# Clinical and Pathological Features of Immune-Mediated Necrotising Myopathies in a Single-Centre Muscle Biopsy Cohort

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## Abstract

# Objective

Immune-mediated necrotising myopathy (IMNM) is a recently entitled novel subset of idiopathic inflammatory myopathies (IIM) characterized by significant elevated creatine kinase (CK) level, muscle weakness and predominant muscle fibre necrosis in muscle biopsy. This study aimed to investigate the clinical and pathological characteristics of patients with IMNM in our single-centre muscle biopsy cohort.

# Methods

A total of 860 patients who had muscle biopsy reports in our centre from May 2008 to December 2017 were enrolled in this study. IMNM was diagnosed in according with 2018 European Neuromuscular Centre (ENMC) clinicopathological diagnostic criteria for IMNM.

# Results

The muscle biopsy cohort consisted of 531 patients with IIM (61.7%), 253 patients with non-IIM (29.4%), and 76 undiagnosed patients (8.8%). Among IIM patients, polymyositis (PM), dermatomyositis(DM), amyopathic dermatomyositis, juvenile DM, and inclusion body myositis were 182(21.2%), 236(27.4%), 83(9.7%), 18(2.1%) and 3(0.3%), respectively. In PM subgroup, 59 patients met serological and pathological characteristics of IMNM according to 2018 ENMC criteria including 29 anti-SRP-positive patients,10 anti-HMGCR-positive patients and 20 MSA-negative patients. Limb girdle muscular dystrophy (LGMD) 2B and lipid storage myopathy (LSM) were 29 and 16 respectively, which present similar manifestations of IMNM with elevated CK levels and muscle weakness among non-IIM group. IMNM patients had older age of onset (mean: 42.25 vs 21.66 and 24.56, $p$<0.0001), shorter duration of diseases (mean: 22.56 vs 66.69 and 48.94, $p$<0.0001) and more frequent of dysphagia (33.9% vs 3.4% and 6.3%, $p$<0.0001) compare to patients with LGMD 2B and LSM. Muscle biopsy from IMNM patients showed frequent muscle fibre necrosis (96.6% vs 72.4% and 56.3%, $p$<0.0001), overexpression of MHC-I on sarcolemma (81.4% vs 37.9% and 12.9%, $p$<0.0001) and $CD4^+$ T cell endomysial infiltration (89.9% vs 53.6% and 50%, $p$<0.0001) compared with LGMD 2B and LSM patients.

# Conclusions

It is easy to distinguish IMNM from other subtype of IIM according to clinical symptoms and MSAs profiles. However, distinguishing IMNM from disorders clinically similar non-IIM need to combine with clinical, serological and pathological features.

## Background

Idiopathic inflammatory myopathies (IIMs) are a group of heterogeneous autoimmune diseases characterised by inflammatory infiltration of skeletal muscle, elevated creatine kinase (CK) levels, and muscle weakness [1, 2]. Conventionally, early IIM is classified into dermatomyositis (DM) and polymyositis (PM), based on the presence or absence of a rash [3, 4]; however, subsequent studies have found that the pathological characteristics of PM and DM are completely different. The invasion of non-necrotic muscle fibres by cytotoxic $CD8^+$ T cells and upregulation of major histocompatibility complex I (MHC-I) on the sarcolemma are key pathological diagnostic features of PM [5]. However, more studies have found that typical pathological characteristics of the $CD8^+$ T/major histocompatibility complex (MHC-I) are not common in PM and that PM has been overdiagnosed [6]. Therefore, in 2004, the European Neuromuscular Centre (ENMC) proposed a new subclass of IIM with pathological manifestations of myocyte necrosis and less inflammation, called immune-mediated necrotising myopathy (IMNM) [7]. IMNM diagnostic criteria were revised by ENMC in 2017, and myositis-specific antibody (MSA) profiles were given consideration in the IMNM criteria. Thus, patients with anti-signal recognition particle (SRP) or anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) antibodies can be diagnosed with IMNM, although IMNM cannot be excluded in seronegative patients [8]. Therefore, seronegative IMNM patients are clinically easily confused with PM. Additionally, it is difficult to distinguish IMNM from other myopathies, such as muscular dystrophy and congenital myopathy, based on the pathological characteristics of myocyte necrosis. Therefore, it is meaningful to analyse the clinical and pathological characteristics of IMNM in detail, especially the differences between IMNM and similar myopathies, such as PM and muscular dystrophy.

In this study, we investigated the clinical and pathological characteristics of IMNM in a single-centre muscle biopsy cohort and analysed the differences between IMNM and its mimics.

# Methods

## *Patients*

This study enrolled 860 patients who underwent muscle biopsy in the Department of Rheumatology of China-Japan Friendship Hospital between May 2008 and December 2017. All patients were re-diagnosed according to the 2017 European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) criteria for IIM [9] and 2018 ENMC criteria for IMNM [8]. Clinical and laboratory data of all the recruited patients were collected retrospectively. Muscle strength was measured by the Medical Research Council Manual Muscle Testing Scale (grade 0-5), and severe muscle weakness was defined as a grade $\leq 3$ for muscle strength [10]. The categories and diagnosis strategy for non-IIM were based on the diagnostic criteria accordingly (see Additional file 1). All patients had assigned information consent. This study was approved by the Research Review Committee and Ethical Review Committee of the China-Japan Friendship Hospital (approval number: 2019-SDZL-3).

## *Detection of MSA and myositis-associated antibodies (MAA)*

Sera obtained from patients were stored at -80°C. MSA, including anti-SRP, anti-EJ, anti-Jo-1, anti-OJ, anti-PL-12, anti-PL-7, anti-Mi-2, anti-MDA5, anti-TIF1γ, anti-NXP2, and anti-SAE, as well as MAA, including anti-Ku, anti-PM-Scl 100, anti-PM-Scl 75, and anti-Ro-52, were detected by immunoblots (Euroimmun, Lübeck, Germany). Anti-HMGCR autoantibodies were tested using an enzyme-linked immunosorbent assay (Inova Diagnostics Inc., San Diego, CA, USA).

## *Muscle biopsy*

Muscle biopsy specimens from all patients were obtained using open-muscle biopsy. Fresh muscle biopsy specimens were cut into 7-μm frozen sections using cryostat frozen sections (Thermo Cryotome E) and stained using haematoxylin-eosin, periodic acid-Schiff, oil red O, modified Gomori's trichrome, NADH-tetrazolium reductase, succinate dehydrogenase, cytochrome C oxidase, and myosin ATPase. Immunohistochemistry staining for dysferlin, dystrophin, α-sarcoglycans to δ-sarcoglycans, α-dystroglycans and β-dystroglycans, MHC-I, CD4, CD8, CD20, and CD68, and major attack complex (MAC) was performed using the avidin-biotin-peroxidase complex method as previously described [11]. All reagents used were purchased from Abcam (Cambridge, UK).

## *Genetic testing*

Patients with suspected hereditary myopathy determined by clinical and pathological evidence were required to undergo genetic testing by next-generation sequencing (NGS). Genomic DNA was extracted from peripheral blood or muscle tissues using standard procedures. Proband-only targeted NGS was performed by a commercial company (MyGenostics, Inc., Beijing, China) according to the manufacturer's instructions, using a clinical exome capture panel containing 4231 disease-causing genes. Sanger sequencing with specific primers was performed to confirm the variants detected by NGS [12].

## *Statistical analyses*

Statistical analysis was performed using SPSS software (version 24.0; IBM Corp., Armonk, USA). Categorical variables were expressed as percentages and absolute frequencies, and continuous features were reported as mean±standard deviation or median (interquartile range). Comparisons among different groups were performed using Student's t test, Mann–Whitney U test, chi-square test, or Fisher's exact test where appropriate. If overall $p<0.05$, pairwise comparisons were performed, and Bonferroni correction was used. Bonferroni-adjusted $p<0.017$ was considered significantly different between the two groups.

# Results

# Classification and distribution of diseases in the muscle biopsy cohort

Our muscle biopsy cohort consisted of 531 IIM patients (61.7%), 253 non-IIM patients (29.4%), and 76 undiagnosed patients (8.8%) with a total of 860. The mean age of onset was (41.32±16.52) years, with disease course of (32.29±53.82) months. The majority of the patients were women (M:F = 310:550). IIM cases were classified as PM (n=185), DM (n=236), amyopathic DM (ADM) (n=83), juvenile DM (n=18), other types of juvenile myositis (n=9), and inclusion body myositis (n=3). In the PM subgroup, 59 patients were reclassified as IMNM according to the 2018 ENMC criteria (Table 1). Taking MSA profiles into consideration to classify PM further, 22 patients were defined as having strict PM without any MSA or skin rash.

Table 1
Classification and distribution of diseases in muscle biopsy cohort

| Classification of muscular diseases | Frequency | Proportion (%) |
|---|---|---|
| Idiopathic inflammatory myopathy | 531 | 61.7 |
| Polymyositis | 182 | 21.2 |
| Immune-mediated necrotising myopathy | 59 | 6.9 |
| Other polymyositis | 123 | 14.3 |
| Dermatomyositis | 236 | 27.4 |
| Amyopathic dermatomyositis | 83 | 9.7 |
| Juvenile dermatomyositis | 18 | 2.1 |
| Juvenile myositis other than juvenile dermatomyositis | 9 | 1.0 |
| Inclusion body myositis | 3 | 0.3 |
| Non-idiopathic inflammatory myopathy | 253 | 29.4 |
| Muscular Dystrophy | 66 | 7.7 |
| Limb grindle muscular dystrophy 2B | 29 | 3.4 |
| Other types muscular dystrophy | 37 | 4.3 |
| Metabolic myopathy | 21 | 2.4 |
| Lipid storage myopathy | 16 | 0.7 |
| Mitochondrial myopathy | 3 | 0.3 |
| Glycogen storage disease | 2 | 0.2 |
| Endocrine myopathy | 13 | 1.5 |
| Myopathies associated with hypothyroidism | 9 | 1.0 |
| Hypokalemic periodic paralysis | 3 | 0.3 |
| Hypophosphorus rickets | 1 | 0.1 |
| Neurogenic myopathy | 19 | 2.2 |
| Other CTD accompanied skeletal muscle symptoms | 62 | 7.2 |
| Myopathy induced by external factors | 25 | 2.9 |
| Asymptomatic hyperCKemia | 47 | 5.5 |
| Undiagnosed | 76 | 8.8 |

CTD, connective tissue diseases; CK, creatine kinase

| Classification of muscular diseases | Frequency | Proportion (%) |
|---|---|---|
| Total | 860 | 100 |

CTD, connective tissue diseases; CK, creatine kinase

# Clinical and pathological characteristics of IMNM in the IIM group

The IIM group included 236 DM (44.4%), all with muscle weakness and skin involvement, consistent with the diagnostic criteria for DM. The most common MSAs in DM were anti-MDA5 (19.9%), anti-TIF1-$\gamma$ (18.6%), and anti-animo-tRNA-synthetase (ARS) antibodies (11.9%). However, DM also included four anti-SRP patients and three anti-HMGCR patients given the presence of heliotrope's rash and Gottron's sign. ADM accounted for 15.6% in IIM given the absence of muscle manifestations. Therefore, IMNM was easier to distinguish from DM and ADM according to the clinical symptoms and MSA profiles. Only three patients had inclusion body myositis in our cohort, with higher onset age and longer disease duration. Compared with strict PM, IMNM had higher prevalence of severe muscle weakness (42.4% vs 18.2%, $p$<0.01) and higher CK level [2242 (894, 5486) vs 392 (52, 570), $p$<0.01]. However, other PM had higher frequency of fever, myalgia, arthritis, and skin involvement and interstitial lung diseases (ILD) than IMNM (28.5% vs 8.5%, 33.3% vs 10.2%, 47.2% vs 10.2%, and 54.4% vs 6.8%, respectively, $p$<0.01). Notably, 36.6% of other PMs were positive for anti-ARS antibodies, which may have attributed to the high prevalence of extramuscular manifestations (Table 2).

Table 2
Clinical characteristics of IMNM and other types of IIM

| Characteristics | IMNM(n=59) | PM other than IMNM(n=123) | DM(n=236) | ADM(n=83) | IBM(n=3) |
|---|---|---|---|---|---|
| Female | 38(64.4) | 81(65.9) | 168(71.2) | 64(77.1) | 1(33.3) |
| Age of onset | 42.25±14.62 | 47.06±13.22 | 46.87±13.67 | 48.01±11.63 | 55±11.13 |
| Duration (months) | 23.34±27.24 | 21.76±37.34 | 21.60±48.82 | 22.02±36.72 | 70±45.03 |
| Fever | 5(8.5) | 35(28.5) | 71(30.1) | 18(21.7) | 0 |
| Loss of weight | 16(27.1) | 37(30.1) | 71(30.1) | 21(25.3) | 0 |
| Muscle weakness | 53(89.9) | 81(65.9) | 236(100) | 0 | 3(100) |
| Severe muscle weakness | 25(42.4) | 13(10.6) | 56(23.7) | 0 | 1(33.3) |
| Dysphagia | 20(33.9) | 28(22.8) | 73(30.9) | 10(12) | 0 |
| Muscular atrophy | 1(1.7) | 0 | 2(0.8) | 0 | 0 |
| Myalgia | 19(32.2) | 57(46.3) | 132(55.9) | 21(25.3) | 0 |
| Arthralgia | 6(10.2) | 41(33.3) | 93(39.4) | 32(38.6) | 0 |
| Skin involvement | 6(10.2) | 58(47.2) | 236(100) | 83(100) | 1(33.3) |
| Heliotrope rash | 0 | 0 | 190(80.5) | 59(71.1) | 0 |
| Mechanics' hands | 0 | 16(13) | 97(41.1) | 26(43.4) | 0 |
| Gottron's sign | 0 | 0 | 165(69.9) | 67(80.7) | 0 |
| V sign | 5(8.5) | 18(14.6) | 137(58.1) | 53(63.9) | 1(33.3) |
| Shawl sign | 3(5.1) | 14(11.4) | 100(100) | 40(48.2) | 0 |
| Raynaud phenomenon | 1(1.7) | 11(8.9) | 14(5.9) | 12(14.5) | 0 |
| Interstitial lung diseases | 4(6.8) | 67(54.4) | 121(51.3) | 46(55.4) | 0 |
| Malignant tumor | 3(5.2) | 5(4.2) | 24(10.3) | 5(6) | 0 |

IIM, idiopathic inflammatory myopathies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; ANA, antinuclear antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

| Characteristics | IMNM(n=59) | PM other than IMNM(n=123) | DM(n=236) | ADM(n=83) | IBM(n=3) |
|---|---|---|---|---|---|
| Other connective tissue diseases | 4(6.8) | 29(23.6) | 27(11.4) | 7(8.4) | 0 |
| ALT (0-40U/L) | 105(54,227) | 44(28,85) | 42(25,87) | 28(18,49) | - |
| AST (0-40U/L) | 80(36,151) | 36(21,74) | 43(23,96) | 25(17,43) | - |
| LDH (100-250IU/L) | 514(313,773) | 291(225,404) | 278(210,424) | 207(171,278) | - |
| CK (26-200IU/L) | 2242(894,5486) | 300(55,1519) | 58(32,130) | 299(93,282) | - |
| ANA (>1:40) | 34(57.6) | 71(57.7) | 109(48.2) | 41(49.4) | 0 |
| MSA | | | | | |
| Anti-MDA5 | 0 | 4(3.3) | 47(19.9) | 19(22.9) | 0 |
| Anti-NXP2 | 0 | 10(8.1) | 20(8.5) | 2(2.4) | 0 |
| Anti-TIF1-$\gamma$ | 0 | 2(1.6) | 44(18.6) | 11(13.3) | 0 |
| Anti-Mi-2 | 0 | 5(4.1) | 13(5.5) | 5(6) | 0 |
| Anti-SAE | 0 | 1(0.8) | 3(1.3) | 3(3.6) | 0 |
| Anti-ARS | 0 | 45(36.6) | 28(11.9) | 14(16.9) | 0 |
| Anti-SRP | 29(49.2) | 0 | 4(1.7) | 0 | 0 |
| Anti-HMGCR | 10(16.9) | 0 | 3(1.3) | 0 | 0 |
| MAA | | | | | 0 |
| Anti-Ro-52 | 13(22) | 28(23) | 57(24.3) | 16(19.3) | 0 |
| Anti-Ku | 1(1.8) | 2(1.7) | 2(0.8) | 1(1.3) | 0 |
| Anti- PM/Scl | 1(1.8) | 1(0.9) | 2(0.9) | 0 | 0 |
| Anti-AMA-M2 | 1(1.8) | 1(0.9) | 3(1.3) | 0 | 0 |

IIM, idiopathic inflammatory myopathies; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; ANA, antinuclear antibodies; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

# Clinical and pathological characteristics of IMNM compared with non-IIM

Limb-girdle muscular dystrophy (LGMD) 2B and lipid storage myopathy (LSM) are the two most common non-IIM disorders that can be definitively diagnosed in our muscle biopsy cohort. They accounted for 11.5% and 6.3% of non-IIM cases, respectively. LGMD 2B and LSM patients shared similar clinical and

laboratory features of muscle weakness and elevated CK with IMNM. However, IMNM patients had a higher onset age (42.25±14.62 vs 21.66±7.86 and 24.56±10.78, $p$<0.0001), shorter duration (22.56±27.24 vs 66.69±67.67 and 24.56±10.78, $p$<0.0001), and more frequent dysphagia (33.9% vs. 3.4% and 6.3%, $p$<0.0001) compared with LGMD 2B and LSM patients. The prevalence of upper limb weakness (55.9% vs. 43.8% vs. 24.1%, $p$<0.05), proximal dominance (84.7% and 68.8% vs. 27.6%, $p$<0.05), neck weakness (20.3% and 43.8% vs. 3.4%, $p$<0.05), and severe muscle weakness (39% and 31.3% vs. 13.8%, $p$<0.05) were higher in IMNM and LSM than in LGMD 2B. The highest peak CK value was observed for LGMD 2B [7036 (3098, 9866)]. LSM patients had the highest level of LDH [808 (341, 1248)] among the three groups. In addition, the prevalence of ANA (>1:40) was higher than that in IMNM compared to LGMD 2B and LSM (58.2% vs. 0 and 6.25%, $p$<0.01). MSA was positive in 67.8% (40/59) of IMNM patients, including 29 cases with anti-SRP-positive and 10 with anti-HMGCR-positive. Nevertheless, no LGMD 2B and LSM patients had MSA positivity (Table 3).

Table 3
Comparison of clinical and laboratory characteristics between IMNM, LGMD 2B and LSM

| Characteristics | IMNM(n=59) | LGMD 2B(n=29) | LSM(n=16) | P |
|---|---|---|---|---|
| Female | 38(64.4) | 22(75.9) | 7(43.8) | 0.098 |
| Age of onset | 42.25±14.62 | 21.66±7.86 | 24.56±10.78 | <0.0001* |
| Late onset (≥40) | 30(50.8) | 0 | 1(6.3) | <0.0001* |
| Duration (months) | 22.56±27.24 | 66.69±67.67 | 48.94±79.07 | 0.001* |
| Muscle weakness | 53(89.8) | 27(93.1) | 15(93.8) | 0.818 |
| Lower limb weakness | 42(71.2) | 17(58.6) | 11(68.8) | 0.493 |
| Upper limb weakness | 33(55.9) | 7(24.1) | 7(43.8) | 0.019† |
| Lower limb dominant | 43(72.9) | 24(82.8) | 16(100) | 0.051 |
| Proximal involvement | 41(69.5) | 15(51.7) | 12(75) | 0.175 |
| Distant involvement | 33(55.9) | 13(44.8) | 6(37.5) | 0.343 |
| Proximal dominant | 50(84.7) | 8(27.6) | 11(68.8) | <0.0001† |
| Severe muscle weakness | 27(39) | 4(13.8) | 5(31.3) | 0.042† |
| Asymmetric | 0 | 10(34.5) | 0 | 0.007† |
| Neck involvement | 12(20.3) | 1(3.4) | 7(43.8) | 0.004† |
| MMT8 | 64(49,76) | 76(70,80) | 69(62.5,78) | <0.0001** |
| Dysphagia | 20(33.9) | 1(3.4) | 1(6.3) | 0.001* |
| Muscular atrophy | 7(11.9) | 8(27.6) | 2(12.5) | 0.156 |
| Myalgia | 20(33.9) | 7(24.1) | 10(62.5) | 0.059 |
| ALT (0-40U/L) | 105(54,227) | 90(64,157) | 74(46,150) | 0.575 |
| AST (0-40U/L) | 80(36,151) | 68(43,95) | 62(36,209) | 0.956 |
| LDH (100-250IU/L) | 514(313,773) | 343(280,455) | 808(341,1248) | 0.006‡ |

IMNM, immune-mediated necrotising myopathy; LGMD, limb-girdle muscular dystrophy; LSM, lipid storage myopathy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase; CK, creatine kinase; ANA, antinuclear antibodies. * Bonferroni P<0.017 between IMNM and LGMD2B or LSM; † Bonferroni P<0.017 between LGMD 2B and IMNM or LSM; ** Bonferroni P<0.017 between IMNM and LGMD2B; ‡ Bonferroni P<0.017 between LSM and IMNM or LGMD 2 B,# Bonferroni P<0.017 between IMNM, LGMD2B, and LSM pairwise.

| Characteristics | IMNM(n=59) | LGMD 2B(n=29) | LSM(n=16) | P |
|---|---|---|---|---|
| CK (26-200IU/L) | 2242(894,5486) | 4383(1557,6485) | 857(325,1618) | 0.001# |
| Peak CK (26-200IU/L) | 5959(2755,10200) | 7036(3098,9866) | 1444(665,2980) | 0.007# |
| ANA (>1:40) | 33/57(58.2) | 0 | 1(6.25) | <0.0001* |

IMNM, immune-mediated necrotising myopathy; LGMD, limb-girdle muscular dystrophy; LSM, lipid storage myopathy; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactic dehydrogenase; CK, creatine kinase; ANA, antinuclear antibodies. * Bonferroni P<0.017 between IMNM and LGMD2B or LSM; † Bonferroni P<0.017 between LGMD 2B and IMNM or LSM; ** Bonferroni P<0.017 between IMNM and LGMD2B; ‡ Bonferroni P<0.017 between LSM and IMNM or LGMD 2 B,# Bonferroni P<0.017 between IMNM, LGMD2B, and LSM pairwise.

The main pathological features of IMNM muscle were fibre necrosis (96.6%), MHC-I overexpression on the sarcolemma (81.4%), and $CD4^+$ T cell infiltration (89.9%). However, muscle fibre necrosis was also observed in LGMD 2B (72.4%) and LSM (56.3%) patients. IMNM patients showed more frequent $CD4^+$ T cell perimyosial expression than LGMD 2B and LSM patients (32.2% vs 7.1% and 12.5%, $p<0.01$), although the expression of $CD4^+$ T cells on endomysia was not different among the three groups. MHC-I expression also occurred in 37.9% of LGMD 2B patients and 12.5% of LSM patients, although diffuse MHC-I expression was only observed in IMNM (23.7%) patients. MAC deposition was not a specific pathological performance of IMNM, which also occurred in 64% of LGMD 2B patients. However, only 20% of LSM patients present with MAC deposition (Table 4 and Figure 1).

Table 4
Comparison of pathological characteristics between IMNM, LGMD 2B and LSM

| Characteristics | IMNM (n=59) | LGMD 2B(n=29) | LSM (n=16) | P |
|---|---|---|---|---|
| Muscle fibre necrosis | 57(96.6) | 21(72.4) | 9(56.3) | <0.0001* |
| Mild necrosis | 27(45.8) | 17(58.6) | 7(43.8) | |
| Severe necrosis | 30(50.8) | 4(13.8) | 2(12.5) | <0.0001* |
| Connective tissue proliferation | 25(42.4) | 11(37.9) | 0 | 0.007† |
| MHC-I expression on sarcolemma | 48(81.4) | 11(37.9) | 2(12.5) | <0.0001* |
| Focal expression | 30(50.8) | 11(37.9) | 2(12.5) | |
| Diffuse expression | 14(23.7) | 0 | 0 | <0.0001* |
| $CD4^+$ T cell | 53(89.9) | 15/28(53.6) | 8(50) | <0.0001* |
| Endomyosial | 34(57.6) | 15/28(53.6) | 7(43.8) | 0.611 |
| Perimyosial | 19(32.2) | 2/28(7.1) | 2(12.5) | 0.019* |
| $CD8^+$ T cell | 40(67.8) | 14/28(48.3) | 5(31.3) | 0.021 |
| Endomyosial | 25(42.4) | 14/28(50) | 5(31.3) | 0.480 |
| Perimyosial | 11(18.6) | 2/28(7.1) | 1(6.3) | 0.222 |
| $CD68^+$ macrophage | 42/57(73.7) | 14/25(56) | 10/15(66.7) | 0.284 |
| Endomyosial | 32/57(56.1) | 14/25(56) | 10(66.7) | 0.748 |
| Perimyosial | 15/57(26.3) | 2/25(8) | 1/15(6.7) | 0.063 |
| MAC | 38/57(66.7) | 16/25(64) | 3/15(20) | 0.004† |
| Sarcolemma of non-necrotic muscle fiber | 29/57(50.9) | 15/25(60) | 1/15(6.7) | 0.003† |
| Capillaries | 21/57(36.8) | 5/25(20) | 2/15(14.3) | 0.126 |

IMNM, immune-mediated necrotising myopathy; LGMD, limb-girdle muscular dystrophy; LSM, lipid storage myopathy; MHC, major histocompatibility complex; MAC, major attack complex. *P<0.017 between IMNM and LGMD 2B or LSM; † P<0.017 between LSM and IMNM or LGMD2B

# Discussion

IMNM is a subclass of IIM characterised by myocyte necrosis, which has been gradually recognised in recent years. Proximal muscle weakness and elevated CK levels are common features of muscular disorders [13]. Based on clinical symptoms alone and without considering muscle biopsy, autoantibody screening, or even genetic testing, it is difficult to distinguish IMNM from other subtypes of IIM as well as some non-IIM [14].

In our muscle biopsy cohort, 61.7% of patients had IIM. In the IIM group, DM was relatively easy to distinguish from IMNM by the presence of typical rashes (heliotrope sign and Gotrron sign) and DM-associated MSAs. In addition, 42.4% of IMNM cases presented with severe muscle weakness, which is higher than that in DM, which can also differentiate IMNM from DM.

According to the 2017 EULAR/ACR diagnostic criteria, 34.3% of IIM patients were diagnosed with PM. However, according to the 2018 ENMC criteria, 32.4% of PM patients could be diagnostically refined as IMNM, and only 3.8% of them met the pathological characteristics of typical PM. Anti-SRP and anti-HMGCR antibodies were the main MSAs of IMNM, although nearly one-third of PM patients were positive for anti-ARS antibodies, which may have contributed to the high prevalence of fever, arthritis, and ILD in PM [15].

LGMD 2B and LSM were the most common non-IIMs that shared similar manifestations with IMNM in our cohort [16, 17], in line with the high prevalence in the Chinese population [18]. However, IMNM has an older age of onset, while the other genetic myopathies have a younger onset age. In addition, the disease course of IMNM is shorter than that of hereditary myopathy. Demographic characteristics seem to vary according to the underlying aetiology. Middle-aged onset and subacute duration suggest IIM; however, young patients present with slowly progressive proximal muscle weakness that can be difficult to differentiate clinically from LGMD. Mohassel et al. [19] reported an anti-HMGCR-positive IMNM case with a more indolent disease course but favourable clinical response to immunotherapy, which is easy to confuse with muscular dystrophy. Tanboon et al. [20] also reported that concurrent anti-HMGCR antibodies and gene mutations indicated the possibility of co-occurrence of IMNM and muscular dystrophy. Thus, testing for these antiantibodies should be an essential part of the evaluation of children with symptoms resembling hereditary muscular disorders. In addition, upper limb weakness and dysphagia are more common in IMNM than in LGMD and LSM, although cervical flexor weakness is more common in LSM. Asymmetric muscle weakness is present only in LGMD 2B patients [21]. The above information reminds muscle specialists, neurologists, or rheumatologists about the necessity of a comprehensive and systemic examination of whole-body muscle strength for muscular diseases.

IMNM, LGMD 2B, and LSM had a significant elevation of CK in this study, although the highest level of peak CK appeared in LGMD 2B. A previous study reported that CK levels in IMNM are always up to 10-15 times the upper normal level, although in LGMD 2B, it can increase more than 20 times. The study also shows that significant CK elevation indicates a higher probability of muscular dystrophy than IMNM [22]. The level of LDH in LSM patients can reach 808 (341, 1248) U/L, with the highest value of up to 2433 U/L, which is higher than that in the other groups. Zhang et al. also observed predominantly higher levels

of LDH in LSM [23]. The reason for this is still unclear. LDH has isoforms of liver and muscle. The abnormally high level of LDH in LSM patients may be due to the presence of lipid or glucose metabolic dysfunction and increased liver types. Therefore, identifying the isoforms may help determine its source and distinguish IMNM from metabolic myopathy.

Muscle fibre necrosis is not a specific manifestation of IMNM, which also occurs in patients without IIM. However, the proportion and degree of fibre muscle necrosis were significantly higher than in non-IIM, and diffuse expression of MHC-I and $CD4^+$ T cell perimyosial infiltration were more specific in IMNM. MAC deposition is less common in LSM, indicating that the complement pathway is less involved in the pathogenesis of LSM. Histochemical staining of dysferlin, ORO, and PAS in patients with suspected muscular dystrophy and metabolic myopathy are helpful for clinicians to exclude IMNM from muscular dystrophy and metabolic myopathy [24–26].

One limitation of this study is that a large proportion of patients with CK elevation and/or muscle weakness cannot be definitively diagnosed despite undergoing autoantibodies, muscle biopsy, and even genetic testing. There is also current clinical confusion regarding the diagnosis of muscular diseases.

# Conclusion

This study investigated the distribution of various types of myopathies and analysed the characteristics of IMNM in a single-centre muscle biopsy cohort. It is still important for rheumatologists to distinguish IMNM from non-IIM and obtain an accurate diagnosis. In doing so, detailed analysis of the clinical and pathological characteristics of IMNM in detail is useful, especially the differences between IMNM and similar myopathies, such as PM and muscular dystrophy.

# Abbreviations

ACR
American College of Rheumatology
ARS
anti-animo-tRNA-synthetase
CK
creatine kinase
DM
dermatomyositis
ENMC
European Neuromuscular Centre
EULAR
European League Against Rheumatism
HMGCR
anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase

IIMs
idiopathic inflammatory myopathies
ILD
interstitial lung diseases
IMNM
immune-mediated necrotising myopathy
LGMD
limb girdle muscular dystrophy
LSM
lipid storage myopathy
MAA
myositis-associated antibodies
MAC
major attack complex
MHC-I
major histocompatibility complex I
MSA
myositis-specific antibody
NGS
next-generation sequencing
PM
polymyositis
SRP
anti-signal recognition particle

# Declarations

*Ethics approval and consent to participate:* This study was approved by the Research Review Committee and Ethical Review Committee of the China-Japan Friendship Hospital (approval number: 2019-SDZL-3). All participants had signed informed consent. All methods used in this study were carried out in accordance with the Declaration of Helsinki.

*Consent for Publication:* Not applicable.

*Availability of data and materials:* The data that support the findings of this study are available from the corresponding author but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of China-Japan Friendship Hospital.

*Competing interests:* The authors declare that they have no competing interests.

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*Authors' contributions:* HY. Yang collected and analysed data, drafted the manuscript; X. Lu conceived the hypothesis, analysed data and critically revised the manuscript and gave final approval; GC. Wang, QL. Peng revised the manuscript; XL. Tian, LN. Zhang, WL. Li, QY. Liu, collected and interpreted data. All authors have read and approved the final manuscript.

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# Figures

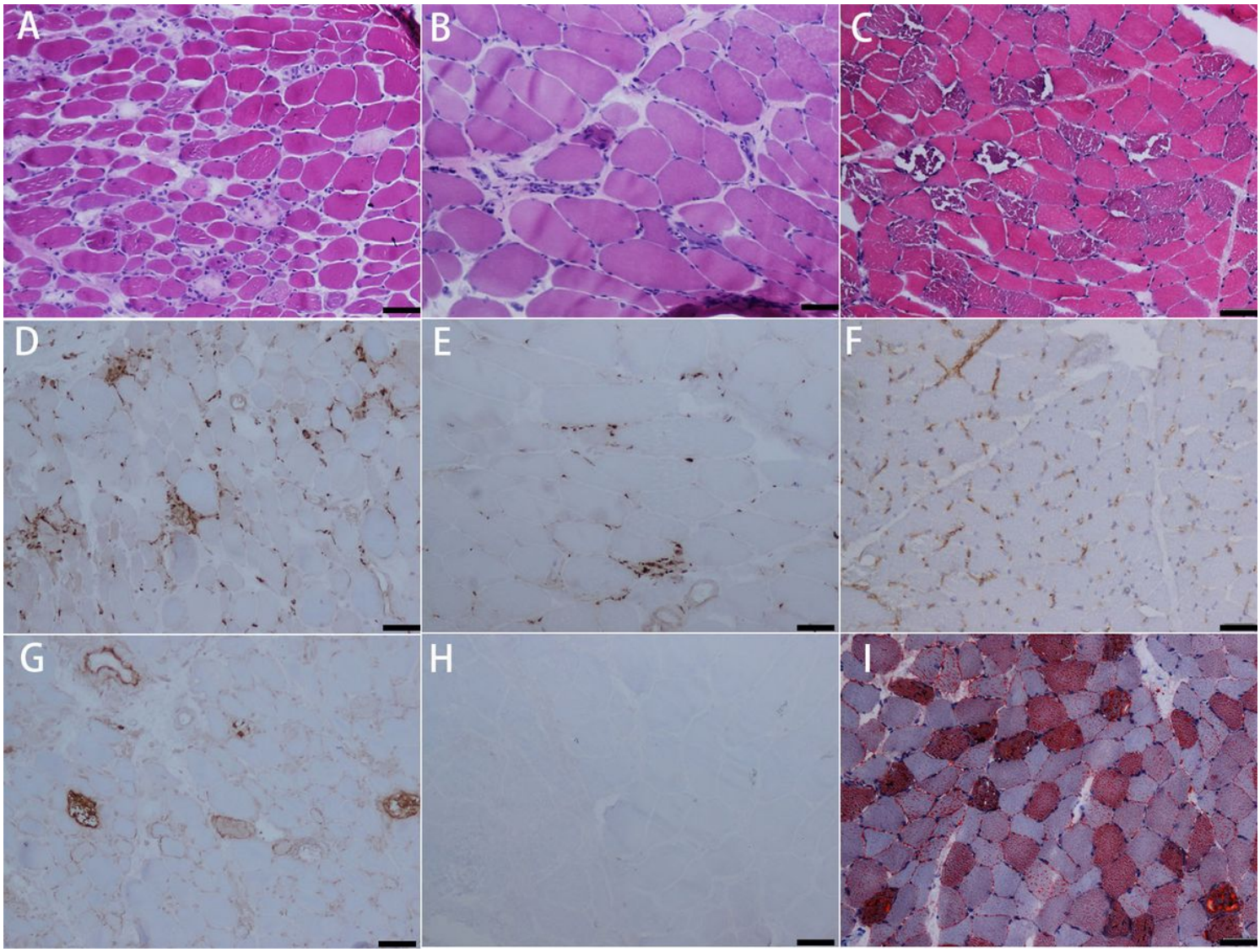


**Figure 1**

Pathological features of IMNM (a, d, g), LGMD 2B (b, e, h) and LSM (c, f, i). In IMNM patients, necrotic muscle fibres were distributed in endomysia (a), and $CD68^{+}$ macrophages (d) and MAC (g) expressed on necrotic myofibres. Scattered necrotic and regenerative myocytes were observed in patients with LGMD 2B (b), with $CD68^{+}$ macrophages expression (e). However, the expression of dysferlin was completely absent in LGMD 2B patients (h). Vascular muscle fibres and many atrophic fibres were found in patients with LSM (c). MHC-I occurred in vascular muscle fibres (f), and lipid droplet deposition was observed using oil red O staining (i) in LSM patients. (a-c) Haematoxylin and eosin staining; d-i, immunohistochemistry staining. Scale bar is 50 μm.

IMNM, immune-mediated necrotising myopathy; LGMD, limb-girdle muscular dystrophy; LSM, lipid storage myopathy.

# Supplementary Files

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