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Myositis Autoantibodies: A Comparison of Results from the Oklahoma Medical Research Foundation Myositis Panel to the Euroimmun Research Line Blot

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The ability to accurately assay a broad range of well-defined autoantibody specificities in myositis patients is imperative for clinical phenotyping and patient care. In myositis research, the systematic serotyping of patient cohorts is often performed using various research assays, and understanding the relationship between research testing and clinical testing is paramount to advancing the field of myositis. An increasingly popular research multiplex antibody assay platform was developed by Euroimmun (EUROLINE Autoimmune Inflammatory Myopathies 16 Ag IgG, Lubeck, Germany) for myositis-specific and associated autoantibodies. Prior research has suggested that the inter-rater reliability of Euroimmun compared to in-house immunoprecipitation methods (considered the gold standard) is reasonable, with the possible exception of TIF1γ [1–4]. In this communication, we share our experience comparing serological results obtained for clinical purposes with a commercial myositis autoantibody panel (the traditional Oklahoma Medical Research Foundation Myositis panel, “OMRF”) and serologies obtained for research purposes using the Euroimmun Myositis panel.

The OMRF myositis autoantibody panel utilizes several different assays to read out antibodies. These include immunodiffusion, indirect immunofluorescence (ANA), immunoprecipitation of ^{35}S-methionine-labeled proteins from cell extracts and RNA-immunoprecipitation. The assay is performed at the Clinical Immunology Laboratory in Oklahoma City. It tests for autoantibodies recognizing Jo-1, Mi2, SRP, PM/Scl, PL-7,

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PL-12, Ku, EJ, and OJ. Results are provided as negative, positive, or weak positive/indeterminate. Weak positive and indeterminate results were excluded from this analysis (10 values). The Euroimmun Autoimmune Inflammatory Myopathies 16 Ag platform tests for Mi2α, Mi2β, PM/Scl75, PM/Scl100, Ku, Jo-1, SRP, PL-7, PL-12, EJ, OJ, TIF1γ, MDA-5, NXP-2, SAE, and Ro52. Based on the Euroimmun manufacturer package insert, different thresholds correspond with borderline (8–14), low positive (≥15, or +), moderately positive (≥36, or ++) and strongly positive (≥71, or +++) results. Borderline Euroimmun results were considered negative for this analysis. Intra-assay reproducibility was previously established both by Euroimmun as well as by our own lab with excellent agreement. Sensitivity and specificity were subsequently calculated for the OMRF and Euroimmun platforms along with the mean ± standard deviation (SD) of Cohen's kappa statistic in order to measure inter-rater agreement using Stata version 14 (College Station, Texas).

We performed Euroimmun testing on serum samples from all consented patients in The Johns Hopkins Myositis Cohort. The subset of patients (dermatomyositis, polymyositis, or immune-mediated necrotizing myopathy, n = 281) was subsequently identified with clinical OMRF myositis autoantibody testing performed on a serum sample taken on the same day as that used for Euroimmun testing. The importance of matching the bleed date has become increasingly appreciated with studies reporting a change in autoantibody titer over time [5–7]. At the time of antibody testing, patients had a mean age of 52 (±14), and the majority of patients were female (71%). The racial composition of the cohort was 72% Caucasian, 13% African American, 4% Asian, and 11% unknown. The mean disease duration from symptom onset to antibody testing was 3.9±5.1 years, and the majority of patients were on immunosuppressive treatment at the time of antibody testing.

A total of 154 patients (55% of samples tested) had at least one antibody specificity by Euroimmun using the standard positive cutoff of ≥15. Of note, this low prevalence of seropositive patients is likely explained by the fact that several other prominent myositis antibody specificities such as HMGCR, TIF1γ, SAE, NXP-2, and MDA-5 were not included in this study (see below). Using the cutoff of ≥15 on Euroimmun, a total of 15 patients had more than one autoantibody (excluding co-positivity of Mi2α-Mi2β and PM/Scl75-PM/Scl100). The number of patients tested, the number of positive results by each assay, and the corresponding sensitivities, specificities, and kappa statistics are presented in Table 1. The inter-assay agreement using different cutoffs for the Euroimmun assay was calculated using kappa statistics and was found to be highest overall for the moderate cutoff (0.73±0.18, 0.78±0.13, and 0.71±0.27 for thresholds of ≥15/+, ≥36/++, and ≥71/+++, respectively). The lowest threshold (≥15/+) resulted in the best kappa statistic for anti-Jo-1, -Mi2α, -Mi2β, -PM/Scl100, and -Ku autoantibodies. The moderate positive threshold of ≥36/++ resulted in the best kappa statistic for anti-SRP -EJ, and -PL-12. The highest cut-off ≥71/+++ had the best kappa statistic for anti-PM/Scl75 and -PL-7.

To better understand discordant results (patients who were positive by Euroimmun but negative by OMRF), we describe the clinical phenotype of 19 patients who had antisynthetase antibodies in Table 2. Among these 19 Euroimmun positive patients, only 5 (26%) had a clinical picture consistent with the antisynthetase syndrome, and those that did

were often positive for other antibody specificities. Interestingly, many of these patients were amyopathic DM patients.

Our findings will enable researchers to correctly interpret serologies acquired by research testing using the Euroimmun platform. Our study highlights the importance of carefully defined cutoffs for assigning positive antibody status. That is, different autoantibody specificities may require different thresholds to define a positive result. The sensitivity of the Euroimmun assay readout appears to depend on the defined cut-off value. It is also possible that the increased sensitivity may come from measuring different epitopes that are not tested for by OMRF. However, data in Table 2 suggest that this increased sensitivity does not translate to clinically-meaningful results. One important distinction between the OMRF and Euroimmun assays is that Euroimmun reports antibody specificity to individual subunits for Mi-2 (α/β) and PM/Scl (75/100), while OMRF does not. OMRF utilizes immunoprecipitation of ^{35}S-methionine-labeled lysate proteins for both anti-Mi-2 and anti-PM/Scl. Analysis of these immunoprecipitates by fluorography shows a series of multiple, distinctive protein bands that are readily recognizable to an experienced investigator. The presence of multiple bands with these autoantibodies (since both antigens are multi-protein complexes) allows definitive identification of the autoantibody (Personal Communication from Dr. Ira Targoff).

A limitation of this data is the inability to assess some of the more common myositis autoantibodies that were not consistently ordered at our center as part of the comprehensive OMRF panel (anti-TIF1γ, -NXP-2, and –MDA-5). Furthermore, this study included too few patients with some autoantibodies (e.g. anti-PL-7, -PL-12, -EJ and -OJ) to make any robust conclusions about the performance of the assays. Based on these results, we conclude that the same threshold may not be appropriate for determining a positive antibody result for all specificities tested using the Euroimmun panel and that sensitivity analyses should be conducted with different titer cut-offs.

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Table 1.

Comparison between OMRF and Euroimmun antibody assays. All positives by OMRF were also positive by Euroimmun≥15. EBO=Euroimmun; OMRF=Oklahoma Medical Research Foundation; #=number, NA=not applicable given there were zero positive OJ results on OMRF.

Autoantibody (EUROIMMUN vs OMRF)	#of patients tested	#of OMRF positive	#of positives on EBO	Cut-off (>=15)/+			#of positives on EBO	Cut-off (>=36)/++			#of positives on EBO	Cut-off (>=71)/+++		
				Sensitivity	Specificity	Kappa		Sensitivity	Specificity	Kappa		Sensitivity	Specificity	Kappa
Jo1	281	29	31	96.6	98.6	0.93	27	89.7	99.6	0.92	24	79.3	99.6	0.85
Mi2	280	20												
Mi2a	280		20	94.7	99.6	0.94	11	55.0	100.0	0.69	2	10.5	100.0	0.18
Mi2b	280		30	85.0	95.0	0.65	18	60.0	97.7	0.60	8	40.0	100.0	0.55
Mi2a *or* Mi2b	280		33	100.0	94.9	0.73	22	80.0	97.6	0.74	8	40.0	100.0	0.55
M12a *and* Mi2b	280		17	80.0	99.6	0.85	7	35.0	100.0	0.50	2	10.0	100.0	0.17
SRP	280	18	27	94.4	96.2	0.74	20	94.4	98.6	0.89	13	70.6	99.6	0.79
PM-Scl	279	12												
PM75	279		26	83.3	94.0	0.50	13	75.0	98.5	0.71	7	58.3	100.0	0.73
PM100	279		15	83.3	98.1	0.73	10	66.7	99.2	0.72	4	25.0	99.6	0.36
PM75 *or* PM100	279		33	100.0	92.1	0.50	16	83.3	97.7	0.70	8	58.3	99.6	0.69
PM75 *and* PM100	279		8	66.7	100.0	0.79	7	58.3	100.0	0.73	3	25.0	100.0	0.39
PL7	280	5	15	100.0	96.4	0.49	9	100.0	98.5	0.71	5	100.0	100.0	1.00
PL12	281	3	7	100.0	98.6	0.59	4	100.0	99.6	0.86	4	100.0	99.6	0.86
Ku	279	3	5	100.0	99.3	0.75	5	100.0	99.3	0.75	5	100.0	99.3	0.75
EJ	281	2	3	100.0	99.6	0.80	2	100.0	100.0	1.00	2	100.0	100.0	1.00
OJ	281	0	2	NA	NA	NA	0	NA	NA	NA	0	NA	NA	NA

Table 2.

Patients who were positive by Euroimmun (≥15) but negative by OMRF testing. Clinical features of antisynthetase syndrome are displayed. Several patients were also found to be positive for other autoantibody specificities on EBO.

Patient positive by EBO≥15 but negative by OMRF	Inflammation on muscle biopsy	Fever (>100.4F)	Interstitial lung disease	Mechanic's hands	Raynaud's phenomenon	Inflammatory arthritis	Additional antibody specificities observed on EBO
	N, (%)	N, (%)	N, (%)	N, (%)	N, (%)	N, (%)	
PL7 (N=10)	5 (50)	1 (10)	3 (30)	3 (30)	6 (60)	2 (20)	Jo1 (3), PL12 (1), Ku (1)
PL12 (N=4)	2 (50)	0 (0)	2 (50)	1 (25)	2 (50)	2 (50)	Jo1 (2), PL7 (1)
OJ (N=2)	0	0	0	0	0	0	SRP (1)
Jo1 (N=2)	0	0	0	0	0	0	SRP (1), Ku (1)
EJ (N=1)	1 (100)	0	0	0	0	1 (100)	SRP (1)