Standardisation of myositis-specific antibodies: where are we today?

With interest we read the recent article by Espinosa-Ortega *et al*¹ comparing a line immunoassay (LIA) and immunoprecipitation (IP) for the detection of autoantibodies associated with idiopathic inflammatory myopathies (IIM). Historically, most of the clinical associations of myositis specific antibodies (MSAs) and myositis associated antibodies (MAAs) have been established using IP. Consequently, it is important to compare newer technologies such as LIA and dot blots (DB) to IP, which does not imply that IP is correct in all instances or that IP should be regarded as the 'gold standard'.

In a recent study that compared LIA and IP, poor agreement was found for several MSAs.² In the letter by Espinosa-Ortega *et al*, the authors made a similar analysis, comparing IP and LIA in a cohort of 110 patients with IIM. For the LIA, the study also included controls (n=60). A strength of the study is the use of both protein and RNA IP performed in a reference centre for IP. In the interest of assay evaluation and standardisation, it is valuable to provide data showing a more statistics-based approach for method comparison. Due to the rarity of several of the MSAs, large cohorts with thousands of patients are needed to carefully validate the positive agreement between methods. The small number of positive samples results in large 95% CIs (table 1), which makes it difficult to draw true conclusions from the results. For example, in the article of Espinosa-Ortega *et al*, the 95% CI derived from *Kappa* statistics for anti-TIF1 γ ranged from 0.29 (poor) to 0.83 (excellent). Table 1 summarises the

results obtained by Espinosa-Ortega *et al*¹, Cavazzana *et al*.² and Mahler *et al*.⁴

In addition, several interesting observations merit further discussion. First, the observation that anti-Mi-2 antibodies do not reflect the known clinical phenotype with skin involvement is interesting and might be related to the assay used. In two recent studies it has been demonstrated that the prevalence of anti-Mi-2 antibodies in polymyositis might be assay dependent.^{3,4} Second, for TIF1 γ it is important to specify how positivity by IP is defined. The original description of anti-TIF1 γ with IP reported a double band (p140/p155), corresponding to TIF1 α (140 kDa) and TIF1 γ (155 kDa). Some laboratories might report both, as based on IP, or only anti-TIF1 γ , as based on either the 155 kDa IP band or a secondary test. Most commercial *in-vitro* diagnostic assays, including LIAs, detect only anti-TIF1 γ , which might result in different results. While anti-TIF1 γ is associated with cancer, anti-TIF1 α co-occurs with anti-TIF1 γ and anti-Mi-2 autoantibodies.⁵ As a matter of fact, about 80% of patients with anti-Mi-2 antibodies also express anti-TIF1 α autoantibodies.⁵ In these patients the clinical phenotype matches the co-occurring MSA, rather than having a separate phenotype. In this context, it should be noted that there is a large variation in anti-TIF1 γ reactivity between different LIA methods, with, for example, the Euroimmun LIA detecting more anti-TIF1 γ reactivity than the AlphaDia DB.⁶

Espinosa-Ortega *et al* emphasise the importance of anti-TIF1 γ due to its association with cancer; however, other markers such as anti-MDA5 autoantibodies are equally important due to the association with rapidly progressive interstitial lung disease. In addition,

Table 1 Agreement between LIA and IP derived from three studies

	Espinosa-Ortega <i>et al</i>	Cavazzana <i>et al</i>	Mahler <i>et al</i>	Combined	
MSA	DP/IP/LP/DN <i>Kappa</i> (95% CI)				Interpretation (<i>kappa</i>)
Jo-1	11/1/7/91 0.69 (0.50 to 0.89)	3/5/7/42 0.21 (−0.11 to 0.52)	NA	14/6/14/133 0.52 (0.33 to 0.70)	Minimal–Moderate
TIF1 γ	1/2/0/107 0.56 (0.29 to 0.83)	4/0/2/51 0.78 (0.49 to 1.00)	11/4/4/138 0.71 (0.51 to 0.90)	16/6/6/296 0.71 (0.55 to 0.86)	Weak–Strong
SRP	3/1/0/106 0.85 (0.57 to 1.00)	0/3/7/47 −0.08 (−0.15 to −0.01)	14/1/8/134 0.73 (0.56 to 0.89)	17/5/15/287 0.60 (0.44 to 0.76)	Weak–Moderate
MDA5	1/2/0/107 0.49 (−0.12 to 1.00)	3/1/2/51 0.64 (0.26 to 1.00)	14/1/5/137 0.80 (0.65 to 0.96)	18/4/7/295 0.75 (0.61 to 0.89)	Moderate–Strong
PM/Scl	2/3/1/104 0.48 (0.05 to 0.92)	NA NA	NA NA	2/3/1/104 0.48 (0.05 to 0.92)	NA
Ku	3/0/1/106 0.86 (0.57 to 1.00)	NA NA	NA NA	3/0/1/106 0.86 (0.57 to 1.00)	NA
SAE	3/0/1/106 0.86 (0.57 to 1.00)	0/2/1/54 −0.02 (−0.06 to 0.01)	15/0/3/139 0.90 (0.79 to 1.00)	18/2/5/299 0.83 (0.70 to 0.95)	Moderate–Almost perfect
NXP2	0/0/2/108 0.00	5/3/2/47 −0.62 (0.31 to 0.92)	13/2/0/142 0.92 (0.81 to 1.00)	18/5/4/297 0.79 (0.65 to 0.92)	Moderate–Almost perfect
Mi-2	2/0/6/102 0.38 (0.00 to 0.76)	3/0/5/49 0.51 (0.45 to 0.87)	12/3/9/133 0.62 (0.43 to 0.82)	17/3/20/284 0.56 (0.41 to 0.72)	Weak–Moderate
EJ	0/0/0/110 NA	1/2/0/54 0.49 (−0.11 to 1.00)	7/3/1/146 0.76 (0.54 to 0.99)	8/5/1/310 0.72 (0.50 to 0.93)	Weak–Almost perfect
OJ	0/1/0/109 0.00	0/2/0/55 0.00	NA	0/3/0/164 NA	NA
PL-7	0/0/0/110 NA	0/0/2/55 0.00	12/3/0/142 0.88 (0.74 to 1.00)	12/3/2/307 0.82 (0.67 to 0.97)	Moderate–Almost perfect
PL-12	0/0/1/109 0.00	0/0/3/54 0.00	14/1/5/137 0.80 (0.65 to 0.96)	14/1/9/300 0.72 (0.56 to 0.89)	Weak–Strong

95% CIs were reconstructed based on the given prevalence.

DN, double negative; DP, double positive; IP, Immunoprecipitation positive; LP, line immunoassay positive; MSA, myositis specific antibody.

Correspondence

Table 2 Overview of myositis specific antibodies and contribution to clinical decisions.

MSA	Criticality/comment	Relevance/criticality
HMGCoA reductase	The presence of anti-HMGCR antibodies predicts poor response to corticosteroid and immunosuppressant therapy. This anticipation is relevant for the clinician.	Relevant, not critical; however, false negative might lead to omission of biopsy
TIF1y	Strong association with malignancy in elderly patients. Extensive screening for malignancy is necessary prior to treatment of IIM.	Critical, false negative result might lead to delayed diagnosis of cancer
SRP	Associated with severe treatment resistant myopathy, leading to long term immunosuppressive therapy. The presence of SRP antibodies predicts poor response to corticosteroid and immunosuppressant therapy. This anticipation is relevant for the clinician.	Relevant, not critical; however, false negative might lead to omission of biopsy
MDA5	Positive result should trigger screening for ILD and if confirmed more aggressive treatment and clinical vigilance	Critical, False negative result may lead to less intensive (respiratory) monitoring with a delay
PM/Scl	In general, associated with a milder disease course	Not critical, low relevance
Ku	Often associated with SLE and/or SSc. Requires monitoring and treatment to coexisting SLE and/or SSc, especially when other antibodies are present (eg, anti-dsDNA). ¹²	Relevant, not critical
SAE	Severe cutaneous disease that classically precede DM with severe dysphagia and systemic symptoms.	Relevant, not critical
NXP2	Juvenile DM, diffused calcinosis. Cancer associated DM triggers consideration for screening for concurrent malignancy prior to treatment initiation.	Critical, similar to TIF1y, however, less pronounced
Mi-2	In general, associated with a milder disease course. If present without other MSA, reassures relatively mild disease phenotype.	Relevant, not critical; false positive can lead to a wrong perception of milder disease (no monitoring for ILD)
Jo-1, EJ, OJ, PL-7, PL-12	Positive result triggers the clinician to screen for ILD and to prospectively follow-up pulmonary function; often requires long-term immunosuppressive treatment <ul style="list-style-type: none"> ► Predicts better therapeutic response to rituximab¹³ ► Influences patient management when pulmonologists identify these antibodies in patients with unexplained ILD ► Consider immunosuppressant strategies ► Follow-up for appearance of extra-pulmonary manifestations of the antisynthetase syndrome¹⁴ 	Relevant, not critical; False negative results might have a negative effect on optimal therapy choice in severe myositis (more arguments for rituximab) and patients with unexplained ILD (as single manifestation) might experience delay in diagnosis.

DM, dermatomyositis; IIM, idiopathic inflammatory myopathies; ILD, interstitial lung disease; MSA, myositis specific antibodies; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

the consequences for false positive or false negative results can be different depending on the autoantibody (see [table 2](#)).

Similar to a previous study reporting variability between blot assays for anti-Jo-1 antibodies,⁶ the current letter also reports higher than expected variability for anti-Jo-1 antibodies which is related to the known limitation of IP for the detection of anti-Jo-1 antibodies, due to the relatively thin uncharacteristic band in IP (in contrast to other aminoacyl-tRNA synthetases) and to the co-migration with the IgG heavy chain.⁷

IP analysis of RNA components to confirm the presence of tRNA is helpful; however, it does not reveal the tRNA identity; it only indicates IP of 'some' tRNA and cannot distinguish between histidyl-tRNA and other tRNAs. Practically, if a band corresponding to a ~50 kD protein is observed on IP, and tRNA by RNA analysis is present, it is reasonable to report anti-Jo-1 antibody positivity.⁷ Seven out of 18 anti-Jo-1 positive samples, as detected by LIA were negative by IP. Three of them tested positive by a fluorescence enzyme immunoassay (FEIA), one borderline and one negative. It appears that IP misses true positive anti-Jo-1 samples that are detected by LIA and FEIA. This last observation is in line with the concept that IP should not be regarded as a gold standard in all instances. Although known and used for decades, IP is by no means standardised across different laboratories which can lead to significant variability. Consequently, site to site comparison studies for IP are needed, followed by a standardisation approach.

As pointed out above, the rarity of several of the MSAs requires large cohorts with thousands of patients in order to carefully validate agreement (especially positive per cent agreement) between methods. A viable and more realistic approach is to select positive samples from clinically well-characterised patients

and complement those with also well characterised controls and test this cohort with various methods and platforms, including IP and LIA. Although this introduces a biased inclusion criterion, it might provide more meaningful data. Along those lines, a close collaboration between clinicians, patient groups, research networks and kit manufacturers are mandatory to make serum samples available for validation, calibration and quality control. An alternative approach for calibration and quality control is the generation of human or humanised monoclonal antibodies. Standardisation is imperative for the consideration of additional MSAs (in addition to anti-Jo-1) as part of future classification criteria for IIM.^{8–10} To conclude, we thank the authors for conducting this study and encourage future studies with larger patient cohorts (such as MyoNet/EuroMyositis), ideally including different novel methods for the detection of MSA next to IP.¹¹

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Contributors All authors contributed to the correspondence.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests MM is employee of Inova Diagnostics. However, no product of the company is mentioned in the correspondence.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

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To cite Mahler M, Vulsteke J-B, Bossuyt X, *et al.* *Ann Rheum Dis* Epub ahead of print: [please include Day Month Year]. doi:10.1136/annrheumdis-2019-216003

Received 8 July 2019

Accepted 10 July 2019

Ann Rheum Dis 2019;0:1–3. doi:10.1136/annrheumdis-2019-216003

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