N-CAM expression: The study of muscle disease in a tertiary center of Thailand

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Research

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Abstract

Background

The neural cell adhesion molecule (N-CAM), also called CD56, is a cell-surface glycoprotein that mediates intercellular adhesive interactions in the nervous system. N-CAM is expressed in neuromuscular endplates, nerves, satellite cells, and embryonic muscle, but it is lost as development proceeds and is nearly absent from adult muscle. N-CAM re-expression was detected in a denervated, regenerating and degenerated muscle fibres. Muscle disease or myopathy is diagnosed based on the presence of denervated, regenerating and degenerated fibres on muscle biopsy combined with clinical information. Some regenerating fibres looked like normal fibre and were challenging to identify but showed positive N-CAM staining.

Objectives

To explore the expression of N-CAM in muscle disease.

Methods

N-CAM expression and interpretation were performed in a 3-year retrospective study of 75 muscle biopsies diagnosed with myopathies. The pathological findings and clinical information were also thoroughly reviewed.

Results

Of 75 myopathy patients, 41 (55%) cases were female, and 34 (45%) cases were male. The mean age was 35 years. The range of age was 3 months to 83 years. The expression of N-CAM and clinical information, including pathological findings, were analyzed using Fisher's exact versus chi-square tests. N-CAM expression data was performed in 75 samples, 35 (46.67%) were positive for N-CAM, and 40 (53.33%) were negative for N-CAM. The diagnosis of the muscle disease was nonspecific myopathy with 35 (46%) cases, inflammatory myopathy with 17 (23%) cases, neurogenic myopathy with 9 (12%) cases, muscular dystrophy with 7 (9%) cases, non-diagnostic myopathy with 5 (7%) cases, and mitochondrial myopathy with 2 (3%) cases. The inflammatory myopathy showed positive N-CAM in 15 out of 17 cases with statistical significance (p-value < 0.001, ORs (95% CI) 14.250 (2.960-68.606)). N-CAM was also positive in muscular dystrophy, neurogenic myopathy, and nonspecific myopathy but was not statistically significant in p-value. No positive N-CAM was found in mitochondrial myopathy and non-diagnostic myopathy.

Conclusion

N-CAM expression accompanied by the pattern of staining could be considered to help narrow down the differential diagnosis of myopathies. We recommend further study in a larger group.

1. Introduction

Muscle disease or myopathy is a neuromuscular disorder in which the primary symptom is muscle weakness due to dysfunction of muscle fibre. The condition can be acquired, familial, and congenital disorders of skeletal muscle. Common muscle diseases include muscular dystrophy, congenital myopathy, mitochondrial myopathy, inflammatory myopathy, and neurogenic myopathy[1]. Diagnosis of muscle disease requires all modalities; clinical examination, serologic markers, electrodiagnostic test, pathologic findings from the muscle biopsy specimen, and molecular genetic study to make a final diagnosis[2, 3]. Muscle biopsy is a useful tool for the diagnostic evaluation of muscle disease. Still, the pathological findings can vary from subtle myopathic changes to normal[2, 3]. Immunohistochemistry (IHC) is an ancillary tool in muscle diseases diagnosis[2, 3, 4]. The neural cell adhesion molecule (N-CAM), also called CD56, is a cell-surface glycoprotein that was discovered in the embryonic brain and which mediates intercellular adhesive interactions in the nervous system. It is also expressed in neuromuscular endplates, nerves, satellite cells, and embryonic muscle fibres to regulate nerve-muscle interaction[5, 6, 7, 8, 9].

N-CAM is expressed in some phases of fetal myogenesis, but is lost as development proceeds and is nearly absent from adult muscle[10]. N-CAM is expressed in satellite cells to activate and proliferate muscle fibre. It is believed that N-CAM is associated with the recruitment of stem cells after muscle injury and regeneration of muscle fibre[5, 7, 11, 12, 13]. After muscle fibre denervation or injury, it is re-expressed again in degenerated and regenerating fibres[5, 7, 11, 12, 13]. Many previous studies found that N-CAM is also presented in degenerated fibres of myopathy[14] and showed a possible association between these fibres in mitochondrial myopathies, inflammatory myopathies, and muscular dystrophies[14, 15, 16].

This study aims to explore the expression, including staining severity of N-CAM in common muscle diseases as a complement for diagnostic evaluation.

2. Materials And Methods

2.1 Muscle specimens

A retrospective study was performed on all muscle biopsy specimens from 2017–2019 in Ramathibodi Hospital. They included 75 clinical myopathy cases who had a muscle biopsy. The diagnosis criteria were based on both clinical correlation and histologic findings. Clinical features and patient characteristics including age, sex, serum creatine phosphokinase level, and specimen site were obtained from the patient's medical record.

2.2 Histologic evaluation

Muscles from biceps brachii or quadriceps femoris in each case were collected as 2 cm-long specimens.

The specimens were divided into three parts. The first part was prepared fresh for the snap-frozen section technique. The second part was performed in 10% neutral buffered formalin fixation. The third part was fixed in 3% glutaraldehyde for electron microscopic study.

The series of routine histochemistry stains included haematoxylin and eosin (H&E), enzymatic histochemistry, and immunohistochemistry. The muscle biopsies were evaluated and correlated with clinical information to diagnosis. (Fig. 1)

In our study, we used only biopsied muscle from snap frozen section to explore the N-CAM expression. The diagnosis of muscle diseases from clinical and histopathology findings was categorized as inflammatory myopathy, neurogenic myopathy, muscular dystrophy, mitochondrial myopathy, nonspecific myopathy and non-diagnostic specimen.

2.3 Immunohistochemical study

The muscle biopsy specimens were snap-frozen in isopentane and liquid nitrogen. Fresh muscle specimens were rapidly frozen in isopentane (− 150 °C) and cooled in liquid nitrogen (− 80 °C). Cryostat sections (10 µm) were cut and dried on glass slides at room temperature. No fixation or pretreatment was performed before the IHC analysis. Samples were incubated for 40 min with primary antibodies diluted in Bond Primary Antibody Diluent (Leica). Visualization with DAB and subsequent counterstaining with Mayer's haematoxylin were performed using the Bond Polymer Refine Detection System (Leica). Sections were then dehydrated, cleared, and mounted. The anti-CD56 (MRQ-42) rabbit monoclonal primary antibody (1:200, Cell Marque, MilliporeSigma, Rocklin, CA, USA) was optimized for use as a fully automated IHC assay on the BenchMark ULTRA (Ventana Medical Systems Inc., Tucson, AZ). Briefly, the primary antibody was applied for 1 hour at 36 °C; amplification was done for 12 minutes amplifier/12 minutes multimer (OptiView Amplification Kit (Ventana Medical Systems Inc.), and counterstained for 16 minutes with hematoxylin II and post-counterstained with bluing reagent for 4 minutes. Palatine tonsil tissues were used as the internal control.

2.4 Interpretation

The N-CAM positivity was shown by membrane staining in regenerating fibres. Generally regenerating fibres were easily identified by enlarged nuclei with a bluish stain sarcoplasm on H&E staining, as shown in Fig. 2A. The bluish stain is due to the increased concentration of RNA within the cell[2]. Some regenerating fibres looked like normal fibre and were challenging to identify but showed positive N-CAM staining. The N-CAM immunohistochemical results were divided into three groups. The strongly positive had intensely diffuse membrane staining, as shown in Fig. 2B. The weakly positive fibres had faint membrane staining, as shown in Fig. 2C. The negatively stained fibres show a complete loss of membrane staining, as shown in Fig. 2D.

2.5 Statistical analysis

The relationship between N-CAM expression and common muscle diseases were evaluated using Fisher's exact versus chi-square tests. Odds ratios (ORs) were estimated in myopathies which correlated with N-CAM expression.

The p-values less than 0.05 were considered statistically significant and ORs more than 1.0 indicated an increased risk among the compared muscle diseases, whereas ORs less than 1.0 indicated a decrease in risk in each muscle disease. All data were analyzed by using SPSS (version 25.0.0.0).

3. Results

3.1 Patient characteristic

Of 75 patients, 41 (55%) cases were female, and 34 (45%) cases were male. The mean age was 35 years. The specimens were collected from quadriceps femoris in 59 (78.67%) cases and collected from biceps brachii in 16 (21.33%) cases. (Fig. 3).

3.2 Common muscle diseases

The most common diagnosis was nonspecific myopathy with 35 (46%) cases, and the second most common diagnosis was inflammatory myopathy with 17 (23%) cases. There were 9 (12%) neurogenic myopathy cases, 7 (9%) muscular dystrophy cases, 5 (7%) non-diagnostic myopathy cases, and 2 (3%) cases of mitochondrial myopathy. (Fig. 4)

3.3 Correlation between muscle diseases and N-CAM expression

The result of N-CAM expression showed positive staining in 35 cases and negative staining in 40 cases.

The inflammatory myopathy showed positive N-CAM in 15 out of 17 cases with statistical significance (p-value < 0.001, ORs (95% CI) 14.250 (2.960-68.606)). N-CAM was also positive in muscular dystrophy, neurogenic myopathy, and nonspecific myopathy but was not statistically significant in p-value. No positive N-CAM was found in mitochondrial myopathy and non-diagnostic myopathy. (Tables 1 and 2)

Table 1
Categorization of muscle disease and its correlation with N-CAM expression

N-CAM expression	Categorization of muscle diseases (n = 75)					
	Muscular dystrophy n. (%)	Neurogenic myopathy n. (%)	Inflammatory myopathy n. (%)	Mitochondrial myopathy n. (%)	Nonspecific myopathy n. (%)	Non-diagnostic specimen n. (%)
Positive	6 (85.71%)	4 (44.44%)	15 (88.24%)	0 (0.00%)	10 (28.57%)	0 (0.00%)
Negative	1 (14.29%)	5 (55.56%)	2 (11.76%)	2 (100.00%)	25 71.43%)	5 (100.00%)

Table 2
Categorization of muscle disease and N-CAM expression

N-CAM expression	Categorization of muscle disease (n = 35)					
	Muscular dystrophy	Neurogenic myopathy	Inflammatory myopathy	Mitochondrial myopathy	Nonspecific myopathy	Non-diagnostic specimen
Present	6 (85.71%)	4 (44.44%)	15 (88.24%)	-	10 (28.57%)	-
p-value	0.030	0.887	< 0.001	-	0.003	-
ORs	8.069	0.903	14.250	-	0.240	-
(95% CI)	(0.920 – 0.732)	(0.223–3.666)	(2.960-68.606)		(0.091–0.635)	

We also correlated the positive group with the presence of regenerating fibres in histology. All positive N-CAM cases showed regenerating fibres in the muscle biopsy on the haematoxylin and eosin-stained slides review. The strongly positive N-CAM mostly appeared in inflammatory myopathy and muscular dystrophy. While the weakly positive N-CAM appeared in neurogenic myopathy and nonspecific myopathy. (Table 3)

Table 3
Categorization of muscle disease and characteristics of N-CAM expression

Characteristics of N-CAM expression	Categorization of muscle disease (n = 35)					
	Muscular dystrophy n. (%)	Neurogenic myopathy n. (%)	Inflammatory myopathy n. (%)	Mitochondrial myopathy n. (%)	Nonspecific myopathy n. (%)	Non-diagnostic specimen n. (%)
Stained grade	1 (16.67%)	4 (100.00%)	4 (26.67%)	-	8 (80.00%)	-
Low	5 (83.33%)	0 (0.00%)	11 (73.33%)	-	2 (20.00%)	-
High	0 (0.00%)	0 (0.00%)	0 (0.00%)	-	0 (0.00%)	-
Degenerated fibres	6 (100.00%)	4 (100.00%)	15 (100.00%)	-	10 (100.00%)	-
Absent						
Present						

4. Discussion

In this study, we showed that inflammatory myopathy was the only muscle disease that had a strong association with positive immunohistochemistry for N-CAM in muscle fibres (15/17 cases, 43%, p-value < 0.01) with an odds ratio of 14.250 (95% CI = 2.960-68.606), because pathogenesis in inflammatory myopathy is either antibody/immune complex mediated or cytotoxic T-cell mediated, which results in necrosis and regeneration of muscle fibres[7]. Regenerating fibre which appears as a morphologically normal fibre on haematoxylin and eosin stain and other histochemistry can be highlighted by immunohistochemistry against N-CAM.

We also found that only inflammatory myopathy and muscular dystrophy groups had strong-intensity immunohistochemistry against N-CAM (73% and 83%, respectively). This pattern of staining could be found in regenerating fibres of muscular dystrophy, even though the overall percentage of positive N-CAM in muscular dystrophy was insignificant (6/7 cases, 17%, p-value = 0.03).

In the mitochondrial myopathy group, our study only had 2 cases, and all cases showed negative immunohistochemistry for N-CAM. These results are limited for evaluation due to the small sample size of the specimen. The small sample size and unknown underlying pathomechanism may be the reason for the discrepancy with the previous studies.

A literature review revealed the case series of positive N-CAM in regenerating muscle fibre and fetal muscle fibre and negative N-CAM in normal adult muscle fibre[13], and the case series of positive N-CAM in inflammatory myopathy, neurogenic myopathy and muscular dystrophy[15, 16]. Nevertheless, Heuss D et al. found N-CAM positive cases in mitochondrial myopathy[14], while we found negative expression in both two mitochondrial myopathies in our study. The different results could be due to the small number of cases. (Table 4)

N-CAM positive fibre has been included as one of the suggestive muscle biopsy findings for dermatomyositis according to the 239th European Neuromuscular Centre (ENMC) international workshop classification in 2018[17]. (Table 5)

Table 4
Literature reviews of N-CAM expression compared to our study

Authors	Study design	Positive N-CAM	Negative N-CAM
Mechtersheimer G. et al, 1992 [13]	Case series (fresh tissue from biobank)	- Fetal muscle fibres - Regenerating muscle fibres - Satellite cells	- Adult muscle fibres
Heuss D. et al, 1995 [14]	Case series in mitochondrial myopathies	- All mitochondrial myopathies cases (n = 14) (with ragged-red fibres)	- Control (n = 8)
Winter A. et al, 1999 [15]	Case series	- Muscular dystrophy: 30.1 ± 6.8% of fibres (n = 14) - Inflammatory myopathy: 20.8 ± 6.3% of fibres (n = 29) - Neurogenic myopathy: 11.9 ± 11.6% of fibres (n = 39)	- Control < 1% (n = 4)
Casciola-Rosen L. et al, 2005 [16]	Case series in inflammatory myopathy	- Dermatomyositis: 35.5 ± 10.1 fibres/400x (n = 11) - Polymyositis: 26.9 ± 6.9 fibres/400x (n = 11)	- Control 5.2 ± 1.1 fibres/400x (n = 5)
Our study, 2020	Retrospective in 75 muscle biopsy specimens	- Inflammatory myopathy (n = 15; 88.24%) - Muscular dystrophy (n = 6; 85.71%) - Neurogenic myopathy (n = 4; 44.44%) - Nonspecific myopathy (n = 10; 28.57%)	- mitochondrial myopathy (n = 2) - non-diagnostic specimen (n = 5)

Table 5 The ENMC 2018 dermatomyositis classification criteria[17].

ENMC 2018 dermatomyositis classification criteria

1. A DM classification can be made if the following clinical and skin biopsy features are present*:

(a) Clinical exam findings (at least two of the following): Gottron's sign, Gottron's papules, and/or heliotrope rash.

(b) Skin biopsy findings: interface dermatitis.

2. A DM classification can be made if the following clinical features are present along with either DM muscle features** or a DM-specific autoantibody:

(a) Clinical exam findings (at least one of the following): Gottron's sign, Gottron's papules, and/or heliotrope rash.

*Based on established skin criteria that allow classification of clinically amyopathic DM.

**DM muscle features

1. Proximal muscle weakness.

2. Elevated muscle enzymes.

3. Suggestive DM muscle biopsy findings: lymphocytic infiltrates (often perivascular), evidence of perifascicular disease (perifascicular predominant fibres that are pale on COX staining and/or positive on NCAM staining).

4. Definitive DM muscle biopsy findings: perifascicular atrophy and/or perifascicular MxA overexpression with rare or absent perifascicular necrosis.

The DM muscle features requirement is met if patients have: (a) 1 and 2, (b) 1 and 3, (c) 2 and 3, or (d) 4

5. Conclusion

In conclusion, inflammatory myopathy showed a statistically significant correlation with positive N-CAM expression. The strongly positive N-CAM was found in inflammatory myopathy and muscular dystrophy. The weakly positive N-CAM was found in neurogenic myopathy and nonspecific myopathy. No case of mitochondrial myopathy or non-diagnostic myopathy reveal N-CAM expression. All positive N-CAM cases had regenerating muscle fibres on muscle biopsy on thorough review with haematoxylin and eosin-stained slides. N-CAM expression accompanied by the pattern of staining could be considered to help narrow down the differential diagnosis of myopathies. We recommend further study in a larger group.

Abbreviations

N-CAM
Neural cell adhesion molecule
IHC
immunohistochemistry
H&E
haematoxylin and eosin
ORs
Odds ratios

ENMC
European Neuromuscular Centre
DM
Dermatomyositis

Declarations

Ethics approval and consent to participate

The protocol for this study was approved by the Ethical Clearance Committee on Human Rights Related to Research Involving Human Subjects of the Faculty of Medicine Ramathibodi Hospital, Mahidol University (MURA) (COA no. 2019/721). All procedures performed in this study that involved human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standard. Written informed consent was obtained from all individual participants included in this study.

Consent for publication

Not applicable

Availability of data and materials

All data are presented in this paper, and there are no additional supporting files. The haematoxylin and eosin-stained and immunohistochemical slides are stored at the Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University.

Competing interests

Not applicable

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Authors' contributions

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Contributions

The authors made substantial contributions to the conception, design of the work, the acquisition, analysis, interpretation of data, drafted the work or substantively revised it, and approved the submitted version.

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Figures

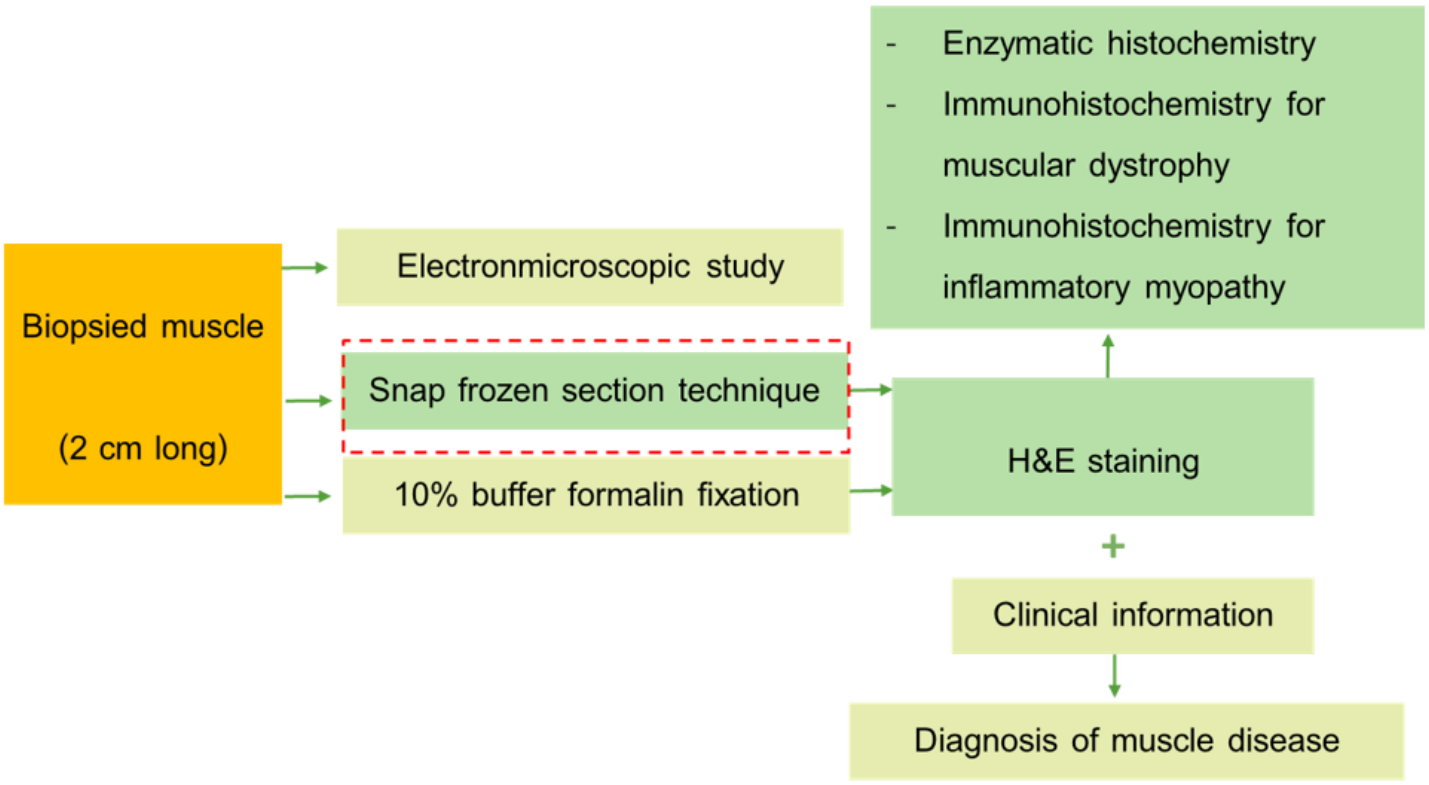

Figure 1

Illustrative flow chart of muscle biopsy in Ramathibodi Hospital

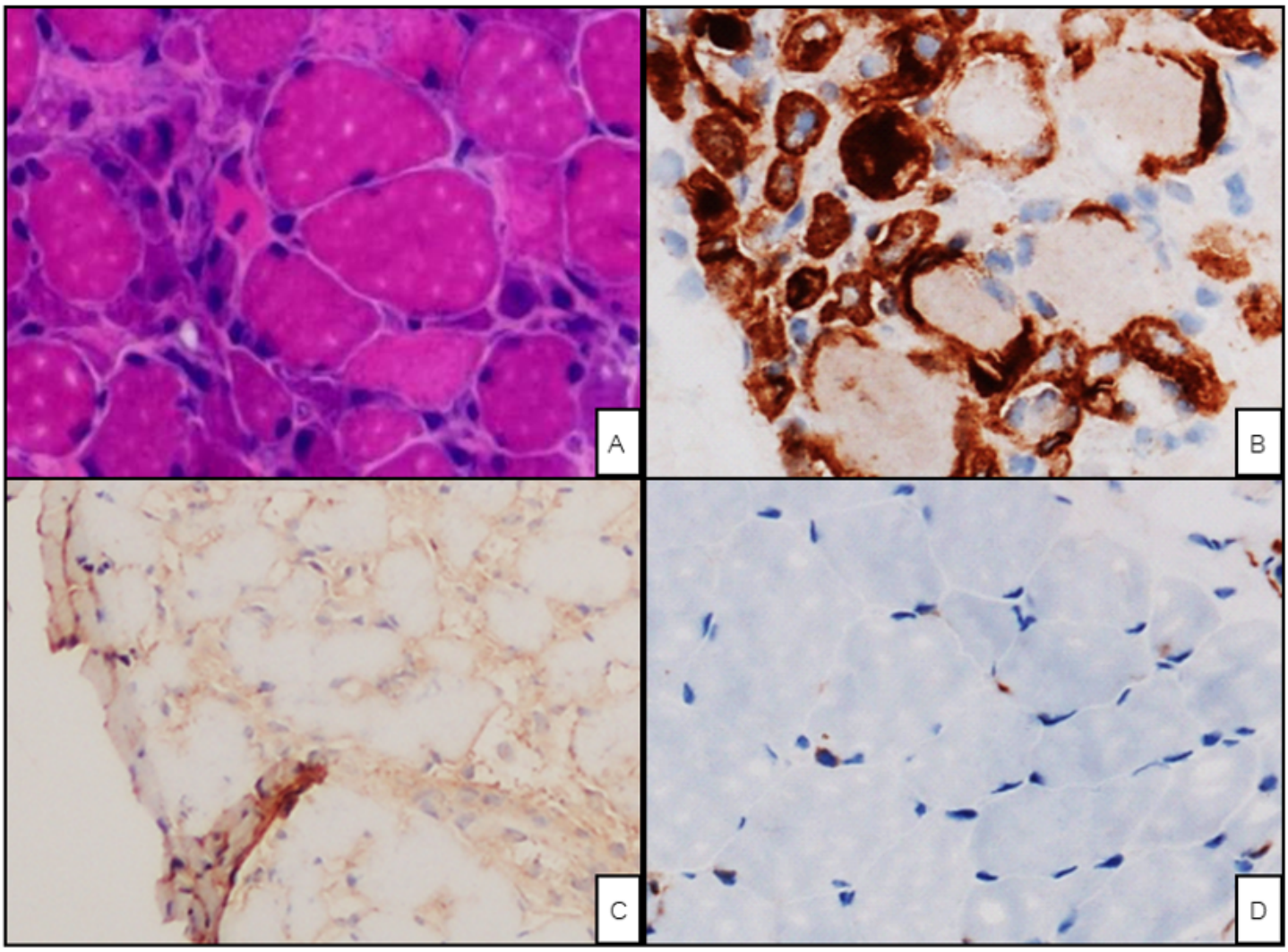

Figure 2

N-CAM interpretation. A, H&E shows regenerating muscle fibres. B, N-CAM was strongly expressed on the sarcolemma of all regenerating fibres. C, Scattered regenerating fibres (N-CAM weakly positive). D, No regenerating fibre (N-CAM negative).

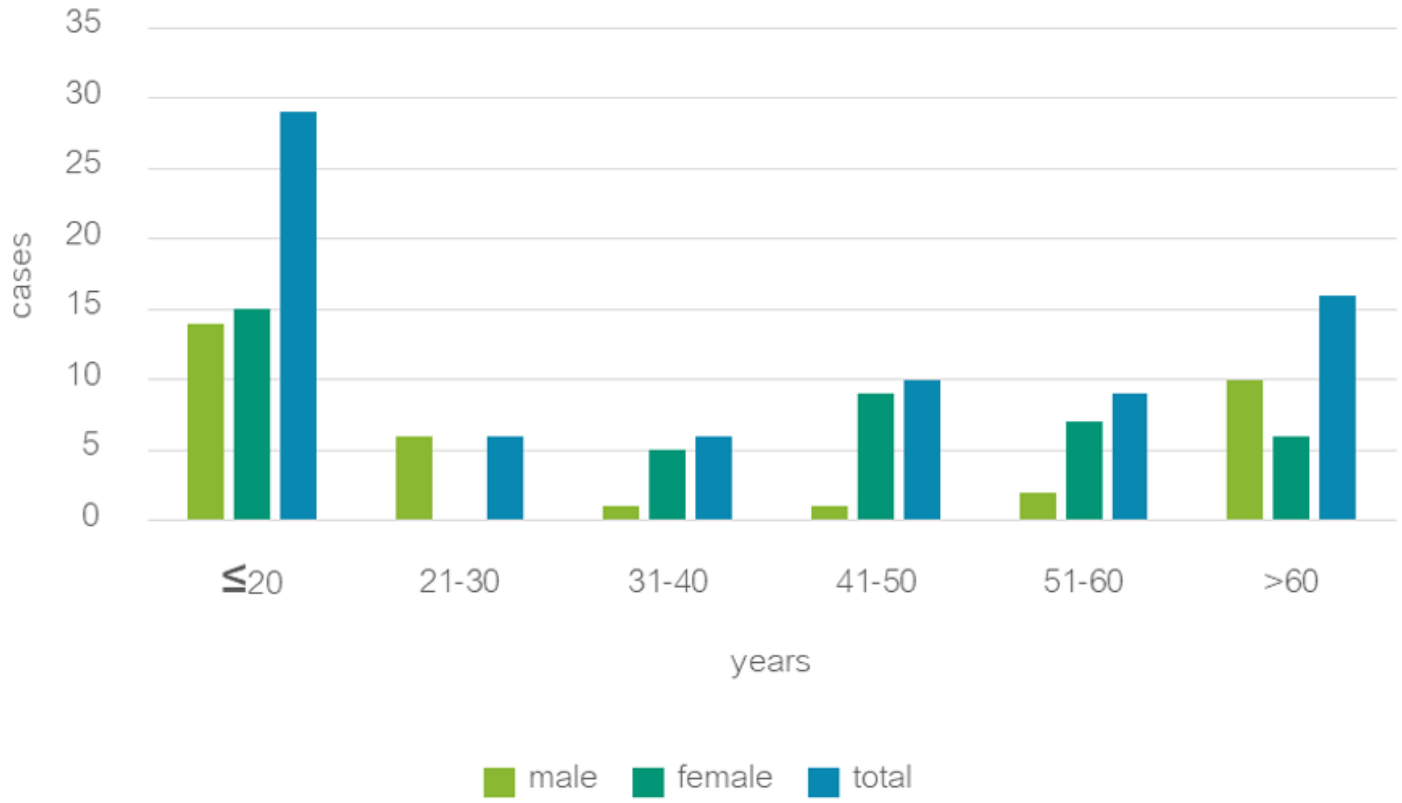

Figure 3

Demographic data of the patient, patient characteristics

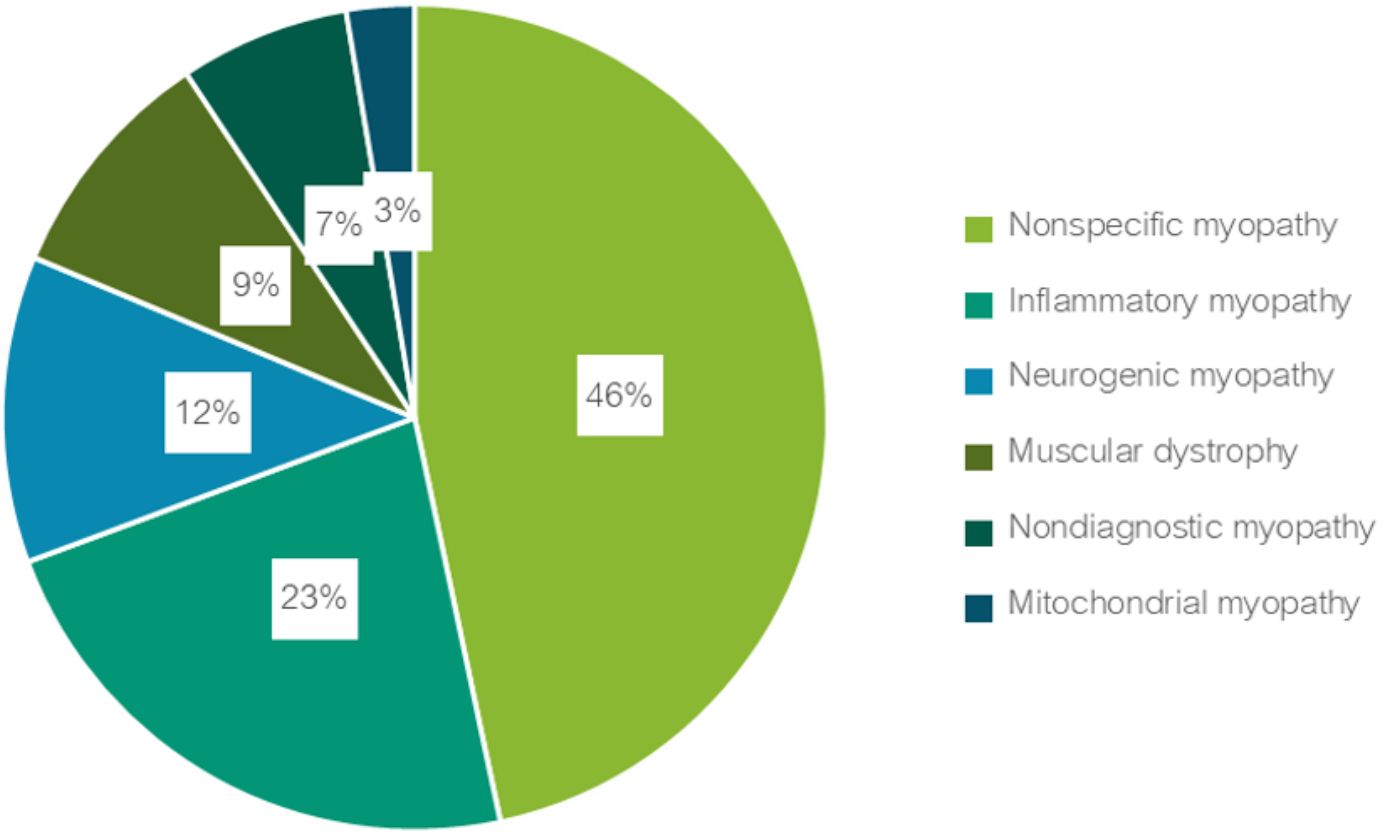

Figure 4

Demographic data of the patient, categorization of common muscle diseases