Autoantibody testing in patients with myositis: clinical accuracy of a multiparametric line immunoassay

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Increasing evidence assesses the value of serum autoantibody testing in the diagnostic workup of idiopathic inflammatory myopathies (IIM) (1-3). Myositis autoantibodies are categorised into two groups, basing on specificity: myositis-specific antibodies (MSA) and myositis-associated antibodies (MAA). Besides established MSA, *i.e*. anti-aminoacyl tRNA synthetases, anti-SRP and anti-Mi-2 antibodies, novel autoantibodies have been identified, which target constitutive proteins involved in gene transcription, post-translational modifications, or innate immunity, such as transcription intermediary factor 1-gamma (TIF1-γ), nuclear matrix protein 2 (NXP2), melanoma differentiation associated protein 5 (MDA5), and SUMO activating enzymes (SAE) (4-6). The MAA, including anti-PM/Scl and anti-Ku, mostly occur in myositis-overlap syndromes (3, 7).

Since our paper in 2010 (8), which assessed the diagnostic accuracy of a line immunoassay (LIA) for the detection of 7 MSA/MAA targets, *i.e*. Jo-1, PL-7, PL-12, PM/Scl, Ku, Mi-2 and Ro52, commercial LIA has been implemented by virtue of human recombinant autoantigens' disposal. Nowadays, a wide spectrum of myositis-related autoantibodies can be determined by last generation LIA, which needs validation before inclusion in clinical setting.

We assessed the clinical accuracy of a multiparametric LIA for the simultaneous detection of 15 myositis autoantibodies in patient's serum. We tested sera from 267 IIM patients, 55 healthy subjects, and 203 disease controls (11 non-autoimmune myopathy, 27 muscular dystrophy, 9 undifferentiated connective tissue disease, 89 systemic lupus erythematosus, 35 systemic sclerosis, 22 Sjögren's syndrome and 10 arthropathy). Patients with IIM were classified according to Bohan and Peter criteria (9): 115 polymyositis, 88 dermatomyositis (DM), 52 overlap myositis, 12 cancer-associated myositis. The study was approved by the Local Ethics Committees according to the Helsinki Declaration, and written informed consent was obtained from each patient. IgG autoantibodies towards myositis antigens were detected by LIA (Euroimmun, Lübeck, Germany). Test nitrocellulose strips are pre-coated with parallel lines of affinity-purified mammalian Jo-1 or recombinant full-length SRP, PL-7, PL-12, EJ, OJ, Mi-2, Mi-2β, TIF1-γ, MDA5, NXP2, SAE1, Ku, PM-Scl75 and PM-Scl100 proteins, derived from respective human cDNAs. Statistical analysis was performed using Fisher's exact test.

Overall, MSA and/or MAA positivity by LIA was found in 166/267 (62%) IIM patients and in 59/258 (23%) controls ($p<0.0001$, Odds ratio 5.5, 95% Confidence Interval 3.7-8.1, + Likelihood ratio 2.7). MSA positivity was found in 48/258 (19%) controls, MAA positivity in 40/258 (15.5%) ones. Forty-five (27%) myositis sera had more than one autoantibody: a MSA/MAA association was observed in 80% of cases. Novel autoantibodies overall accounted for a 24% increment in sensitivity compared with the assay confined to 7 analytes. The test's specificity was: 100% for anti-Jo-1, anti-OJ and anti-SAE1; 99% for anti-PL-7 and anti-PM-Scl100; 98% for anti-EJ, anti-TIF1-γ and anti-MDA5; 96.5% for anti-PL-12 and anti-Mi-2β; 95% for anti-SRP, anti-Mi-2α and anti-NXP2; 94% for anti-PM-Scl75; 92% for anti-Ku. Anti-Mi-2β and anti-PM-Scl100 antibodies were more specific than the antibodies against Mi-2α or PM-Scl75 subunits. The ability of each antibody marker to distinguish myositis from controls is presented in Table I. Notworthy, the clinical accuracy of each antibody can vary widely, from very good for anti-Jo-1, anti-Mi-2α/β, anti- TIF1-γ, anti-MDA5, anti-SAE1, and anti-PM/Scl100, to less good for anti-SRP, anti-NXP2, anti-PM/Scl75 and anti-Ku.

Our data support the clinical utility of implemented LIA as a confirmatory test for the diagnosis of myositis. It can represent a powerful alternative to throughoutput yet complex gold standard immunoprecipitation. Novel putative DM-specific antibodies were almost restricted to DM. The association of anti-TIF1-γ with CAM was confirmed (10), being observed in 4 out of 7 patients with cancer-associated DM, thus providing a tool for the diagnosis of prognostically unfavourable DM. Crucial for the testing accuracy is the quality and epitope integrity/availability of blot-immunobilised antigens. Improvement of the test's accuracy, expecially pertaining to anti-SRP and anti-NXP2 antibodies, is recommended by the manufacturer.

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Table I. Clinical accuracy of each MSA or MAA for IIM *versus* controls.

	Sensitivity %	Specificity %	+LR	PPV %	NPV %	*p*-value	OR (95% CI)
Anti-Jo-1	19	100	nd	100	54	<0.0001	123 (7.5-2006)
Anti-SRP	8	95	1.6	63	50	n.s.	1.7 (0.8-3.4)
Anti-ARS non Jo-1	7	95	1.5	60	50	n.s.	1.5 (0.7-3.1)
Anti-Mi-2 α/β (DM)*	18	96.5	5.1	64	86	<0.0001	10.6 (4.5-24.8)
Anti-TIF1- (DM)*	17	98	8.5	85	64	<0.0001	10.04 (2.8-35.3)
Anti-MDA5 (DM)*	11	98	5.7	77	65	0.0052	6.3 (1.7-23.5)
Anti-SAE1 (DM)*	6	100	nd	100	61	0.0037	20.7 (1.1-372)
Anti-NXP2	3	95	0.5	50	35	n.s.	0.55 (0.2-1.5)
Anti-Ku	8	92	1	50	49	n.s.	0.96 (0.5-1.8)
Anti-PM-Scl75	8	95	1.3	58	50	n.s.	1.3 (0.7-2.6)
Anti-PM-Scl100	7	99	5.8	86	50	0.001	6.1 (1.8-21.1)

*Findings are referred to the target form of IIM, DM in these cases.
MSA: myositis-specific antibody; MSA: myositis-associated antibody; IIM: idiopathic inflammatory myopathy; ARS: aminoacyl-tRNA synthetase; DM: dermatomyositis; +LR: positive likelihood ratio; PPV: positive predictive value; NPV: negative predictive value; *p*: statistical significance; OR: odds ratio; CI: confidence interval; nd: not determined; n.s.: not significant.

References

1. BARSOTTI S, TERENZI R, LA PAGLIA GM, BELLUCCI E, BALDINI C, NERI R: One year in review 2015: idiopathic inflammatory myopathies. *Clin Exp Rheumatol* 2015; 33: 593-601.
2. FERNANDEZ C, BARDIN N, MAUES DE PAULA A *et al*.: Correlation of clinicoserologic and pathologic classifications of inflammatory myopathies. *Medicine* 2013; 92: 15-24.
3. ALLENBACH Y, BENVENISTE O: Diagnostic utility of auto-antibodies in inflammatory muscle diseases. *J Neuromusc Dis* 2015; 2: 13-25.
4. GHIRARDELLO A, BASSI N, PALMA L *et al*.: Autoantibodies in polymyositis and dermatomyositis. *Curr Rheumatol Rep* 2013; 15: 335-44.
5. BODOKI L, NAGY-VINCZE M, GRIGER Z, BETTERIDGE Z, SZÖLLŐSI L, DANKÓ K: Four dermatomyositis-specific autoantibodies-anti-TIF1γ, anti-NXP2, anti-SAE and anti-MDA5-in adult and juvenile patients with idiopathic inflammatory myopathies in a Hungarian cohort. *Autoimmun Rev* 2014; 13: 1211-9.
6. TARRICONE E, GHIRARDELLO A, RAMPUDDA M, BASSI N, PUNZI L, DORIA A: Anti-SAE antibodies in autoimmune myositis: identification by unlabelled protein immunoprecipitation in an Italian patient cohort. *J Immunol Methods* 2012; 384: 128-34.
7. RANQUE B, BÉREZNÉ A, LE-GUERN V *et al*.: Myopathies related to systemic sclerosis: a case-control study of associated clinical and immunological features. *Scand J Rheumatol* 2010; 39: 498-505.
8. GHIRARDELLO A, RAMPUDDA M, EKHOLM L *et al*.: Diagnostic performance and validation of autoantibody testing in myositis by a commercial line blot assay. *Rheumatology* (Oxford) 2010; 49: 2370-4.
9. BOHAN A, PETER JB: Polymyositis and dermatomyositis (first of two parts). *New Engl J Med* 1975; 292: 344-7.
10. FIORENTINO DF, CHUNG LS, CHRISTOPHERSTINE L *et al*.: Most patients with cancer-associated dermatomyositis have antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1γ. *Arthritis Rheum* 2013; 65: 2954-62.