High prevalence of necrotising myopathy pattern in muscle biopsies of patients with anti-Jo-1 antisynthetase syndrome

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Abstract

Objetive

Until now, researchers have not provided a well-defined muscle histological pattern for antisynthetase syndrome (ASSD). Therefore, we aimed to analyse the muscle biopsies of patients with anti-Jo-1 ASSD.

Methods

This study included 26 patients with anti-Jo-1 ASSD admitted for investigation of the disease and obligatorily with muscle impairment, from 2010 to 2021, whose serial frozen muscle sections were analysed.

Results

Patients' mean age at disease diagnosis was 42.8±11.6 years, and the female gender was most predominant. Concerning muscle biopsies, cell infiltrates were present in 76.9% of the samples, and they were mainly located at the endomysium area (70%), with a predominance of macrophages (92.9%). Fibre muscle necrosis was present in 92.3% and was diffused in 54.2%. Expression of MHC-I was seen in all samples. Samples were mostly marked by the presence of CD68+ and discreet/low $CD4^+$ and $CD8^+$ staining, which is consistent with a higher predominance of observed necrosis and macrophage cell infiltrates. In general, 38.5% of patients had a necrotising myopathy pattern in muscle biopsies, whereas 34.6% and 26.9% had a general inflammatory myopathy pattern and non-specific myopathy, respectively. This necrotising myopathy pattern was not associated with the demographic, clinical, or laboratory data.

Conclusion

Our data show that almost 40% of patients with well-defined anti-Jo-1 ASSD with objective muscle impairment have a necrotising myopathy pattern in their muscle biopsies. Although this pattern is more classically related to immune-mediated necrotising myopathies, in association with clinical manifestations and the presence of anti-Jo-1 autoantibodies, this characteristic may lead to ASSD diagnosis.

Key words

antisynthetase syndrome, inflammatory myopathies, muscle biopsies, myositis

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Introduction

Antisynthetase syndrome (ASSD) is a rare systemic autoimmune myopathy that is defined by the presence of anti-aminoacyl-tRNA synthetase (anti-ARS) autoantibodies. Clinically, ASSD is characterised by myositis, arthritis, and interstitial pneumopathy, in addition to "mechanic's hands", Raynaud's phenomenon, or fever (1-4). Myositis has a broad phenotypic spectrum among individuals with ASSD, ranging from increased serum levels of muscle enzymes in asymptomatic patients or isolated myalgia to severe muscle weakness in bedridden patients (5).

Until now, a well-established definition and validated criteria of a histological pattern for ASSD have not existed. Scarce case reports have shown a diffuse necrosis in muscle biopsies among patients with anti-ARS positive autoantibodies (6-9). However, these studies have not given details as to which criteria were used to classify these patients as having ASSD, clinical and laboratory manifestations, and/or defined exclusion criteria.

A retrospective study (10) analysed a representative sample of muscle biopsies (n=53) among patients with anti-Jo-1 positive ASSD, and suggested that these patients have perifascicular necrosis, sarcolemma complement deposition, and inflammation located mainly in the perimysium (with extension into the endomysium area) and/or around vessels. Similarly, another study (11) suggested that perifascicular necrosis is a pathological feature not only in muscle biopsies among ASSD patients with anti-Jo-1 positive autoantibody, but also those with other anti-ARS autoantibodies, such as anti-OJ and anti-PL-7. Finally, Stenzel *et al.* (12) observed in 21 patients the presence of necrotising perimysium myositis, in addition to distinctive myonuclear actin filament inclusions and rod formations. However, as a limitation, these studies (10-12) included patients with anti-ARS positive autoantibodies, without determining if they met previous well-defined and homogeneous criteria in order to unequivocally classify them as having ASSD. Furthermore, a well-defined criterion to categorise a patient with ASSD is of immediate importance, as the presence of an anti-ARS autoantibody alone can be an epiphenomenon (13) or even have another myopathy that is not ASSD (14). Working to define the inclusion and exclusion criteria of patients is of paramount importance in order to mitigate the selection bias, increase the homogeneity of the sample and enrich the literature for further studies and literature review.

Therefore, to fill the gaps in the current literature, we aimed to evaluate and describe the histopathological findings of the muscle biopsy of patients with well-defined anti-Jo-1 positive ASSD admitted for investigation of the disease and obligatorily with muscle impairment (clinical and laboratory).

Materials and methods

Study design

This single-centre retrospective cohort study included adult patients with ASSD, from 2010 to 2021. A local ethical committee approved the study (CAAE 39974020.4.0000.0068).

Patients

All patients were initially admitted to our tertiary service to investigate the clinical manifestations of muscle weakness associated with non-normal serum levels of skeletal muscle enzymes. During the additional clinical and laboratory investigations, the patients fulfilled the ASSD criteria proposed by Behrens Pinto *et al.* (3) that included the presence of anti-ARS autoantibodies (anti-Jo-1) associated with the presence of at least two of the following parameters: muscle (mandatory), lung or joint involvement. In addition, the presence of fever, "mechanic's hands," or Raynaud's phenomenon may be present.

Exclusion criteria

The absence of muscle biopsy for revaluation; presence of anti-HMGCR and anti-SRP autoantibodies; to have other systemic autoimmune diseases (overlap syndrome), other causes of myositis (infectious, drugs, medications and metabolic), thyroid disorder (hyper or hypothyroidism) uncompensated by the time of biopsy, previous use of statins or fibrates, or cancer (current or previous).

Patients' demographic data

We retrieved the following data from the electronic medical records with pre-standardised and parameterised information: patient age at disease diagnosis, gender, ethnicity, duration between symptoms' onset and disease diagnosis, cumulative dose of corticosteroids before biopsy and diagnosis, clinical symptoms (muscle, lung, and joints) and laboratory data.

Muscle involvement was defined by the elevation of muscle enzymes (creatine phosphokinase - CPK or an aldolase increase >50% compared with upper normal values) associated with clinical muscular weakness and/or complementary imaging exam (electromyographic [EMG] and/or muscle magnetic resonance) compatible with muscle involvement. Muscle biopsy was performed only for patients with defined muscle involvement. Clinical and objective muscle weakness was verified and defined by a rheumatologist (classified by Medical Research Council - MRC). To be considered compatible with myopathic pattern, the EMG needed to show a small, spontaneously discharging potentials – fibrillation at rest and polyphasic potential (15). Joint involvement was defined by the presence of arthritis (inflammatory joint pain, swelling and/or tenderness is required). Pulmonary involvement was defined by the presence of symptoms as exercise intolerance (change of functional class) and/or dyspnoea associated with at least one altered complementary exam: computed tomography with signs of alveolitis/fibrosis, incipient pneumopathy, ground-glass opacities with or without bronchiectasis or pulmonary fibrosis and honeycombing areas on high-resolution and or plethysmography with analysis of forced vital capacity (FVC), forced expiratory volume during the first second (FEV1), and diffusing capacity for carbon monoxide (DLCO); The presence of constitutional symptoms such as fever (axillary temperature >37.8ºC objectively measured, not otherwise explained); Involuntary weight loss of >10% of weight in the last 6 months of the inclusion of patients in the study was also evaluated; Palm plantar hyperkeratosis (mechanic's hands); Raynaud's phenomenon.

Laboratory data

The follow parameters were analysed in all patients: CPK (normal range: 32–294 U/L), aldolase (range <7.6 U/L), antinuclear antibodies (ANA), anti-Ro-52; anti-SRP, anti-ARS autoantibodies (anti-Jo-1, OJ, EJ, PL-7, PL-12), anti-Ku, anti anti -PM/Scl, and anti -Mi-2 (Myositis Profile Euroline Blot test kit, Euroimmun, Lübeck, German) according to the manufacturer's protocol. Anti-HMGCR autoantibody was performed by an Enzyme-Linked Immunosorbent Assay (ELISA) test (MyBioSource, CA, USA). The results were considered positive if the bands showed moderate or strong reactions.

Muscle biopsies

Muscle biopsy was performed for diagnostic investigation, at biceps brachii or vastus lateralis muscle, and the rest of the material is stored in liquid nitrogen at - 170ºC. These biopsies were guided by the imaging method (area of greater muscle oedema and areas of less fat replacement or hypo/atrophic) and performed on muscles considering clinically compromised (paretic [MRC 4-2], but non-plegic [MRC 1] limbs). If EMG were performed less than one month of the biopsy, the limb contralateral to the site of the EMG were elected for the biopsy. The diagnostic routine includes serial and 5 μm - thick cross sections to perform histological, histochemical and immunohistochemical reactions. For the present study slides of muscle biopsies that have already been processed and stained by the method of haematoxylin and eosin (H&E) and acid phosphatase processed according to the technique standardised by Dubowitz (16) will be reviewed, and also immunohistochemistry. The expressions of CD4 and CD8 were identified by immunohistochemistry using the EnVision-AP technique (Dako En Vision System, alkaline phosphatase, Dakopatts), CD68 cells were identified by immunohistochemistry, using the LSAB+ system (Dako, A/S Denmark), while class I MHC expressions were identified using the immunoperoxidase technique StreptABComplex/HRP-Duet immunohistochemical reaction (StreptABComplex/HRP Duet, mouse/rabbit; Dakopatts). The parameters analysed in the histology will be:

a) All samples will be re-evaluated and analysed for the degree of inflammatory process (endomysium, perimysium and/or perivascular); presence and distribution of necrosis of muscle fibres.
b) Qualitative analysis of muscle fibre size variation, perifascicular atrophy (atrophy of one to two fibre layers in the periphery of the fascicle) and inflammatory infiltrate will be performed. The inflammatory infiltrate was classified by the predominant cell type: macrophage or lymphocytic; the distribution and location of the infiltrate was classified as perivascular, perimysium and endomysium. The term "perimysium" describes changes in the connective tissue between the fascicles (17).
c) Necrosis was defined by pale and/or hyalinised staining, visualised on haematoxylin and eosin staining. Regenerating fibres were identified by increased basophilia with H&E staining, as well as large vesicular nuclei (18).
d) Additionally, a semiquantitative analysis will be performed to assess inflammation and necrosis in the fibres. Such assessment was determined using a scale from 0 to 3: absent: 0; mild: 1, moderate: 2, intense: 3.
e) MHC I expressions and reactions with monoclonal antibodies to CD4, CD8 and CD68 will be analysed and classified semi-quantitatively as: (-): absence of positive cells; (+) 0 to 25%; (++) 26 to 50%; (+++): 51 to 75%; (++++): 76 to 100% of cells with positivity in the analysed structures.
f) For vascular evaluation, the entire sample will be qualitatively evaluated for the presence of perivascular infiltrate (perimysium) and vascular ectasia (endomysium);
g) All slides will be analysed by two blinded and independent investigators (LMBS and IBPB), from the Division of Rheumatology the HCFMUSP, who have undergone extensive training in muscle histology according to the Institution's protocol to develop the skills needed to read muscle biopsy slides. If there is disa-

greement between the evaluators, at the end of the evaluation, a third evaluator (a myology expert) will make a new evaluation (SKS).

After the initial assessment, each sample received a classification of the most predominant histological standard based on the following:

a) General inflammatory myopathy pattern. This biopsy pattern suggests an inflammatory myopathy based on the histological criteria suggested (19, 20): degeneration and regenerative changes, necrosis and phagocytosis; a mononuclear interstitial or perivascular inflammatory infiltrate; and immunohistochemistry with a predominance of $CD4^+$ or $CD8^+$ (18, 21)
b) Necrotising myopathy pattern. This includes abundant myofibre necrosis, degeneration, and regeneration with only minimal, if any, inflammation on muscle biopsy (with a predominance of macrophage) and immunohistochemistry (with a predominance of $CD68^+$) (22).
c) Non-specific myopathy. May have variation in myofibre size, some isolated necrosis and regeneration myofibres, zero or minimal inflammatory infiltration, and unspecific immunohistochemistry.

Statistical analysis

The Shapiro-Wilk test was used to test the data distribution. The results were presented as mean ± standard deviation or median (interquartile 25%-75%) for continuous variables, whereas frequency (%) for categorical variables. Results were compared by *t*-Student or Mann-Whitney tests for continuous variables to determine differences between histological patterns with several parameters analysed in the present study. Differences in categorical variables were calculated by Fisher's exact test and Pearson's x^2. All statistical analyses were performed using the software SPSS, v. 22.0 (Chicago, IL, USA).

Results

A total of 55 patients with ASSD were initially evaluated in the laboratory and clinically. Nine patients did not meet the inclusion criteria because were negative for the anti-Jo-1 autoantibodies (three

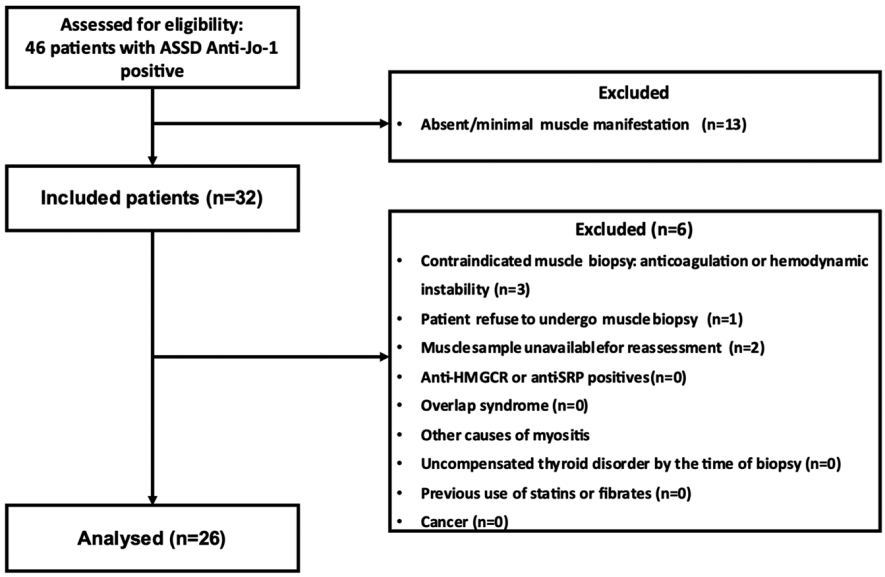

Fig. 1. Flow-chart of the present study.
ASSD: antisynthetase syndrome.

Table I. Patients' general characteristics at disease diagnosis onset.

Parameters	n=26
Age at disease diagnosis (years)	42.8 ± 11.6
Female gender	18 (69.2)
White ethnicity	47 (73.4)
Duration between symptoms' onset and diagnosis (months)	5.0 (3.0-12.0)
Initial clinical features	
Muscle involvement	26 (100)
Upper muscle strength	
V degree	5 (19.2)
IV degree	11 (42.3)
III degree	10 (38.5)
II degree	0
I degree	0
Lower muscle strength	
V degree	1 (3.9)
IV degree	14 (53.8)
III degree	11 (42.3)
II degree	0
I degree	0
Joint involvement	24 (92.3)
Lung involvement	25 (96.2)
Lung computed tomography	
Ground-glass opacity	16 (61.5)
Interstitial lung disease	24 (92.3)
Pulmonary fibrosis	5 (19.2)
Mechanic's hands	21 (88.5)
Raynaud's phenomenon	20 (76.9)
Fever	13 (50.0)
Laboratory parameters	
Antinuclear antibody	21 (80.8)
Anti-Jo-1 antibody	26 (100)
Anti-Ro-52 antibody	10 (38.5)
Maximum creatine phosphokinase (U/L)	4326 (998-9831)
Maximum aldolase (U/L)	40.0 (22.9-93.0)

Results expressed as mean ± standard deviation, median (interquartile 25^{th} - 75^{th}) or frequency (%).

patients had anti-PL7, three had anti-PL12, and three had anti-EJ). Therefore, we assessed that a total of 46 patients with ASSD anti anti-Jo-1 positive were eligible for the present study (Fig. 1). However, 19 patients were excluded: 13 had been admitted in our service with previous ASSD diagnosis, prior treatment, and few clinical (and muscular) manifestations, and 6 had no available muscle biopsies. Therefore, we assessed a total of 26 patients with muscle biopsies. In addition, none of the patients had anti-HMGCR, anti-SRP autoantibodies, or other systemic autoimmune diseases (overlap syndrome), other causes of myositis (infectious, drugs, medications and metabolic), thyroid disorder (hyper or hypothyroidism) uncompensated by the time of biopsy, previous use of statins or fibrates, or cancer (current or previous).

General clinical characteristics and laboratory data

The patients' mean age at disease diagnosis was 42.8±11.6 years, and the female gender and White ethnicity were most predominant (Table I). The duration between symptoms' onset and diagnosis was 5 (3-12) months. All 26 patients had muscle involvement as the initial clinical features, and the majority were associated with a muscle strength of the III or IV degree in both upper and lower limbs. The lung and joint involvements were observed in 96.2% and 92.3% of the patients, whereas "mechanic's hands," Raynaud's phenomenon, and fever were observed in 88.5%, 76.9%, and 50.5% of the cases. In the laboratory, the presence of ANA was observed in 80.8% of cases, and the maximum serum levels of CPK and aldolase were 4326 U/L and 40.0 U/L, respectively.

Muscular histological parameters

The variability of muscle fibre diameter was present in all samples, and inflammatory cell infiltrates were apparent in 76.9% of the samples (Table II). They were mainly located in the endomysium area (70%), and macrophages were more predominant. Perimysium and diffuse infiltrates (perimysium and endomysium areas) were also seen, but they were less prevalent (5% and 25%, respectively). It is important to notice that even though perivascular infiltrates and perifascicular atrophy were observed in 15.4% of patients, these features were not evidenced concomitantly in the same muscular sample/patient, as these two characteristics are usually related to dermatomyositis, especially when associated (23). Also 92.3% had muscle necrosis, predominantly diffuse necrosis (54.2%). The MHC I expression was seen in all samples (Table III), mostly classified as intense staining. It was highly marked by the presence of CD68$^+$ and discreet/low CD4$^+$ and CD8$^+$ staining (mostly absent or mild samples), which is consistent with the higher predominance of necrosis and macrophage cell infiltrates visualised in the samples.

Table II. Histological analysis of muscle biopsies.

Histological parameters	n=26	
Muscle fiber diameter change	26/26	(100)
Inflammatory infiltrate	20/26	(76.9)
Isolated endomysium infiltrate	14/20	(70.0)
Lymphocytic	8/14	(57.1)
Macrophagic	13/14	(92.9)
Isolated perimysium infiltrate	1/20	(5.0)
Lymphocytic	1/1	(100)
Macrophage	1/1	(100)
Diffuse infiltrate	5/20	(25.0)
Lymphocytic	1/5	(20.0)
Macrophage	5/5	(100)
Perivascular infiltrate	4/26	(15.4)
Lymphocytic	3	(75.0)
Macrophage	1	(25.0)
Perifascicular atrophy	4/26	(15.4)
Fibre muscle necrosis	24/26	(92.3)
Perifascicular area	1/24	(4.2)
Endomysium area	10/24	(41.7)
Diffuse	13/24	(54.2)
Histological standard		
Inflammatory myopathy	9	(34.6)
Necrotising myopathy	10	(38.5)
Nonspecific myopathy	7	(26.9)

Results expressed as frequency (%).

Table III. Immuno-histochemistry staining.

Parameters	n (%)
CD4$^+$	n=18
None	5 (27.8)
Mild	11 (61.1)
Moderate	2 (11.1)
Intense	0
CD8$^+$	n=19
None	13 (68.4)
Mild	5 (26.3)
Moderate	0
Intense	1 (5.3)
CD68$^+$	n=19
None	0
Mild	8 (42.1)
Moderate	7 (36.8)
Intense	4 (21.1)
MHC I	n=19
None	0
Mild	2 (10.5)
Moderate	5 (26.3)
Intense	12 (63.2)

Results expressed as frequency (%).

Myopathological pattern

In general, 38.5% of the patients had a necrotising myopathy pattern in muscle biopsies, 34.6% had muscle biopsies suggestive of an inflammatory myopathy pattern, and 26.9% had nonspecific myopathy (Table III).

Figure 2 illustrates each one of these myopathological patterns. Six patients had biopsies performed without previous treatment (prednisone or immunosuppressor) (Table IV). Although 20% of the patients with necrotising patterns and 40% with other patterns (inflammatory and non-specific) had not undergone treatment by the time of the biopsy, this fact does not influence the histological features, as they were similar in both groups.

The demographic, duration between symptoms' onset and diagnosis, clinical and laboratory data (including degree of muscle strength, serum levels of CPK, and aldolase), and treatment (Table IV) were comparable between patients, whose muscle biopsies were compatible with the necrotising myopathy pattern *versus* other patterns. Likewise, the histological features were similar in both groups, except for the absence of general lymphomononuclear infiltration (including in the perimysium area) and macrophage infiltration in the perivascular area in patients with a necrotising myopathy pattern.

Discussion

In the present study, we analysed a representative sample of muscle biopsies among patients with well-established ASSD. Our data showed a higher prevalence of the necrotising myopathy pattern with diffuse fibre necrosis, highly marked by the presence of CD68$^+$ and

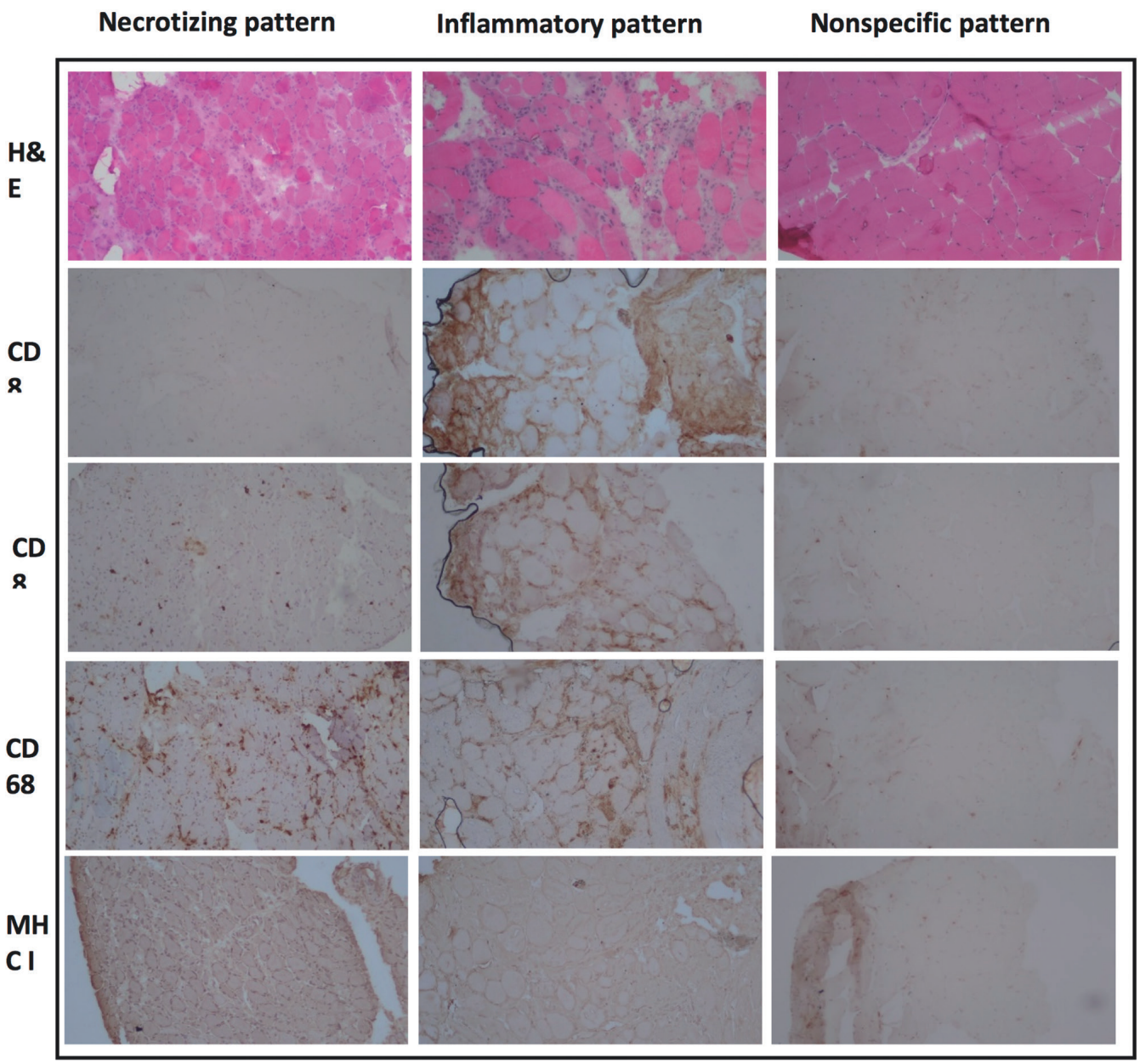

Fig. 2. Myopathological patterns described in the present study.
Figure shows necrotising myopathy, inflammatory myopathy, and non-specific myopathy patterns.
Stains: Haematoxylin & Eosin (H&E), $CD4^+$, $CD8^+$, $CD68^+$, and MHC I. 10x magnification.

discreet/low $CD4^+$ and $CD8^+$ staining in muscle biopsies. In addition, we did not find an association with the necrotising myopathological pattern and demographic characteristics, clinical manifestations, and laboratory data (including muscle impairment), regardless of the use of glucocorticoids or immunosuppressive drugs at the time of biopsy.

Unlike scarce studies analysing muscle biopsies, whose patients had anti-ARS positive autoantibodies, the present study included initially well-defined ASSD patients, and, secondarily, we assessed their muscle biopsies, based on strict inclusion and exclusion criteria. We mitigated the selection bias and increased sample specificity by minimising the chance of these patients having other non-ASSD disorders. Since this is a rare disease, and given the scarcity of data in the literature, it is to be expected that studies on the area will have a small number of participants without, conversely, losing a representative sample.

This is because the anti-Jo-1 ASSD patients are the most common, it is the most studied autoantibody in ASSD (24, 25), and it is associated with a higher frequency of muscle involvement when compared to non-anti-Jo-1 patients (24, 26-28). The non-anti-Jo-1-ARS autoantibody is less prevalent in ASSD and has a higher heterogeneity in clinical manifestations (specifically in muscle impairment) (2). For instance, anti-PL-7 and anti-PL-12 are more associated with interstitial pneumopathy and a lower incidence of myositis (29), similar to what is observed with anti-KS autoantibody (30). The presence

Table IV. Comparison between patients with muscle biopsies with necrotising myopathy pattern and other patterns by the time of the muscle biopsy.

	NM pattern (n=10)	Other patterns (n=16)	*p*-value
Age	39.4±11.3	44.9±11.5	0.243
Female	7 (70.0)	11 (68.8)	>0.999
White ethnicity	6 (60.0)	13 (81.3)	0.369
Duration between symptoms' onset and diagnosis (months)	4.0 (3.0-12.0)	5.0 (3.0-5.0)	0.737
Constitutional symptoms	8 (80.0)	12 (75.0)	
Muscle strength			
Upper limb			
V degree	1 (10.0)	1 (6.3)	0.150
IV degree	3 (30.0)	11 (68.8)	
III degree	6 (60.0)	4 (25.0)	
Low limb			
V degree	0	1 (6.3)	0.300
IV degree	4 (40.0)	10 (62.5)	
III degree	6 (60.0)	5 (31.3)	
Systemic involvement	5 (50.0)	8 (50.0)	>0.999
Joint	10 (100)	14 (87.5)	0.508
Lung	9 (90.0)	16 (68.8)	0.385
Interstitial pneumopathy	9 (90.0)	15 (93.8)	>0.999
(Ground-glass opacification)	6 (60.0)	10 (62.5)	>0.999
Basal fibrosis	3 (30.0)	2 (12.5)	0.340
Raynaud's phenomenon	8 (80.0)	12 (75.0)	>0.999
Mechanics' hands	8 (80.0)	15 (93.8)	0.538
CPK	4101 (1238-7743)	4220 (790-11492)	0.623
Aldolase	37.5 (17.4-43.0)	39.6 (21.0-141.0)	>0.999
Treatment			
None	2 (20.0)	4 (25.0)	0.664
Prednisone			
Current use by	8 (80.0)	12 (75.0)	>0.999
Cumulative dose (mg)	1010 (205-3165)	2400 (100-6500)	0.551
IS/IM/Biol	4 (40.0)	4 (25.0)	0.664
Lymphomononuclear infiltration			
General infiltration	0	12 (75.0)	-
Endomysium area	1 (10.0)	8 (50.0)	0.087
Perimysium area	0	1 (6.3)	>0.999
Perivascular area	1 (10.0)	2 (12.5)	>0.999
Macrophagic infiltration			
General infiltration	9 (90.0)	12 (75.0)	0.617
Endomysium area	9 (90.0)	9 (56.3)	0.099
Perimysium area	4 (40.0)	2 (12.5)	0.163
Perivascular area	0	10 (62.3)	-
Perifascicular atrophy	1 (10.0)	3 (18.8)	>0.999
Perifascicular necrosis	6 (60.0)	8 (50.0)	0.701
Endomysium necrosis	10 (100)	13 (81.3)	0.262

Biol: biological; CPK: creatine phosphokinase; IM: immunomodulator; IS: immunosuppressive; NM: necrotising myopathy.
IS/IM/Biol: adalimumab, azathioprine, cyclophosphamide, cyclosporine, intravenous immunoglobulin, mycophenolate mofetil, leflunomide.

of anti-EJ and anti-OJ has been related in association with myositis (31), but its prevalence in ASSD is low (31). So, to maintain a more homogeneous group, as non-anti-Jo-1 antibodies are less related to muscle manifestation, only patients with well-defined anti-Jo-1 ASSD admitted for investigation of the disease and obligatorily with muscle impairment (either clinical or laboratory manifestation) were included; the majority of patients had muscle strength grade III and IV by the time of the muscle biopsy.

The patients' mean age at ASSD diagnosis was 42.8 years, and the female gender and White ethnicity were most predominant, similar to that reported in previous studies (1, 2, 32, 33). Because the course of anti-Jo-1 positive ASSD is very variable (32), ASSD diagnosis is often delayed by being frequently misdiagnosed as another disease (similar to polymyositis, dermatomyositis, rheumatoid arthritis, non-specific interstitial pneumopathy, and others) at the earliest months/years of the onset of the disease (2, 32, 34). Despite that, in our sample, the time between the symptoms' onset and disease diagnosis at 5 (3.0-12.0) months was similar to the average time described in the literature (1, 2, 32). This is important because even with a more specific inclusion criterion (at least two manifestations of the classic triad), we did not find any retardation in diagnosing the disease or, consequently, any delay in performing the muscle biopsies.

Concerning the myopathological findings, the variability of muscle fibre diameter and the upregulation of class I MHC molecules on the surface of muscle fibres were present in all samples. These abnormalities are often observed in the muscle samples of inflammatory myopathies, even though they are unspecific (35). Most patients had inflammatory macrophagic infiltrates, which were predominantly located in the endomysium area, and they had a significant presence of fibre muscle necrosis, proposing a necrotising myopathy pattern. Despite the fact that this pattern is classically described in patients with immune-mediated necrotising myopathies (12), some studies have provided indirect evidence that ASSD may have a necrotising pattern in muscle biopsy (6-9, 22).

Classically, signs and symptoms may help to differentiate between both diseases (ASSD and MNIM) as either clinical manifestation (lung, joints, and cutaneous impairment in ASSD; isolate myositis for IMNM (1, 12, 22) or laboratory manifestation (presence of an anti-ARS autoantibody for ASSD, and presence of an anti-SRP or anti-HMGCR autoantibodies for IMNM) (1, 12, 21). Even though histology analyses might be crucial for a correct and definitive diagnosis of the various inflammatory myopathies, especially in ASSD, clinical manifestations may precede other symptoms by years, and patients can present only with muscle

manifestation by the disease onset (2). Although both diseases (IMNM and ASSD) may present a predominantly necrotising pattern in muscle biopsy, the presence of inflammation per se seems to be more prominent in ASSD than IMNM (10). The distribution of necrotic fibres may help to differentiate between both diseases; in this present study, it was mainly located in the endomysium area. In IMNM, necrosis tends to be more diffused (22).

We did not find any association between age, gender, ethnicity, clinical manifestations, or laboratory data when comparing necrotising myopathy to other histological standards. Researchers have previously reported the lack of association between clinical features and histopathological parameters (36-38) due to the fact that histological inflammatory infiltrates can occur in foci, justifying a dissociation between clinical parameters and histological characteristics. Also, the previous use of glucocorticoid and/or immunosuppressive drugs did not influence the histological pattern because the cumulative dose was similar in the groups.

The main limitation of this study is its retrospective design (39); however, because of the rarity of ASSD, we believe this will add relevant insights into the still scarce data concerning the histology of ASSD, as muscle biopsy remains one of the pillars for a correct diagnosis of the idiopathic inflammatory myopathies' subtypes (40). Such information is of paramount importance for the development of a validated diagnostic criteria for the disease in the future. We rigorously included patients with at least two symptoms known to be classic for the disease, thus reducing the selection bias and increasing sample specificity. We fully acknowledge limitations arising from the fact that not all patients had abstained from therapy at the time of the muscle biopsy; raising the possibility that may have influenced the histology presentation. Despite concerns that the early introduction of glucocorticoid may interfere with the inflammatory process in the muscle tissues, previous use of glucocorticoid does not seem to influence the presence or degree of inflammatory infiltrates in muscle biopsies in patients with clinical and laboratory disease activity (36, 37, 41).

In conclusion, our data demonstrate a representative necrotising myopathy pattern in patients with well-defined anti-Jo-1 positive ASSD. This histological characteristic, although more classically related to IMNM, if correlated with a suggestive clinical feature, may lead to ASSD diagnosis. Clarifying the still cloudy pattern for ASSD myopathological may help to identify the disease and enable an earlier diagnosis, thus improving prognosis and patients' outcomes.

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