Deep Pathological Phenotyping of Dermatomyositis with Different Autoantibodies

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Research Article

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Abstract

Objective

Dermatomyositis with different myositis-specific autoantibodies has distinctive clinical presentations. Pathological variation of patients with different antibodies has not been fully understood.

Methods

A retrospective review of muscle pathological features was performed in dermatomyositis patients with known myositis-specific antibodies.

Results

A total of 46 dermatomyositis patients with one myositis-specific autoantibody (anti-MDA5 11, anti-Mi-2 10, anti-NXP2 13, anti-TIF1γ 8, anti-SAE 4) were included and the pathological severity score was evaluated. Patients with anti-Mi-2 demonstrated higher pathological severity scores and apparent sarcolemmal complement deposition, which was in consistency of more severe muscle weakness and higher level of muscle enzymes. In contrast, patients with anti-MDA5 generally had minimal pathological changes in muscle with less inflammatory cell infiltration, fewer membrane attack complex deposition, and milder myxovirus resistance protein A upregulation. Patients with anti-SAE had more inflammatory cell infiltration and MAC deposition compared to anti-MDA5 group. Muscle pathological scores varied largely in patients with anti-NXP2 and anti-TIF1γ.

Conclusion

The muscle pathological features varies among dermatomyositis with different autoantibodies, which further indicates the heterogeneity of dermatomyositis.

Introduction

Dermatomyositis (DM) is an idiopathic inflammatory myopathy with a range of muscle and skin manifestations. Muscle involvement usually manifests as proximal muscle weakness, with or without myalgias or tenderness. Typical skin lesions in DM include heliotrope rash, periorbital edema, Gottron papules, Gottron sign, V-sign and shawl sign. Two special subtypes of DM, the amyopathic variant and dermatomyositis sine dermatitis, have been described in the 2017 UELAR/ACR DM criteria[1]. In addition to muscle and skin manifestations, interstitial lung disease, joint disease and malignancy are may also present in DM patients. The inconsistent clinical findings result in challenges in diagnosis and treatment of DM patients.

In the last decades, about 20 myositis-specific antibodies (MSA) and myositis-associated antibodies (MAA) were discovered [2]. DM is associated with five MSAs including anti-melanoma differentiation associated gene 5 (MDA5), anti-mitochondrial M2-associated protein (Mi-2), anti-nuclear matrix protein 2 (NXP2), anti-transcription intermediary factor 1 (TIF1γ) and anti-small ubiquitin-like modifier activating enzyme (SAE) antibodies. These autoantibodies are associated with distinct clinical features [3]. For example, anti-MDA5 dermatomyositis is more associated with mucocutaneous ulceration, mild muscle weakness and progressive interstitial lung disease[4]. The anti-Mi-2

patients is reported to be associated with typical dermatomyositis skin lesion, high levels of muscle enzymes and good response to therapy[3, 5]. The anti-NXP2 and anti- TIF1γ dermatomyositis is associated with a higher risk of malignancy[6, 7]. The anti-SAE antibody was found exclusively in adult DM patients, and the common symptoms include a diffuse dark red skin rash and mild muscular weakness, but dysphagia occurred more frequently[8]. All these serological findings induce a new clinic-serological classifications of IIMs based on MSA and MAA[2].

Although different MSAs are associated with distinct clinical features, the pathological phenotype of different MSA subgroups remains unclear. Recently a surrogate marker of induce type I interferon (IFN1) pathway, myxovirus resistance protein A (MxA), has been recognized as a specific marker for DM with much higher sensitivity than perifascicular atrophy (PFA), the classic pathological feature of DM. [9] Anti-MDA5 antibodies were reported to be associated with relatively normal muscle biopsy without PFA, while anti-Mi-2 patients demonstrated classic PFA with perifascicular necrosis and MxA upregulation[10]. In this study we aim to study the detailed muscle pathology profiles of different MSA subgroups, which may help revealing different underlying pathogenesis of muscle damage.

Patients And Methods

Patients, clinical data and muscle imaging

DM patients diagnosed in Huashan Hospital, Fudan University and Tongji Hospital, Huazhong University of Science and Technology from 2016 to 2019 were included in this study. The diagnosis of DM was according to the European Neuromuscular Centre (ENMC) criteria in 2004[11]. Clinical data, laboratory tests and treatment status at time of biopsy were recorded. Muscle strength was evaluated according to MMT-8 scores (scale 0–80) at the time of diagnosis [12]. Magnetic resonance imaging (MRI; T1- and T2-weighted and short T1 inversion recovery) was used to scan the muscles in the bilateral lower extremities before a biopsy[13]. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committees of Huashan Hospital (2018 – 153) and Tongji Hospital (TJ-C20121221). Informed consents were obtained from all the patients.

Autoantibody testing

Serum from patients with dermatomyositis were sent to Oumeng V Medical Laboratory for screening autoantibodies using ELISA test as described previously [14]. Briefly, the assay of the MSAs and MAAs subsets was performed with EUROLINE autoimmune inflammatory myopathy Ag (IgG) test kit (EUROIMMUN, Germany). The positive control was provided by the kit while the negative control was the sample buffer. EUROBlotOne (EUROIMMUN, Germany) was used to detect the signal intensity, and the cutoff threshold was above 25.

Muscle pathology

Muscles with moderate muscle weakness (MRC 3/5 or 4/5), or with edema on MRI, or with myopathic changes on electromyography were chosen for biopsy. Muscle biopsies were obtained using standard techniques and were then snap-frozen by immersion in liquid nitrogen-cooled isopentane. Frozen sections were stained with Haematoxylin & Eosin (H&E), Gomori-modified trichrome (MGT), NADH tetrazolium reductase (NADH-TR), periodic acid-Schiff (PAS), oil red O (ORO) and ATPase staining (pH 4.6 and pH 9.6). The following primary antibodies were used for immunohistochemistry with standard procedures: anti-MHC-I (1: 300; Abcam); anti-human C5b-9 (1:50; Dako); anti-human MxA (1:500; Millipore); anti-human CD4 (1:50; Dako); anti-human CD8 (1:50; Dako); anti-human CD20 (1:50; Dako); anti-human CD68 (1:50; Dako). Monoclonal antibody against dysferlin (NCL-Hamlet; 1:40; Novocastra) was

also used to exclude dysferlinopathy. The Novolink Polymer DS kit (Leicabiosystems) were applied according to the manufacturer's protocol.

Pathological scoring

A semiquantitative scoring system called the MIC Pathological Scale (Table 1) was adopted from Lucy Wedderburn [15]. Briefly, severity of pathology was assessed in 3 domains: muscle fiber, inflammatory and connective tissue domain. Components of the muscle fiber domain used to determine abnormalities were PFA, sarcoplasmic MxA overexpression, assessment of the number of necrotic and regenerating fibers within fascicles and in peri-fascicular regions, and presence of internal nuclei. PFA was defined by the presence of at least one muscle fascicle possessing a cluster of small fibers that occupied more than 60% of the fibers along the edge of the fascicle[9]. The components of inflammatory domain included the numbers of inflammatory cells infiltration on CD4, CD8, CD20 and CD68 staining, overexpression of MHC class I and the deposition of MAC. Excess fibrosis was assessed on H&E and MGT stains in perimysial and endomysial regions. The total score includes assessment in 3 domains and the values range from 0–24, with higher scores indicating more severe pathology. All biopsy assessments were performed by two independent observers (Zeng L, Ma X).

Statistical analysis

All statistical analyses were carried out using Prism (Graphpad, La Jolla, CA). Qualitative variables were expressed as percentages and frequencies while quantitative variables were expressed as median, first and third quartiles. A factorial analysis of variance (ANOVA) was performed using the nonparametric Kruskal-Wallis test to evaluate the main effects of MSA subgroups on clinical and pathological scores. Post-hoc comparisions were conducted using Dunn's Multiple Comparision test to identify pairs of MSA subgroups whose clinical or pathological scores were significantly differed from each other. The Chi square test were used to compare categorical findings between different autoantibodies. For all analyses, values of $p < 0.05$ were considered significant.

Results

Demographic and clinical features

A total of 46 DM patients (anti-MDA5 11, anti-Mi-2 10, anti-NXP2 13, anti-TIF1γ 8, anti-SAE 4) were included (Table 2). The median age at onset was 48 years and the median time from onset to biopsy was 4 months. Most of the patients showed preferential involvement of proximal muscle. Total MMT scores varied from 30 (severe) to 80 (normal) with the median of 63. The most frequent biopsy site was quadriceps, followed by biceps brachii, gastroenemius, tibialis anterior and deltoid muscles. More than half of the DM patients (52.2%) were on steroids use at the time of biopsy while the median duration from steroids use to biopsy was 2 months (0.875-5.5 months).

The clinical features of different subgroups were compared (Fig. 1). Anti-SAE patients had a later onset compared with the anti-MDA5 group. Interestingly, patients with anti-Mi-2 autoantibodies were more likely to have severe muscle weakness than patients with anti-MDA5, anti-NXP2 and anti-TIF1γ patients ($p < 0.05$). No significant difference was found between other groups. Patients with anti-Mi-2 autoantibody had significantly higher levels of creatine kinase (CK) compared with the anti-MDA5, anti-TIF1γ and anti-SAE groups. The anti-TIF1γ group had intermediate CK levels, while anti-MDA5 and anti-SAE groups had normal or slightly elevated serum CK levels. Patients with anti-NXP2 had a wide range of CK levels. Similar trends were observed for the level of lactate dehydrogenase (LDH) and CK/LDH ratio.

MRI of the thigh muscles was performed in 32 patients (anti-MDA5 5, anti-Mi-2 6, anti-NXP2 11, anti-TIF1γ 7, and anti-SAE 3) using a 1.5-T machine (GE; Signal). Representative MRI imaging for DM with anti-Mi-2, anti-MDA5 and anti-SAE are shown in Fig. 2. Patients with anti-Mi-2 exhibited diffuse edema on STIR images, especially the anterior compartments of the thigh. In contrast, fascial edema or patchy distribution were detected in patients with anti-MDA5 and anti-SAE autoantibodies. In the anti-NXP2 and anti-TIF1γ groups, a wide range from foggy distribution to diffuse involvement were observed (Supplementary Fig. 1).

Muscle pathological features among DM subgroups

The pathological score in each domain were compared (Fig. 3, Table 3). In the muscle fiber domain, more severe muscle fiber pathology was demonstrated in anti-Mi-2 group compared with anti-MDA5 group ($p < 0.001$). The anti-Mi-2 group showed obvious PFA (9 of 10, or 90%), perifascicular (90%) or non-perifascicular (80%) necrosis and regeneration of muscle fiber (70%). In anti-Mi-2 group, all samples showed perifascicular sarcoplasmic MxA expression (100%). In contrast, most patients with anti-MDA5 autoantibody had very mild, minimal myofiber abnormalities with little degeneration or regeneration (18.2%). Only 36.4% (4/11) of anti-MDA5 patients demonstrated PFA, meanwhile, sarcoplasmic MxA expression was found only in 45.5% (5/11) of anti-MDA5 autoantibody positive groups. The MxA expression often highlighted the perifascicular areas, but a scattered distribution pattern was observed exclusively in anti-MDA5 groups (Fig. 4).

In the inflammatory domain, high level of inflammation was demonstrated in anti-Mi-2 and anti-SAE group compared with anti-MDA5 group ($p < 0.01$). The anti-Mi-2 group showed obvious infiltration of CD8 positive T cells (9/10), CD20 positive B cells (4/10) and CD68 positive macrophages (7/10). In anti-SAE group, 3/4 of the samples displayed CD8 positive T cells and CD20 positive B cells in the endomysial tissue (Supplementary Fig. 2). In contrast, anti-MDA5 group had little or no inflammatory infiltrates. A few CD4 + T cells (3/11) and CD68 + macrophages (1/11) were seen, while CD8 + T cells and CD20 + B cells were not present in these samples. Almost 90% (39/46) samples showed MHC-I upregulation in cytosol or sarcolemmal membrane. Even in anti-MDA5 group, 10 of 11 cases showed increased MHC-I expression. There were also significant differences in the MAC deposition among groups. All anti-Mi-2 samples showed MAC deposition on capillaries, and 2 samples even showed sarcolemmal deposition, while only 3 of 11 cases showed capillary MAC deposition in the anti-MDA5 group (Fig. 4). In the connective tissue domain, no significant difference between groups were seen.

Overall, patients with anti-Mi-2 demonstrated higher pathological severity scores, both in the myofiber domain and inflammation domain. In contrast, MDA5 associated myopathies generally had minimal myofiber abnormalities with little or no inflammatory infiltration, scattered MxA upregulation and few complement deposition on capillaries. Patients with SAE had more inflammatory cell infiltration and MAC deposition compared to MDA5 group. NXP2 and TIF1 γ group showed a wide range in muscle pathological scores.

Discussion

In this study we comprehensively compared the pathological features in DM patients with different autoantibodies. Certain features were relatively common among most DM patients regardless the autoantibody. PFA was found in 52.2% of all DM patients, while sarcoplasmic MxA expression was found in 67.4% of all DM patients. The sensitivity of MxA in our cohort was lower than previous report (67.4% vs 77%) [9], which might be due to more anti-MDA5 case enrollment in our study (24% vs 17.5%) [9]. A significant proportion of DM samples showed MHC-I upregulation and complement deposition, 84.8% and 67.4% respectively which were another important pathological features of DM [10].

Notably, the pathological features also showed certain divergence in different subgroups in our cohort. Anti-Mi-2 DM patients had higher prevalence of PFA, fiber necrosis and regeneration while anti-MDA5 groups had minimal myofiber abnormalities without classic PFA. One possible mechanism for PFA is the vascular insufficiency, supported by frequent MAC capillary deposition in the perifascicular area [16]. However, we observed not only perifascicular capillary MAC deposition but also sarcolemmal MAC deposition. In addition, we confirmed that Mi-2 patients also had higher number of CD8, CD20 and CD68-positive cell infiltration than anti-MDA5 group, in consistent with previous report [17]. CD68-positive cells could secret a lot of cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β)[18], which further exacerbate perimysial inflammation. The above findings, together with higher level of muscle enzymes and edema of thigh, might explain the more severe muscle weakness in anti-Mi-2 patients. Of note, although patients with anti-Mi-2 had more severe muscle weakness, laboratory testing and pathological scores, some research showed anti-Mi-2 patients have significantly low risk of interstitial lung diseases and malignancy, the prognosis is favorable with good response to treatment [19, 20].

As a distinctive subtype of DM, anti-MDA5 group has been linked with clinical amyopathic DM and progressive interstitial lung disease [21]. In consistent with the mild muscle weakness, low or nearly normal muscle enzymes, anti-MDA5 DM patients generally had minimal myofiber abnormalities with little or no inflammatory infiltration. The MDA5 molecular is one of the viral RNA sensors that indicate IFN1 pathway activation, thereby suppressing virus replication[22]. Interestingly, accumulating pathological evidence suggests that IFN1 signatures are high in serum and affected skin[23, 24], but significantly lower in muscles in anti-MDA5 DM patients compared to other DM subgroups[25]. In our research, negative or scattered distribution of myofiber MxA expression in MDA5 subgroup further suggested the low activation of IFN1 pathway in muscle tissue, as MxA is a surrogate marker for IFN1 pathway activation. We speculate that selectively recruitment of IFN1 in different tissues of MDA5 patients might contribute to this particular pathological feature.

Different from anti-Mi-2 and anti-MDA5 subgroups, patients with anti-TIF1γ and anti-NXP2 have a wide range in muscle strength, enzyme levels, muscle imaging and histopathological scores. In one study from adult TIF1γ positive patients indicated that capillary MAC deposition is associated with paraneoplastic myositis [26]. However, our data didn't show significant difference on C5b-9 immunohistochemical profile between anti-TIF1γ group and other groups. Some studies suggest anti-NXP2 dermatomyositis tends to be associated with perimysial angiopathy and shows microinfarction and capillary loss in pathology[27, 28]. However, in our cohort microinfarction is present in only 4/13 of the patients (data not shown), this may be due to steroid use before biopsy.

This study has several limitations. First, this study involves a relatively small number of patients, especially for rare antibodies such as anti-SAE. Second, although all pathological staining were performed at Huashan hospital, the protocol of clinical data collection and biopsy site were inevitably variable. Nevertheless, half of the patients had already received immunotherapy before biopsy, therefore, the clinical data do not reflect these patients' conditions prior to treatment. Further prospective multicenter study would be helpful in understanding the characteristics among different MSA subgroups.

In conclusion, we established semi-quantifying Pathology Scale that could help deep phenotyping the muscle pathology in DM. Our results demonstrated the divergence of pathological features among different MSA DM subgroups, which further indicated different underlying pathogenic process of muscle damage.

Abbreviations

DM

dermatomyositis; PFA:perifascicular atrophy; MSA:myositis- specific antibodies; MAA:myositis-associated antibodies; MDA5:antimelanoma differentiation associated gene 5; Mi-2:antimitochondrial M2-associated protein; NXP2:nuclear matrix protein 2; TIF1γ:transcription intermediary factor 1; SAE:small ubiquitin-like modifier activating enzyme; H&E:Haematoxylin & Eosin; MGT:Gomori-modified trichrome; NADH-TR (tetrazolium reductase); PAS:periodic acid-Schiff; ORO:oil red O; MxA:myxovirus resistance protein A; CK:creatine kinase; LDH:lactate dehydrogenase; MAC:membrane attack complex; MHC:major histocompatibility complex; TNF-α:tumor necrosis factor alpha; IL-1β:interleukin-1 beta

Declarations

Ethics approval and consent to participate

Declarations: All methods in this study were carried out in accordance with the Declaration of Helsinki, The study was approved by the Ethics Committees of Huashan Hospital (2018-153) and Tongji Hospital (TJ-C20121221). Informed consents to participate were obtained from all the patients.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Study design: LZ, CZ, JL, WZ; Data collection: LZ, XM, JL, MG, ZW, CS, JX, SL, JL, QK, BB; Statistical analysis: LZ, XM, WZ; Data interpretation: LZ, CZ, JL, WZ; Manuscript preparation: LZ, CZ, WZ; Literature Search: LZ, WZ; Funds Collection: WZ, LZ; all authors read and approved the final manuscript.

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Tables

Table 1

MIC Pathological Scale for DM muscle pathology scoring

Domain	Score	Definition and instruction
Muscle fiber domain		
Perifascicular atrophy (PFA)*	0,1,2	Score 0: no staining; score 1: present in one or 2 fascicles; score 2: present in 3 or more fascicles;
Necrosis/ regeneration in peri-fascicular area	0,1,2	Includes: punch-out cells, myofibrillar infarction, and/ or pallor, myophagocytosis. For each of perifascicular and non-perifascicular, score 0: none; score 1: any of the features present in one or 2 fascicles; score 2: present in 3 or more fascicles;
Necrosis/ regeneration in non-perifascicular area	0,1,2	
Internal myonuclei in normal myofibers	0,1	Score 0: < 3% fibers;score 1: > 3% fibers
MxA over-expression	0,1,2	Score 0: no staining; score 1: stained fibers < 5 layers in periphery fascicles; score 2: diffuse distribution or > = 5 layers
Inflammatory domain		
CD4 infiltration	0,1,2	Score for CDs infiltrating cells as follows: if none, or < 4 cells in x20 field = score 0; if > 4 cells in a x20 field and/or 1 cluster = score 1; if > 2 clusters in whole biopsy, and or > 20 cells in a x20 field = score 2;
CD8 infiltration	0,1,2	
CD20 infiltration	0,1,2	
CD68 infiltration	0,1,2	
MHC-I over-expression	0,1,2,3	Score 0: no staining; score 1: focal overexpression; score 2: diffusely overexpression in sarcolemmal membrane; score 3: diffusely overexpression in cytosol.
MAC deposition	0,1,2	Score 0: no staining; score 1: capillary labelling; score 2: sarcolemmal labelling;
Connective tissue domain		
Endomysial fibrosis	0,1	Score 0: absence; score 1: present of any
Perimysial fibrosis	0,1	

*PFA: the presence of at least one muscle fascicle possessing a cluster of small fibers that occupied more than 60% of the fibers along the edge of the fascicle

MAC: membrane attack complex; MHC: major histocompatibility complex; MxA: myxovirus resistance protein A.

Table 2

Demographic and clinical features of all 46 DM patients

Clinical Features	
Male: Female	19:27
Age at onset (years)	48 (29.5–59.5)*
Time from onset to biopsy (months)	4 (1.25–6.5)*
MMT-8 at biopsy	63 (51.5–72)*
On steroids at biopsy, n (%)	24 (52.2)
Time from steroids use to biopsy (months)	2 (0.875-5.5)*
Biopsy site, n (%)	
Quadriceps	21 (45.7)
Biceps	9 (19.6)
Gastroenemius	6 (13)
Tibialis anterior	5 (10.9)
Deltoid	2 (4.3)
Unknown	3 (6.5)
Myositis-specific autoantibodies, n (%)	
Anti-MDA5	11 (23.9)
Anti-Mi-2	10 (21.8)
Anti-NXP2	13 (28.3)
Anti-TIF1γ	8 (17.4)
Anti-SAE	4 (8.6)

*IQR: interquatile range.

Table 3
Pathological characteristics of dermatomyositis with different antibodies

Pathological feature	Total n = 46	Anti-MDA5 n = 11	Anti-Mi-2 n = 10	Anti-NXP2 n = 13	Anti-TIF1γ n = 8	Anti-SAE n = 4	Significant differences*			
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	MDA5 vs Mi-2	MDA5 vs SAE	Mi-2 vs NXP2	MDA5 vs Tif1γ
PFA	24 (52.2)	4 (36.4)	9 (90)	6 (46.2)	3(37.5)	3 (75)	*		*	
Necrosis/ regeneration in peri-fascicular area	20 (43.5)	2 (18.2)	9 (90)	4 (30.8)	5 (62.5)	0 (0)	***		**	*
Necrosis/ regeneration in non- peri-fascicular area	21 (45.7)	1 (9.1)	8 (80)	5 (38.5)	5 (62.5)	2 (50)	**		*	*
Internal myonuclei	7 (15.2)	1 (9.1)	2 (20)	0 (0)	3 (37.5)	1 (25)				
MxA overexpression	31 (67.4)	5 (45.5)	10 (100)	7 (53.8)	6 (75)	3 (75)	**		*	
Endomysial fibrosis	12 (26.1)	3 (27.3)	3 (30)	2 (15.4)	1 (12.5)	2 (50)				
Perimysial fibrosis	26 (58.7)	4 (36.4)	7 (70)	6 (46.2)	6 (75)	3 (75)				
CD4 infiltration	19 (41.3)	3 (27.3)	5 (50)	5 (38.5)	4 (50)	2 (50)				
CD8 infiltration	24 (52.2)	0 (0)	9 (90)	8 (61.5)	4 (50)	3 (75)	****	**		**
CD20 infiltration	13 (28.3)	0 (0)	4 (40)	5 (38.5)	1 (12.5)	3 (75)	*	**		
CD68 infiltration	17 (37.0)	1 (9.1)	7 (70)	5 (38.5)	3 (37.5)	1 (25)	**			
MHC-I overexpression	39 (84.8)	10(90.9)	10 (100)	8 (61.5)	7 (87.5)	3(75)			*	
MAC deposition	31 (67.4)	3 (27.3)	10 (100)	8 (61.5)	6 (75)	3(75)	***			*

*significant differences: *p<0.05; **p<0.01,***p<0.001;****p<0.0001

Figures

Fig 1

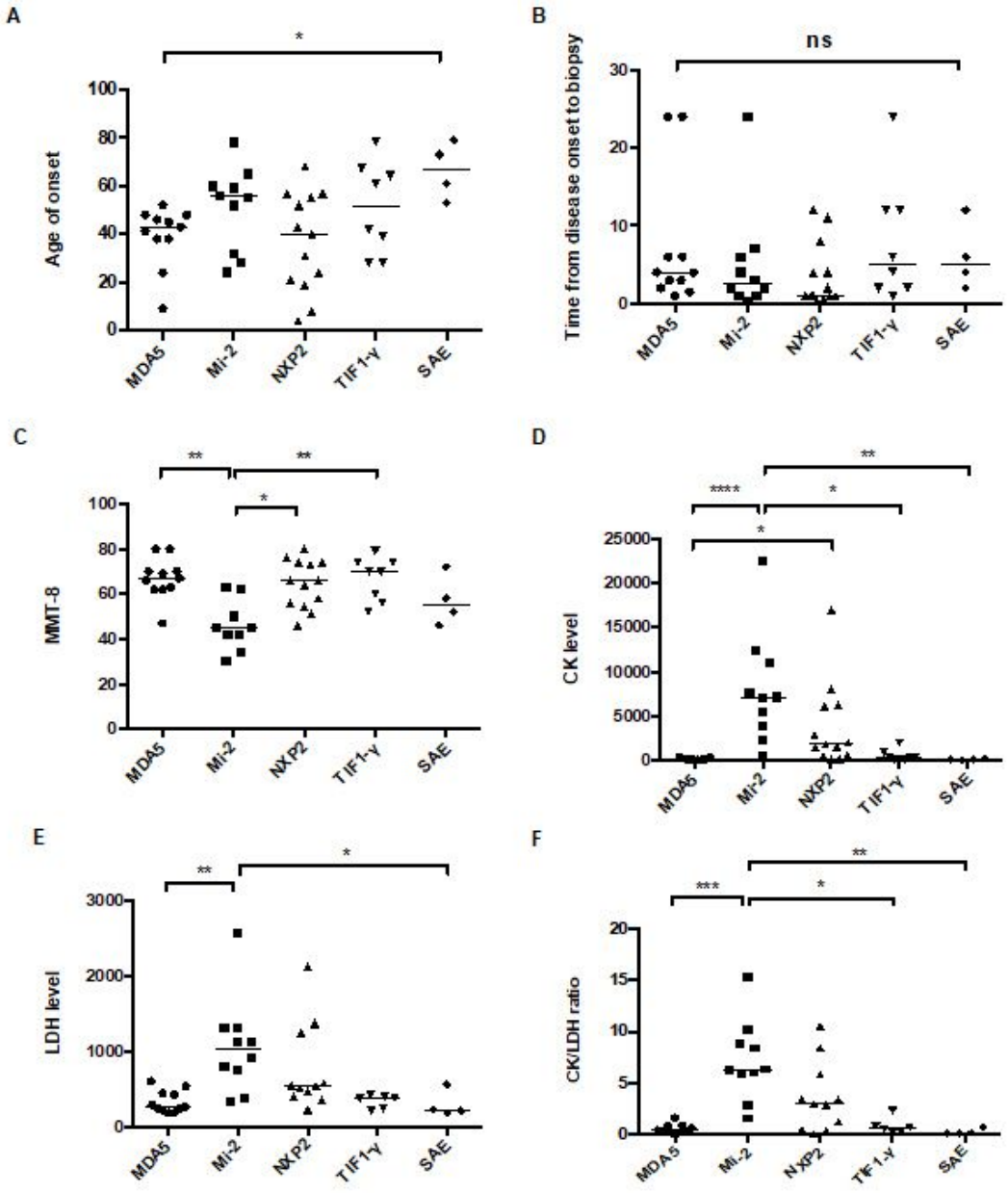

Figure 1

Clinical and serological characteristics among autoantibody groups. The distribution of age of onset (A), the duration from disease onset to biopsy (B), the muscle strength on biopsy (C) and muscle derived enzymes (D-F) were determined across MSA subgroups. Kruskal-Wallis ANOVA with Dunn's Multiple comparison analysis indicated that the average age of DM onset among anti-SAE cases was significantly older than anti-MDA5 group (A). The duration from disease onset to biopsy were not significantly differently distributed across the MSA subgroups (B). Muscle strength differed between anti-Mi-2 and anti-MDA5, anti-Mi-2 and anti-NXP2, anti-Mi-2 and anti-TIF1γ groups (C). The anti-Mi-2 group had significantly higher median levels of CK, LDH, and CK/LDH ratio compared with other groups (D-F). Each symbol indicates an individual case, bars show the median. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 and ns = non significance.

Fig 2

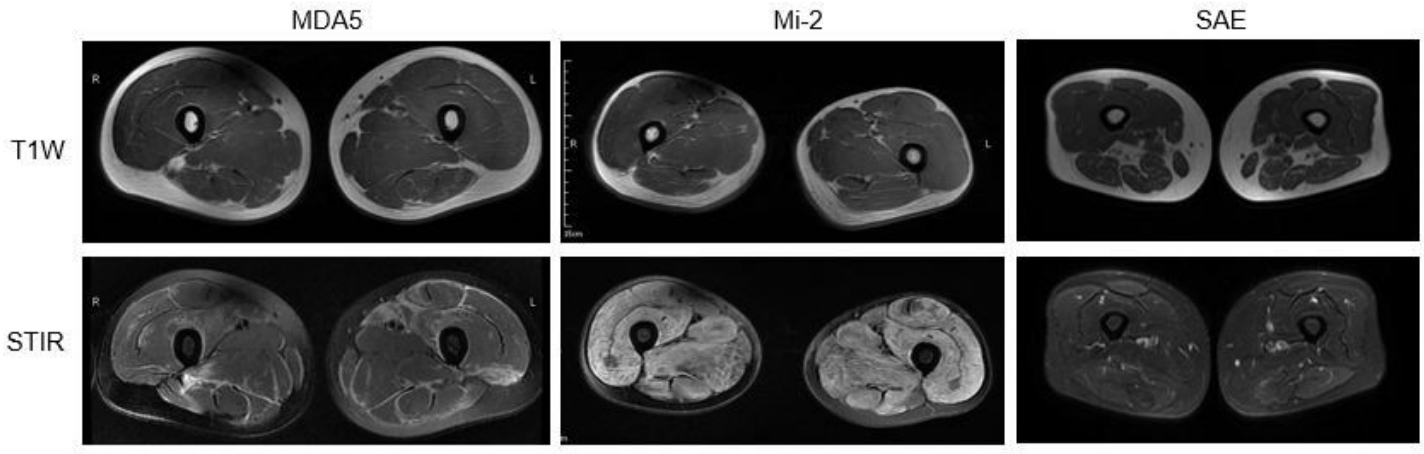

Figure 2

Representative muscle MRI of the thigh in patient with Mi-2, MDA5 and SAE autoantibodies. Anti-Mi-2 cases exhibited diffuse edema on STIR images, while MDA5 and SAE groups showed fascial edema or patchy distribution of edema.

Fig 3

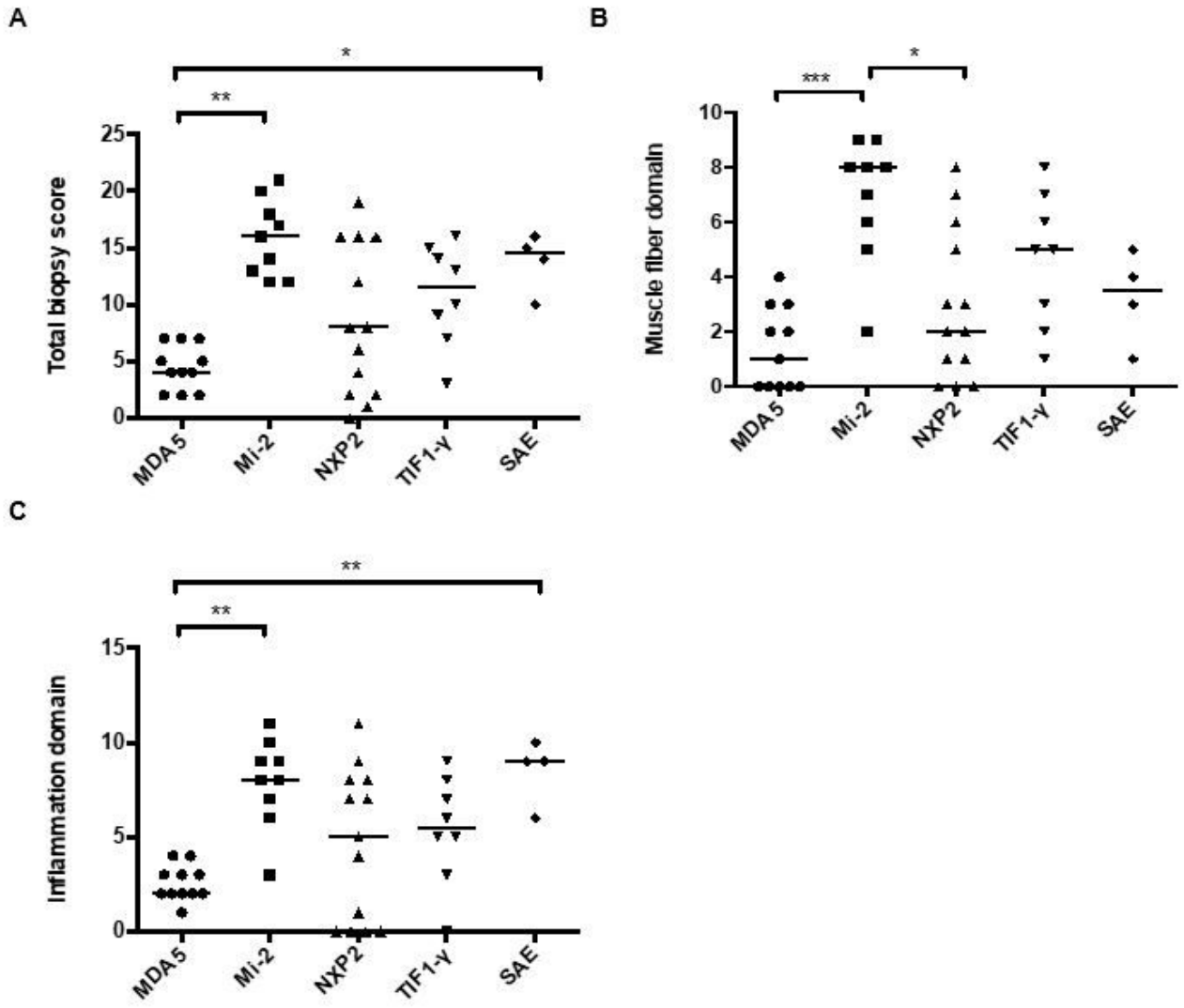

Figure 3

Pathological features among autoantibody groups. The distribution of total biopsy score (A), the muscle fiber domain (B) and the inflammation domain (C) was determined across MSA subgroups. Kruskal-Wallis ANOVA with Dunn's Multiple comparison analysis indicated that the total biopsy scores differed between the anti-Mi-2 and anti-MDA5, anti-MDA5 and anti-SAE cases. The muscle fiber domain scores differed between the anti-Mi-2 and anti-MDA5, anti-Mi-2 and anti-NXP2 groups. The inflammation domain scores differed between anti-MDA5 and anti-Mi-2, anti-MDA5 and anti-SAE cases. Each symbol indicates an individual case, bars show the median. *p<0.05, **p<0.01, ***p<0.001.

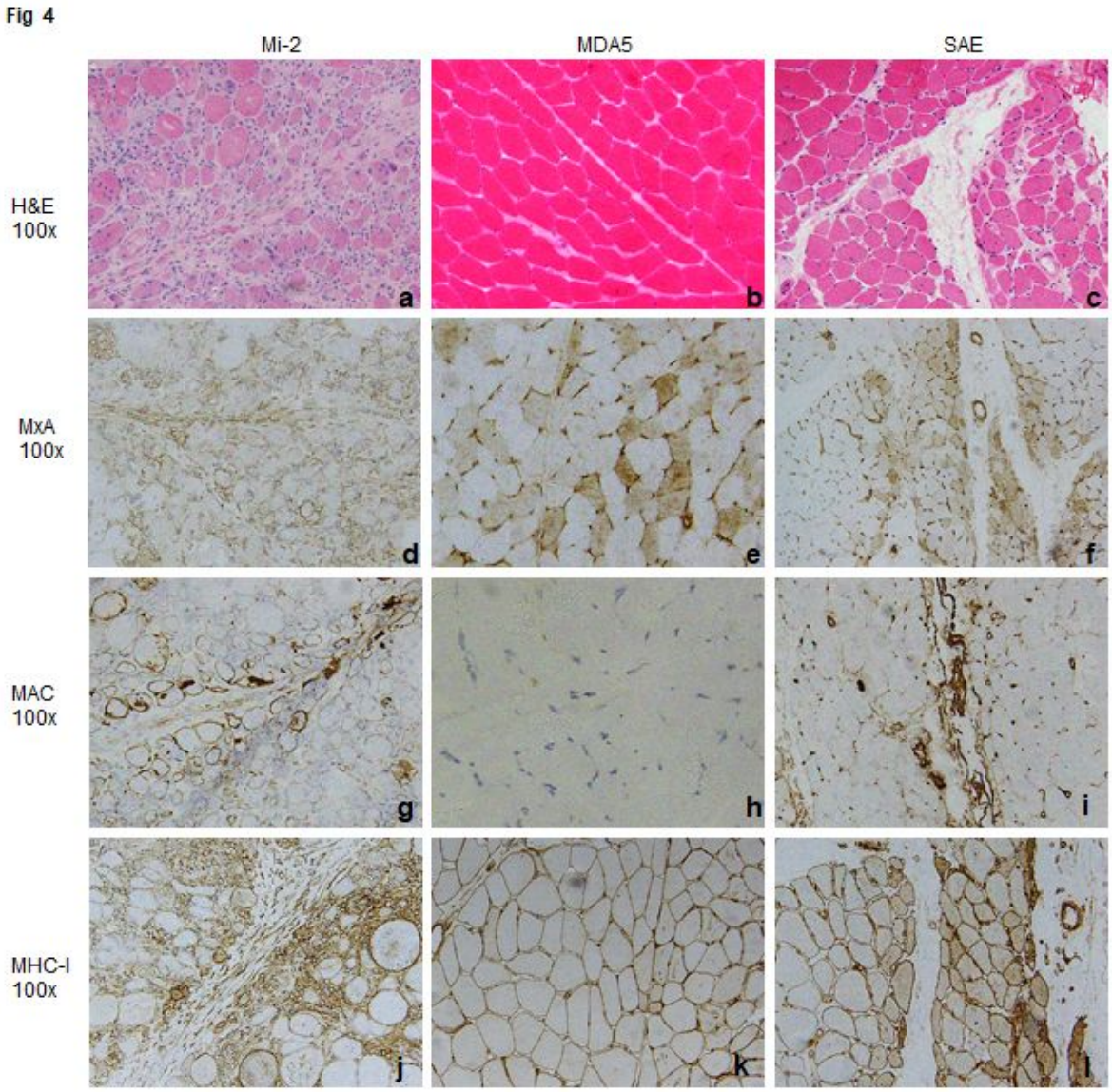

Figure 4

Representative pathological findings for a patient with Mi-2, MDA5 and SAE autoantibodies. Mi-2 group and SAE group typically had high level of myofiber pathology, while MDA5 group had mild appearance with minimal myofiber abnormality and no inflammation. a, b, c – H&E; d,e,f – MxA; g,h,i –MAC; j, k, l -MHC-I. The original magnification is X100.

Supplementary Files

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