Antisynthetase antibodies in clinical laboratories: the importance of clinical correlation and indirect immunofluorescence. Response to: Comment on: 'Idiopathic inflammatory myopathies and antisynthetase syndrome: contribution of antisynthetase antibodies to improve current classification criteria' by Greco *et al*' by Knitza *et al*

We have recently published a retrospective two centres study of 37 patients with clinical suspicion of idiopathic inflammatory myopathies (IIM) or antisynthetase syndrome (ASSD), and anti-aminoacyl-transfer RNA synthetase (ARS) autoantibodies in the myositis immunoblot. In it, we discussed the role of the ARS in the IIM and the ASSD classification criteria, and a possible overlapping between both of them.¹

Regarding the detection of ARS, previous studies have shown differences in the specificity between different commercial assays; thus, in agreement with the highly appropriate commentaries raised by Knitza *et al* on our report, we consider that a careful interpretation of them is mandatory.^{2,3} In this way, in a recent review Damoiseaux *et al* proposed that to safeguard a high specificity of myositis-specific autoantibodies in multispecific-assays, it could be useful¹: to establish adequate cut-off values in the immunoblot²; to correlate the results with another monospecific-assay (ie, ELISA or immunoprecipitation test) or³ with the HEp-2 indirect immunofluorescence assay (IIFA); and⁴ to correlate them also with the clinical information.² Additionally, it is interesting to mention that recent recommendations of the International Consensus on Antinuclear Antibodies Patterns (ICAP), describes that not all ARS produce an IIFA pattern and that they are more likely to present AC-19 (dense fine speckled) and AC-20 (fine speckled) patterns.⁴

As for the daily practice application of these methodological aspects in our laboratories. (1) The blot assay used was the Euro-line myositis profile three by Euroimmun, and the manufacturer's reference ranges were respected.¹ (2) All cases with anti-Jo1 positivity were validated by ELISA; nevertheless, as in most

clinical laboratories, we do not have ELISA for other than anti-Jo1 ARS or immunoprecipitation test availability. (3) All cases were correlated with IIFA; however, due that it is not sufficiently sensitive for the spectrum of ARS, cases with clinical suspicion of IIM or ASSD were included in our series even without presenting a cytoplasmic pattern.² (4) In our laboratories, the myositis immunoblots are performed only under an adequate clinical suspicion; thus, 33 of 37 cases (89.2%) presented at least one of the clinical manifestations included in the classic triad of the ASSD (table 1).

Concerning the IIFA's, it is interesting to mention that we observed differences in how our laboratories reported them. In one centre they were reported as positive (title $\geq 1/160$) or negative, without discriminating its pattern; thus, only six of 10 analysed cases were reported as positive, including those that fulfilled Solomon's ASSD criteria (n=2). In the other centre, whose Autoimmunity Laboratory has been externally certified by the UK National External Quality Assessment Service for the last 15 years, the IIFA reports were more rigorous. In it, all cases (n=27) presented positive IIFA (title $\geq 1/80$): 19 cases (70.4%) with a cytoplasmic speckled pattern or AC-19/AC-20 pattern if were performed after the first ICAP publication in 2015 (10 of them presented an associated nuclear pattern), and eight cases (29.6%) presented only a nuclear pattern. Evaluating the ASSD diagnosis criteria, 25 cases (92.6%) fulfilled those of Connors', and 15 of them (55.5%) also met Solomon's criteria. Correlating these, 13 of 27 cases with clinical suspicion of IIM or ASSD and positive ARS (48.1%) presented a cytoplasmic speckled IIFA pattern and also fulfilled Solomon's criteria; representing the 68.4% of the cases with these IIFA patterns and the 86.6% of those that met Solomon's criteria. The other two cases that fulfilled Solomon's criteria (13.3%) presented only a nuclear pattern.

To conclude, in line with previous studies, our results suggest that an adequate ASSD clinical suspicion and the presence of cytoplasmic speckled patterns in the IIFA, can safeguard the specificity of the ARS detected by myositis immunoblots, and also increases the probability of fulfilling Solomon's ASSD criteria. Additionally, the differences observed in the IIFA reports between our centres highlights the importance of the International Autoantibody Standardization and the ICAP initiatives^{4,6};

Table 1 Clinical manifestations and ASSD diagnosis criteria fulfilment in patients with positive antisynthetase antibodies*

	Anti-JO1 (n=17)	Anti-PL12 (n=8)	Anti-PL7 (n=4)	Anti-EJ (n=4)	Anti-OJ (n=4)	Total (n=37)
Clinical manifestations included in ASSD diagnosis criteria						
Arthritis†	13 (76.5)	3 (37.5)	1 (25)	1 (25)	3 (75)	21 (56.7)
Interstitial lung disease†	10 (58.8)	3 (37.5)	3 (75)	1 (25)	3 (75)	20 (54.1)
Muscle weakness†	8 (47.1)	5 (62.5)	2 (50)	1 (25)	2 (50)	18 (48.6)
Raynaud phenomenon	7 (41.2)	2 (25)	1 (25)	2 (50)	2 (50)	14 (37.8)
Mechanic hands	7 (41.2)	2 (25)	0 (0)	0 (0)	2 (50)	11 (29.7)
Fever without known cause	5 (29.4)	1 (12.5)	1 (25)	0 (0)	1 (25)	8 (21.6)
Sum of classic triad components						
3/3	2 (11.8)	1 (12.5)	0 (0)	0 (0)	1 (25)	4 (10.8)
2/3	11 (64.7)	3 (37.5)	2 (50)	0 (0)	2 (50)	18 (48.6)
1/3	3 (17.6)	2 (25)	2 (50)	3 (75)	1 (25)	11 (29.7)
0/3	1 (5.9)	2 (25)	0 (0)	1 (25)	0 (0)	4 (10.8)
ASSD diagnosis criteria						
SOLOMON	11 (64.7)	2 (25)	1 (25)	0 (0)	3 (75)	17 (45.9)
CONNORS	16 (94.1)	7 (87.5)	3 (75)	4 (100)	4 (100)	34 (91.9)

*Results are expressed as number (percentage).

†Clinical classic triad components.

ASSD, antisynthetase syndrome.

whose implementation in clinical laboratories could facilitate the development of multicentre studies, and consequently the evaluation of low-frequency antibodies and also of different IIFA patterns to be considered in future IIM and ASSD classification criteria.

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