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Original article

Severe muscle damage with myofiber necrosis and macrophage infiltrates characterize anti-Mi2 positive dermatomyositis

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Abstract

Objective. The aim of our study was to investigate clinical and histopathological findings in adult DM patients positive for anti-Mi2 (anti-Mi2+) antibodies compared with DM patients negative for anti-Mi2 (anti-Mi2–).

Methods. Clinical data of adult DM patients, who fulfilled EULAR/ACR 2017 classification criteria, were gathered from electronic medical records of three tertiary Rheumatology Units. Histopathological study was carried out on 12 anti-Mi2+ and 14 anti-Mi2– muscle biopsies performed for diagnostic purpose. Nine biopsies from immune mediated necrotizing myopathy (IMNM) patients were used as control group.

Results. Twenty-two anti-Mi2+ DM [90.9% female, mean age 56.5 (15.7) years] were compared with 69 anti-Mi2– DM patients [71% female, mean age 52.4 (17) years]. Anti-Mi2+ patients presented higher levels of serum muscle enzymes than anti-Mi2– patients [median (IQR) creatine-kinase fold increment: 16 (7–37)*vs* 3.5 (1–9.9), $P < 0.001$] before treatment initiation. Moreover, a trend towards less pulmonary involvement was detected in anti-Mi2+ DM (9.1% *vs* 30.4%, $P = 0.05$), without any case of rapidly progressive interstitial lung disease. At muscle histology, anti-Mi2+ patients showed more necrotic/degenerative fibres than anti-Mi2– patients [mean 5.3% (5) *vs* 0.8% (1), $P < 0.01$], but similar to IMNM [5.9% (6), $P > 0.05$]. In addition, the endomysial macrophage score was similar between anti-Mi2+ and IMNM patients [mean 1.2 (0.9) *vs* 1.3 (0.5), $P > 0.05$], whereas lower macrophage infiltration was found in anti-Mi2– DM [mean 0.4 (0.5), < 0.01].

Conclusions. Anti-Mi2+ patients represent a specific DM subset with high muscle damage. Histological hallmarks were a higher prevalence of myofiber necrosis, endomysial involvement and macrophage infiltrates at muscle biopsy.

Key words: DM, antibodies, muscle biopsy

CLINICAL SCIENCE

Rheumatology key messages

- Anti-Mi2 positive dermatomyositis represents a specific subset characterized by a severe muscle damage.
- Muscle biopsies of anti-Mi2 positive dermatomyositis show myofiber necrosis comparable to immune mediate necrotizing myopathy.
- Infiltrates of macrophages at endomysium distinguish anti-Mi2 positive dermatomyositis from other DM subsets.

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Introduction

DM is a rare inflammatory disease included in the spectrum of idiopathic inflammatory myopathies (IIMs) and characterized by specific skin manifestations such as heliotrope rash, Gottron's papules, V-neck and shawl signs [1]. These patients may present also with interstitial lung disease (ILD) and arthritis, or develop malignancies, but in some cases muscle involvement is not clinically observed [1, 2]. Recently, new classification criteria were developed through a joint process involving the EULAR and ACR. The new criteria use a probability-score approach and a classification tree for subclassification of patients with IIM, including amyopathic DM [3]. Several autoantibodies have been described in patients with IIMs in general and with DM in particular [4]. Although the pathogenic role of these autoantibodies is unknown, they appear closely associated with specific clinical phenotypes and prognostic evolution [4]. Anti-Mi2 antibodies recognize a component of the nucleosome remodelling-deacetylase complex involved in transcription regulation that has been found markedly overexpressed in muscle biopsies obtained from patients with DM [5], especially in regenerating myofibers [6]. Interestingly, Mi2 antigens were present in 60% of muscle biopsy from DM patients, but only 20% of DM patients have anti-Mi2 antibodies [5]. Whether and how Mi2 protein contributes to generate an immune response remains unknown.

Prior reports have characterized IIM patients carrying anti-Mi2 antibodies as having mild muscle disease along with typical DM skin manifestations and infrequent ILD, while the association with malignancies remains unclear [7–13]. Even fewer studies have described muscle biopsies from anti-Mi2 positive (+) DM. These works have found a higher prevalence of primary inflammation (i.e. the invasion of non-necrotic fibres by mononuclear cells) [10] and more prominent muscle necrosis [12] in anti-Mi2+ DM. The aim of this study was to further characterize anti-Mi2+ DM, evaluating clinical, prognostic and histopathologic features in a multicentre Italian cohort of adult patients. Herein, we performed a detailed qualitative and quantitative muscle analysis based both on morphological, inflammatory and vascular domains to improve the knowledge of histologic hallmarks of anti-Mi2+ DM.

Materials and methods

Patients

We performed a multicentre retrospective observational cohort study including DM patients recruited in three tertiary Rheumatology Units (Rheumatology Unit, Policlinico of Bari, Italy; Rheumatology and Clinical Immunology Unit, ASST Spedali Civili of Brescia, Italy; and Division of Rheumatology, Policlinico S. Matteo of Pavia, Italy) in a time frame from 2010 to 2019. All patients included in the present study fulfilled 2017 EULAR/ACR classification criteria [3] with a score of $\geq$7.5 without biopsy or $\geq$8.7 with muscle biopsy, corresponding to 'definite IIM' (probability of $\geq$90%). Patients with autoantibodies against Aminoacyl tRNA Synthetase (ARS) (e.g. Jo1, PL-7, PL-12, OJ or EJ) or against PM/Scl proteins or who fulfilled criteria for other connective tissue diseases were excluded from the analysis because it is now considered as a distinct disease [14, 15]. Cancer-associated myopathy (CAM) was defined as neoplasia detection before or after 3 years DM onset [16]. Patients with normal muscle strength and no increase of creatine-kinase (CK) levels were classified as clinically amyopathic DM (CADM) [3]. All muscle samples analysed in this study were performed for diagnostic purposes from patients attending the outpatient clinic of Bari University (Italy). Muscle samples of patients with active disease (clinical, instrumental or laboratory) at the time of biopsy were selected for histological analysis. All patients had given informed consent for muscle biopsy as part of the diagnostic workout and for their medical records to be used for research purposes. Ethical approval was not required for the use of routinely collected anonymized data in this observational study.

Clinical data

Clinical and demographics data analysed in the present study were retrospectively obtained by individual electronic medical records reviewed by rheumatologists with recognized expertise in the diagnosis and management of IIM. Disease onset was considered from the observation of the first constitutional (fever), muscle (weakness), lung, joint or skin symptom/sign related to DM. Onset symptoms/signs were defined as concomitant when they occurred less than three months apart. The following data were recorded. Demographics: age, gender, ethnicity, age at disease onset and diagnosis, outcome at last follow-up visit (alive/death) and cause of death. Skin manifestations: heliotrope rash, Gottron's papules/sign, facial rash, 'V-neck'sign, 'shawl' sign, periungual hyperaemia, calcinosis, skin ulcers, Raynaud's phenomenon, mechanic's hands. Other clinical manifestations: fever, arthritis, serositis and dysphagia (the latter was confirmed with fiberoptic endoscopic evaluation of swallowing). Treatments administered during follow-up were also recorded. ILD was defined by high-resolution CT scan of the chest. Rapidly progressive-ILD (RP-ILD) was defined as a critical condition characterized by severe hypoxaemia (PaO2/FiO2 ratio $\leq$200) that worsened within 3 months from the onset of ILD upon exclusion of other possible causes (pulmonary infections, heart failure, embolism). Muscle involvement was defined as the presence of at least one condition among muscle enzymes' elevation, proximal muscle weakness on manual muscle testing (MMT-8) [17], myositis at muscle biopsy, presence of typical EMG alterations, skeletal muscle oedema on MRI [18]. Finally, MMT-8 assessment was recorded at baseline and after 1-year follow-up, if available, in patients who presented muscle involvement.

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Serological data

Maximum levels of creatine kinase (CK), lactate dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were recorded before treatment initiation. CK level was also recorded after 1-year follow-up. Because routine analyses were carried out in different laboratories, to avoid bias due to different normal value ranges, CK, LDH, GOT and GPT were normalized and expressed as fold of increase from the upper normal limit (UNL, international units). Myositis-specific antibodies (MSA) (Jo1, PL7, PL12, EJ, OJ, Mi2 α/β, TIF1γ, MDA5, NXP2, SAE1/2, SRP) and myositis-associated antibodies (Ku, PM-Scl 100/75, Ro-52) were searched by the same line blot assay, performed according to the manufacturer's recommendations (Euroline Autoimmune Inflammatory Myopathies, Euroimmun, Germany). Twenty out of 22 DM patients positive for anti-Mi2 antibodies underwent a second confirmation test among the following: immunodot assay (MYO12D-24, D-Tek, Belgium) for 14 patients, CIE for three patients and immunoprecipitation for three patients [19]. ANA were assayed using IIF methods on HEp-2 substrate cells. ANA titres ≥1/160 were considered positive. DM patients with confirmed serum anti-Mi2 antibodies were included in anti-Mi2+ group, while patients with DM who did not fulfil any exclusion criteria were included in the anti-Mi2 negative(–) group.

Histological and immunohistochemical analysis

A total of 26 diagnostic muscle biopsies were available for histological characterization, 12 from Mi2+ DM and 14 from Mi2– DM. As control group, nine specimens from immune mediated necrotizing myopathies (IMNM) positive for anti-SRP (three patients) or anti-HMGCR (six patients) were selected.

Muscle samples were obtained with open surgery by a dedicated surgeon (D.D.) and soon after fresh-frozen in isopentane pre-cooled in liquid nitrogen. All frozen samples were analysed in the Department of Neurophysiopathology (University of Bari), following standardized procedures and according to a routine protocol [20]. A complete description of histological procedures and stains, image acquisition and analysis of muscle biopsies have been provided in Supplementary Data S1, available at *Rheumatology* online.

Statistical analysis

The Kolmogorov–Smirnov test was used to evaluate the distribution of continuous variables. Demographics and disease characteristics were evaluated using standard descriptive statistics. Categorical variables were expressed as number or percentage; continuous variables as mean (S.D.) or median and interquartile range (IQR). Comparisons between groups were performed by Fisher's exact test, Student's *t test*, Mann–Whitney test or Kruskal–Wallis test followed by post-hoc tests with Bonferroni correction, when appropriate. A paired *t* test was used to determine whether there was a statistically significant change in CK level and MMT-8 at baseline and after 1-year follow-up among anti-Mi2+ patients. A *P*-value <0.05 was considered statistically significant. Spearman's test was used to investigate possible correlation between CK and histological findings. Survival at 5 years from disease onset was estimated using Kaplan–Meier (K–M) life-table method and differences between groups were compared using the log-rank test. The SPSS IBM Software (v. 21.0. Armonk, NY, USA) was used for statistical analysis.

Results

Demographic and clinical analysis

Of 437 patients affected with IIM, 91 (21%) DM patients were included in this analysis and formed the patient cohort (Fig. 1). Twenty-two (24%) patients were positive for anti-Mi2 antibodies (anti-Mi2+ group), whereas 69 (76%) DM patients were negative (anti-Mi2– group). Among anti-Mi2– patients, 11 were positive for anti-MDA5, eight for anti-NXP2, eight for anti-SAE and 10 presented positivity for anti-TIF1γ. Finally, 32 DM patients were negative for MSA. Skin rash was the most prevalent symptom reported by patients at onset, occurring in 77% of anti-Mi2+ and 57% of anti-Mi2– patients ($P = 0.21$) at disease onset. Fever was not complained of by anyone from the anti-Mi2+ patients, but was the onset symptom in seven (10%) anti-Mi2– DM. No differences were found for proximal muscle weakness (32% *vs* 38%), arthritis (5% *vs* 4%), dysphagia (0% *vs* 3%) and ILD (0% *vs* 4%) at onset between anti-Mi2+ and anti-Mi2– patients. All clinical and demographic characteristics of our cohort during follow-up are reported in Table 1. ANA titre ≥1/160 was found in 100% of anti-Mi2+ DM and in 60.9% of anti-Mi2– patients ($P < 0.001$). No anti-Mi2+ patients were additionally positive for anti-Ro52, which was detected in 19 (27.5%, $P < 0.01$) anti-Mi2– DM, isolated in five patients and co-expressed with anti-MDA5 in eight patients or anti-TIF1γ in three patients or anti-SAE in three patients.

The main clinical characteristic of anti-Mi2+ patients was muscle involvement. All anti-Mi2+ patients showed at least one laboratory, instrumental (MRI/EMG) or histological sign of muscle disease and none of them fulfilled the criteria for CADM. The latter was diagnosed in 10 (14.5%) anti-Mi2– patients (six anti-MDA5, two anti-SAE, one anti-TIF1γ and one MSA negative). Moreover, serum muscle enzymes before treatment were significantly higher in anti-Mi2+ group (Table 1). MMT-8 at first clinical evaluation tended to be lower in anti-Mi2+ than anti-Mi2– patients [mean (s.d.) 68.1 (7.7) *vs* 72 (8.5), $P = 0.15$]. Prevalence of CAM was not significantly different between the two groups; three (13.6%) cases were recorded in anti-Mi2+ patients (one breast cancer, one lung cancer and one endometrial cancer) and 13 (18.8%, $P = 0.75$) cases in anti-Mi2– patients. As expected, CAM showed the highest prevalence in anti-

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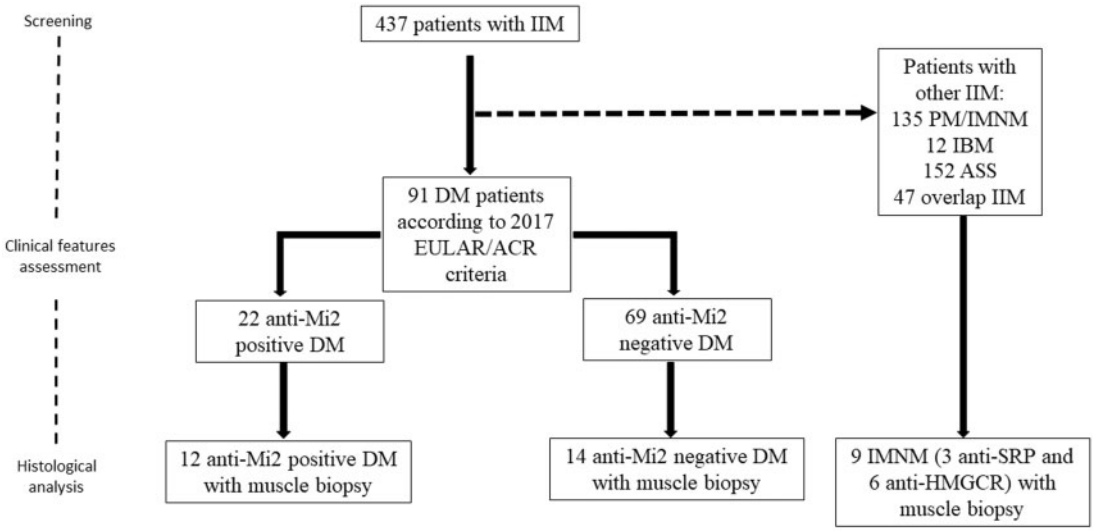
Fig. 1 Flow chart of the study population



ASS: anti-synthetase syndrome; IIM: idiopathic inflammatory myopathy; IMNM: immune mediated necrotizing myopathies.

TIF1γ DM (6/10 patients, 60%) followed by MSA negative DM (6/32 patients, 18.8%). A trend towards a lower prevalence of ILD was found in anti-Mi2+ patients (9.1% *vs* 30.4%, $P = 0.05$), with no case of RP-ILD compared with six (8.7%) patients recorded in anti-Mi2– group ($P = 0.32$). Of these, four RP-ILD patients were positive for anti-MDA5, one for anti-SAE and one was negative for MSA. No other clinical differences were found between the two groups. All DM patients were treated with at least one immunosuppressive drug during the study follow-up, without any difference between anti-Mi2+ and anti-Mi2– patients (Table 1).

Survival analysis and outcomes

Global survival at 5 years from disease onset was 85.7% for the whole cohort. No difference was found between anti-Mi2+ and anti-Mi2– patients (Fig. 2), despite a trend towards a better survival being seen for the anti-Mi2+ group (95.5% *vs* 82.6%, log-rank 1.96, $P = 0.16$). Thirteen patients died during the time-frame of the survey. One patient with anti-Mi2 antibodies died because of lung cancer after 23 months. The main cause of death in anti-Mi2– group was RP-ILD, that was the direct cause of death during the first 7 months of disease in four patients. The other eight deaths in the anti-Mi2– group were caused by major cardiovascular events (two patients), infections (two patients), related cancer complications (one patient), whereas the cause of death was unknown in three patients. Clinical response in terms of CK levels (18 patients) and MMT-8 (13 patients) was available in anti-Mi2+ patients after 1-year follow-up. We observed a decrease of CK levels from median (IQR) 15-fold UNL (5.4–32.7) to 0.6-fold UNL (0.4–1.1) ($P < 0.01$) along with an increase of muscle strength by MMT-8 from mean (s.d.) 68 (8) to 78 (2) ($P < 0.001$) (Fig. 3).

Histological findings

Twelve diagnostic muscle biopsies from anti-Mi2+ patients were available for histological analysis and were compared with 14 biopsies from anti-Mi2– patients and nine biopsies from patients affected with IMNM (six anti-HMGCR+ and three anti-SRP+ patients). All clinical and histological data of patients at time of biopsy are shown in Table 2. The majority of patients were not taking an immunosuppressant at the time of the biopsy. Immunosuppressive treatment was started soon after muscle biopsy. CK levels were higher in anti-Mi2+ than in anti-Mi2– patients ($P < 0.05$), but lower than the IMNM ones ($P < 0.01$). The hallmark of DM is perifascicular atrophy and was found in 100% of anti-Mi2+ patients and 71.4% of anti-Mi2– patients, but not in IMNM ($P < 0.001$). Moreover, considering only biopsies with perifascicular atrophy, anti-Mi2+ muscle specimens presented a higher proportion of fascicles with perifascicular atrophy than anti-Mi2– specimens, but not significant [mean (s.d.) 70% (30%) *vs* 51% (19%), $P = 0.13$]. The extent of myofiber degeneration/necrosis was higher in anti-Mi2+ patients than in anti-Mi2– patients [mean (s.d.) 5.3% (5.2%) *vs* 0.8% (1%), $P < 0.01$] and was similar to that of IMNM patients [5.9% (6.5%), $P = 0.78$]. Of note, a different distribution of myofiber alterations was detected. Anti-Mi2+ patients had both perifascicular and endomysial myofibers degeneration/necrosis. The latter was less evident in anti-Mi2– patients, where perifascicular atrophy prevailed over myofibers necrosis. Finally, a scattered endomysial distribution of degeneration/necrosis myofibers was detected in IMNM patients (Fig. 4a–c). The percentage of regenerating ($CD56^+$)

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Table 1 Demographic and disease characteristics observed in anti-Mi2 positive and anti-Mi-2 negative DM patients during follow-up

Variables	Overall (*n* 91)	Anti-Mi2+ (*n* 22)	Anti-Mi2– (*n* 69)
Demographics			
Onset age, mean (s.d.), years	53.4 (17)	56.5 (15.7)	52.4 (17.3)
Diagnostic delay, months, median (IQR)	3 (1–9)	3.5 (1–10.5)	3 (1–7.5)
Follow-up, months, median (IQR)	36 (13–97)	28.5 (16–80)	38 (12.5–104)
Female, *n* (%)	69 (75.8)	20 (90.9)	49 (71)
Caucasian, *n* (%)	87 (95.6)	21 (95.5)	66 (95.7)
CADM, *n* (%)	10 (11)	0 0	10 (14.5)
CAM, *n* (%)	16 (17.6)	3 (13.6)	13 (18.8)
Skin features			
Heliotrope rash, *n* (%)	70 (76.9)	17 (77.3)	53 (76.8)
Facial rash, *n* (%)	68 (74.7)	18 (81.8)	50 (72.5)
Gottron's papules/sign, *n* (%)	67 (73.6)	15 (68.2)	52 (75.4)
Shawl sign, *n* (%)	63 (69.2)	14 (63.6)	49 (71)
Mechanic's hands, *n* (%)	6 (6.6)	1 (4.5)	5 (7.2)
Periungual telangiectasia, *n* (%)	34 (37.4)	10 (45.5)	24 (34.8)
Calcinosis, *n* (%)	10 (11)	1 (4.5)	9 (13)
Skin ulcers, *n* (%)	16 (17.6)	3 (13.6)	13 (18.8)
Raynaud's phenomenon, *n* (%)	15 (16.5)	4 (18.2)	11 (15.9)
Lung features			
ILD, *n* (%)	23 (25.3)	2 (9.1)	21 (30.4)
RP-ILD, *n* (%)	6 (6.6)	0 0	6 (8.7)
Dyspnoea, *n* (%)	27 (29.7)	5 (22.7)	22 (31.9)
Serositis, *n* (%)	2 (2.2)	0 0	2 (2.9)
Muscle features			
Muscle involvement, *n* (%)	86 (94.5)	22 (100)	64 (92.8)
Proximal weakness, *n* (%)	75 (82.4)	18 (81.8)	57 (82.6)
EMG alterations, *n* (%)[a]	75 (89.3)	20 (100)	55 (85.9)
Thigh MRI oedema, *n* (%)[a]	31 (83.8)	8 (88.9)	23 (82.1)
Histologic alterations, *n* (%)[a]	33 (94.3)	12 (100)	21 (91.3)
MMT-8 at first visit, mean (s.d.)[b]	71.1 (8.4)	68.1 (7.7)	72 (8.5)
Other features			
Fever, *n* (%)	18 (19.8)	2 (9.1)	16 (23.2)
Arthritis, *n* (%)	16 (17.6)	4 (18.2)	12 (17.4)
Dysphagia on FEES, *n* (%)	24 (26.4)	4 (18.2)	20 (29)
Laboratory			
ANA titre $\geq$1/160, *n* (%)	64 (70.3)	22 (100)	42 (60.9)***
Ro52, *n* (%)	19 (20.9)	0 0	19 (27.5)**
CK maximum level, median (IQR)[c]	6 (1.6–16)	16 (7–37)	3.5 (1–9.9)***
GOT maximum level, median (IQR)[c]	2.5 (1.3–5)	3.8 (2–6.7)	2.1 (1.1–4.7)*
GPT maximum level, median (IQR)[c]	1.9 (1.1–3.2)	2.6 (1.8–4.3)	1.7 (1–2.7)**
LDH maximum level, median (IQR)[c]	2.1 (1.4–3.6)	3.6 (2.2–5.8)	1.9 (1.4–2.7)**
Treatments			
Corticosteroids, *n* (%)	88 (96.7)	22 (100)	66 (95.7)
Methotrexate, *n* (%)	58 (63.7)	13 (59.1)	45 (65.2)
Azathioprine, *n* (%)	45 (49.5)	14 (63.6)	31 (44.9)
Mycophenolate, *n* (%)	16 (17.6)	2 (9.1)	14 (20.3)
Cyclophosphamide, *n* (%)	11 (12.1)	2 (9.1)	9 (13)
Rituximab, *n* (%)	11 (12.1)	2 (9.1)	9 (13)
IVIG, *n* (%)	27 (29.7)	4 (18.2)	23 (33.3)

Data are expressed as mean (s.d.), median and interquartile range (IQR) or frequencies with percentages (%). [a]EMG, MRI and histology results were available for 84, 37 and 35 patients, respectively. [b]MMT-8 was available for 14 anti-Mi2+ patients and 43 anti-Mi2– patients. Patients with CADM have been excluded in MMT-8 count. [c]Maximum levels of CK, GOT, GPT and LDH before treatment are expressed as x-fold of increment from upper normal limit. *P <0.05, **P <0.01, ***P <0.001 *vs* anti-Mi2+. CADM, clinically amyopathic DM; CAM, cancer associated myositis; CK, creatine kinase; FEES, fiberoptic endoscopic evaluation of swallowing; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ILD, interstitial lung disease; LDH, lactate dehydrogenase; Mi2+, anti-Mi2 positive; Mi2–, anti-Mi2 negative; MMT-8, manual muscle testing score on eight muscle groups; RP-ILD, rapidly progressive ILD.

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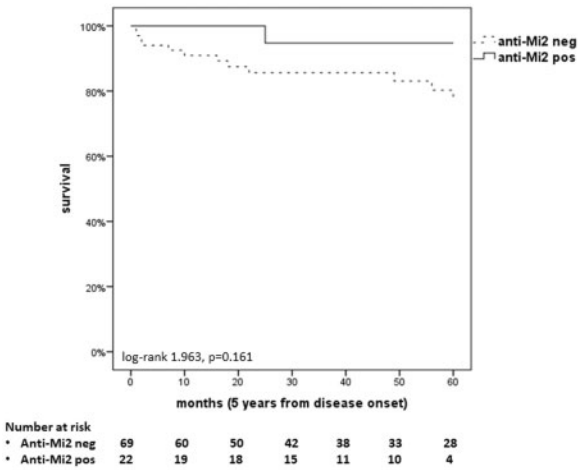
fibres was higher, but not statistically different, in anti-Mi2+ than in anti-Mi2– patients [mean (s.d.) 4.6% (6.6%) *vs* 1.6% (2.2%), P =0.09], whereas IMNM patients showed the highest percentage of myofibers in regenerating/immature state [13.5% (12.9%), P <0.05]. Sarcolemmal C5b-9 deposition was sporadic in DM patients. In fact, only two (16.7%) anti-Mi2+ patients and one (7.1%) anti-Mi2– patient showed this finding, whereas exclusive sarcolemmal C5b-9 deposits were found in six (66.7%) IMNM patients (P <0.05). Sarcoplasmic C5b-9 deposition is a non-specific feature related to whatever necrotic damage. Accordingly, we found a higher prevalence of sarcoplasmic C5b-9 deposition in anti-Mi2+ (n = 8, 66.7%) and IMNM (n = 6, 66.7%) patients than in anti-Mi2– DM patients (n = 2, 14.3%, P =0.01) (Fig. 4d–f). Endomysial capillary deposition of C5b-9 was a rare finding in our cohort and it was found in one (8.3%) anti-Mi2+ and two (14.3%) anti-Mi2– patients, but never detected in IMNM patients.

FIG. 2 Kaplan–Meier analysis of 5-years survival since DM presentation, comparison between anti-Mi2+ and anti-Mi2– patients

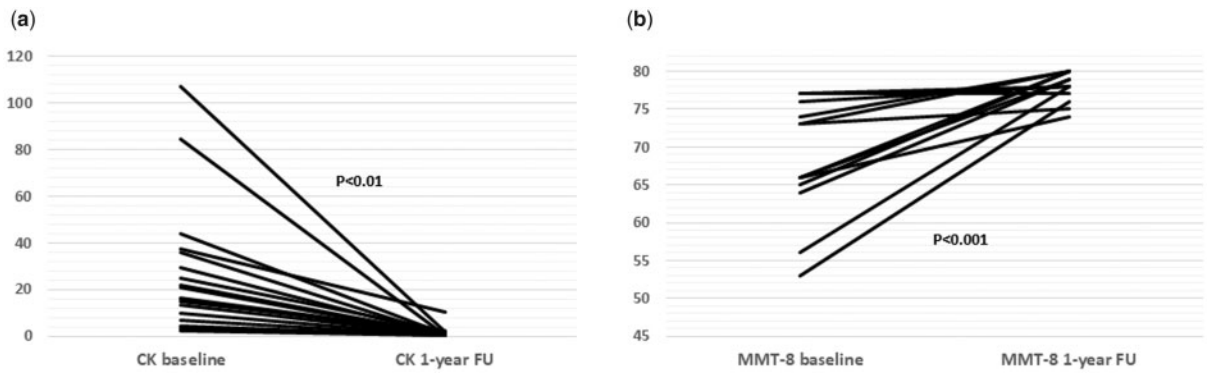


MHC-I was overexpressed on sarcolemma or sarcoplasma in the majority of biopsies without difference among the three groups (58.3% of anti-Mi2+, 64.3% of anti-Mi2– and 66.7% of IMNM patients, P =0.91), but a perifascicular reinforcement was only observed in DM patients (Fig. 4g–i). On the contrary, sarcolemmal MHC-II was faint in all biopsies. Inflammatory domain was ranked using the scoring system developed for juvenile DM [21]. The score for immunolocalization of macrophages ($CD68^+$ cells) infiltrating endomysium was significantly higher in anti-Mi2+ patients or IMNM patients, than in anti-Mi2– DM (P <0.01) (Fig. 4j–l and Table 2). No difference among the three groups was found for macrophages score in perimysium, as well as for $CD4^+$, $CD8^+$or $CD20^+$ lymphocytes score, both in endomysium and in perimysium. Finally, we detected a lower, but not significant, endomysial capillary density in anti-Mi2+ patients [mean (s.d.) 156.8 (19.9) per mm^2] than in anti-Mi2– DM [189.1 (54) per mm^2, P =0.06]. On the contrary, we observed higher endomysial capillary density in IMNM patients [272.6 (56.3) per mm^2, P <0.001] (Fig. 4m–o and Table 2). No difference was found for capillary density in perimysial sites between the three subsets of IIM. Spearman's test showed a significant direct correlation between CK levels and percentage of myofibers in degeneration/necrosis (r = 0.68, P <0.001), regenerating myofibers (r = 0.48, P <0.01), and sarcoplasmic complement deposition (r = 0.55, P <0.01). No further significant correlations were found between CK levels and other histological parameters.

Discussion

In this study we have shown that anti-Mi2+ DM patients are characterized by an inflammatory myopathy with a severe muscular involvement together with the typical DM rash. A major finding of our study is that the high degree muscle damage in anti-Mi2+ DM patients has been confirmed through a careful histological analysis and compared with anti-Mi2– DM tissue controls. Anti-Mi2+ muscle biopsies showed percentages of myofibers

FIG. 3 Changes of creatine kinase and manual muscle testing (MMT-8) after 1-year follow-up of anti-Mi2+ patients



Creatine kinase (CK) levels are expressed as x-fold of increment from upper normal limit. FU: follow-up

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TABLE 2 Clinical and histologic characteristics of anti-Mi2 positive, anti-Mi2 negative DM and immune mediated necrotizing myopathy patients

Variables	Anti-Mi2+ (*n* 12)	Anti-Mi2– (*n* 14)	IMNN (*n* 9)	*P*-value
Demographic and clinical characteristics at time of biopsy				
Age, mean (s.d.) years	59 (17.9)	55.3 (16.3)	57.9 (16.2)	0.84
Disease duration from symptom onset, months, median (IQR)	6.5 (2.3–6.5)	4 (1–6.5)	6 (4–22.5)	0.10
Immunosuppressive therapy, *n* (%)[a]	1 (8.3)	2 (14.3)	1 (11.1)	0.89
CK level, median (IQR)[a]	8 (2.3–21)	2.3 (1–8.1)*	23 (10–37.7)**	0.006
Muscle analysed				
Deltoid, *n* (%)	2 (16.7)	3 (21.4)	1 (11.1)	0.47
Quadriceps, *n* (%)	7 (58.3)	10 (71.4)	6 (66.7)	
Triceps, *n* (%)	2 (16.7)	0 0	0 0	
Biceps, *n* (%)	1 (8.3)	1 (7.1)	2 (22.2)	
Muscle fibres domain				
Perifascicular atrophy, *n* (%)	12 (100)	10 (71.4)	0 0**	<0.001
Myofiber in degeneration/necrosis, mean (s.d.)	5.3% (5.2)	0.8% (1)**	5.9% (6.5)	0.015
Regenerating fibres, mean (s.d.)	4.6% (6.6)	1.6% (2.2)	13.5% (12.9)*	0.016
Sarcolemmal C5b-9 deposition, *n* (%)	2 (16.7)	1 (7.1)	6 (66.7)*	0.004
Sarcoplasmic C5b-9 deposition, *n* (%)	8 (66.7)	2 (14.3)*	6 (66.7)	0.01
MHC-I, *n* (%)	7 (58.3)	9 (64.3)	6 (66.7)	0.92
MHC-II, *n* (%)	1 (8.3)	00	2 (22.2)	0.43
Vascular domain				
Endomysial capillaries/mm^2, mean (s.d.)	156.8 (19.9)	189.1 (54)	272.6 (56.3)**	<0.001
Perimysial capillaries/mm^2, mean (s.d.)	3.5 (2.3)	4.5 (2.9)	4.7 (3.4)	0.52
Endomysial C5b-9 capillary deposition, *n* (%)	1 (8.3)	2 (14.3)	0 0	0.49
Perimysial C5b-9 capillary deposition, *n* (%)	8 (66.7)	12 (85.7)	7 (77.8)	0.59
Inflammatory domain				
Primary inflammation, *n* (%)	6 (50)	4 (28.6)	4 (44.4)	0.51
Perivascular inflammation, *n* (%)	9 (75)	8 (57.1)	4 (44.4)	0.35
CD68+ endomysial score, mean (s.d.)	1.2 (0.9)	0.4 (0.5)*	1.3 (0.5)	0.005
CD68+ perimysial score, mean (s.d.)	1.3 (0.9)	1.2 (0.7)	1.3 (0.7)	0.82
CD8+ endomysial score, mean (s.d.)	0.1 (0.3)	0.2 (0.6)	0.4 (0.5)	0.13
CD8+ perimysial score, mean (s.d.)	0.1 (0.3)	0.1 (0.3)	0.2 (0.4)	0.51
CD4+ endomysial score, mean (s.d.)	0.1 (0.3)	0 (0)	0 (0)	0.38
CD4+ perimysial score, mean (s.d.	0.1 (0.4)	0.1 (0.4)	0 (0)	0.50
CD20+ endomysial score, mean (s.d.)	0.1 (0.3)	0.1 (0.4)	0 (0)	0.50
CD20+ perimysial score, mean (s.d.)	0.1 (0.8)	0.2 (0.6)	0 (0)	0.46

Data are expressed as mean (S.D.), median with first and third quartile (IQR) or frequencies with percentages (%). [a]CK is expressed as x-fold of increment from upper normal limit. [b]Any immunosuppressive treatment including glucocorticoids administered as a single bolus of 2 mg/kg body weight or as a dosage equivalent to prednisone of 20 mg/die for ≥2 weeks. Post-hoc tests: *P <0.05, **P <0.01 *vs* anti-Mi2+ patients. CK, creatine kinase; IMNM, immune mediated necrotizing myopathy; Mi2+, anti Mi2 positive; Mi2–, anti-Mi2 negative.

in degeneration/necrosis that were comparable to those observed in IMNM. Characteristic of anti-Mi2+ myopathy was also a meaningful endomysial involvement with scattered macrophage infiltrates, similar to that observed in patients with IMNM but not present in the other anti-Mi2– DM subsets. Despite some analogies between anti-Mi2+ and IMNM muscle specimens, the perifascicular atrophy together with MHC-I overexpression of perifascicular area, and the reduction of density of endomysial capillaries have been confirmed as DM-specific features, also in anti-Mi2+ patients.

A few studies have investigated morphological and immunohistochemical findings through different DM subsets. A possible correlation between DM immunotype and muscle pathology has been observed in anti-MDA5 [22], anti-NXP2 [23] and anti-TIF1ɣ[16] positive DM. Previous studies have provided evidence that the invasion of non-necrotic fibres by mononuclear cells and a prevalent necrotizing myositis were histological hallmarks of anti-Mi2+ patients [10, 12, 24]. In addition, the distribution of macrophages found in our study seems to be characteristic, because they usually infiltrate the perimysium of DM patients [25, 26] but in our study a substantial invasion of endomysium by macrophages in anti-Mi2+ DM has been also observed, as sometimes reported in anti-Mi2+ juvenile DM [27]. Usually, endomysial macrophages are abundantly seen in IMNM biopsies [28–30] and can range from an early pro-inflammatory

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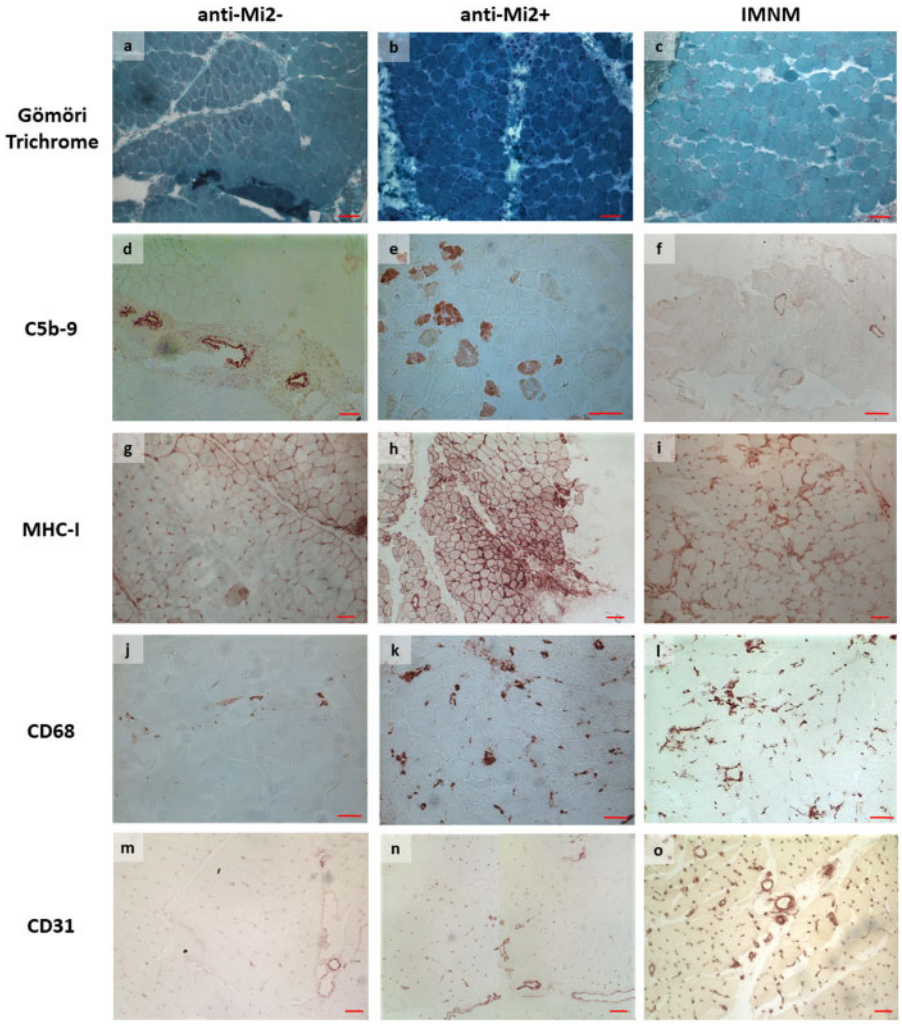
FIG. 4 Representative histopathological characteristics of anti-Mi2 positive(+) DM, anti-Mi2 negative(–) DM, and IMNM muscle biopsies



(**a**) Perifascicular atrophy and myofiber vacuolation in an anti-Mi2– patient. (**b**) Perifascicular atrophy in an anti-Mi2+ patient. Myofiber degeneration/necrosis appears located both in perifascicular and endomysial sites. (**c**) Scattered myofiber necrosis in an IMNM patient. (**d**) MAC deposition on perimysial capillaries in an anti-Mi2– patient. (**e**) Sarcoplasmic MAC deposition on necrotic myofiber in an anti-Mi2+ patient. (**f**) Sarcolemmal MAC deposition on several myofibers in an IMNM patient. (**g–h**) MHC-I staining with perifascicular reinforcement in anti-Mi2– and anti-Mi2+ DM. (**i**) Scattered diffuse myofiber and capillary immunostaining for MHC-I in an IMNM patient. (**j**) CD68+ macrophages accumulate in perivascular and perimysial areas of an anti-Mi2– patient. (**k–l**) Macrophages infiltrates in endomysium of representative anti-Mi2+ and IMNM patients. (**m–o**) CD31 immunostaining of endothelial cells showing muscle capillary distribution. Note the relative defect of blood vessels in anti-Mi2– (**m**) and anti-Mi2+ (**n**) patients compared with the IMNM patient (**o**). IMNM: immune mediated necrotizing myopathies; MAC: membrane attack complex (C5b-9); MHC-I: major histocompatibility complex-I. Scale bars =100 μm.

M1 polarization [28, 29], to a later M2 phenotype sustaining tissue repair and regeneration [30, 31]. To date, there are no studies that investigated the macrophage phenotype in anti-Mi2+ DM. Nevertheless, the detection of myofiber necrosis and regeneration prompts us to speculate that endomysial macrophages might play similar functions both in anti-Mi2+ DM and IMNM. Further studies are needed to better understand the role of tissue macrophages in anti-Mi2+ DM patients, focusing on a possible imbalance of pro-inflammatory M1 over anti-inflammatory M2 phenotype. Sarcolemmal complement deposition is believed to be a hallmark of IMNM [28, 32], and some authors described this finding also in anti-Mi2+ DM [27, 33]. We did not find a significant difference for sarcolemmal MAC deposition between anti-Mi2+ and anti-Mi2– patients, although two anti-Mi2+ patients showed sarcolemmal MAC deposition also in our cohort.

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On the clinical side, prior reports showed that anti-Mi2 positivity was found in 10–30% of DM and associated with pathognomonic skin lesions [7, 34, 35], low occurrence of pulmonary involvement [9, 13], high achievement of disease remission [9, 10] and raised levels of muscle enzymes[9, 10, 12]. Herein, we have confirmed that higher levels of muscle enzymes represent a characteristic feature of anti-Mi2+ DM, whereas a trend towards lower frequency of pulmonary involvement has been observed also in our cohort. Despite the severe muscle involvement at the onset, anti-Mi2+ patients showed a good response to immunosuppressive therapy as evidenced by the quick reduction of CK levels and the recovery of muscle strength. We observed a good prognosis at 5-years follow-up, as only one patient with lung cancer died in anti-Mi2+ group. This finding is consistent with previous studies where anti-Mi2 DM patients without malignancies seemed to have a better prognosis [12, 13], as expected because of the low prevalence of ILD and no case of RP-ILD in our analysis.

Currently, whether the presence of anti-Mi2 antibodies in DM patients represents a higher risk of malignancy is still a matter of debate and data in literature are conflicting [7, 9–13]. Our study did not detect difference of CAM prevalence between anti-Mi2+ and anti-Mi2– patients. Nevertheless, a recent study has confirmed that DM patients with anti-Mi2 have an increased risk of cancer when compared with age- and sex-matched people [12]. Therefore, careful screening for cancer should be recommended in all DM patients, including patients with anti-Mi2 antibodies.

Some limitations of our study need to be mentioned. First, the retrospective nature of the study. Nevertheless, we do believe that this type of study is a necessary starting point when rare diseases are explored. Second, the small number of muscle biopsies analysed. Muscle biopsy is an invasive procedure that is not routinely performed in all patients. However, all muscle tissues of our study were obtained with open surgery from patients with active disease, analysed with a standardized protocol and scored according to recent literature [20–21]. We used a biopsy scoring tool validated for juvenile DM that performs equally well in muscles from different sites [36]. The suitability of this score for adult DM patients is corroborated by the fact that a quite similar score has been preliminarily proposed for adult patients [37]. Third, the use of commercial line blot assay for detection of MSA/myositis associated antibodies. Immunoprecipitations (IP) represents the gold standard for the detection of MSA/myositis-associated antibodies, but IP cannot be routinely applied in daily clinical practice and require a high level of expertise. Nevertheless, the presence of anti-Mi2 antibodies has been confirmed at least by another test in 20 out of 22 patients. Furthermore, the strict inclusion criteria of the study enrolling only patients with DM, a diagnosis strictly associated with anti-Mi2 antibodies, made our cohort quite reliable.

In conclusion, our study of a cohort from an Italian geographic area appears to be worthy to confirm that DM patients with anti-Mi2 antibodies represent an IIM subgroup characterized by severe muscle involvement at onset. Histological hallmarks of this disease are high prevalence of myofiber necrosis and endomysial inflammation with scattered macrophage infiltrates.

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Criterion 1: (a) Substantial contributions to study conception and design; and/or (b) Substantial contributions to acquisition of data; and/or (c) Substantial contributions to analysis and interpretation of data. Criterion 2: Drafting the article or revising it critically for important intellectual content. Criterion 3: Final approval of the version of the article to be published. M.F., F.G., F.I.: Criteria 1a, 1 b, 1c, 2 and 3. L.C., F.F., M.G., A.A., A.L., M.T., D.D, M.F., G.Z., L.M., L.C., L.M.: Criteria 1 b, 1c, 2 and 3.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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