Quick reference guide to interpretation of Myositis Specific Antibodies for healthcare professionals

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Key points:

1. Myositis Specific Antibodies (MSA) are positive in 60% of the IIM. A negative MSA does not rule out a diagnosis of juvenile onset IIM.
2. MSAs can help categorise disease phenotypes and may therefore help in discussion regarding prognosis, or identify groups as risk of certain features such as interstitial lung disease or calcinosis.
3. MSAs may be measured by different techniques including line blot, dot blot, commercial multiplex assays, ELISA, gel precipitation methods, and radio-immunoprecipitation. Results may vary according to which technique is used.
4. Myositis Specific Antibodies (MSA) and Myositis Associated Antibodies (MAA) can co-exist together, but it is extremely unusual to have more than 1 MSA positive in any one patient. Consider further testing at a specialist lab (e.g. University of Bath Lab, UK) if the result does not match what is expected clinically.
5. Some MSAs have different associations in childhood onset disease vs. adult onset disease. For example, in childhood onset IIM, TIF1γ and NXP2 are not associated with malignancy. Other antibodies (eg. Anti-synthetase antibodies) have similar associations, but are less common in juvenile onset IIM.
6. MSA may be positive despite a negative ANA antibody. It is helpful to be aware of which MSAs are associated with a positive ANA and which ones are not (cytoplasmic) – see details below.
7. Myositis Associated Antibodies (MAA) tend to be seen in overlap syndromes. It is possible to have a positive MAA and a positive MSA. Some test results may help guide prognosis – eg. Ro-52 positive MAA in a patient with positive anti-synthetase antibodies may increase risk of ILD.

For reference, please refer to the following sections:

A. MSA / MAA antibodies and associations in juvenile onset IIM
B. Hep-2 staining patterns corresponding to different auto-antibody specificities in IIM
C. Methods for detecting MSA and key points to be aware of.
D. MSA that may change / decrease over time with treatment:
E. Links to educational tools for more information on MSA.
F. Useful references.

A. MSA / MAA antibodies and associations

Adapted & updated from Wu Q, Wedderburn LR, McCann LJ. Juvenile dermatomyositis: Latest advances. Best Pract Res Clin Rheumatol. 2017 Aug;31(4):535-557.

	Autoantibody	**Autoantigen target**	**Frequency in juvenile population**	**Clinical features in juvenile population**
MSA	Anti-TIF1γ (Anti-p155/140)	Transcriptional intermediary factor 1	18-35% Highest prevalence in white race Younger age group (median age = 7 years)	• Risk of cutaneous ulceration / worse cutaneous disease, lipodystrophy, contractures and chronic disease course. • Greater muscle weakness (CMAS)
	Anti-NXP2 (Anti-MJ)	Nuclear matrix protein 2	15-25% Highest prevalence in white race Younger age group (median age = 6 years)	• Younger age of disease onset • Greater muscle weakness, dysphagia, dysphonia • Increased risk of calcinosis • Severe disease course, persistent disease activity.
	Anti-MDA5	Melanoma differentiation-associated gene 5	6-38%	• Mild disease • Increased risk of cutaneous and oral ulceration • Arthritis • ILD (rapidly progressive in Japanese / Korean / Chinese cohorts)
	Anti-Mi-2	Nucleosome-remodeling deacetylase complex	4-10% Larger proportion of non-white patients Mostly Hispanic Older age group (median age = 11 years)	• Marked muscle disease early in disease course, but decreased odds of remaining on treatment over time • Dysphagia • Oedema • Cutaneous features

	Anti-SAE	Small ubiquitin-like modifier activating enzyme	6-8% in European cohorts ~ 2% in Asian cohorts	• Predominant cutaneous involvement • Amyopathic at onset
	Anti-synthetase antibodies: ASA (Anti-Jo-1, Anti-PL-12, Anti-EJ, Anti-KS, Anti-PL-7, Anti-OJ, Anti-Ha, Anti-Zo)	Aminoacyl tRNA synthetases (ARS)	2-5% Larger proportion of non-white patients Older age of onset (median age = 14 years)	• Anti-synthetase syndrome: ILD, Raynaud's, arthritis, fever, mechanics hands & rash • Lipoatrophy • Anti-Jo-1: Increased frequency of myositis, arthralgia & mechanic's hands • Non-Jo-1 ASA-positivity: Increased frequency of DM skin lesions, fever & ILD
	Anti-SRP	Signal recognition particle	2% JDM Increased prevalence in Black race Older age of onset (median age = 15 years)	• More likely to be classified as JPM • Severe necrotising myopathy • Chronic disease course (treatment resistant) • Dysphagia • Raynaud's • Arthritis • Proximal & distal muscle weakness, very high CK • Increased risk of cardiac involvement in some studies
	Anti-HMGCR	3-hydroxy-3-methylglutaryl-coenzyme A reductase	~ 1% JDM	• More likely to be classified as JPM • Increased risk of muscle weakness, & dysphagia

MAA	Anti-Ro (SSA)	52 or 60kDa ribonucleoproteins (hYRNA)	6% JDM 14-25% myositis overlap	• Associated with poorer prognosis with decreased functional status with long-term follow up • Associated with lung disease
	Anti-La (SSB)	Ribonucleoprotein	2-12% myositis overlap	• No significant associations described in IIM
	Anti-U1-RNP	U1 ribonucleoprotein (snRNP)	3-8% JDM / JPM 25-40% myositis overlap	• Polymyositis / polymyositis overlap phenotype • Older age at disease onset. Less likely to be weak • Arthritis, Raynaud's and sclerodactyly
	Anti PM-Scl	Nucleolar multi-protein complex	3-5%	• Myositis overlap, most commonly scleroderma (SSc) overlap features • Risk of ILD, arthritis, Raynaud's • Association described with calcinosis & lipoatrophy
	Anti-Ku	p70/p80 heterodimer, DNA-associated proteins	9-19% patients with myositis overlap	• CTD overlap • Increased arthralgia, Raynaud's, ILD & musculoskeletal manifestations

MSA: myositis specific antibodies; MAA: myositis associated antibodies; ILD: interstitial lung disease; IIM: idiopathic inflammatory myopathies; JPM: juvenile polymyositis; JDM: juvenile dermatomyositis

B. Hep-2 staining patterns corresponding to different auto-antibody specificities in IIM

Adapted from 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 Feb;52(1):1-19.

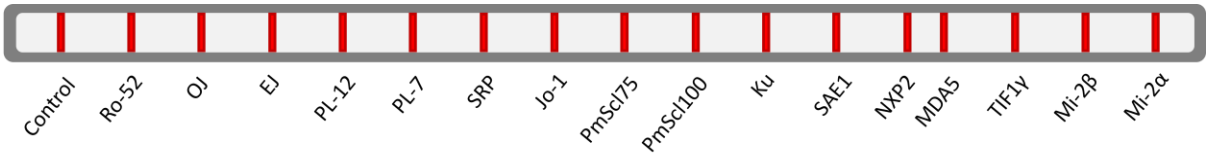
Hep-2 cell immunofluorescence pattern	**MSA / MAA**
Nuclear - Speckled	U1 RNP U1 / U2 RNP Ku
Nuclear - Fine speckled	Mi-2 TIF1γ SAE MJ / NXP2
Nuclear - Multiple dots	MJ / NXP2
Nuclear - Cajal body	SMN
Nucleolar	PM-Scl U3RNP
Cytoplasm	ARS SRP MDA5
Negative	MDA5 ARS

C. Methods for detecting MSA and key points to be aware of:

Method	***Line blot***	***ELISA***	***Immunoprecipitation***
Strengths	Cheap to perform, with rapid results. Tests multiple MSA at same time.	Fast and accurate. Produces a quantitative result.	- Accurate. Considered the reference standard. Can detect novel autoantibodies
Weaknesses	False +ve MSA in 12-13% cases.	Multiple assays needed to test for all MSA.	- Additional testing required to confirm NXP2 and MDA5 which produce very similar patterns (140kDa band). Limited availability. Low-throughput (up to 6 weeks to return result) - Expensive
MSA not reliably detected by this method.	Does not reliably detect TIF1γ or rare ARS		- Does not detect anti-Ro-52, anti-HMGCR or anti-CN1a.

The Euroimmun line blot is currently the most widely used assay in the UK for detecting MSA

The 16Ag strip includes the following MSA: (a strip including anti-CN1a is also available and used in some centres).



Note: there are 2 antigens for anti-PmScl (75 and 100) and anti Mi-2 (α and β)

Top tips for interpretation of line blot:

Poor agreement between line blot and immunoprecipitation (Cohens K <0.8) was seen for

Anti-Mi-2
Anti-TIF1γ – only 60% of anti-TIF1γ positives were detected by line blot
Anti-PmScl75
Anti-EJ
Anti-OJ – none were detected by line blot

Reference:

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 Aug 1;59(8):2109-2114.

Multiple MSA specificities strongly suggest the presence of at least one false positive result

If suspicious of false positives/negatives, consider additional testing using an alternative method. Use the clinical phenotype and ANA pattern as a guide. Repeating the same type of test is rarely helpful.

Remember key autoantibodies **NOT** tested for by this assay

Anti-U1RNP (tested for by ANA line blot)

Anti-U3RNP (tested for by SSc/nucleolar profile blot) – is there a clumpy nucleolar staining pattern on ANA?

Anti-HMGCR – available via Oxford. Does the patient have IMNM, very high CK, resistant disease and/or minimal/atypical skin rash?

Anti-CN1a – the significance of this autoantibody in JDM is not clear:
One study identified anti-CN1a in 27% of JDM patients (using immunoblot). Anti-CN1a positivity was associated with more severe disease.
A second study (using ELISA) did not find anti-CN1a in any JDM patients

References:

Yeker RM, Pinal-Fernandez I, Kishi T, et al. Childhood Myositis Heterogeneity Collaborative Study Group. Anti-NT5C1A autoantibodies are associated with more severe disease in patients with juvenile myositis. Ann Rheum Dis. 2018 May;77(5):714-719.

Rietveld A, Wienke J, Visser E, et al. Juvenile Dermatomyositis Research Group and the Dutch Myositis Consortium. Anti-Cytosolic 5'-Nucleotidase 1A Autoantibodies Are Absent in Juvenile Dermatomyositis. Arthritis Rheumatol. 2021 Jul;73(7):1329-1333.

D. MSA that may change / decrease over time with treatment:

MSA titre often changes over time. In adults, small studies have shown a relationship between the titres of anti-Jo-1, anti-MDA5, anti-HMGCR and anti-SRP autoantibodies with disease activity measures. Anti-MDA5 has been shown to be useful in predicting response to treatment in Japanese children with JDM.

It is likely to be a change in titre from previous that is most relevant to disease activity, rather than the absolute value/level (akin to anti-dsDNA in lupus). Some patients become MSA negative over time/with treatment.

This data is not yet considered sufficiently robust to recommend monitoring of MSA titre.

Currently, repeat MSA testing is rarely clinically indicated.

E. For more information on MSA:

- A narrated PowerPoint presentation and podcast on MSAs are available on the Paediatric Rheumatology European Society (PReS) website https://www.pres.eu/working-parties/jdm-working-party.html
- JDM Cohort and Biomarker Study (JDCBS) website https://juveniledermatomyositis.org.uk

F. Further useful references on MSA:

McHugh NJ, Tansley SL. Autoantibodies in myositis. Nat Rev Rheumatol. 2018 Apr 20;14(5):290-302

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.

This guide is correct at the time of writing.
Please note that assays and corresponding guidance may change in the future.