Immunopathological Characterization of Muscle Biopsy Samples from Immune-Mediated Necrotizing Myopathy Patients

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF **Qiong Wang**
ACF **Yue Li**
AE **Suqiong Ji**
ADF **Fang Feng**
ADEG **Bitao Bu**

Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, P.R. China

Corresponding Author: Bitao Bu, e-mail: bubitao@tjh.tjmu.edu.cn
Source of support: Departmental sources

Background: Immune-mediated necrotizing myopathy (IMNM) is a relatively new proposed category of idiopathic inflammatory myopathies (IIMs), characterized by the presence of abundant necrotic muscle fibers, myophagocytosis, and sparse inflammatory infiltrates. The aim of our study was to analyze the immunopathological characteristics of IMNM by detecting biopsy samples from a cohort of patients, and to delineate the pathways involved in the pathogenesis.

Material/Methods: A retrospective evaluation of muscle biopsy samples, clinical and laboratory data, and immunohistochemical analysis of macrophages MHC-I and MAC, was performed for all patients diagnosed as having IMNM but without a prior exposure to statins.

Results: Immunohistochemical analysis revealed the presence of CD68^{+} macrophages mainly in the necrotic muscle fibers and the endomysial connective tissue. MHC-I and MAC positively stained not only the necrotic fibers or vessels but also the non-necrotic ones.

Conclusions: Our data describe general immunological features in IMNM patients, which may be helpful in serving as biomarkers, aid in diagnostic decisions, and provide clues into the underlying mechanisms involved in this disease.

MeSH Keywords: **Complement Membrane Attack Complex • Macrophages • Major Histocompatibility Complex • Myositis**

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/907380

📄 1865   ▦ 2   📊 2   📖 27

Background

Immune-mediated necrotizing myopathy (IMNM) is a heterogeneous group of muscular diseases with specific muscular pathological and immunological features and is considered one of the idiopathic inflammatory myopathies [1–4], including polymyositis, dermatomyositis, and inclusion body myositis. To date, the recognition of IMNM is based on clinical features, pathological characteristics, and specific antibodies.

Although the pathogenesis of IMNM has not been fully elucidated, several studies have suggested some biomarkers and pathological mechanisms indicating the involvement of an autoimmune-mediated process [3–16]. Some studies have shown that major histocompatibility complex class I (MHC-I), the membrane attack complex (MAC, C5b-9), and macrophages were involved in the pathogenesis of IMNM [1,3,4,16,17]. In addition, the presence of IMNM-associated antibodies, such as the signal recognition particle (SRP), 3-hydroxy-3-methylglutarylcoenzyme A reductase (HMGCR), and histidyl-tRNA synthetase (Jo-1) antibodies, have been intensively described for this disease [3,12,16]. However, the underlying disimmunity and the characteristics of INMN patients in Chinese populations are yet to be described. Here, we aimed to assess muscle tissues in a cohort of 40 Chinese patients with IMNM, focusing on clinical patterns, immunological profiles, inflammatory infiltrates, and the important biomarkers expressed in the biopsied muscles.

Material and Methods

Patients and biopsy specimens

The biopsied muscles, as well as clinical and laboratory data obtained from 40 patients who were diagnosed with IMNM at Tongji Hospital between January 2012 to December 2014 were retrospectively analyzed. The diagnosis of IMNM was made based on the clinical features and muscle pathologic changes, according to the classification criteria proposed by the European Neuromuscular Centre (ENMC) in 2004 [1]. Other differentiated idiopathic inflammatory myopathies (polymyositis, dermatomyositis, and inclusion body myositis), as well as infectious myopathy, were excluded from the study. Informed consents were signed by all the patients after the Ethics Committee of Tongji Hospital approved the study. Magnetic resonance imaging (MRI) was used to scan the muscles in the extremities before a biopsy. All the biopsy specimens were cryopreserved immediately in liquid nitrogen, and then stored at −80°C. Transverse 7-μm cryostat sections were stained with hematoxylin and eosin (H&E), and enzymatic histopathology was applied for the preliminary diagnosis of myopathy.

Immunohistochemical analysis

To examine inflammatory infiltrates and study the immunological characteristics, muscle sections were stained with mouse monoclonal antibodies against either CD68, or MHC-I, or MAC, and visualized by IHC and immunofluorescence. The sections were fixed in ice-cold acetone, then treated with 3% H_2O_2, blocked with 10% fetal bovine serum albumin (BSA), and incubated with the above-mentioned primary antibodies overnight at 4°C. After a washing step, appropriate horseradish peroxidase-binding anti-mouse secondary antibodies or fluorescently labeled antibodies were added and the samples were incubated for 60 min at room temperature. Then, 3, 3'-diaminobenzidine (DAB) was used during a 5-min incubation period, and hematoxylin used to stain the nuclei. Omission of primary antibodies in the control sections resulted in the absence of any positive staining. Finally, the results were observed using a microscope (BX51, Olympus) under light or fluorescent conditions.

Semiquantitative evaluation of immunohistochemical staining

A semiquantitative scoring system reported by Corinna Preusse [4] was adopted with some modifications.

The infiltration with CD68+ macrophages was defined as the number of positive cells in necrotic cells and endomysium visually counted separately in 6 randomly selected high-power fields. Then, the average was graded as follows: <5 positive cells, almost no staining (–); 5–20 positive cells, less staining (+); 21–50 positive cells, more staining (++); and more than 50 positive cells, abundant staining (+++).

Similarly, The MHC-I expression and MAC deposition were defined as the negative staining (–), slightly positive staining (+), moderately positive staining (++), and strongly positive staining (+++) within different areas. All sections were evaluated by 2 independent observers. The averages were calculated.

Statistics

Demographics, clinical data, and results are presented as descriptive statistics. Data are expressed as mean or mean ±SD.

Results

Analysis of clinical data

There was no significant difference in female/male ratio, which was 1: 1 (19: 21). The mean age was 38.1±15.9 years. The disease duration ranged from 3 days to 24 months (mean: 6.4 months). None of the patients had ever been exposed to a

Table 1. No. (sex/age), duration (months), symptoms of onset, CK value, treatment, outcome.

Case (age/sex)	Disease duration (months)	Symptoms of onset	CK value (U/l)	Treatment	Outcome
1 (19/F)	12	Weakness	6344	PSL, ATP	Improve
2 (23/F)	4	Weakness	21294	PSL, MTX	Remission
3 (13/F)	0.3	Weakness, myalgia	6673	PSL	Improve
4 (19/M)	2	Weakness, dysphagia	14102	PSL	Improve
5 (48/M)	0.5	Weakness, myalgia, dysphagia, dyspnea	25419	PSL	Improve
6 (24/M)	1	Weakness, myalgia	271	PSL	Remission
7 (47/F)	6	Weakness	7535	PSL, CTX	Remission
8 (20/F)	2	Dyspnea	10923	PSL, TAC	Remission
9 (52/F)	24	Weakness, amyotrophy	2430	PSL, ATP	Remission
10 (33/F)	8	Weakness, amyotrophy	16	PSL, TAC	Remission
11 (46/F)	2	Weakness, myalgia	8252	PSL, MTX, ATP	Remission
12 (54/F)	12	Weakness, dyspnea	1099	PSL, MTX	Remission
13 (12/M)	2	Weakness, myalgia	30836	PSL	Remission
14 (49/M)	2	Weakness	14255	PSL	Remission
15 (32/M)	2	Weakness, myalgia	4803	PSL	Fluctuation
16 (64/M)	1	Weakness	3806	PSL	Remission
17 (46/F)	12	Weakness	1541	PSL	Remission
18 (34/F)	12	Weakness	1104	PSL	Remission
19 (39/F)	6	Weakness, dysphagia,	638	PSL	Remission
20 (26/M)	6	Weakness,	1241	PSL	Remission
21 (37/M)	4	Weakness, amyotrophy	1088	PSL, ATP, IVIG	Fluctuation
22 (28/F)	4	Weakness	12335	PSL, ATP/TAC	Improve
23 (32/M)	12	Weakness, myalgia	10177	PSL	Remission
24 (66/F)	4	Weakness	4974	PSL	Remission
25 (36/M)	24	Weakness, amyotrophy	3202	PSL	Remission
26 (22/M)	1	Weakness, myalgia	11057	PSL	Remission
27 (30/M)	6	Weakness	1592	PSL	Remission
28 (15/M)	0.1	Weakness, myalgia	13470	PSL	Remission
29 (18/M)	2	Weakness, myalgia	1601	PSL, TAC, IVIG	Fluctuation
30 (55/F)	1	Weakness, myalgia	4026	PSL, CTX	Remission
31 (60/M)	0.3	Weakness, myalgia	9187	PSL	Remission
32 (31/F)	3	Weakness, myalgia	6318	PSL	Remission
33 (58/F)	12	Weakness	418	PSL, IVIG, TAC	Remission
34 (45/F)	2	Weakness, myalgia, dysphagia	1790	PSL, TAC	Remission
35 (42/M)	24	Weakness, amyotrophy	32	PSL	Remission
36 (65/M)	2	Weakness, myalgia	8463	PSL	Remission
37 (59/M)	1	Weakness, amyotrophy	2281	PSL, ATP	Remission
38 (21/M)	12	Weakness	18640	PSL	Remission
39 (54/F)	24	Weakness, amyotrophy	5288	PSL, CTX	Exacerbation
40 (50/M)	2	Weakness	3295	PSL, IVIG, TAC	Fluctuation

F – female; M – male; CK – creatine kinase; PSL – prednisolone; ATP – azathioprine; MTX – methotrexate; CTX – cyclophosphamide; TAC – tacrolimus; IVIG – intravenous immune globulin.
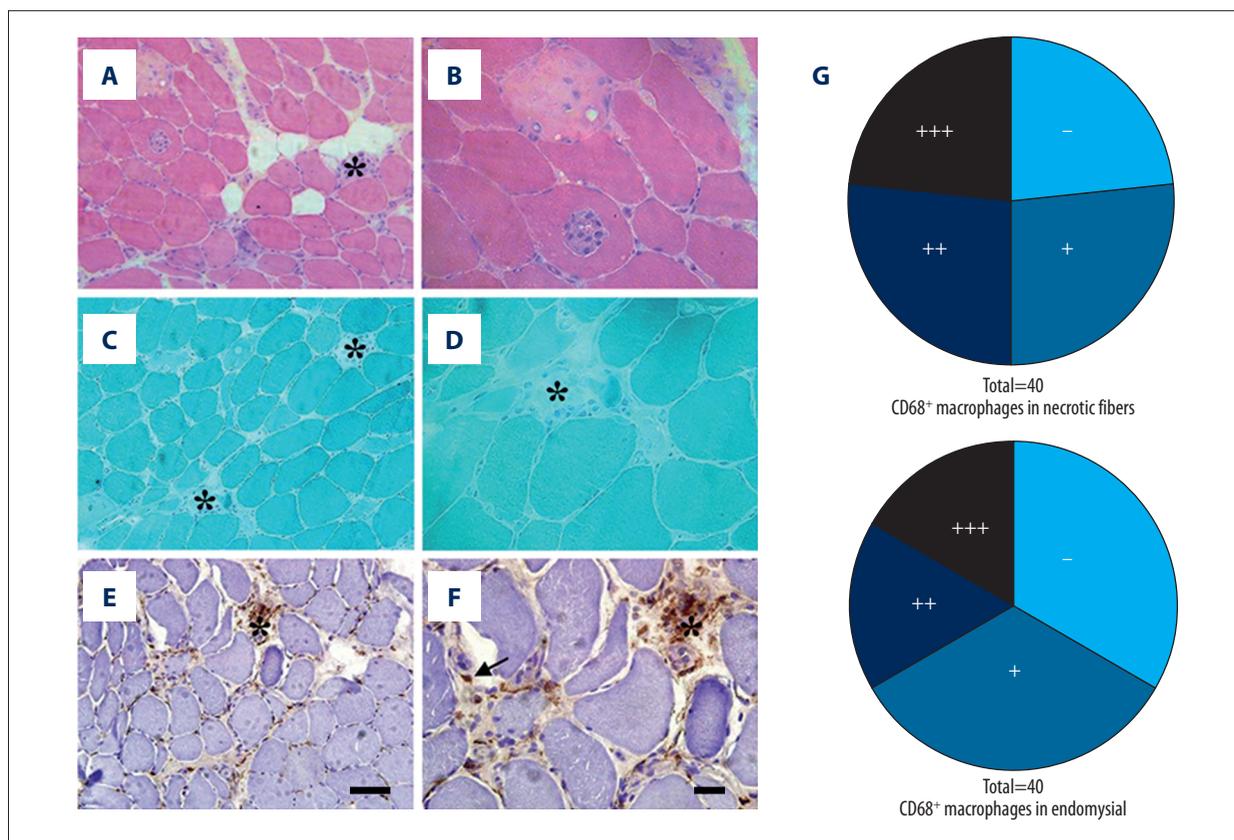
Figure 1. Morphological characterization of IMNM by hematoxylin and eosin (H&E) (**A, B**) and Gömöri trichrome (**C, D**) staining showing necrosis and myophagocytosis of muscle fibers. Many CD68+ macrophages are found in IMNM (**E, F**). (**G**) Two pie charts show the distribution of CD68+ macrophages in necrotic fibers and the endomysial tissue. **Asterisk** indicates necrotic fibers; **arrows** designate positive staining of macrophages. Scalebar: 50 µm for panels **A, C, E**; and 20 µm for panels **B, D, F**.

myofiber toxic drugs or statins or had previously been diagnosed with a tumor. In addition, there were 11 patients who already presented a chronic course (longer than 1 year) before they came to our hospital. Limb weakness was the initial presentation in all the patients. Among them, 14 patients complained of myalgia, 6 patients had muscle wasting and 4 had severe dysphagia and/or dyspnea. Furthermore, co-existing diseases were identified in some cases, including connective tissue disease (9 cases), interstitial pulmonary disease (2 cases), hepatic dysfunction (5 cases), chronic renal insufficiency (1 case), and myasthenia gravis (1 case). Laboratory investigations revealed that creatine kinase levels were elevated in 38 patients (mean: 7045.4, min: 16, max: 30836U/l), except for 2 cases who displayed the normal range (3–190 U/l). Half of the patients showed elevated levels of antinuclear antibodies (such as anti-SSA and anti-RNP) and myositis-associated antibodies (such as anti-Ro-52). There were 39 patients who responded well to the treatments (prednisolone or in combination with other immunosuppressants), and only 1 case experienced exacerbating symptoms, possibly due to renal failure. The demographics and clinical data for the 40 patients are summarized in Table 1.

Predominant infiltration with macrophages in the muscle biopsies

The aim of this study was to distinguish IMNM from other myopathies by routine histochemical staining (Figure 1A–1D) in combination with clinical data analysis. Many researchers believe that IMNM is characterized by sparse infiltration of B cells and T cells, but infiltration with macrophages has been frequently observed in muscle biopsies [1]. In our study, the CD68+ macrophages were mainly accumulated in the necrotic muscle fibers and the endomysial tissue (Figure 1E, 1F). In contrast, in more than three-quarters (31: 9) of the samples displayed CD68+ macrophages in the necrotic fibers, and over three-fifths (27: 13) of the samples in the endomysial tissue (Figure 1G). In addition, a few CD4+ T cells were seen in the perimysium (16/40), while CD8+ T cells were scarce (1/40) but without CD20+ B cells in these samples.

Prominent expression of MHC-I and obvious deposition of MAC on capillaries and necrotic fibers in muscle biopsies

In our samples, we observed an upregulation of MHC-I expression in both the vessels and non-necrotic fibers
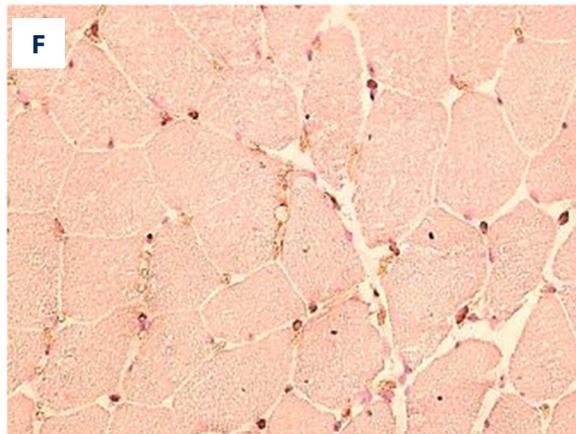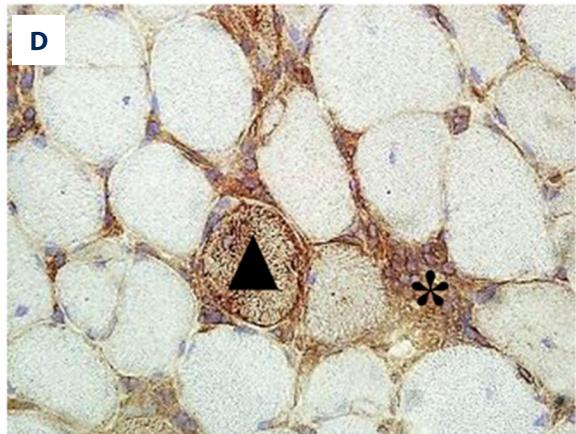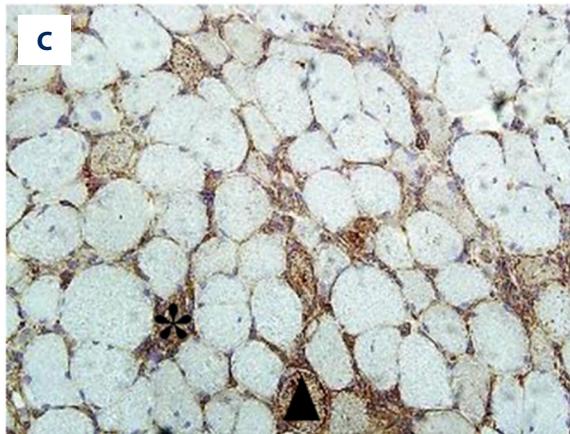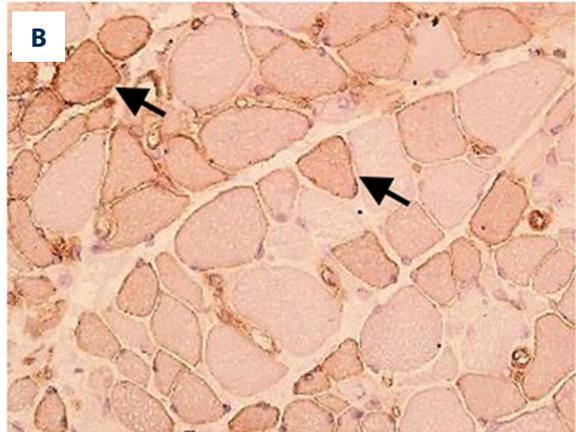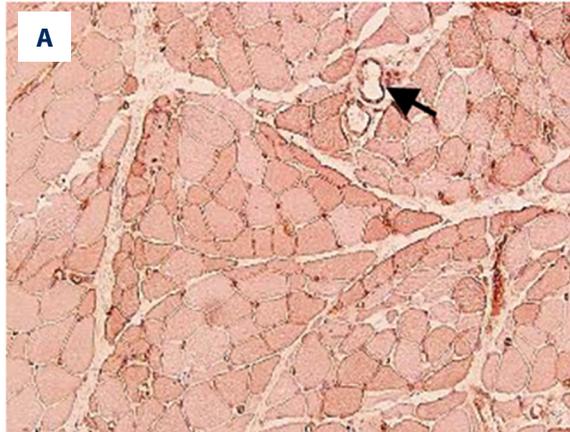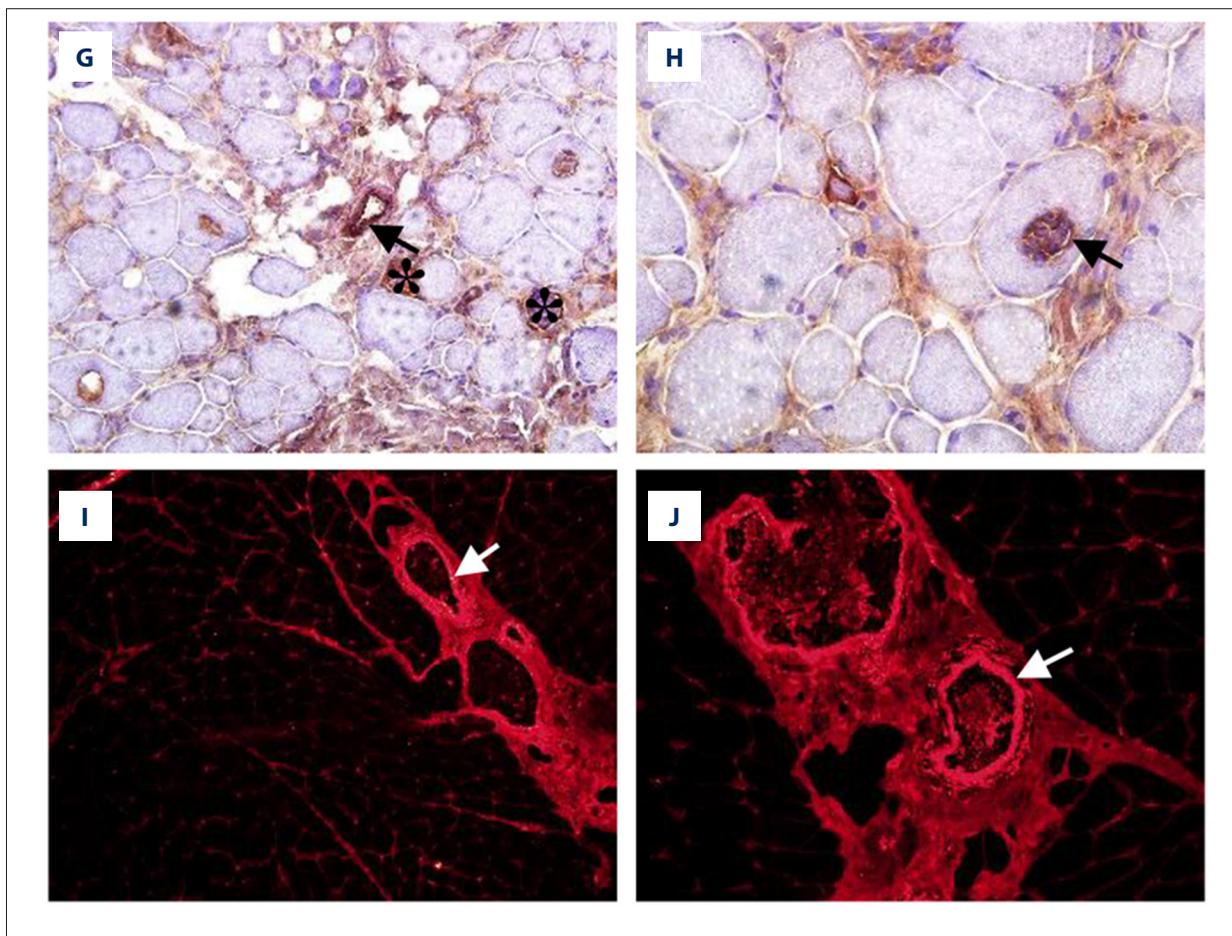
Figure 2. Expression of MHC-I and MAC deposition in IMNM. MHC-I is upregulated in vessels (**A**) and normal fibers (**B**) marked by arrows, necrotic fibers and regenerative fibers (**C, D**) compared with the normal muscle sample (**E, F**). MAC is deposited on endomysial vessels (arrows) and necrotic fibers (**G**), normal fibers (arrows) (**H**) and perimysium vessels (arrows) (**I, J**). MAC deposition is shown in red. **Asterisk** indicates necrotic fibers; **arrows** designate positive immunoreactivity; the **triangle**, indicates the regenerative fibers. Scalebar: 50 µm for panels **A, C, E, G, I**; and 20 µm for panels **B, D, F, H, J**.

(Figure 2A, 2B); and in the necrotic as well as the regenerative fibers (Figure 2C, 2D). All samples showed abnormal expression of the MHC-I molecule to various degrees (Table 2) compared with the normal muscle samples (Figure 2E, 2F).

The deposition of membrane attack complex (MAC, C5b-9) was another important pathological feature of IMNM. The MAC deposition was evident on the small blood vessels (Figure 2G, 2I, 2J), consistent with previous reports [1,4]. Notably, the same IMNM cases displayed substantial MAC deposition on the necrotic fibers (Figure 2G), as well as the non-necrotic ones (Figure 2H). The detailed information for all the muscle biopsies is shown in Table 2.

Discussion

As a newly identified clinical entity, the immunopathological profiles of IMNM have not been fully elucidated [4,17,18]. The

Table 2. Illustration of a semiquantitative scale based on immunohistochemical results of MHC- I and MAC.

	−	+	++	+++
MHC- I				
Necrotic fibers	13	14	7	5
Regenerative fibers	26	9	5	0
Normal fibers sarcolemmal	3	31	4	2
Perimysium vessels	33	3	2	2
MAC				
Vessels	3	31	5	2
Necrotic fibers	5	14	15	6
Normal fibers	31	5	2	2

A definition of the scale is given in materials and methods (−, negative; +++, strongly positive staining).

findings in the cohort of 40 IMNM patients without exposure to statins suggest that in the development of IMNM, not only the complement system is activated, but that macrophages may also play an important role.

Because of the abundant myofiber necrotic debris, myophagocytosis is a scavenging process often seen in multiple myopathies, such as in necrotizing myopathy, autoimmune myopathies, toxic and paraneoplastic myopathies, and in some muscular dystrophies. Therefore, immunostaining with MHC-I and MAC is of further diagnostic value. The abnormal overexpression of MHC-I and the MAC deposition on different areas in muscle tissues such as in the non-necrotic, necrotic fibers, and endomysial vessels indicate the possibility of an immune-mediated inflammatory process [4]. Furthermore, macrophages, as the predominant cells of infiltration in IMNM, were widely detected in the necrotic fibers and endomysial tissue, while the predominant inflammatory cells in other idiopathic inflammatory myopathies were T cells or B cells or/and macrophages [19]. In addition, the majority of these macrophages had a proinflammatory phenotype, as indicated by the presence of the classically activated CD68+ M1 macrophages. Interestingly, endophagocytosis of the normal-looking myofibers was frequently observed (9/40) (Figure 2H), suggesting the very early involvement of macrophages, before myofiber necrosis occurs. However, the detailed pathogenesis of the activated macrophages in IMNM, whether they simply act as scavengers of the necrotic debris resulting in imbalanced homeostasis, or whether they play a key role in upstream pathways leading to necrosis, needs to be investigated more thoroughly in future studies.

The up-regulation of MHC-I in inflammatory myopathies has been extensively reported, with various results in different studies [3,4,9]. Although some researchers did not report MHC-I overexpression in the sarcolemmal tissue of IMNM patients [3], others have shown very intense immunostaining against MHC-I on non-necrotic fibers [4,9], with the latter being supported by our latest observations. In addition, several muscle biopsy specimens in our study have shown an obvious up-regulation of the molecules on the perimysium vessels and regenerative fibers, which has not been described before. The pivotal role of up-regulation of MHC-I has already been suggested in a mouse model, showing that the self-sustaining autoimmune myositis can be successfully induced by conditional up-regulation of MHC-I, in combination with the production of myositis-specific antibodies [20]. It is important to further evaluate the involvement of MHC-I on regenerating muscle fibers in IMNM.

The deposition of MAC in IMNM samples has also been reported on a variety of tissue structures in different studies, including the typical deposition on myosial capillaries [1]. A previous study has observed an increased deposition only in necrotic myofibers [3]. However, there was an indication that MAC deposition on the sarcolemmal surface of non-necrotic fibers constituted a common feature of anti-HMGCR myopathy [9]. In contrast, our study showed that MAC was not only deposited strongly on the vessels, but also on the necrotic fibers and to a lesser extent on non-necrotic fibers. The different patterns of deposition sites of MAC in different reports may be due to sampling from different subtypes of IMNM. In the development of myofiber necrosis, the involvement of MAC suggests activation of the complement system, which could be elicited by proinflammatory cytokines [21–25]. Inhibiting activation of the complement system may be a method to reduce necrosis and to reduce the number of refractory cases in disease treatments, such as in the eculizumab treatment of dermatomyositis [26,27].

Our study did not establish any association between the muscle pathology and the myositis-specific antibodies in the cohort of patients with IMNM. We speculate that our cohort included patients with different subtypes of IMNM and different immunopathological profiles, such as the SRP-abs or other myositis-specific antibodies. Given the heterogeneity of the immunological features, the pathways or cell types involved in the necrotic process might be distinct. In future studies, enrollment of IMNM patients with similar immunological characteristics is the key to minimizing variability in the results.

Conclusions

In summary, our data describes the general immunopathological features of IMNM, the abnormal expression of MHC-I, and the deposition of MAC on the vessels, necrotic fibers, and non-necrotic fibers. Further studies on the role of macrophages are needed to elucidate the mechanism(s) of IMNM development.

References:

1. Hoogendijk JE, Amato AA, Lecky BR et al: 119th ENMC international workshop: Trial design in adult idiopathic inflammatory myopathies, with the exception of inclusion body myositis, 10–12 October 2003, Naarden, The Netherlands. Neuromuscul Disord, 2004; 14(5): 337–45

2. Mehndiratta P, Mehta S, Manjila SV et al: Isolated necrotizing myopathy associated with ANTI-PL12 antibody. Muscle Nerve, 2012; 46(2): 282–86

3. Hengstman GJ, ter Laak HJ, Vree Egberts WT et al: Anti-signal recognition particle autoantibodies: Marker of a necrotising myopathy. Ann Rheum Dis, 2006; 65(12): 1635–38

4. Preusse C, Goebel HH, Held J et al: Immune-mediated necrotizing myopathy is characterized by a specific Th1-M1 polarized immune profile. Am J Pathol, 2012; 181(6): 2161–71

5. Klein M, Mann H, Plestilova L et al: Increasing incidence of immune-mediated necrotizing myopathy: single-centre experience. Rheumatology (Oxford), 2015; 54(11): 2010–14

6. Pinal-Fernandez I, Parks C, Werner JL et al: Longitudinal course of disease in a large cohort of myositis patients with autoantibodies recognizing the signal recognition particle. Arthritis Care Res (Hoboken), 2017; 69(2): 263–70

7. Lu X, Peng Q, Wang G: Discovery of new biomarkers of idiopathic inflammatory myopathy. Clin Chim Acta, 2015; 444: 117–25

8. Ambang T, Tan JS, Ong S et al: Clinicopathological features of telbivudine-associated myopathy. PLoS One, 2016; 11(9): e0162760

9. Chung T, Christopher-Stine L, Paik JJ et al: The composition of cellular infiltrates in anti-HMG-CoA reductase-associated myopathy. Muscle Nerve, 2015; 52(2): 189–95

10. Loganathan P, Oddis CV, Aggarwal R: Immune-mediated statin myopathy. Expert Rev Clin Immunol, 2016; 12(1): 33–38

11. Mammen AL: Necrotizing myopathies: beyond statins. Curr Opin Rheumatol, 2014; 26(6): 679–83

12. Musset L, Allenbach Y, Benveniste O et al: Anti-HMGCR antibodies as a biomarker for immune-mediated necrotizing myopathies: A history of statins and experience from a large international multi-center study. Autoimmun Rev, 2016; 15(10): 983–93

13. Ohnuki Y, Suzuki S, Shiina T et al: HLA-DRB1 alleles in immune-mediated necrotizing myopathy. Neurology, 2016; 87(18): 1954–55

14. Picard C, Vincent T, Lega JC et al: Heterogeneous clinical spectrum of anti-SRP myositis and importance of the methods of detection of anti-SRP autoantibodies: A multicentric study. Immunol Res, 2016; 64(3): 677–86

15. Shovman O, Gilburd B, Chayat C et al: Anti-HMGCR antibodies demonstrate high diagnostic value in the diagnosis of immune-mediated necrotizing myopathy following statin exposure. Immunol Res, 2017; 65(1): 276–81

16. Mescam-Mancini L, Allenbach Y, Hervier B et al: Anti-Jo-1 antibody-positive patients show a characteristic necrotizing perifascicular myositis. Brain, 2015; 138(Pt 9): 2485–92

17. Basharat P, Christopher-Stine L: Immune-mediated necrotizing myopathy: Update on diagnosis and management. Curr Rheumatol Rep, 2015; 17(12): 72

18. Rinnenthal JL, Goebel HH, Preusse C et al: Inflammatory myopathy with abundant macrophages (IMAM): The immunology revisited. Neuromuscul Disord, 2014; 24(2): 151–55

19. Dalakas MC: Pathogenesis and therapies of immune-mediated myopathies. Autoimmun Rev, 2012; 11(3): 203–6

20. Nagaraju K, Raben N, Loeffler L et al: Conditional up-regulation of MHC class I in skeletal muscle leads to self-sustaining autoimmune myositis and myositis-specific autoantibodies. Proc Natl Acad Sci USA, 2000; 97(16): 9209–14

21. Cong L, Pu CQ, Shi Q et al: Complement membrane attack complex is related with immune-mediated necrotizing myopathy. Int J Clin Exp Pathol, 2014; 7(7): 4143–49

22. Cheng SC, Sprong T, Joosten LA et al: Complement plays a central role in *Candida albicans*-induced cytokine production by human PBMCs. Eur J Immunol, 2012; 42(4): 993–1004

23. Tramontini NL, Kuipers PJ, Huber CM et al: Modulation of leukocyte recruitment and IL-8 expression by the membrane attack complex of complement (C5b-9) in a rabbit model of antigen-induced arthritis. Inflammation, 2002; 26(6): 311–19

24. Viedt C, Hansch GM, Brandes RP et al: The terminal complement complex C5b-9 stimulates interleukin-6 production in human smooth muscle cells through activation of transcription factors NF-kappa B and AP-1. FASEB J, 2000; 14(15): 2370–72

25. Kilgore KS, Schmid E, Shanley TP et al: Sublytic concentrations of the membrane attack complex of complement induce endothelial interleukin-8 and monocyte chemoattractant protein-1 through nuclear factor-kappa B activation. Am J Pathol, 1997; 150(6): 2019–31

26. Takada K, Bookbinder S, Furie R et al: A pilot study of eculizumab in patients with dermatomyositis. Arthritis and Rheumatism, 2002; 46(9): S489

27. Reis ES, Mastellos DC, Yancopoulou D et al: Lambris, applying complement therapeutics to rare diseases. Clin Immunol, 2015; 161(2): 225–40