Original Article

Comparison of Muscle Biopsy Features with Myositis Autoantibodies in Inflammatory Myopathies: A Pilot Experience

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Abstract

Background: Idiopathic inflammatory myopathies (IIM), also called autoimmune myositis, are heterogeneous. These include dermatomyositis (DM), inclusion body myositis, immune mediated necrotizing myopathy (IMNM), anti-synthetase syndrome (ASS), and overlap polymyositis. Classification of IIM has evolved from clinical to clinico-pathologic to the recent clinico-sero-pathologic with the discovery of myositis-specific antibodies (MSA) and myositis-associated antibodies. The various antibodies have shown association with specific phenotypes. **Objective:** To analyze muscle biopsy features with respect to each MSA and MAA to understand the frequency of findings in each entity. **Materials and Methods:** Biopsy-proven cases of IIM where myositis profile was available were included in the study after obtaining Institutional Ethics Committee (IEC) approval. In addition to the stains and enzyme histochemistry, immunohistochemistry with MHC class I and II and MxA was performed. Features like perifascicular atrophy, perifascicular necrosis, scattered necrosis, inflammation, etc. were analyzed. Myositis profile was performed by line-blot technique using a 16-antigen panel. Cases were divided into different autoantibody subgroups. Various clinical, demographic, and muscle biopsy features were studied with respect to each MSA and MAA. **Results:** There were a total of 64 cases. Mi2 (N = 18) was the most common autoantibody. Some of the salient observations included PFA with perivascular inflammation in Mi2; pediatric cases and microinfarcts in NXP2; no PFA or inflammation in MDA5; perifascicular necrosis in JO1; extensive necrosis with sparse inflammation in SRP; more inflammation in overlap myositis; MxA positivity in DM; and absent in ASS. **Conclusion:** This is a pilot study documenting differences in biopsy phenotype with each MSA and MAA which is comparable to the literature. These findings can be used to characterize IIM in seronegative biopsies.

Keywords: Inflammatory myopathy, muscle biopsy, myositis antibodies

Introduction

Autoimmune myositis, also called as idiopathic inflammatory myopathies (IIM), are a heterogeneous group of diseases involving muscular as well as extramuscular manifestations including cutaneous, lung, and joints among others. The major subgroups of IIM include dermatomyositis (DM), inclusion body myositis (IBM), immune-mediated necrotizing myopathy (IMNM), and anti-synthetase syndrome (ASS).[1,2] Others include overlap myositis and nonspecific myositis. Polymyositis (PM) is of late not considered as a distinct entity.[2] Various autoantibodies associated with IIMs have been discovered, beginning with anti-Mi2, back in 1976.[3]

There has been a constant stride to classify this polyphenotypic group of diseases into clinically meaningful, pathologically informative, and diagnostically reproducible entities. The classification schemas and diagnostic criteria are constantly being revised. However, some of the classification schemas are widely accepted among others.

The classification of autoimmune myositis has evolved over the decades from clinical to clinico-pathologic and to the recent clinico-sero-pathologic. This is reflected in various diagnostic schema beginning with Bohan and Peter in 1975, Dalakas in 1991, and the various revisions of European Neuromuscular Centre (ENMC) classification criteria.[4-9] The inclusion of serologic features into diagnostic criteria provides a noninvasive yet robust technique for diagnosis.

Several myositis-specific (MSA) and myositis-associated (MAA) antibodies have been detected in the sera of myositis patients. The various antibodies have been shown to be associated with specific phenotypes, organs involved, and severity.[10]

Over time, the biotechnological advancements have paved way for various platforms for the detection of

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autoantibodies.[11] Of these, the immunoblot technique is relatively simple to perform, reproducible, and widely available with promising results. Ready-to-use panel of antigens is now available in the form of strip tests to this end, which is being incorporated into routine practice for diagnosis of DM, IMNM, and ASS cases.

However, about one-third of myositis cases are sero-negative.[12] The antibodies can also fluctuate during the disease course and in response to treatment.[13,14] Muscle biopsy is required in such cases as well as in cases with atypical presentation to establish a diagnosis, in addition to diagnosis of IBM.

In this study, we compare the muscle biopsy features with the respective autoantibodies. This is a pilot experience to explore the morphologic spectrum of IIM with respect to each antibody.

Materials and Methods

This was a retrospective study done in the department of Pathology, Nizam's Institute of Medical Sciences (NIMS) after obtaining approval from the institutional ethics committee (64th ESGS No. 1406/2022). The study group included all the patients with clinical diagnosis of IIM in whom muscle biopsy was performed and the myositis profile was positive. All such cases from January 2019 to December 2022 were included in the study. The muscle biopsy interpretation as well as myositis profile for all the cases was performed at our hospital in department of pathology. The clinical and demographic features, Creatine phosphokinase and Electroneuromyography (CPK and ENMG) results were obtained from patient request forms.

Muscle biopsy

The biopsies were snap frozen followed by enzyme histochemistry, routine, and special stains. The enzymes included Adenosine triphosphatase preincubatedat pH 9.4 and 4.6, Nicotinamide adenine dinucleotide-Tetrazoliumreductase, Succinate dehydrogenase (SDH), Cytochrome oxidase (COX), and combined COX-SDH, whereas the stains included routine Hematoxylin and Eosin (H and E) along with special stains like Modified Gomoritrichrome and Masson trichrome.

The following features were evaluated in these biopsies:

1. Perifascicular atrophy (PFA)
2. Perifascicular necrosis (PFN)
3. Scattered necrotic fibers
4. Groups of necrotic fibers forming microinfarcts
5. Type of inflammatory infiltrate: lymphocytes or macrophages
6. Location of inflammation: whether endomysial or perivascular
7. Any other significant findings.

Immunohistochemistry (IHC): Major histocompatibility complex (MHC) class I (Dako; 1:100 dilution) and class II (Dako; 1:20) and Myxovirus resistance protein 1 (MxA) (Cell signaling technology; 1:150).

Interpretation of IHC

MHC Class I and Class II: Staining along sarcolemma in muscle fibers was noted; endothelial cells were used as internal control.

MxA: Sarcoplasmic staining was considered as positive with the following patterns:

Perifascicular = Positivity in perifascicularfibers only.

Diffuse = Positivity in all the fibers across a fascicle.

Scattered = Positivity in individual fibers scattered randomly in a fascicle.

Negative = No positive staining in any fibers.

Myositis antibody profile: This was performed on a semi-automated machine with ready-to-use strips employing line-blot technique (Euroimmune). A standard 16-antigen panel is used to detect antibodies in patients' sera. The presence and strength of each antibody are noted as follows:

1. (+) Borderline
2. + Positive
3. ++ Positive
4. +++ Positive.

The various clinical, demographic, and muscle biopsy features were studied with respect to each MSA and also MAA. Currently 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR) testing platforms are not available in India; hence, this particular autoantibody could not be incorporated in our study.

Inclusion criteria: Cases diagnosed as inflammatory myopathy with muscle biopsy and positive myositis profile (strength 1+ and more).

Exclusion criteria:

1. Cases of inflammatory myopathy lacking either muscle biopsy or myositis profile.
2. Negative and borderline cases on myositis profile.

Sensitivity and specificity of MxA was calculated in DM biopsies using Jo1 positive ASS cases as control.

Results

There were a total of 1,272 muscle biopsies performed during the study period of which 110 were diagnosed as inflammatory myositis. Of these, myositis profile was positive in 64 cases which were included in the study. Among the MSAs, Mi2 cases accounted for the majority (N = 18) followed by Jo1 (N = 9) and Signal recognition particle (SRP) (N = 9). Of the remaining 46 patients, 35 were seronegative group (negative myositis antibody profile) and in 11 patients the myositis profile showed multiple (more than two) antibodies all of which were borderline positive. These 11 patients are not included in the final analysis.

There was a wide age distribution of patients ranging from 4 to 75 years with 49 female and 15 male patients (male:female = 1:3.3). There were four pediatric

patients (6.25%) of which three showed NXP2 positivity. The various clinical and biochemical parameters associated with different antibodies are depicted in Table 1. The histopathological and IHC features of the biopsies pertaining to different antibody classes are detailed in Table 2.

Anti-Mi2 autoantibody (n = 18)

All the patients in this subgroup were adults >18 years with 13 females and five males. Mean CPK was 3,000 IU. These patients presented with features of proximal myopathy and cutaneous involvement was seen in nine. Four patients were mentioned to have bulbar muscle involvement in the form of dysphagia and one had subcutaneous calcification. The muscle biopsies showed PFA in 10 of which seven had perivascular inflammation. Perifascicular necrosis was seen in one biopsy. The biopsies which did not show perifascicular pathology (N = 8) showed fiber degeneration, necrosis, and regeneration along with perivascular inflammation in two, only fiber necrosis and regeneration in four, whereas one biopsy showed only type 2 fiber atrophy. Two of the biopsies without PFA or inflammation showed mitochondrial abnormalities in the form of blue ragged fibers on SDH. These fibers accounted for 8%-10% of the fibers.

COX deficiency in perifascicular fibers was seen in 10 biopsies and these fibers stained blue on combined COX-SDH stain.

Table 1: The myositis profile of the study group along with clinical and biochemical parameters of each autoantibody class of IIM

Antibody No. of cases	Age (years) range	Age (years) median	CPK (IU/L) mean	Skin	Myopathy No. %	Myalgia	Bulbar muscle	ILD	Calcinosis	Others
Mi2α/β (18)	23-65	44.5	3,000	9	18 100%	5	4		1	Arthralgia 4
TiF1 γ (3)	48-75	61	500	2	3 100%	2	1			
MDA5 (2)	18-49	-	500	2	1 50%	1	1			
NXP2 (5)	4-52	11	1,500	1	5 100%	3	2			
Jo1 (9)	23-53	35	20,000	2	9 100%	2		2		
PL7 (2)	60-63	-	1,700	1	2 100%			1		
SRP (7)	25-56	48	11,000	1	7 100%	4	2			Cancer 1
Ro52 (9)	14-52	38	3,500	4	9 100%	3	4	1		Arthralgia 2 SLE 1
Ku (4)	38-57	47.5	2,000	1	4 100%		1			Arthralgia 1
PM/Scl (5)	24-43	35	2,500	2	5 100%	1	1			Arthralgia 1

CPK, creatine phosphokinase; ILD, interstitial lung disease; SLE, systemic lupus erythematosus

Table 2: Histopathologic and IHC features of each antibody class of IIM

Antibody No. of cases	PFA	PFN	Necrotic Fibers		Micro-infarcts	Inflammation		MHC I	MHC II	MxA			
			Scattered	Extensive		PV	EM			PF	Focal	Diffuse	Negative
Mi2α/β (18)	10	2	11	4	1	8	2	6/6		7/16	2/16	2/16	5/16
TiF1 γ (3)	1		1	1		1					1/1		
MDA5 (2)	Nil	Nil	2		1	Nil	Nil			1/2	1/2	Nil	Nil
NXP2 (5)	3	1		4	3	2				1/5	4/5	Nil	Nil
Jo1 (9)	1	2	4	2		3	3		8/9				8/8
PL7 (2)						1	1		½				
SRP (7)			2	4		1	1	4/7					
Ro52 (9)	1		5	3		5	1						
Ku (4)	1		3			2							
PM/Scl (5)	1	1	3	1		2	1						

PFA, perifascicular atrophy; PFN, perifascicular necrosis; PV, perivascular; EM, endomysial; MHC, major histocompatibility complex; MxA, Myxovirus resistance protein 1; PF, perifascicular. The number of biopsies positive for each parameter are mentioned; denominator is included to show the total number of cases in which the respective IHC was performed in each antibody class

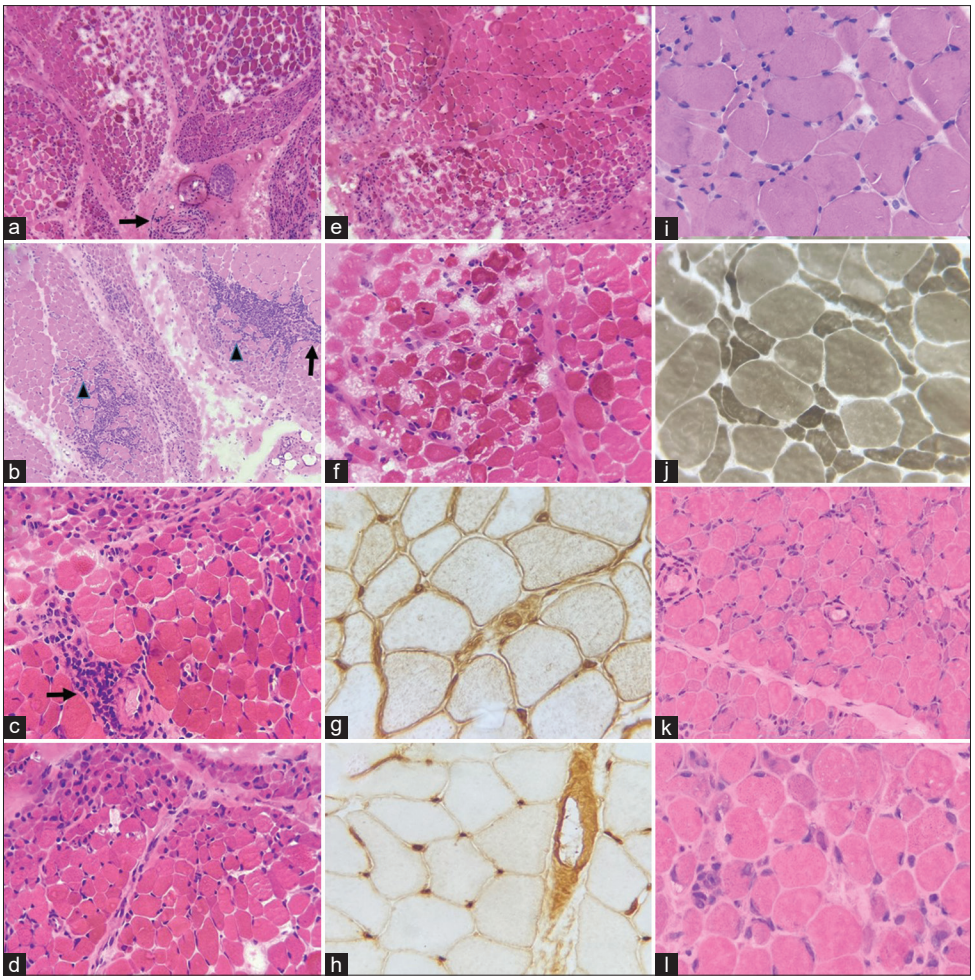

Figure 1: Showing dermatomyositis cases of various autoantibody positivity: Anti-Mi2 cases (a-d) showing perifascicular atrophy in all along with perivascular inflammation in A to C (arrow); the infiltrate is extending into the endomysium surrounding the non-necrotic fibers in B (arrow head) (a, b = H and E x 40; c, d = H and E x 100). Anti-NXP2 cases (e-h) showing perifascicular atrophy (e) and extensive necrosis forming microinfarcts (e, f); IHC with MHC class I shows sarcolemmal positivity (g) as opposed to control showing only positivity in vessels (h) (e = H and E x 40; f = H and E x 100; g, h = DAB x 400). Anti-TIF 1γ case (i and j) showing nonspecific atrophy of type II fibers (i = H and E x 400; j = ATPase at pH 9.4 x 400). Anti-MDA5 case (k and l) showing only scattered regenerating fibers; there is no PFA, necrosis or inflammation (k = H and E x 100; l = H and E x 400)

MHC class I IHC was positive in all the six biopsies where it was performed.

The biopsy features are shown in Figure 1.

MxA was performed on 16 biopsies. Perifascicular pattern of staining was the common staining pattern (N = 7). Diffuse and focal patterns of staining were seen in two biopsies each. MxA was negative in five biopsies. The various patterns of staining are shown in Figure 2. Of these five MxA negative biopsies, one showed perifascicular MHC class I positivity, one had MHC class I positivity along with PFA, and two had PFA and perivascular inflammation. The remaining one biopsy was type 2 fiber atrophy without either PFA or perivascular inflammation.

Of the eight patients in whom clinical follow-up details were available, six (75%) had clinical improvement and two patients expired.

Anti-TiF1 gamma autoantibodies (n = 3)

These were two female and one male patients with a median age of 61 years. One patient had skin rash. The mean CPK was 500 IU. Perifascicular atrophy was seen in one biopsy with perivascular inflammation and many necrotic fibers. The biopsy of a 75-year-old patient showed marked fiber necrosis and regenerating fibers in absence of PFA or inflammation. The third biopsy showed only type 2 fiber atrophy. All three patients showed a significant improvement in muscle weakness following immunosuppressive treatment.

MHC I staining was positive in one of the three biopsies. MxA was performed on only one biopsy which showed focal positivity.

Anti-NXP2 autoantibodies (n = 5)

There were four females and one male in this group. Three of the five patients were children without any skin rash. Subcutaneous

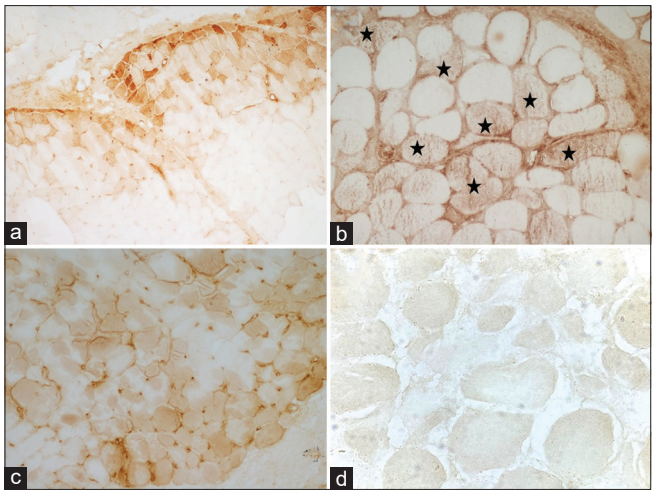

Figure 2: Showing different patterns of MxA staining on IHC: Perifascicular (a), focal (b), diffuse (c) and negative (d) staining (a = DAB x 100; b-d = DAB x 400)

calcification was reported in three patients. Muscle biopsies showed PFA in three and perifascicular necrosis in one. Two biopsies with PFA also showed perivascular inflammation. Four of the biopsies showed significant fiber necrosis forming microinfarcts [Figure 1].

MHC I staining was positive in three of the five biopsies. MXA staining showed focal pattern in four and perifascicular in one of the biopsies.

Anti-MDA5 autoantibodies (n = 2)

These were two adult patients, both female, with proximal myopathy and normal CPK. PFA and inflammation was absent. The biopsies were nonspecific with few scattered necrotic and regenerating fibers [Figure 1].

MHC I was positive in one of the two biopsies. MxA showed one focal and one perifascicular pattern of staining indicating a strong association of Nuclear matrix protein (NXP)-2 with MxA.

Anti-Jo1 autoantibodies (n = 9)

These were nine adult patients, five females and four males, with markedly elevated CPK (mean 20,000 IU/L). Interstitial lung disease (ILD) at presentation was documented in two patients. Muscle biopsies showed PFA in one, whereas perifascicular necrosis in two [Figure 3]. Of the remaining six biopsies, two showed perivascular as well as endomysial inflammation with significant fiber regeneration and scattered necrosis. Two biopsies showed sarcoplasmic rimmed vacuoles with fiber degeneration and regeneration. One of the biopsy showed angulated atrophic fibers and grouping indicating neurogenic atrophy. One biopsy showed only scattered necrotic fibers.

MHC class II staining was found to be positive in five biopsies and it was concentrated more in the perifascicular region [Figure 3].

MxA was negative in all the eight biopsies [Figure 2].

Anti-PL7 antibodies (n = 2)

Of the two patients in this subgroup, ILD and skin rash were noted at presentation in one patient. The same patient showed many foci of endomysial and perivascular inflammation in absence of PFA. The other patient had only proximal myopathy with only type II fiber atrophy on muscle biopsy examination.

MHC II was positive in the perifascicular fibers in one case where it was performed.

Anti-SRP autoantibodies (n = 7)

All the patients in this group were adults with six females and one male. Proximal myopathy and myalgias was the predominant clinical manifestation with mean CPK levels of 11,000 IU/L. Necrosis was the only conspicuous finding in the muscle biopsy of these patients which was marked involving the entire fascicles in four of the biopsies, whereas the necrotic fibers were scattered in remaining three [Figure 3]. Only one of the biopsy showed mild perivascular inflammation.

The expression of MHC class I was focal in four and absent in three.

We did not have any anti-small ubiquitin-like modifier activating enzyme (SAE) positive cases during the study period.

Anti-Ro 52 autoantibodies (n = 9)

Eight were adult females and one was a 14-year-old boy. Mean CPK was 3,500 IU/L. Skin rash was present in five, ILD in one, bulbar muscle weakness in four, and arthralgia in one patient. Five of the nine biopsies showed significant inflammation [Figure 3], four perivascular and one endomysial. Necrotic and regenerating fibers were seen in all. One biopsy showed PFA. Only one of the biopsies showed blue ragged fibers on SDH.

Anti-Ku autoantibodies (n = 4)

All were adults, three females and one male, with a mean CPK of 2,000 IU/L. All the patients presented with proximal myopathy. Skin rash, arthralgia, and bulbar muscle involvement were present in one each. Three of four showed necrotic and regenerating fibers with perivascular inflammation in two of these and focal PFA in one. One of these also showed central cores on SDH. The remaining one biopsy showed very significant fiber changes in the form of atrophy, hypertrophy, and splitting. It was difficult to differentiate these features from muscular dystrophy. This patient had received steroids in the past. However, further genetic work or follow-up was not available in this patient.

Anti-PM/Scl (n = 5)

All patients in this group were adults, four females and one male, with median age 35 years. Mean CPK was 2,500 IU/L. Presenting symptom was proximal myopathy in all, with arthralgia and myalgia in one each. One had bulbar muscle involvement. Muscle biopsies showed scattered necrotic fibers in three, perivascular

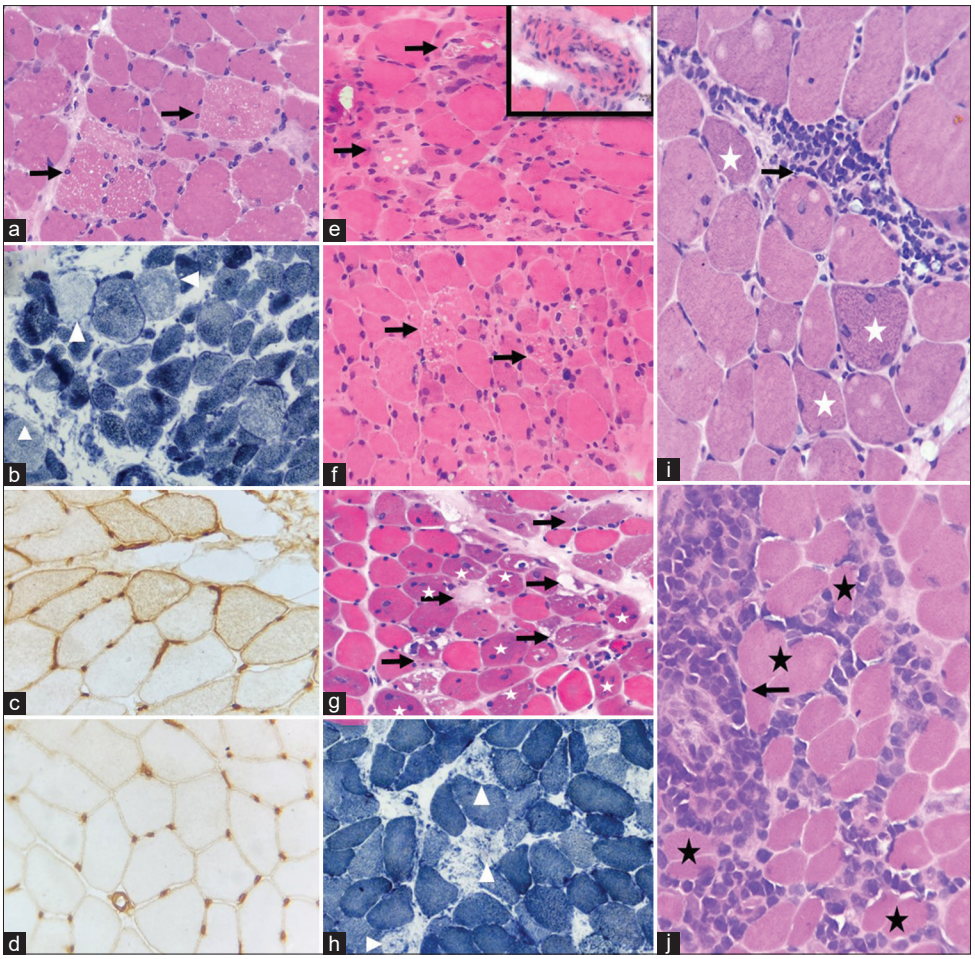

Figure 3: Showing other autoimmune myositis cases other than dermatomyositis: ASS cases (a-d) showing necrotic fibers (arrow) concentrated in the perifascicular area (a = H and E x 400; b = SDH x 400); IHC with MHC class II shows sarcolemmal positivity showing a gradient in staining from periphery of the fascicle toward the center (c = DAB x 400) as opposed to control showing positivity in vessels (d = DAB x 400). Anti-SRP positive IMNM (e-h) cases showing randomly scattered single necrotic fibers (arrow) throughout the fascicle with conspicuously absent inflammation (inset shows a perimysial arteriole which is devoid of perivascular inflammation); basophilic regenerating fibers (asterix) are seen around the necrotic fibers (g); the necrotic fibers have a moth-eaten appearance on SDH (arrow head) (H) (e-g = H and E x 400; h = SDH x 400). Ro-52 positive overlap myositis cases (i and j) showing dense perimysial inflammation (arrow) along with scattered regenerating fibers (white asterix) (i = H and E x 400), and perimysial perivascular inflammation (arrow) which is seen extending into the endomysium surrounding non-necrotic fibers (black asterix) (j = H and E x 400)

inflammation in two, and endomysial inflammation around non-necrotic fibers in one. One of these three also showed PFA. One biopsy showed features of neurogenic atrophy with angulated atrophic fibers and no inflammation.

Seronegative Group (n = 35)

This group included total 35 patients including dermatomyositis (DM) (12), non-specific myositis (5), inclusion body myositis (6), necrotizing myopathy (8), and whereas four biopsies showed only type 2 fiber atrophy. The clinical features, disease duration, CPK, and other associated features of all these patients are depicted in Table 3 and the detailed biopsy features are enlisted in Table 4. The DM included three juvenile and nine adult patients (two overlap myositis) with a mean CPK of 2,356 IU/L. Perifascicular atrophy was dominant in this group (11) of which myxovirus positivity was identified in 10. Long-term follow-up was available in only six patients of whom five recovered and one had a relapse of myositis after a year of first episode. Five biopsies were classified as nonspecific myositis as per ENMC 2014 criteria. These were elderly patients with proximal muscle weakness. The biopsies showed mostly perivascular inflammation in absence of PFA. Myxovirus expression was identified in two of these biopsies indicating these actually were DM cases. IBM is an important seronegative entity which can only be diagnosed on muscle biopsy. These were six patients with dominant distal weakness and median age at diagnosis of 51 years. These biopsies showed significant fiber size variation particularly fiber hypertrophy and splitting unlike the other groups of IIM. The inflammation was endomysial around non-necrotic fibers with focal myophagocytosis. Rimmed vacuoles were seen in all and mitochondrial blue and red ragged fibers were identified in five biopsies. Necrotizing myopathy was another distinct entity of the seronegative group of which four were statin induced. The MHC 1 expression was inconsistent in these biopsies. A 40/F patient in this group had

Table 3: Biopsy Features in the seronegative gpatients

Biopsy Diagnosis	PFA	PFN	Necrotic Fibers		Micro-infarcts	Inflammation		MHC I	MHC II	MxA			
			Scattered	Extensive		PV	EM			PF	Focal	Diffuse	Negative
DM (12)	11	1	9	2	1	8	2	12/12 Diffuse 12/15 Perifascicular 3/15	Focal 3/12	5/15	3/12	2/12	2/15
Non Specific Myositis (5)	-	-	4	1	-	4	1	5/5 Diffuse 3/8 Focal 1/8 Perifascicular 1/8	-	1/8	1/8		
IBM (6)	-	-	3	-	-	1	6	6/7	-	-	-	-	-
Necrotizing Myopathy (8)	-	-	2	6	4	2	1	2/8 Focal		-	1/8	-	-
Type 2 atrophy (4)	-	-	-	-		-	-	3/4 diffuse		-	-	-	-

Table 4: Clinical and demographic features of the patients in seronegative group

Type of Myositis Mean Disease Duration	Age (years) range	Age (years) median	CPK (IU/L) mean	Skin	Myopathy No. %	Myalgia	Bulbar muscle	ILD	Calcinosis	Others
DM (12) 9 months	11-55	21	2,536	4	12 100%	5	3	2	1	Arthralgia 4 SLE1 Scleroderma 1
Nonspecific Myositis (5) 6 months	48-65	50	7,854	-	5 100%	2	2	-	-	DM 2 HTN1 Polycythemia 1
IBM (6) 18 months	40-68	51	2,856	-	6 100% Distal weakness	-	-	-	-	-
Necrotizing myopathy (8) 18 days	31-65	55	33,128	-	8 100% AKI 3	5	2	-	-	DM, HTN, CAD treated with Rosuvastatin 4
Type 2 Atrophy (4) Two months	23-45	35	859	2	4 100%	2	2	2	-	SLE 2

presented with an acute onset myopathy following an episode of fever associated with rash and CPK of 7,800 IU/L. This biopsy showed dominant necrosis and microinfarcts with minimal inflammation without PFA. MxA showed classic perifascicular expression in non-necrotic fibers indicating that this could be a DM similar to that seen in the NXP-2/TTF-γ group. The biopsy features are shown in Figure 4. The patient responded to treatment. A repeat myositis profile six months after treatment remained negative. Six of the eight necrotizing myopathy patients showed improvement after intravenous steroids and immunoglobulins; however, two patients with severe rhabdomyolysis and Acute kidney injury (AKI) succumbed to illness.

Four other patients including two known systemic lupus erythematosus ones presented with subacute onset of proximal weakness, elevated CPK. The biopsies showed only type 2 fiber atrophy. Two of these patients were already treated with steroids at the time of biopsy. MHC class 1 antigen showed diffuse expression in three indicating a possible myositis in these.

Comparison of the seronegative and seropositive group

The age at presentation, clinical phenotype, and associated conditions were comparable in both the groups. The clinical and demographic features were similar. Immunohistochemistry with MHC class 1 and class 2 antigen helped in reclassifying two cases of nonspecific myositis as DM and ASS, respectively. Myxovirus expression was consistent in the DM group with PFA. This IHC also helped in reclassifying one patient of necrotizing myopathy as DM with necrosis. The group of IBM is a distinct clinicopathologic entity which cannot be compared to the seropositive group. The only known antibody association of IBM is cytosolic 5′-nucleotidase 1A which is not available in the 16-antigen immunoblot panel. Similar is the case with HMGCR; unavailability of which in the blot panel creates a seronegative group in the necrotizing myopathy cases particularly the ones which are statin mediated.

Discussion

Although several MSA and MAA have been incorporated into the diagnostic criteria of IIMs, the exact mechanism by which

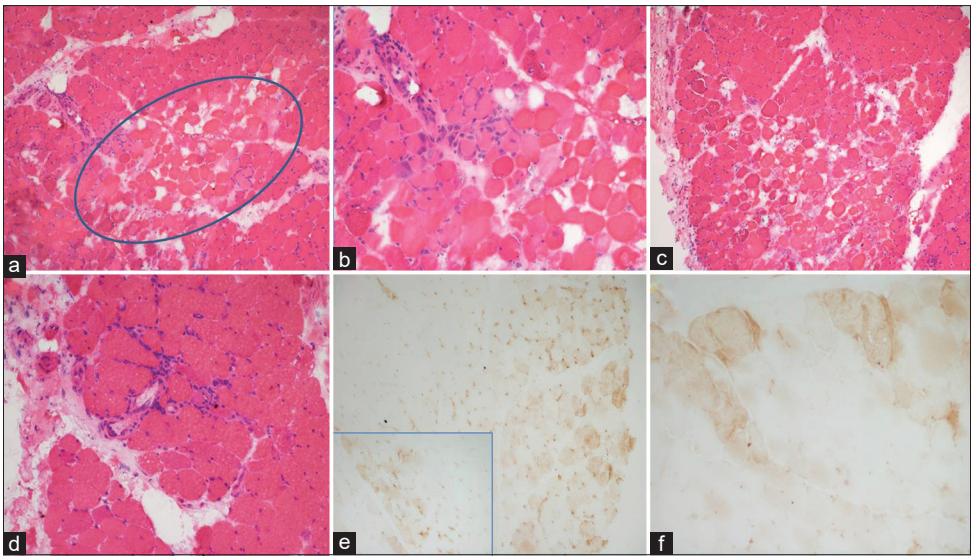

Figure 4: A case with fascicular necrosis (circle) with minimal inflammation (a-d) (a,c= H and E x 40; b,d = H and E x 100). Subsequent IHC with MX1 (e and f)was positive (e = DAB x 40; f = DAB x 400) and the case was revised as dermatomyositis

they cause organ damage is enigmatic. There have been several possible explanations to consider their putative pathogenic nature. These include variable expression of these antibodies in different organs, fluctuation of serum levels correlating with disease activity and clinical remissions,[13,14] and *in vitro* studies demonstrating pathologic changes in mice following passive transfer of autoantibodies.[15] But whether there is a direct cause-and-effect relationship between these autoantibodies and target organ damage or these are just an epiphenomenon in the inflammatory process is yet to be elucidated.

Nevertheless, the association of different MSA and MAA with specific clinical phenotypes is well documented and they are invaluable not just in diagnosis but also in prognostication sometimes.[10] However, the literature comparing the muscle biopsy features with the myositis antibody subtypes is limited and relatively recent. But this comparison is essential as it helps in understanding of the pathogenesis and provides objective evidence to the disease severity in many cases. It may also offer an explanation to the differential response to treatment and refractoriness to therapy in different patients.

Cutaneous involvement is a mandatory feature for DM diagnosis as per ENMC 2018 criteria. In our cohort, skin rash at presentation was absent in four patients. However, these patients had definitive histopathologic features on muscle biopsy including PFA and perivascular inflammation along with MHC class I and MxA positivity on IHC. These patients classify as dermatomyositis *sine* dermatitis. But this entity has not been accommodated in the 2018 ENMC-DM diagnostic criteria. We opine that skin manifestations may not be essentially present at the time of initial clinical presentation and this should not prevent from making a diagnosis of DM when other characteristic features are present.

Recent molecular advances including transcriptomics and gene expression profiling have revealed that DM is a type I interferonopathy,[16] while in ASS type II interferon pathway predominates over type I.[17] The diagnosis of ASS depends on serology, which otherwise mimics DM or PM clinically. The muscle biopsy features also may overlap with DM which include PFA, necrotic, and regenerating fibers. PFA was seen in one and PFN in two biopsies, while perivascular and endomysial inflammation were seen in three cases each of ASS in our cohort. We observed that PFA was more frequent in biopsies with mi-2 and NXP-2 similar to that reported in literature.[2]

Myxovirus resistance protein 1 (MxA) has been identified as the most reliable marker of type I interferon pathway,[18] which is now available on IHC. MxA has been shown to be very specific for DM in the studies by Uruha *et al.* and Inoue *et al*.[18-20] MxA is now included as a separate criteria for definitive diagnosis by 2018 ENMC-DM classification.[9] But they have taken only perifascicular pattern of MxA staining into consideration. In our study, although perifascicular localisation was the predominant pattern, diffuse staining of all the fibers and scattered staining of some fibers were seen in two cases each. These patterns were also reported by other studies in DM cases.[19] We want to report that the staining pattern of MxA is difficult to interpret in comparison to MHC, particularly for focal positive cases. The sarcoplasmic staining intensity is variable and positivity in focal and less intense cases can be missed. There is perhaps a need to score the staining to improve its reproducibility. Interestingly, MxA by IHC was conspicuously absent in all our ASS cases. MxA in our study was positive in 79% (19/24) of the dermatomyositis and none of the ASS cases, which gives a sensitivity of 61.5% and specificity of 100%. This is comparable to the existing literature where MxA has shown 98%-100% specificity and 71%-77% sensitivity.[18,19] Hence, MxA positivity can be used to identify DM against its close mimic ASS, whenever there is histologic ambiguity.

Perifascicular necrosis, which is a characteristic feature of ASS, is expected to be rare or absent in DM according to ENMC 2018 criteria.[9] This finding was however reported in dermatomyositis biopsies, especially anti-Mi2 cases.[21] Two Mi2 cases showed perifascicular necrosis in our study.

Muscle fiber damage in IIM is complement mediated. The location of necrotic fibers can be attributed to the site of C5b-9 deposition. Although both DM and ASS are characterized by perifascicular changes, the pathogenesis of perifascicular pathology in DM is different from that of ASS. DM is primarily vascular pathology characterized by membrane attack complex (MAC) deposition on perimysial vessels. This results in ischemic changes in perimysial water shed areas in the form of PFA. Whereas, in ASS, perifascicular necrosis is the predominant finding which can be explained by MAC deposition in perifascicular fibers.[22] In addition, CD8+ infiltrates are also seen in perimysial and perifascicular endomysium in ASS, correlating with MHC-II distribution in this area.[23]

In NXP2 cases, MAC staining is reported on capillaries as well as sarcolemma. Microinfarctsare reported in anti-NXP2 cases particularly in cases with acute presentations and children.[9] The underlying pathomechanism for this acuteness of ischemia causing muscle infarction is not clear.

In anti-MDA5 DM patients, Allenbach *et al.*[24] have identified expression of nitric oxide synthetase2 (NOS2) and heat shock protein 70 (HSP 70) in the muscle fibers which are thought to be cytoprotective. This is probably the reason for muscle sparing in anti-MDA5 subset of DM.

Among the different antibody subgroups of DM, muscle fiber necrosis and serum CPK levels are highest in anti-Mi2 cases compared to others. Interestingly, sarcolemmal MAC has been reported predominantly in anti-Mi2 DM cases compared to other antibody groups, being least in anti-MDA5 cases.[9] This is consistent with the amyopathic presentation of MDA5-positive cases which also shows minimal changes on muscle biopsy.

Within the same MSA, there have been phenotypic variations of patients from different geographic regions. For example, clinically apparent muscle involvement in anti-MDA5 cases is more in the west compared to Chinese and Japanese patients.[25,26] Similarly, the incidence of cancer and ILD which is considered rare in anti-Mi2 cases was significant in his series of French cohort by Dr Landon Cardinal.[9] Some of the MSAs have shown associations with specific Human leucocyte antigen (HLA) loci.[27,28] There was only one case of malignancy in our cohort which was a carcinoma lung, noted in IMNM with anti-SRP antibodies. ILD was also infrequent in our series. The variation in clinical phenotype, disease severity, and response to therapy within the same antibody subgroup of IIM could be due to genetic and ethnic differences.

The necrosis in IMNM is attributed to Chaperon-assisted selective autophagy and endoplasmic reticulum (ER) stress response.[29] This is probably the reason for sparse inflammation in IMNM muscle biopsies. Only two IMNM muscle biopsies showed inflammation in our study of the total seven anti-SRP cases. This was mild, perivascular in one and endomysial in one. Immune-mediated damage in these cases is established by overexpression of MHC and MAC on muscle fibers. In our study, we found MHC I upregulation on scattered fibers.

Presence of fiber necrosis alone does not qualify for a diagnosis of IMNM as necrotic fibers are seen in other IIM types as well. Recognising seronegative IMNM patients, which are a distinct subset of IMNM, is important as it is a severe phenotype of IIM. Although an association with statin exposure has been reported initially with HMGCoR antibodies, subsequent literature showed many statin-naive patients, especially young, with anti-HMGCoR positive IMNM. HMGCoR antibodies are routinely tested using enzyme-linked immunosorbent assay. Our cohort of IMNM cases included only anti-SRP positive cases, which did not have a history of statin intake.

The autoantibodies are also known to be of prognostic significance and can guide treatment. Anti-Mi2 is known to show good response to corticosteroids with positive prognosis. Liang *et al.*[30] studies 40 patients of anti-mi2b DM who showed low frequency of ILD and malignancy, good treatment response, and favorable outcome. The response to treatment also varies according to the underlying clinical manifestation in one serotype. The musculoskeletal features show good response to steroids as against ILD in Ku-positive DM/PM patients. Yoshifuji H *et al.*[31] studies 41 patients of Jo-1 positive PM/DM. They compared treatment response in seropositive versus negative groups. The response to steroids for ILD was significantly better in the positive group; however, recurrences were more frequent. The long-term pulmonary function (2 years) was not different in both the groups.

MDA-5 is known to have amyopathic presentations. These patients have been reported to develop rapidly progressive ILD refractory to immunosupression.[26] Early mortality has been reported in 45% of anti-MDA5 positive DM in a study by Nakashima *et al.*[32] highlighting the poor prognosis.

Among the IMNM group associated with HMGCR, steroid refractoriness has been shown in statin naïve and younger patients.[33] Similar observations of steroid unresponsiveness and increased mortality have been published in association with SRP.[34]

Conclusion

Our study helps in understanding the biopsy variations associated with different subtypes of autoantibodies. The study also highlights the entire spectrum of pathologic features seen in biopsies of inflammatory myopathies. Demonstration of specific tissue changes is a robust way of confirming the pathologic and myositis causing nature of these autoantibodies. Perifascicular atrophy which is a defining feature of DM is common with mi-2 and NXP-2 is rare in Jo-1 and is not associated with other antibodies. Extensive necrosis defines IMNM, however can be

observed in TIF-1 gamma and NXP-2–associated IIM. MxA staining helps in confirming the diagnosis of DM; however, staining interpretation is difficult in focal positive cases. It is possible to extrapolate this pathologic understanding for a confirmed diagnosis of myositis in seronegative cases. The biopsy features along with IHC with MHC Class 1 and 2 antigen and myxovirus help in reclassifying the seronegative cases. We concur with the clinico-sero-pathologic approach for the diagnosis of IIM. Isolating one from other can lead to misdiagnosis.

Limitations

Retrospective nature of the study, nonavailability of certain myositis autoantibodies particularly HMGCoA, and lack of complete follow-up data are certain limitations of our study.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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