Detection of myositis-specific antibodies: additional notes

With interest we read the recent article by Vulsteke *et al*[1] showing data derived from an evaluation of three immunoassay systems for the detection of autoantibodies associated with autoimmune inflammatory myopathies (AIM). As stated by the authors, careful evaluation of autoantibody assays for the detection of myositis-specific (MSA) and myositis-associated (MAA) antibodies is of utmost importance since some of these are included or being considered for the AIM classification criteria.[2–4] The biomarkers are also relevant for establishing the diagnosis and stratification into specific disease subsets.

The authors compared the performance of three test systems and used primarily clinical diagnoses and features as comparators. In the interests of assay evaluation and standardisation, it is valuable to also provide data showing a more comprehensive statistics-based approach for method comparison. However, this might be linked to the small number of AIM patients tested (n=144) and the small number of positive cases for many of the markers, which represents a limitation of this evaluation and most other studies on MSA and MAA. Although some clinical associations yield statistical significance using P values, verifying significance might be relevant by using Benjamini-Hochberg or Bonferroni correction.

When performing clinical evaluations on AIM, two important aspects to consider are the relatively low prevalence of most MSA and the composition of the control population. Although the differential diagnosis of other systemic autoimmune rheumatic diseases (SARD) is important, there are some challenges. When considering patients with SARD as controls, it is important to rule out overlap syndromes.[5] One example is the association of AIM with interstitial lung disease, which can occur in myositis and in other SARD and especially systemic sclerosis.[6] The differences observed for anti-Jo-1 antibodies are surprising and concerning since those antibodies have been measured for many years,[5] and proficiency testing programmes have shown mostly consistent results (eg, https://www.immqas.org.uk).

Historically, most of the clinical associations of MSA and MAA have been established using immunoprecipitation (IP). Consequently, it is important to also compare newer technologies such as line immunoassays (LIA) and dot blots (DB) with IP, as also stated by Lundberg *et al*.[3] Of relevance, in a recent study comparing LIA and IP, poor agreement was found for several MSAs.[7] This observation does not imply that IP is correct in all instances or that IP should be regarded as the 'gold standard', however, such inter-technology comparative data are invaluable.

To address the significant subjectivity of interpreting LIA and DB assays, automated scanning systems have been developed and introduced for LIA and DB.[8 9] A 'semi-quantitative' approach using scanning systems allows for the analysis of discrepant results considering the antibody levels (titres). One significant limitation of LIA and DB is the lack of analyte specific controls and proper calibration. Consequently, studies of run-to-run and also lot-to-lot variability are required to assess the reliability of the assays and to exclude inter-manufacturer variability that may be attributed to limited precision and reproducibility. Ideally, those studies should contain sufficient samples around the cut-off and follow Clinical and Laboratory Standards Institute guidelines (https://clsi.org/). Along those lines, a close collaboration between patient groups, research networks and kit manufacturers is mandatory to make serum samples available for calibration and quality control. An alternative approach is the generation of human or humanised monoclonal antibodies that can be used in a similar manner. In conclusion, we thank the authors for conducting this study and encourage future studies with larger patient cohorts (such as the MyoNet or EuroMyositis) that will eventually provide sufficient evidence to include more MSA into the classification criteria.

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REFERENCES

1 Vulsteke JB, De Langhe E, Claeys KG, *et al*. Detection of myositis-specific antibodies. *Ann Rheum Dis* 2019;78:e7.
2 Lundberg IE, Tjärnlund A, Bottai M, *et al*. 2017 European League against rheumatism/American college of rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann Rheum Dis* 2017;76:1955–64.
3 Lundberg IE, Bottai M, Tjärnlund A. Response to: 'Performance of the 2017 European league against rheumatism/American college of rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies in clinical practice' by Hočevar et al. *Ann Rheum Dis* 2018;77:e91.
4 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:e77.
5 Mahler M, Miller FW, Fritzler MJ. Idiopathic inflammatory myopathies and the anti-synthetase syndrome: a comprehensive review. *Autoimmun Rev* 2014;13:367–71.
6 Sánchez-Cano D, Ortego-Centeno N, Callejas JL, *et al*. Interstitial lung disease in systemic sclerosis: data from the spanish scleroderma study group. *Rheumatol Int* 2018. 10.1007/s00296-017-3916-x. [Epub ahead of print 10 Jan 2018].
7 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.
8 Mahler M, Meroni PL, Bossuyt X, *et al*. Current concepts and future directions for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies. *J Immunol Res* 2014;2014:1–18.
9 Ghirardello A, Rampudda M, Ekholm L, *et al*. Diagnostic performance and validation of autoantibody testing in myositis by a commercial line blot assay. *Rheumatology* 2010;49:2370–4.