibodies had the lowest at 0.65.

- *Local immunoprecipitation vs. central definition*

Among patients who underwent local testing through IP, 31 (55%) tested positive for anti-ARS locally, while 28 (50%) tested positive in central testing (Table IV). This resulted in a sensitivity of 96.4% and a specificity of 85.7%, with a PPV of 0.87 and an NPV of 0.96. Anti-Jo1 PLR could not be determined due to the 100% specificity. In comparison, the non-Jo1 antibody with the highest PLR was anti-EJ, which had a PLR of 5.5. The best overall performance, in terms of sensitivity, specificity, PPV, and NPV, was achieved with anti-PL12 antibodies, but anti-EJ and anti-Jo1 also showed favourable results; however, a lower sensitivity was observed compared to our central testing results and definition. The analysis of the combined anti-ARS results obtained via local IP testing yielded a weighted κ coefficient of 0.82. Notably, anti-PL12 antibodies had the highest κ coefficient at 0.90, followed closely by anti-EJ at 0.88, while anti-Jo1 antibodies had a slightly lower coefficient of 0.82.

- *Unspecified methods vs. central definition*

Among patients who underwent local testing using alternative methods, 23 (40%) were tested positive for anti-ARS locally, while 21 (37%) were tested positive in central testing (Table V). This resulted in a sensitivity of 100% and a specificity of 94.4%, with a PPV of 0.91 and an NPV of 1. Anti-Jo1 and anti-PL12 PLR could not be determined due to the 100% specificity, while anti-EJ had a PLR of 5.5. The best overall performance, in terms of sensitivity, specificity, PPV, and NPV, was observed with anti-Jo1 antibodies. In contrast, the other anti-ARS antibodies displayed good sensitivity and specificity but had lower PPV values. The analysis of the combined anti-ARS results obtained via alternative local methods yielded a weighted κ coefficient of 0.93. Notably, anti-Jo1 antibodies had the highest κ coefficient at 1, indicating strong agreement, while anti-PL7 and anti-PL12 antibodies had coefficients of 0.66.

False negative rate (FNR) and false positive rate (FPR) for each method of local assessment are described in further detail in Supplementary Table S4.

Discussion

The reference standard method for detecting MSAs, including anti-ARS, is

Table III. Calculated sensitivity, specificity, PPV, NPV and weighted ϰ comparing the evaluation of ELISA local testing methods *versus* our gold-standard definition for anti-ARS positivity.

Antibodies	Local detection (%)	Central detection (%)	Sensitivity (%)	Specificity (%)	PPV	NPV	Positive likelihood ratio	Inter-rater agreement Weighted κ
				Local ELISA n=92				
ARS (combined)	56 (61)	55 (60)	96.4	91.9	0.95	0.94	11.9	0.89 (95%CI 0.79 – 0.98)
Jo1	47 (51)	47 (51)	100	100	1	1	∞	1 (95%CI 1 – 1)
Non-Jo1	9 (10)	8 (9)	75	96.4	0.67	0.98	20.8	0.68 (95%CI 0.41 – 0.94)
PL7	1 (1.5)	0	n/a	98.9	n/a	1	n/a	-
PL12	4 (4)	5 (5)	60	98.9	0.75	0.98	54.5	0.65 (95%CI 0.28 – 1)
EJ	3 (3)	2 (2)	100	98.9	0.67	1	90.9	0.79 (95%CI 0.4 – 1)
OJ	1 (1.5)	0	-	98.9	-	1	n/a	-
KS	0 (0)	1 (1)	0	1	-	0.99	n/a	-

Table IV. Calculated sensitivity, specificity, PPV, NPV and weighted κ comparing the evaluation of IP local testing methods *versus* our gold-standard definition for anti-ARS positivity.

Antibodies	Local detection (%)	Central detection (%)	Sensitivity (%)	Specificity (%)	PPV	NPV	Positive likelihood ratio	Inter-rater agreement Weighted κ
				Local Immunoprecipitation n=56				
ARS (combined)	31 (55.3)	28 (50)	96.4	85.7	0.87	0.96	6.7	0.82 (95%CI 0.67 – 0.97)
Jo1	10 (17.9)	13 (23.2)	76.9	100	1	0.93	∞	0.84 (95%CI 0.66 – 1)
Non-Jo1	21 (37.5)	15 (26.8)	93.3	82.9	0.67	0.97	5.5	0.68 (95%CI 0.48 – 0.88)
PL7	2 (3.6)	0	-	96.4	-	1	n/a	-
PL12	5 (8.9)	6 (10.7)	83.3	100	1	0.98	∞	0.9 (95% CI 0.7 – 1)
EJ	11 (17.9)	9 (16.1)	100	81.8	0.81	1	5.5	0.88 (95%CI 0.71 – 1)
OJ	1 (1.8)	0	-	98.2	-	1	n/a	-
KS	3 (5.4)	0	-	94.6	-	1	n/a	-

Table V. Calculated sensitivity, specificity, PPV, NPV and weighted κ comparing the evaluation of local testing obtained through other methods versus our gold-standard definition for anti-ARS positivity.

Antibodies	Local detection (%)	Central detection (%)	Sensitivity (%)	Specificity (%)	PPV	NPV	Positive likelihood ratio	Inter-rater agreement Weighted κ
				"Other" - Local other/unknown testing methods n=57				
ARS (combined)	23 (40.4)	21 (36.8)	100	94.4	0.91	1	17.9	0.93 (95%CI 0.83-1)
Jo1	19 (33.3)	19 (33.3)	100	100	1	1	∞	1 (95%CI 1 – 1)
Non-Jo1	5 (8.8)	2 (3.5)	66.7	94.4	0.4	0.98	11.9	0.65 (95%CI 0.57 – 0.74)
PL7	2 (3.5)	1 (1.8)	100	98.2	0.5	1	55.6	0.66 (95%CI 0.04 – 1)
PL12	2 (3.5)	1 (1.8)	100	98.2	0.5	1	55.6	0.66 (95%CI 0.04 – 1)
EJ	0	0	-	-	-	-	n/a	-
OJ	0	0	-	-	-	-	n/a	-
KS	1 (1.8)	0	-	98.3	-	1	n/a	-

IP. While IP would help avoid issues associated with using immunoassays in clinical practice, IP has been primarily restricted to research settings (12, 20). The validation of widely used immunoassays in large cohorts, such as the CLASS project, is imperative to ensure congruence with the reference standard (21). However, even in cases where IP is utilised, the agreement we observed in our cohort suggests that clinicians should exercise caution in interpreting the results. Given these considerations, it becomes evident that detecting anti-ARS antibodies can pose challenges in our daily clinical practice, necessitating a meticulous interpretation of antibody testing (21).

Our study has demonstrated that local assessment methods for anti-Jo1, encompassing LIA, ELISA, and IP, aligned with a very high level of agreement with the results obtained through central testing and our gold-standard definition. ELISA exhibited the highest degree of concordance for detecting anti-Jo1 antibodies. Hence, clinicans should regard ELISA as a reference technique for determining the presence of anti-Jo1 antibodies (8). These immunoassays are widely accessible, straightforward to perform, and cheaper than radio-labelled assays such as IP. Additional confirmatory tests are not required for patients who test positive for Jo1 antibodies using locally reported methods due to our study's high level of agreement.

Considering all the local testing methods employed by centers participating in the CLASS project, there was a good overall agreement for non-anti-Jo1 antibodies. However, there is a cause for concern due to the observed poor PPV. However, the calculated PLR for non-

anti-Jo1 suggests that detecting these antibodies may be relevant for patients with a high pre-test probability.

The agreement with local IP was not as robust as anticipated due to the relatively low number of samples being assessed with local IP and disagreement observed between central and local results in detecting some anti-ARS such as anti-PL7, anti-OJ and anti-KS. However, correlation coefficients for anti-PL12 and anti-EJ on local IP demonstrate very good agreement, with $\varkappa$ values of 0.90 and 0.88, respectively. For anti-PL12, when utilising our central definition, local IP achieved a PPV of 1.00. However, it had a slightly lower sensitivity at 83.3%. Local IP also performed well for anti-EJ, with a sensitivity of 81.1% and PPV of 0.81, although its performance was lower than that observed for anti-PL12.

Local LIA results demonstrated good agreement for anti-PL12 and anti-EJ antibodies with moderate agreement for anti-PL7. ELISA displayed good agreement for both anti-PL12 and anti-EJ antibodies. However, local LIA only has a PPV of 0.52 for anti-PL7 positive samples. For anti-PL12, ELISA was found to have a higher PPV than local LIA of 0.75 and 0.58, respectively, while identifying over two-thirds of the positive central samples. However, concerningly, the PPV demonstrated for anti-PL12 suggests that up to 25-40% of samples could be false positives. LIA and ELISA had PPV values of 0.64 and 0.67 for anti-EJ antibodies, respectively, indicating that these assays correctly identified two-thirds of locally positive samples according to our central definition. There was no significant correlation with our central definition for the rarer anti-ARS, including anti-OJ and anti-KS. In the case of anti-KS, the negative weighted κ coefficient from LIA suggested a random relationship between local testing and our central definition. However, interpreting these results is challenging due to the limited availability of positive serum samples for anti-OJ, anti-KS, and even anti-Zo.

Experts have raised concerns about interpreting results related to MSAs and anti-ARS antibodies. In an online survey involving 111 members of IMACS across 65 institutions and six continents, 36% expressed a lack of confidence in the MSA testing results from their respective laboratories. Among those who did express confidence, a significant number indicated that their confidence levels fluctuated depending on the specific type of MSA identified (15).

Commercial LIA is increasingly used worldwide between the available assays, allowing the identification of different MSAs with a single assay. Although clinicians can use underlying MSA specificity to define IIM subsets, Lackner *et al*. observed that in a cohort of 249 patients positive for MSAs, only 45 (18%) were diagnosed with a myositis spectrum disorder (13). LIA false positive rates can reach 30% in amyopathic connective tissue disease patients, most associated with weakly positive results (14). Commercial immunoassays can also demonstrate positivity for multiple MSAs despite mainly being mutually exclusive, which can complicate antibody interpretation in the real world (22).

Furthermore, clinicians should exercise greater discretion and consider the degree of clinical suspicion when requesting immunoassays, limiting their use to situations where a positive result significantly alters the diagnosis follow-up plan or influences therapeutic decisions for the patient. Clinicians must recognise that immunoassays, particularly for non-anti-Jo1 antibodies, may yield false positive or negative outcomes, potentially leading to unnecessary follow-up procedures, appointments and treatments (23). Importantly, in challenging cases where there is doubt over the accuracy of an immunoassay, a multidisciplinary process should be utilised, including a laboratory specialist with an interest in autoantibody testing (16).

A two-step approach may be a suitable methodology, commencing with LIA and confirming positivity through ELISA. While clinicians often concentrate on the risk of false positive results, it is important to acknowledge the possibility of false negatives. This could be due to the omission of certain anti-ARS on different LIA testing kits or in the other methods applied, or perhaps because of the existence of as-yet-undetected ARS antibodies. We believe that the forthcoming classification criteria for ASSD, a primary focus of the CLASS project, will provide clinicians with guidance regarding this problematic issue and aid in interpreting MSAs' significance.

This study has limitations that warrant consideration. As previously noted, we could not determine the degree of LIA positivity and whether some positive results in the CLASS cohort were borderline or weakly positive. Secondly, various LIA testing kits may have been employed in other centres, each including different anti-ARS antibodies. We applied stringent criteria for dual positive assays in our central gold standard testing, which also necessitated the exclusion of several samples due to logistical constraints when dual testing was not feasible due to limited serum samples available or funding limitations. Additionally, the limited number of samples and results available for antibodies such as OJ, KS, and Zo made it challenging to draw meaningful interpretations for rarer ARS antibodies. Moreover, anti-OJ can only reliably be detected using IP, most likely as it forms part of a multi-synthetase complex and autoantibodies are directed against conformational or quaternary epitopes; therefore, our gold-standard definition would not have allowed theoretical OJ samples to be included (24-26). However, it is important to note that within our cohort, four local samples tested positive for anti-OJ, but all of them subsequently tested negative in central immunoprecipitation tests conducted on two separate occasions. Our definition of anti-Jo1 positivity accommodated ELISA-positive results, even when IP yielded negative results (19.4%). This factor could account for the significant agreement between anti-Jo1 and the local ELISA results. Lastly, we could not confirm whether multiple testing methods were employed locally to identify the relevant anti-ARS antibodies, as local physicians could only report one testing method. While it appears reasonable that most anti-Jo1 positive patients also underwent ELISA screening tests, as confirmed by cases at our centres, we lacked information from all participating centres.

Conclusion

Our study assesses the real-world performance of identifying anti-ARS in a large cohort of multinational patients with ASSD and real-world controls. We found that the most commonly used technique for identifying MSA was LIA, which was especially demonstrated within Europe, South America and India. While local methods for detecting anti-Jo1 antibodies proved reliable compared to our gold-standard definition and central testing results, the picture differed for non-Jo1 antibodies. We observed that the PPV for non-Jo1 antibodies ranged between 0.5–0.7, with varying levels of agreement compared to our gold-standard and central definitions for the presence of ASSD antibodies. Specifically, anti-PL7 antibodies and the rarer anti-synthetase antibodies, such as anti-OJ, anti-KS, and anti-Zo, exhibited the highest level of disagreement between local and central testing results, however, limited samples' number make interpretation difficult.

Clinicians must be aware of the reduced specificity and the potential for high false positivity when using current real-world methods to identify non-anti-Jo1 antibodies, in contrast to the more reliable detection of anti-Jo1. Accurate identification of anti-ARS is vital, not only due to their inclusion in future ASSD classification criteria but also because it can provide valuable clinical insights into prognosis and disease progression. These insights can significantly influence treatment decisions (15, 22, 27, 28).

Our analysis underscores the importance of not solely relying on serological testing for non-anti-Jo1 assays, except when performed using IP, particularly when the clinical presentation does not align with the diagnosis. This study also highlights the need to develop and validate clinical-serological classification criteria for non-Jo1 ASSD rather than depending solely on serology for diagnosis or classification.

Take home messages

1. What is already known? The recognition and significance of identifying anti-synthetase antibodies as diagnostic and prognostic markers in anti-synthetase syndrome are growing. Commercial immunoassays (ELISA/Line blot) are increasingly used to screen patients for these antibodies. Nevertheless, concerns persist regarding the accuracy of these assays.
2. What does this study add? Our study assesses the real-world performance of locally reported testing methods against our central definition of positivity for anti-aminoacyl tRNA synthetase antibodies using the CLASS database, which, to our knowledge, is the largest database of anti-synthetase syndrome patients.
3. How does this study affect current practice? The use of commercial immunoassays for the assessment of anti-Jo1 is reliable; however, clinicians must interpret positive results for non-anti-Jo1 antibodies with caution. We recommend confirming with the ANA pattern or utilising another confirmatory test if there is doubt from the clinician about the accuracy of the non-anti-Jo1 immunoassay result.

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Competing interests

G. Sambataro has received speaking honoraria by Boehringer Ingelheim outside this work.
C. Selmi has received speaker's fees from Octapharma.
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The other authors have declared no competing interests.

References

1. BETTERIDGE Z, TANSLEY S, SHADDICK G *et al.*: Frequency, mutual exclusivity and clinical associations of myositis autoantibodies in a combined European cohort of idiopathic inflammatory myopathy patients. *J Autoimmun* 2019; 101: 48-55. https://doi.org/10.1016/j.aut.2019.04.001
2. TANSLEY SL, SIMOU S, SHADDICK G *et al.*: Autoantibodies in juvenile-onset myositis: Their diagnostic value and associated clinical phenotype in a large UK cohort. *J Autoimmun* 2017; 84: 55-64. https://doi.org/10.1016/j.jaut.2017.06.007
3. SATOH M, TANAKA S, CERIBELLI A, CALISE SJ, CHAN EK: A comprehensive overview on myositis-specific antibodies: new and old biomarkers in idiopathic inflammatory myopathy. *Clin Rev Allergy Immunol* 2017; 52(1): 1-19. https://doi.org/10.1007/s12016-015-8510-y
4. DOURADO E, BOTTAZZI F, CARDELLI C *et al.*: Idiopathic inflammatory myopathies: one year in review 2022. *Clin Exp Rheumatol* 2023; 41(2): 199-213. https://doi.org/10.55563/clinexprheumatol/jof6qn
5. BOZZALLA-CASSIONE E, ZANFRAMUNDO G, BIGLIA A *et al.*: Anti-Ro52 antibodies positivity in antisynthetase syndrome: a single centre cohort study. *Clin Exp Rheumatol* 2022; 40 (Suppl. 134): S27-31. https://doi.org/10.55563/clinexprheumatol/bjb2gf
6. CAVAGNA L, MELONI F, MEYER A *et al.*: Clinical spectrum time course in non-Asian patients positive for anti-MDA5 antibodies. *Clin Exp Rheumatol* 2022; 40(2): 274-83. https://doi.org/10.55563/clinexprheumatol/di1083
7. FREDI M, CAVAZZANA I, CERIBELLI A *et al.*: An Italian multicenter study on anti-NXP2 antibodies: clinical and serological associations. *Clin Rev Allergy Immunol* 2022; 63(2): 240-50. https://doi.org/10.1007/s12016-021-08920-y
8. TANSLEY SL, LI D, BETTERIDGE ZE, MCHUGH NJ: The reliability of immunoassays to detect autoantibodies in patients with myositis is dependent on autoantibody specificity. *Rheumatology* (Oxford) 2020; 59(8): 2109-14. https://doi.org/10.1093/rheumatology/keaa021
9. AGGARWAL R, ODDIS CV, GOUDEAU D *et al.*: Autoantibody levels in myositis patients correlate with clinical response during B cell depletion with rituximab. *Rheumatology* (Oxford) 2016; 55(6): 991-9. https://doi.org/10.1093/rheumatology/kev444
10. ZANFRAMUNDO G, FAGHIHI-KASHANI S, SCIRE CA *et al.*: Defining anti-synthetase syndrome: a systematic literature review. *Clin Exp Rheumatol* 2022; 40(2): 309-19. https://doi.org/10.55563/clinexprheumatol/8xj0b9
11. TANSLEY S, GUNAWARDENA H: The evolving spectrum of polymyositis and dermatomyositis--moving towards clinicoserological syndromes: a critical review. *Clin Rev Allergy Immunol* 2014; 47(3): 264-73. https://doi.org/10.1007/s12016-013-8387-6
12. CAVAZZANA I, FREDI M, CERIBELLI A *et al.*: Testing for myositis specific autoantibodies: Comparison between line blot and immunoprecipitation assays in 57 myositis sera. *J Immunol Methods* 2016; 433: 1-5.

https://doi.org/10.1016/j.jim.2016.02.017
13. LACKNER A, TIEFENTHALER V, MIRZAYEVA J *et al*.: The use and diagnostic value of testing myositis-specific and myositis-associated autoantibodies by line immuno-assay: a retrospective study. *Ther Adv Musculoskelet Dis* 2020; 12: 1759720X20975907. https://doi.org/10.1177/1759720X20975907
14. TO F, VENTIN-RODRIGUEZ C, ELKHALIFA S, LILLEKER JB, CHINOY H: Line blot immunoassays in idiopathic inflammatory myopathies: retrospective review of diagnostic accuracy and factors predicting true positive results. *BMC Rheumatol* 2020; 4: 28. https://doi.org/10.1186/s41927-020-00132-9
15. TANSLEY SL, SNOWBALL J, PAULING JD *et al*.: The promise, perceptions, and pitfalls of immunoassays for autoantibody testing in myositis. *Arthritis Res Ther* 2020; 22(1): 117. https://doi.org/10.1186/s13075-020-02210-2
16. TIRELLI C, MORANDI V, VALENTINI A *et al*.: Multidisciplinary approach in the early detection of undiagnosed connective tissue diseases in patients with interstitial lung disease: a retrospective cohort study. *Front Med* (Lausanne) 2020; 7: 11. https://doi.org/10.3389/fmed.2020.00011
17. CERIBELLI A, ISAILOVIC N, DE SANTIS M *et al*.: Myositis-specific autoantibodies and their association with malignancy in Italian patients with polymyositis and dermatomyositis. *Clin Rheumatol* 2017; 36(2): 469-75. https://doi.org/10.1007/s10067-016-3453-0
18. ALTMAN DG: Practical statistics for medical research: London: Chapman and Hall; 1991. https://doi.org/10.1201/9780429258589
19. MCHUGH ML: Interrater reliability: the kappa statistic. *Biochem Med* (Zagreb) 2012; 22(3): 276-82. https://doi.org/10.11613/BM.2012.031
20. PALTERER B, VITIELLO G, CARRARESI A, GIUDIZI MG, CAMMELLI D, PARRONCHI P: Bench to bedside review of myositis autoantibodies. *Clin Mol Allergy* 2018; 16: 5. https://doi.org/10.1186/s12948-018-0084-9
21. MAHLER M, BETTERIDGE Z, BENTOW C *et al*.: Comparison of three immunoassays for the detection of myositis specific antibodies. *Front Immunol* 2019; 10: 848. https://doi.org/10.3389/fimmu.2019.00848
22. MCHUGH NJ, TANSLEY SL: Autoantibodies in myositis. *Nat Rev Rheumatol* 2018; 14(5): 290-302. https://doi.org/10.1038/nrrheum.2018.56
23. SHINODA K, OKUMURA M, YAMAGUCHI S *et al*.: A comparison of line blots, enzyme-linked immunosorbent, and RNA-immunoprecipitation assays of antisynthetase antibodies in serum samples from 44 patients. *Intern Med* 2022; 61(3): 313-22. https://doi.org/10.2169/internalmedicine.7824-21
24. HAMAGUCHI Y, KUWANA M, TAKEHARA K: Comparison of anti-OJ antibody detection assays between an immunoprecipitation assay and line blot assay. *Mod Rheumatol* 2017; 27(3): 551-2. https://doi.org/10.1080/14397595.2016.1213947
25. NAKASHIMA R, IMURA Y, HOSONO Y *et al*.: The multicenter study of a new assay for simultaneous detection of multiple anti-aminoacyl-tRNA synthetases in myositis and interstitial pneumonia. *PLoS One* 2014; 9(1): e85062. https://doi.org/10.1371/journal.pone.0085062
26. VULSTEKE JB, SATOH M, MALYAVANTHAM K, BOSSUYT X, DE LANGHE E, MAHLER M: Anti-OJ autoantibodies: rare or underdetected? *Autoimmun Rev* 2019; 18(7): 658-64. https://doi.org/10.1016/j.autrev.2019.05.002
27. MALAVIYA AN: 2017 EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups: little emphasis on autoantibodies, why? *Ann Rheum Dis* 2018; 77(11): e77. https://doi.org/10.1136/annrheumdis-2017-212701
28. LUNDBERG IE, DE VISSER M, WERTH VP: Classification of myositis. *Nat Rev Rheumatol* 2018; 14(5): 269-78. https://doi.org/10.1038/nrrheum.2018.41