Detection of myositis-specific antibodies

It was with much interest that we read the recent European League Against Rheumatism/American College of Rheumatology classification criteria for idiopathic inflammatory myopathies.[1] These criteria include Jo-1 autoantibodies, and the authors discussed that future updates of the criteria should also include the more recently identified myositis-specific autoantibodies.[1 2] The interest in autoantibodies for classification is also illustrated by a recent proposal for a new clinico-serological classification of adult autoimmune myositis, which is based on the association of autoantibodies with distinct clinical phenotypes.[3 4] For example, antibodies to synthetases (eg, Jo-1, PL-7 and PL-12) define the antisynthetase syndrome, anti-MDA-5 antibodies are associated with myositis with overlap features such as interstitial lung disease, anti-TIF-1γ and anti-NXP-2 define a subgroup of dermatomyositis and anti-SRP and anti-HMGCR are associated with necrotising autoimmune myositis.[2]

As autoantibodies play a role in the newly proposed classifications,[1 2] it is expected that measurement of myositis-specific autoantibodies will be increasingly introduced in clinical practice. Most of the myositis-specific autoantibodies have been identified by immunoprecipitation. Alternative, easy-to-use commercial line/dot immunoassays are available. However, such assays are not standardised and may suffer from low specificity.[2] Therefore, these assays need to be further validated.

We evaluated a cohort of 144 patients with inflammatory myopathy (IIM) and 240 controls (blood donors, chronic inflammatory demyelinating polyneuropathy, rheumatoid arthritis, systemic sclerosis, Sjögren's syndrome and systemic lupus erythematosus; 40 of each) for myositis-specific autoantibodies using assays from Alphadia (myositis 12 IgG dot for Bluediver) (Mons, Belgium), Euroimmun (Euroline Autoimmune Inflammatory Myopathies) (Lübeck, Germany) and Trinity Biotech (ImmcoStripe Myositis Advanced LIA) (Buffalo, New York, USA).

The results are shown in table 1. We observed differences in specificity (reactivity in controls) between the manufacturers and between individual antibodies. For example, 2.9% and 2.4% of controls tested positive for anti-Jo-1 by Euroimmun and Trinity, respectively, compared with 0.4% by Alphadia. Overall, Euroimmun and Trinity showed more reactivity in controls than Alphadia, except for anti-SAE for which Euroimmun showed less reactivity in controls. Differences in reactivities between manufacturers were also observed in myositis patients, with the most pronounced difference for anti-TIF-1γ (2.1% with Alphadia versus 12.4% with Euroimmun and 11% with Trinity). It should be noted that even for an established marker such as anti-Jo-1 antibodies, differences between manufacturers were observed in patients with IIM. The likelihood ratio (LR) (prevalence of antibodies in patients divided by prevalence of antibodies in controls) gives a good estimate of how the test result affects the post-test probability (an LR >10 indicates a clinical significant difference in pretest to post-test probability). The LRs are shown in table 1 and further illustrate differences between individual antibodies and between manufacturers.

Table 2 shows the corresponding phenotype of myositis-specific antibodies in patients with IIM. The association between antisynthetase antibodies and interstitial lung disease, arthritis and Raynaud's phenomenon was highly significant for all assays. The association of TIF-1γ antibodies and dermatomyositis was high for two of the three assays tested. The association of other antibodies with certain phenotypes (eg, association of TIF-1γ and NXP-2 with malignancy, of Mi-2 with dermatomyositis, NXP-2 with calcinosis and MDA-5 with amyopathic IIM) were weaker and differed between the assays (table 2), indicating that the assays did not perform similarly.

Taken together, as myositis-specific autoantibodies are included in classification criteria, it is important that clinicians and laboratory professionals are aware of the performance characteristics of the assays used to detect such antibodies. Initiatives to harmonise assays across manufacturers are needed.

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Table 1 Myositis-specific and myositis-associated antibodies in 144 patients with myositis and in 240 controls

	IIM	(n=144)			Contr	(n=240)		BD (n=40)	CIPD (n=40)	RA (n=40)	Scl (n=40)	SjS (n=40)	SLE (n=40)			IIM versus contr
	# Pos	% Pos	# Conc pos	>3× cut-off	# Pos	% Pos	# Conc pos	# Pos	# Pos	# Pos	# Pos	# Pos	# Pos	LR	95% CI	P value
Jo-1 Alphadia	31	21.4	22/25/22	30	1	0.4	1/1/1	0	0	0	1	0	0	53.5	9.1 to 298	<0.0001
Jo-1 EuroImmun	29	20.0	22	24	7	2.9	1	0	0	1	1	2	3	6.9	3.2 to 15.1	<0.0001
Jo-1 Trinity	24	16.6			6	2.5		1	2	0	1	2	0	6.6	2.9 to 15.6	<0.0001
PL-7 Alphadia	1	0.7	0/1/0	1	0	0.0	0/0/0	0	0	0	0	0	0	∞	0.44 to ∞	0.3750
PL-7 EuroImmun	3	2.1	1	1	2	0.8	0	0	0	0	0	1	1	2.6	0.50 to 12.4	0.3680
PL-7 Trinity	1	0.7			2	0.8		0	1	0	0	1	0	0.9	0.11 to 6.3	1.0000
PL-12 Alphadia	3	2.1	1/2/1	2	1	0.4	0/0/1	0	1	0	0	0	0	5.3	0.72 to 34.7	0.1504
PL-12 EuroImmun	3	2.1	1	2	3	1.3	0	0	1	0	0	1	1	1.6	0.39 to 7.1	0.6758
PL-12 Trinity	3	2.1			7	2.9		2	2	0	1	2	0	0.7	0.20 to 2.5	0.7492
EJ Alphadia	3	2.1	2/2/2	2	0	0.0	0/0/0	0	0	0	0	0	0	∞	1.3 to ∞	0.0520
EJ EuroImmun	2	1.4	2	1	0	0.0	0	0	0	0	0	0	0	∞	0.87 to ∞	0.1400
EJ Trinity	2	1.4			2	0.8		0	1	0	0	0	1	1.8	0.30 to 9.4	0.6326
SRP Alphadia	3	2.1	1/3/1	1	0	0.0	0/0/0	0	0	0	0	0	0	∞	1.3 to ∞	0.0520
SRP EuroImmun	4	2.8	1	3	4	1.7	0	2	1	0	0	1	0	1.6	0.46 to 6.0	0.4799
SRP Trinity	2	1.4			2	0.8		0	1	0	0	1	0	1.8	0.30 to 9.4	0.6326
Mi-2 Alphadia	8	5.5	6/7/6	7	0	0.0	0/0/0	0	0	0	0	0	0	∞	3.5 to ∞	0.0003
Mi-2a EuroImmun	8	5.5	7	2	3	1.3	1	0	0	0	0	0	3	4.2	1.3 to 15.3	0.0231
Mi-2b EuroImmun	7	4.8		1	1	0.4		0	0	0	0	0	1	12.0	1.9 to 72.3	0.0052
Mi-2 Trinity	9	6.2			1	0.4		0	0	0	0	0	1	15.5	2.5 to 91.1	0.0008
MDA-5 Alphadia	7	4.8	4/7/4	6	0	0.0	0/0/0	0	0	0	0	0	0	∞	3.1 to ∞	0.0010
MDA-5 EuroImmun	10	6.9	5	7	0	0.0	0	0	0	0	0	0	0	∞	4.4 to ∞	<0.0001
MDA-5 Trinity	10	6.9			5	2.1		0	1	1	0	0	3	3.3	1.2 to 9.2	0.0267
TIF1γ Alphadia	3	2.1	3/3/3	2	2	0.8	0/0/0	0	0	0	2	0	0	2.6	0.50 to 12.4	0.3680
TIF1γ EuroImmun	18	12.4	8	6	3	1.3	0	1	0	1	0	0	1	9.5	3.0 to 29.8	<0.0001
TIF1 (γ+α) Trinity	16	11.0			22	9.2		3	2	1	2	6	8	1.2	0.66 to 2.2	0.5974
HMGCR Alphadia	7	4.8		7	0	0.0		0	0	0	0	0	0	∞	3.1 to ∞	0.0010
Ro52 Alphadia	32	22.1	26/29/26	27	57	23.8	53/57/53	0	0	1	3	36	17	0.9	0.61 to 1.3	0.6160
Ro52 EuroImmun	39	26.9	33	33	79	32.9	70	0	6	2	12	38	21	0.8	0.58 to 1.1	0.1785
Ro52 Trinity	36	24.8			89	37.1		2	9	3	16	39	20	0.7	0.47 to 0.91	0.0095
SAE1/SAE2 Alphadia	5	3.4	2/3/3	3	3	1.3	0/0/0	1	0	0	1	1	0	2.6	0.74 to 10.4	0.1572
SAE1 EuroImmun	4	2.8	2	2	0	0.0	0	0	0	0	0	0	0	∞	1.8 to ∞	0.0193
SAE1 Trinity	6	4.1			3	1.3		0	0	2	0	1	0	3.2	0.93 to 12.0	0.0854
NXP-2 Alphadia	8	5.5	3/4/5	5	1	0.4	0/1/0	0	0	0	0	1	0	13.8	2.2 to 81.7	0.0021
NXP-2 EuroImmun	5	3.4	3	4	3	1.3	0	0	1	1	0	1	0	2.6	0.74 to 10.4	0.1572
MORC3 Trinity	7	4.8			2	0.8		1	0	0	0	1	0	6.0	1.4 to 24.5	0.0297
OJ EuroImmun	0	0.0	0	0	0	0.0	0	0	0	0	0	0	0			
OJ Trinity	0	0.0			0	0.0		0	0	0	0	0	0			

Continued

Table 1 Continued

	IIM	(n=144)			Contr	(n=240)		BD (n=40)	CIPD (n=40)	RA (n=40)	Scl (n=40)	SjS (n=40)	SLE (n=40)			IIM versus contr
	# Pos	% Pos	# Conc pos	>3× cut-off	# Pos	% Pos	# Conc pos	# Pos	# Pos	# Pos	# Pos	# Pos	# Pos	LR	95% CI	P value
PM75 EuroImmun	7	4.8	4	1	13	5.4	5	2	0	0	6	3	2	0.9	0.38 to 2.1	1.0000
Pm-Scl75 Trinity	7	4.8			16	6.7		3	3	1	4	3	2	0.7	0.31 to 1.7	0.5148
PM100 EuroImmun	7	4.8	5	0	8	3.3	5	0	3	0	3	1	1	1.5	0.56 to 3.8	0.5875
PM-Scl100 Trinity	11	7.6			17	7.1		3	5	1	5	2	1	1.1	0.53 to 2.2	0.8416
Ku EuroImmun	4	2.8	3	3	6	2.5	4	0	1	1	1	0	3	1.1	0.34 to 3.6	1.0000
Ku Trinity	3	2.1			5	2.1		0	2	0	1	0	2	1.0	0.27 to 3.7	1.0000
U1 snRNP 68 Trinity	1	0.7			4	1.7		0	0	0	1	0	3	0.4	0.06 to 2.7	0.6544
U1 snRNP A Trinity	3	2.1			11	4.6		0	0	1	2	0	8	0.5	0.14 to 1.5	0.2672
U1 snRNP C Trinity	5	3.4			14	5.8		1	0	2	1	1	9	0.6	0.23 to 1.5	0.3424
U2 snRNP A′ Trinity	2	1.4			4	1.7		0	0	3	0	1	0	0.8	0.18 to 3.8	1.0000
U2 snRNP B″ Trinity	2	1.4			6	2.5		0	0	5	0	1	0	0.6	0.13 to 2.4	0.7154
Fibrillarin Trinity	1	0.7			0	0.0		0	0	0	0	0	0	∞	0.44 to ∞	0.3750
KS Trinity	3	2.1			1	0.4		0	0	0	0	1	0	5.3	0.72 to 34.7	0.1504
CN-1A Trinity	19	13.1			25	10.4		0	3	3	4	8	7	1.3	0.73 to 2.2	0.4080

For each antibody, the number of positive samples (and percentage) is given for myositis patients and controls as well as the number of positive samples with a value higher than 3 times the cut-off (for patients with myositis). The number of concordant (conc) results is given as follows: on the line that describes the Alphadia results, concordance between all three assays (first number), between Alphadia and Euroimmun (second number) and between Alphadia and Trinity (third number) is given. On the line that describes the Euroimmun results, concordance between Euroimmun and Trinity is given. The likelihood ratio (LR) is given as well as the association between each of the antibodies and IIM (evaluated by χ^2 testing or Fisher's exact test (if cell size was <10) using Analyse-it for Excel). Patients with IIM (female/male: 80/64) (median age at diagnosis 53 years; age range 3–84 years) included dermatomyositis (n=57), polymyositis (n=48), antisynthetase syndrome (n=15), necrotising myositis (n=6), clinically amyopathic dermatomyositis (n=7), sporadic inclusion body myositis, overlap and undifferentiated myositis (n=9) and undefined (n=2). Diagnosis was based on a combination of clinically significant muscle weakness, elevated creatine kinase levels, electromyography, muscle biopsy (available in 90 of 144 patients) and/or skin manifestations. Demographic data (female/male, median age (age range)) of the controls were 149/91, 57 years (16–85 years); 17/23, 56 years (18–69 years) for the blood donors; 8/32, 56 years (32–75 years) for the chronic inflammatory demyelinating polyneurophathy (CIPD); 30/10, 57 years (26–75 years) for rheumatoid arthritis (RA); 25/15, 61 years (41–81 years) for systemic sclerosis (SSc); 35/5, 52 years (16–85 years) for Sjögren's syndrome (SjS); and 34/6, 47 years (25–84 years) for systemic lupus erythematosus (SLE).
IIM, inflammatory myopathy.

Table 2 Association of myositis-specific antibodies with clinical phenotype in patients with IIM

		Alphadia					Euroimmun					Trinity				
		Pos	Pos	Neg	Neg		Pos	Pos	Neg	Neg		Pos	Pos	Neg	Neg	
Phenotype	Marker	Pos	Neg	Pos	Neg	P value	Pos	Neg	Pos	Neg	P value	Pos	Neg	Pos	Neg	P value
Malignancy	Tif1-γ	1	2	10	131	0.2135	3	14	8	119	0.1240	4	12	7	121	0.0212
	NXP-2 (MORC3)	2	6	9	127	0.1155	1	4	10	129	0.3318	1	6	10	127	0.4337
DM skin lesions	Mi-2	6	2	53	83	0.0638	6	5	53	80	0.3582	5	4	54	81	0.4873
	TIF-γ	3	0	56	85	0.0667	16	1	43	84	<0.0001	14	2	45	83	<0.0001
	NXP-2 (MORC3)	4	4	55	81	0.7166	3	2	56	83	0.4003	4	3	55	82	0.4449
	MDA-5	5	2	54	83	0.1228	8	2	51	83	0.0159	6	4	53	81	0.3176
	SAE	4	1	55	84	0.1590	4	0	55	85	0.0265	4	2	55	83	0.2273
Amyopathic DM	MDA-5	2	5	5	132	0.0380	2	8	5	129	0.0758	2	8	5	129	0.0758
Calcinosis	NXP-2 (MORC3)	2	6	7	129	0.0801	2	3	7	132	0.0316	7	5	2	130	<0.0001
Inclusion body myositis	CN-1A											1	18	5	120	0.5791
Arthritis	Jo-1	14	17	10	103	<0.0001	13	16	11	104	<0.0001	10	14	14	106	0.0003
	Jo-1, PL7, PL12 or EJ	16	21	8	99	<0.0001	16	21	8	99	0.0001	12	18	12	102	0.0004
ILD	Jo-1	19	12	13	98	<0.0001	19	10	13	100	<0.0001	15	9	17	101	<0.0001
	Jo-1, PL7, PL12 or EJ	22	15	10	95	<0.0001	23	17	9	93	<0.0001	17	13	15	97 97	<0.0001
Raynaud	Jo-1	12	18	13	99	0.0003	13	15	12	102	<0.0001	9	14	16	103	0.0063
	Jo-1, PL7, PL12 or EJ	16	20	9	97	<0.0001	17	22	8	95	<0.0001	11	18	14	99	0.0013

The association was evaluated by χ^2 testing or Fisher's exact test (if cell size was <10) using Analyse-it for Excel.
IIM, inflammatory myopathy.

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