

Supplementary Tables

Table S1. Criteria used to assess MSA pattern compatibility.

Antibody	Accepted AC-pattern(s) ¹ as defined in this study
Anti-Mi2	Nuclear fine speckled (AC-4)
Anti-Jo1	Cytoplasmic dense fine speckled/cytoplasmic fine speckled (AC-19/20)
Anti-EJ	Cytoplasmic dense fine speckled/cytoplasmic fine speckled (AC-19/20)
Anti-PL7	Cytoplasmic dense fine speckled/cytoplasmic fine speckled (AC-19/20)
Anti-NXP2	Nuclear fine speckled (AC-4)/multiple nuclear dots (AC-6)
Anti-TIF1 γ	Nuclear fine speckled (AC-4)
Anti-SAE	Nuclear fine speckled (AC-4)
Anti-MDA5	Cytoplasmic dense fine speckled/cytoplasmic fine speckled (AC-19/20) or negative (AC-0)
Anti-SRP	Cytoplasmic dense fine speckled/cytoplasmic fine speckled (AC-19/20)
Anti-HMGCR	Musset <i>et al.</i> JIM 2014

¹ AC patterns defined according to ICAP nomenclature (www.ANAPatterns.org).

Table S2. Details on MSA positivity in other samples (controls, consecutive patients with IIM excluded and IMID patients with myositis overlap not excluded).

	MSA	MSA blot signal intensity (AU) [and method] ^a AC ANF ICAP pattern	Cohort- clinical diagnosis by the treating clinician	Age (years) and gender	Remark
1	TIF1 γ	16 [EI] AC-4	consecutive cohort-IIM excluded	36 (female)	Mechanic hands without other features of IIM
2	TIF1 γ	12 [EI] AC-2/AC-1? (low positive)	RA cohort	68 (male)	seronegative rheumatoid arthritis
3	TIF1 γ	14 [EI] AC-4 + AC-19	consecutive cohort- IMID with myositis not excluded	58 (male)	Differential diagnosis: granulomatosis with polyangiitis, polyarteritis nodosa
4	TIF1 γ	17 [DT] AC-1 + AC-3	SSc cohort	62 (male)	Limited cutaneous SSc with no arguments for myositis overlap
5	TIF1 γ	12 [DT] AC-3	SSc cohort	70 (female)	Limited cutaneous SSc with no arguments for myositis overlap
6	TIF1 γ	77 [DT] AC-3	SSc cohort	66 (female)	Limited cutaneous SSc with no arguments for myositis overlap
7	TIF1 γ	12 [DT] AC-4 + undefined cytoplasmic staining	SLE cohort	84 (female)	No arguments for myositis overlap
8	SRP	21 [DT] 1x AC-4 + AC-19	consecutive cohort- IMID with myositis not excluded	41 (male)	Steroid responsive Polyarthritis
9	SRP	25 [DT] AC-5 + AC-19	RA cohort	53 (female)	No arguments for myositis overlap
10	SRP	13 [EI] AC-4 + AC-16	RA cohort-myositis not excluded	69 (male)	Myositis overlap not excluded as ILD+ and presence of PM/Scl antibodies No muscle weakness nor CK-elevation No arguments for myositis overlap
11	Mi2	11 [EI] AC-4	SLE cohort	23 (female)	No arguments for myositis overlap
12	Mi2	14 [EI] AC-4 (+ weak AC-19)	consecutive cohort- IMID with myositis not excluded	64 (male)	Generalised osteoarthritis with differential diagnosis of polymyalgia rheumatica/seronegative rheumatoid arthritis
13	SAE	16 [DT] AC-4	consecutive cohort-IIM excluded	45 (male)	Aspecific myalgia without other features of IIM
14	SAE	14 [DT] AC-4	SSc cohort	54 (female)	Diffuse cutaneous SSc with no arguments for myositis overlap
15	SAE	80 [DT] AC-5 + AC-19	RA cohort	69 (male)	No arguments for myositis overlap
16	PL7	56 [DT] AC-19	consecutive cohort- myopathic features without IIM	71 (female)	drug-induced rhabdomyolysis
17	Jo1	23 [EI] AC-20 + AC-4	consecutive cohort- IMID with myositis not excluded	74 (female)	Cutaneous lesions compatible with polyarteritis nodosa, sclerodactyly and scleroderma pattern on videocapillaroscopy
18	Jo1	20 [EI] AC-0 (negative)	consecutive cohort-IIM excluded	20 (female)	Fibromyalgia, shoulder trauma

DT: D-tek MYOS12DIV-24 Blue Diver dot blot; EI: EUROLIne Autoimmune inflammatory myopathies 16 Ag; SSc: systemic sclerosis; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis; IIM: idiopathic inflammatory myopathy; IMID: immune mediated inflammatory disease; ILD: interstitial lung disease; CK: creatine kinase.

^a cut-off values as proposed by the manufacturers (positive if >10AU) are applied.

Table S3. Comparison of the MSA results obtained on 2 lineblots.

	Total MSA (positivity on minimal 1 lineblot) ^a	Kappa agreement in all samples (n=214) ^c	% (n/total) concordant positive findings in all samples (n=214) ^c	% (n/total) concordant positive findings in patients with no clinical IIM diagnosis or myositis overlap syndrome (n=152)
Mi2	6	0.660	50% (3/6)	0% (0/1)
Jo1	5	0.760	60% (3/5)	0% (0/1)
EJ	1	1.000	100% (1/1)	NP
OJ	0	NA	NA	NA
PL7	1	NP	0% (0/1)	NP
PL12	0	NP	NP	NP
NXP2	1	1.000	100% (1/1)	NP
TIF1 γ	9	-0.021	0% (0/9)	0% (0/6)
SAE	5 ^b	0.395	25% (1/4)	0%(0/3)
MDA5	1	NP	0% (0/1)	NP
SRP	3	-0.006	0% (0/3)	0% (0/1)
HMGCR	4	NA	NA	NA

^a Details on the 2 lineblots used: D-tek MYOS12DIV-24 Blue Diver dot blot and EUROLIne Autoimmune inflammatory myopathies 16 Ag, with application of manufacturers' cut-off values for positivity; ^b1 SAE positive sample with missing clinical information; NA: not applicable, as the antigen is only present in 1 system; NP: not performed; ^conly samples with available clinical information taken into account.

Table S4. Detailed overview of the MSA results, clinical features and EULAR score of the IIM patients.

	MSA	MSA titer (AU [and method]) ^a	Clinical Diagnosis	Age (years) and gender	Minimal EULAR/ACR score	Muscle biopsy suggestive for IIM	Prox. Muscle weakness	ILD	RP	Arthritis	Calcinosis	Malignancy	CK	Skin lesions
1	Mi2	100 [DT], 31 [EI]	cDIIM	55 (female)	100%	NP	Yes	No	Yes	No	No	No	Yes	Yes
2	Mi2	61 [DT], 28 [EI]	cDIIM	69 (female)	99%	NP	Yes	No	No	No	No	No	Yes	Yes
3	Mi2	63 [DT]	cDIIM	44 (male)	99%	NP	Yes	No	No	No	No	No	Yes	Yes
4	Mi2	69 [DT], 35 [EI]	cDIIM	56 (female)	68%	NP	Yes	No	No	No	No	No	Yes	Yes
5	EJ	36 [DT], 37 [EI]	cPIIM	48 (female)	6%	Yes	No	No	No	No	No	No	No	No
6	MDA5	12 [DT]	cDIIM	57 (female)	100%	No	Yes	Yes	Yes	Yes	No	No	No	Yes
7	NXP2	56 [DT], 78 [EI]	cDIIM	11 (female)	100%	NP	Yes	No	No	No	Yes	No	Yes	Yes
8	Jo1	74 [DT], 136 [EI]	cDIIM	70 (female)	99%	Yes	Yes	No	No	No	No	No	Yes	No
9	Jo1	73 [DT], 98 [EI]	cPIIM	68 (female)	66%	NP	No	Yes	Yes	No	No	UKN	No	No
10	Jo1	86 [DT], 22 [EI]	cDIIM	58 (female)	97%	Yes	Yes	Yes	Yes	No	No	No	Yes	No
11	HMGCR	91 [DT]	cDIIM	66 (female)	60%	Yes	Yes	No	UKN	No	UKN	Yes	Yes	No
12	HMGCR	55 [DT]	cDIIM	48 (male)	56%	No	Yes	No	No	No	No	No	Yes	Yes
13	HMGCR	33 [DT]	cDIIM	86 (female)	60%	Yes	Yes	No	No	No	No	No	Yes	No
14	HMGCR	70 [DT]	cPIIM	51 (female)	60%	Yes	Yes	No	No	No	No	No	Yes	No
15	SAE	95 [DT], 24 [EI]	cDIIM	73 (female)	99%	NP	No	No	No	No	No	No	No	Yes
16	TIF1 γ	15 [EI]	cDIIM	34 (female)	97%	NP	Yes	No	No	No	UKN	No	No	Yes
17	TIF1 γ	86 [EI]	cDIIM	17 (male)	100%	NP	Yes	No	Yes	Yes	Yes	No	No	Yes
18	None	No MSA	cDIIM	48 (male)	28%	Yes	Yes	No	No	No	No	No	Yes	Yes
19	None	No MSA	cPIIM	42 (male)	22%	No	Yes	No	No	No	No	No	Yes	No
20	None	No MSA	cDIIM	14 (male)	45%	NP	No	No	No	No	No	No	No	Yes
21	None	No MSA	cPIIM	51 (male)	9%	No	Yes	Yes	Yes	No	No	No	No	No
22	None	No MSA	cDIIM	30 (female)	99%	No	Yes	No	No	No	No	No	Yes	Yes
23	None	No MSA	cDIIM	47 (male)	15%	Yes	Yes	No	Yes	No	No	No	Yes	No
24	None	No MSA	cDIIM	51 (female)	23%	Yes	Yes	No	Yes	Yes	No	No	Low+	No
25	None	No MSA	cDIIM	62 (female)	31%	Yes	Yes	No	No	No	No	No	Yes	No
26	None	No MSA	cPIIM	58 (female)	22%	Yes	Yes	No	No	No	No	No	Yes	No
27	None	No MSA	cDIIM	58 (male)	85%	NP	Yes	No	No	No	No	No	Yes	Yes
28	None	No MSA	cPIIM	73 (female)	96%	Yes	Yes	No	No	No	No	No	Yes	No
29	None	No MSA	cPIIM	77 (male)	15%	No	Yes	No	No	No	No	No	Yes	No
30	None	No MSA	cPIIM	66 (female)	23%	NP	No	Yes	Yes	Yes	No	No	Low+	No
31	None	No MSA	cPIIM	75 (male)	24%	NP	No	No	No	No	No	Yes	No	Yes

DT: D-tek MYOS12DIV-24 Blue Diver dot blot; EI: EUROLIne Autoimmune inflammatory myopathies 16 Ag; cDIIM: clinical definite IIM; cPIIM: clinical probable IIM; NP: not performed; UKN: unknown; Calc.: calcinosis; Malign.: malignancy; ILD: interstitial lung disease; RP: Raynaud's phenomenon; CK: creatine kinase; Low+: less than 2x upper limit of normal; ^a cut-off values as proposed by the manufacturers (positive if >10 AU) are applied.