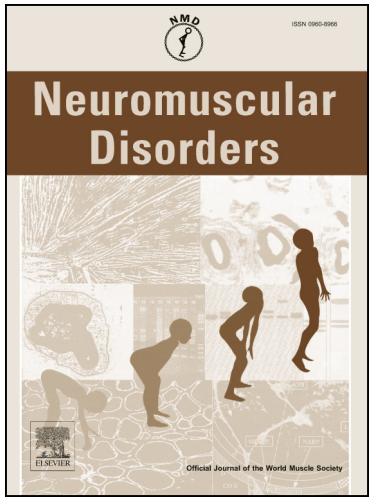

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An analysis of the sensitivity and specificity of MHC-I and MHC-II immunohistochemical staining in muscle biopsies for the diagnosis of inflammatory myopathies

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Abstract

Although there have been several previous reports of immunohistochemical staining for MHC antigens in muscle biopsies, there appears to be a lack of consensus about its routine use in the diagnostic evaluation of biopsies from patients with suspected inflammatory myopathy. Positive MHC-I staining is nonspecific but is widely used as a marker for inflammatory myopathy, while the role of MHC-II staining is not clearly defined. We investigated the sensitivity and specificity of MHC-I and II immunostaining for the diagnosis of inflammatory myopathy in a large group of biopsies from a single reference laboratory. Positive staining for MHC-I was found to have a high sensitivity in biopsies from patients with inflammatory myopathy but a very low specificity, as it was also common in other non-inflammatory myopathies and neurogenic disorders. On the other hand, MHC-II positivity had a much higher specificity in all major subgroups of inflammatory myopathy, especially inclusion body myositis. The findings indicate that the combination of MHC-I and MHC-II staining results in a higher degree of specificity for the diagnosis of inflammatory myopathy and that in biopsies with inflammation, positive MHC-II staining strongly supports the diagnosis of an immune-mediated myopathy. We recommend that immunohistochemical staining for both MHC-I and MHC-II should be included routinely in the diagnostic evaluation of muscle biopsies from patients with suspected inflammatory myopathy. However, as the sensitivity and interpretation of MHC staining may depend on the technique used, further studies are needed to compare procedures in different centres and develop standardised protocols.

Keywords: MHC-I and MHC-II immunohistochemistry; muscle biopsies; inflammatory myopathies, non-inflammatory myopathies; sensitivity, specificity.

1. Introduction

As patients with idiopathic inflammatory myopathies (IIM) may benefit from immune therapies it is crucial to develop diagnostic tools that achieve a high level of sensitivity and specificity. Sets of diagnostic criteria for different types of IIM, based on a combination of clinical and pathological findings, have been proposed for use in clinical trials and research studies [1,2]. However, muscle biopsy is still the definitive diagnostic procedure in clinical practice and should ideally be performed before starting treatment [3]. A major concern is that as the pathology is often patchy the biopsy may not show an inflammatory infiltrate although it may be present in other parts of the muscle. This is a well-known pitfall, especially when the biopsy is performed after treatment has been initiated [4]. In addition, inflammatory infiltrates are nonspecific and may also occur in other myopathies such as dysferlinopathy, facioscapulohumeral dystrophy and other types of muscular dystrophy and myasthenia gravis, and may lead to a mistaken diagnosis of an IIM [5,6]. Other markers of an autoimmune process are therefore necessary to improve the sensitivity and specificity of the muscle biopsy. Vascular membrane attack complex (MAC) and immunoglobulin deposition, and upregulation of major histocompatibility complex (MHC) antigens have been proposed as diagnostic criteria for IIM [2]. A number of previous studies, which have been summarised in Tables 1 and 2, have reported positive immunohistochemical staining for MHC in IIM and other muscle conditions. Some studies have also addressed the diagnostic value of MHC expression with different methodologies and results [7–9].

MHC-I is expressed but is undetectable immunohistochemically in normal muscle fibres and is up-regulated in IIM. MHC-I molecules are necessary for antigen-specific T cell-mediated cytotoxicity and can mediate a response against surface antigens on myofibres [10]. Previous studies have also shown that MHC-I can behave as a pathogenic molecule in its own right since its expression can precede lymphocytic cell infiltration, and transgenic mice overexpressing MHC-I have been shown to develop a severe myopathy even in the absence of inflammation [11–14]. Unlike the inflammatory infiltrates, MHC-I expression is still detectable even after short-term immunosuppressive treatment and in patients with chronic myositis [9,15]. Moreover, MHC-I staining often occurs early, preceding the inflammatory infiltrates, and is present diffusely throughout the biopsy and is thus less likely to be affected by sampling error [16]. Nevertheless, even though it has been considered helpful in distinguishing IIM from other muscle diseases, it is not specific and also occurs in other myopathies [17]. On the other hand, MHC-II expression does not occur constitutively on normal mature muscle fibres, unlike myoblasts in culture which express MHC-II and can behave as antigen-presenting cells [18,19]. Few studies have addressed the diagnostic value of MHC-II expression in IIM and the results of previous studies have varied (Table 1 and 2).

In the present study we analysed the sensitivity and specificity of immunohistochemical staining for MHC-I and MHC-II in the diagnosis of IIM in a large group of muscle biopsies from a single reference centre. We paid particular attention to the contribution of MHC-II staining in improving diagnostic accuracy, as, in our experience, positive MHC-I staining alone is nonspecific.

2. Materials and methods

2.1 Details of cases included

We carried out a prospective survey of diagnostic muscle biopsies from 2000-2013 referred to the Section of Neuropathology at Royal Perth Hospital, which is the State Reference Centre for muscle biopsies and the in-vitro contracture test (IVCT) for malignant hyperthermia (MH). A total of 432 patients were included in the study: 186 cases of IIM and 246 cases of non-inflammatory myopathies (NIM) and other neuromuscular disorders. In addition, 20 biopsies from individuals undergoing investigation for suspected MH, who were MH-negative on the IVCT and had normal muscle histology, comprised the normal control group. The IIM cases included: sporadic inclusion body myositis (s-IBM) 42; dermatomyositis (DM) 33; polymyositis (PM) 12; overlap syndromes 16; immune-mediated necrotising myopathy (IMNM) 16; focal myositis 15; granulomatous myositis 7; unclassified myositis 45 (Figure 1, Tables 3 & 4). The final diagnosis of IIM was based on a combination of clinical and histopathological findings, as well as the subsequent clinical course and response to treatment [2]. In the case of IBM all patients fulfilled the clinical and histopathologic criteria for definite IBM according to Griggs et al [20] and the 2011 proposed ENMC criteria for clinicopathologically defined IBM [21].

The NIM group included: muscular dystrophies and distal myopathies 37; non-immune mediated necrotising myopathies 36; metabolic myopathies 20; non-specific myopathies 93; neurogenic disorders 46; other muscle disorders 14. Details of the cases of necrotising myopathy, muscular dystrophies and distal myopathies are given

in Table 3. The mean (±sd) ages were 59.1±16.1 years in the IIM and 51.9±21.5 years in NIM group. Further details of the age ranges of the different subgroups are provided in Table 4.

2.2 Immunohistochemistry techniques

Needle or open muscle biopsies were mainly from the vastus lateralis, deltoid or gastrocnemius muscles. The muscle tissue was routinely frozen in isopentane cooled with liquid nitrogen and stored at -70°C. Routine staining techniques included haematoxylin and eosin (H&E), modified Gomori trichrome and enzyme histochemistry for mitochondria (NADH, SDH and cytochrome c oxidase). Immunoperoxidase stains for MHC-I and II and C5b-9 (membrane attack complex of complement) and CD31 were performed on 8-µm acetone fixed cryostat sections using the streptavidin–biotin complex technique with diaminobenzidine as a colour indicator. Immunostaining for T- and B-cell subsets was performed on paraffin sections using the streptavidin–biotin complex technique. For MHC-I, the antibody used was a monoclonal mouse anti-human HLA Class 1 antigen clone W6/32 (DAKO-HLA-ABC) code number M 0736 DAKO, Denmark A/S isotype IgG2a kappa in a dilution of 1:200. For MHC-II, a monoclonal mouse anti-HLA-DR, which recognises a human MHC class II antigen and is composed of mouse isotype IgG2a heavy chains and kappa light chains, clone HLA-DR (L246), code no 347360, Becton Dickenson Biosciences, San Jose, was used in a dilution of 1:100. This antibody reacts with a non-polymorphic HLA-DR epitope and does not cross-react with HLA-DQ or HLA-DP. The irrelevant negative control antibody was a monoclonal mouse IgG2a composed of isotype IgG2a kappa, code number X0943 DAKO Denmark A/S. The optimum titration was identified on the basis of the findings

in a preliminary study of 34 biopsies from cases of proven IIM and other myopathies to determine the highest dilution at which there was widespread strong staining of the sarcolemma and sarcoplasm consistent with the distribution previously reported in the literature. The irrelevant control was then used at a similar immunoglobulin concentration of 1mg/l to the selected dilutions (0.9mg/l for W6/32 at 1 in 200 dilution and 0.25 mg/l for L246 at 1 in 100 dilution), corresponding to a dilution of 1 in 200. As a positive comparative control, a CD31 monoclonal mouse anti-human endothelial blood vessel stain, clone JC70A, code M0823, DAKO, Denmark A/S was used to prevent over interpretation of the sarcolemma as being positive, and positive staining of blood vessel endothelial cells acted as an internal positive control.

2.3 Interpretation of biopsies

All biopsies were reported by an experienced myopathologist (VF or RCJ), who was not blinded, and were reviewed by an independent observer (FLM) if there was any uncertainty about the diagnosis. Inter-observer variability in reporting was not formally evaluated. Biopsies were classified as showing definitely positive or negative staining, while biopsies with questionable or faint staining were considered as being negative. Positive staining in necrotic or regenerating muscle fibres was not taken into account in the classification of biopsies. The patterns and distribution of sarcolemmal and sarcoplasmic staining in non-necrotic muscle fibres were interpreted qualitatively. Staining was classified as being 'focal' if it was present in single fibres or small groups of fibres only and 'widespread' if it was present in most or all the fibres in the biopsy. In addition, sarcolemmal staining was classified as being 'complete' or 'incomplete', depending on whether it involved the entire surface of muscle fibres or was patchy in

distribution. No attempt was made to grade the intensity of sarcolemmal or sarcoplasmic staining.

2.4 Data Analysis

Data were initially registered in a database (Microsoft Access 2010; Microsoft Corporation, Redmond, WA). The statistical analysis was done using SPSS V.15.0 software (IBM, Armonk, NY). Sensitivity, specificity, predictive values and receiver operating characteristic (ROC) curves were used to test the discriminatory power of MHC-I and MHC-II staining in the diagnosis of IIM. Patterns of staining and presence of inflammatory infiltrates in the IIM and NIM groups were compared using the Chi square test for independence, the Fisher's exact test and the Z-test for proportions after adjusting the p-values with the Bonferroni correction. Logistic regression analysis was used to assess the relation to prior treatment and years from disease onset. P-values <0.05 were considered statistically significant.

3. Results

3.1 Frequency of MHC-I and MHC-II staining

There was no inflammation or MHC-I or MHC-II staining of muscle fibres in any of the normal control group of biopsies. The proportions of cases with positive staining for MHC-I and MHC-II individually, and of cases with negative staining in the different types of IIM and NIM are shown in Figures 2 & 3. MHC-II staining was less frequent than MHC-I staining and never occurred in the absence of MHC-I staining. Overall, the proportion of cases with positive MHC-I staining was 98.3% in the IIM group and 92.7%

in the NIM group, while MHC-II was positive in 61.7% and 10.1% respectively in the two groups (chi-square, χ^2=125.8; p=0.000). There were no significant differences in the frequency of MHC-I positivity in the different IIM subgroups, whereas for MHC-II positivity, IBM stood out from the other groups with a MHC-II positivity of 100% (chi-square, χ^2=33.40, p=0.000), (Figure 4). When compared with the different IIM subgroups, the proportion of cases with negative MHC-II staining in the NIM group was significantly lower (Z-test; p<0.05). The proportion of MHC-I positive cases in the IIM group was not significantly different from that in the NIM group as a whole or in the dystrophies/distal myopathies, necrotising myopathies, non-specific myopathies or neurogenic subgroups, but was significantly higher than in the metabolic myopathy (Z-test; p<0.05).

The sensitivity, specificity and predictive values of positive MCH-I and MHC-II staining for the diagnosis of IIM are shown in Table 5. MHC-I showed a very high sensitivity (0.984) and low specificity (0.071), while MHC-II showed a high specificity (0.908) and moderate sensitivity (0.605). The ROC curves for the IIM group are shown in Figure 5. When only MHC-I staining was taken into consideration, the area under the curve was 0.53 (standard error, SE: 0.28; p=0.215). The area increased to 0.762 (SE: 0.024; p<0.001) when MHC-II staining was added. In the absence of MHC-I staining, the ROC curve for MHC-II staining showed a similar area (0.754, SE: 0.025; p<0.001).

3.2 Correlation with presence of inflammatory infiltrates

Mononuclear inflammatory infiltrates were present in 147 biopsies (79.9%) in the IIM group and 43 biopsies (17.3%) in the NIM group (Table 6). In the IIM group there was

no association between MHC-I positivity and the presence of an inflammatory infiltrate (chi-square, χ^2=0.591, p=0.446), whereas there was a significant association between MHC-II positivity and infiltrates (chi-square, χ^2=6.041, p=0.014). In the NIM group there was no association between either MHC-I (Fisher's exact test, p=0.374) or MHC-II (chi-square, χ^2=3.255, p=0.071) positivity and the presence of inflammatory infiltrates.

3.3 Patterns of MHC-I and MHC-II staining

The patterns of staining in the IIM and NIM groups are summarised in Tables 7 & 8. Both focal and widespread patterns of sarcoplasmic and sarcolemmal MHC-I staining of non-necrotic fibres were found in both the IIM and NIM groups, but widespread staining was more frequent in the IIM group and focal staining was more frequent in the NIM group. In the case of MHC-II, both widespread and focal patterns of staining were found in the IIM group, while in the NIM group positive MHC-II staining was infrequent, being present in only a small proportion of cases, and sarcolemmal staining was more often focal. A Chi-square test for independence showed that the IIM group had a more widespread and complete pattern of sarcolemmal expression for MHC-I and MHC-II than the NIM group and more frequent widespread sarcoplasmic staining.

3.4 Correlation with disease duration and prior treatment

In the IIM group, disease duration from onset of symptoms to muscle biopsy ranged from 0 to 30 years (2.0 ± 4.1 years). At the time of biopsy 63 patients were on treatment with prednisolone (44), or a combination of prednisolone and methotrexate or azathioprine (19). Logistic regression analysis showed that neither disease duration

nor absence of prior treatment were predictors of MHC-I positivity (χ^2=3.57, p=0.167) and MHC-II positivity (χ^2= 5.75, p=0.056). There was also a lack of correlation between the presence of inflammatory infiltrates and disease duration and prior treatment (χ^2=4.060, p=0.131).

4. Discussion

The diagnosis of IIM has in the past been based largely on the criteria established by Bohan and Peter [22]. New diagnostic criteria have since been proposed [1,2,23,24] but muscle biopsy remains the most sensitive and specific diagnostic tool, as well as the most common cause of misdiagnosis due to misinterpretation [25]. The inclusion of immunohistochemical staining for MHC-I has been recommended for cases of suspected IIM to increase the degree of diagnostic certainty [2,23]. A number of previous studies have demonstrated a high sensitivity of up-regulated MHC-I expression in IIM biopsies, but relatively few studies have investigated the specificity of MHC-I staining compared to large numbers of control NIM biopsies, or have provided sufficient data to define the role of staining for MHC-II. In this study of the largest groups of IIM and NIM biopsies to date, we investigated the sensitivity and specificity of positive MHC-I and MHC-II staining and their combined value in distinguishing IIM from NIM biopsies. Our findings confirm that positive MHC-I staining has a high sensitivity in IIM, but indicate that its specificity for IIM is very low unless it is combined with positive MHC-II staining.

Expression of MHC-I in muscle fibres is associated with antigen processing and presentation and plays an integral part in the pathogenesis of the CD8+ T-cell mediated inflammatory myopathies PM and IBM [26]. However, recent studies have shown that MHC-I overexpression can also mediate muscle fibre damage and dysfunction even in the absence of inflammation, through non-immune mechanisms such as endoplasmic reticulum (ER) stress and induction of the unfolded protein response [11,26,27]. This could explain the incomplete efficacy of immunosuppressive drugs in the treatment of IIM and the disparity between the severity of muscle damage and extent of inflammatory infiltrates in muscle biopsies, as well as the increased MHC-I expression in non-inflammatory myopathies. The demonstration of MHC-I up-regulation and its co-localisation with the ER marker calnexin in myositis biopsies has confirmed that MHC molecules can play a critical role in mediating ER stress by disrupting ER homeostasis [12,28]. Endoplasmic reticulum stress has also been associated with other conditions besides IIM such as myotonic dystrophy, dysferlinopathy, myasthenia gravis, statin-induced myopathy and metabolic myopathies [17,29–32]. It seems that ER stress mechanisms can activate inflammatory responses through a number of different pathways and mediators: e.g. NF-κB, JNK, reactive oxygen species, interleukin-6 and TNF-α [33,34]. Recent studies also suggest that immature muscle precursor cells are a possible source of type I interferon secretion and may be implicated in HLA class I overexpression through the activation of Toll-like Receptor 3 [35].

The finding of upregulation of MHC-I expression in both the IIM and NIM groups may indicate that this is a secondary non-specific and common response to muscle damage

from various causes. However, our findings do not shed any light on whether the increased MHC-I expression may contribute to the muscle fibre injury *per se* in these conditions or is purely a downstream consequence of ER stress. The variable frequency and patterns of MHC-I immunostaining in IIM reported in the literature could be related to methodological differences, in particular variation in antibody dilution (Table 1), and in methods of detection and amplification. Such differences may account for our finding of a higher frequency of MHC-I staining in the NIM group than in previous studies. The use of a more sensitive (low dilution) protocol could result in more widespread MHC-I staining, whereas with higher antibody dilutions, more restricted patchy or perifascicular patterns of staining might be found. A comparison of positive staining rates and staining patterns achieved in different centres using high and low antibody dilutions would be helpful in the standardisation of protocols for MHC immunohistochemical staining. Ultimately, an internationally accepted diagnostic standard based upon a level of sensitivity in terms of false negatives and false positives (for a defined level of strong MHC-I staining) for selected disease groups restricted not only to known IIMs and normal controls but also non-IIM disease controls is necessary to ensure that a "positive" result has equivalent diagnostic significance across multiple centres.

In this study, we found a high specificity (0.891) and moderate sensitivity (0.605) for MHC-II expression in IIM and, as shown in Figure 5, the addition of MHC-II increases the diagnostic power of MHC staining in IIM. The diagnostic value of MHC-II has not been as well established as that of MHC-I. The literature shows inconsistent results, with some studies finding no expression of MHC-II on muscle fibres, while others

found positive expression with varying sensitivities (25%-93%) or specificity (100%) in IIM [7,36]. MHC-II expression is necessary to activate T-helper cells and to initiate an immune response, and muscle cells can act as facultative antigen-presenting cells through the expression of MHC-II molecules [19,37]. Their ability to process and present endogenous antigens via MHC-II molecules is believed to influence the perpetuation or spreading of muscle immune responses through a T-cell stimulatory function [38]. This was shown in sporadic IBM, where β-amyloid targeted for lysosomal degradation via autophagy was associated with MHC-II overexpression.[39] Recent findings suggest that TNF-α is one of the major immune regulators of macroautophagy in inflammatory myopathies, mediating MHC class II expression levels via the delivery of autophagosome contents to the cell surface [39]. Other proinflammatory molecules such as IL-1α, cathepsin S, IFN-γ, ICOS and ICOS-L have also been implicated in MHC-II expression [40–43]. The finding of high specificity of MHC-II expression for IIM may reflect the fact that immune responses dependent on MHC-II molecules do not have a major role in NIM.

The independence of MHC expression in muscle fibres from the presence of adjacent inflammatory cell infiltrates has also been noted in previous studies [15,44–47]. This was observed in both the early and late chronic phases of the disease, and in symptomatic and asymptomatic muscles from the same individuals [48,49]. However, in the present study we found that, unlike MHC-I, there was a significant association between MHC-II expression and the presence of inflammatory infiltrates in the IIM group. It is also well known that there is a poor correlation between clinical symptoms and the presence of inflammatory infiltrates in patients with myositis [15,43,50]

although clearance of infiltrates may correlate with clinical improvement after commencement of corticosteroid therapy [47]. The expression of cytokines such as IL-1α has also been shown to be independent of inflammatory infiltrates or the state of evolution of the disease [15,49]. In addition, we found that the frequency of MHC expression was not affected by disease duration or prior treatment. A previous study also found that MHC overexpression was independent of disease duration and the degree of muscle damage, [51] while two other studies found that MHC-I expression was independent of corticosteroid therapy administered prior to the muscle biopsy and was not affected by the short-term use of immunosuppressive agents [9,52].

In conclusion, our findings provide further evidence that MHC-I expression is non-specific and appears to be a common response to muscle damage resulting from both immunological and non-immunological mechanisms such as ER stress. On the other hand, MHC-II expression has a high specificity for IIM, probably reflecting the fact that immune responses dependent on MHC-II molecules do not play a major role in NIM. Our findings indicate that combining immunohistochemical staining for MHC-I and MHC-II results in a greater degree of specificity for the diagnosis of IIM and suggest that both should be included routinely in the diagnostic evaluation of muscle biopsies from patients with suspected IIM, including cases with a clinical phenotype suggestive of IBM but lacking inflammation or other typical pathological changes. They also indicate that expression of both MHC-I and MHC-II may occur with a variety of pathological processes and cannot *per se* be regarded as an indicator of an immune-mediated myopathy, or be interpreted in isolation without clinical and other pathological information.

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FIGURES LEGENDS

Figure 1:

Title: Diagnostic categories and numbers of cases included in the study.
Footnote: Sporadic inclusion body myositis (s-IBM); DM (dermatomyositis); PM (polymyositis); IMNM (Immune-mediated necrotising myopathies); MD & MD (muscular dystrophies and distal myopathies); NIMNM (non-immune mediated necrotising myopathies).

Figure 2:

Title: Proportions of cases with positive and negative staining for MHC-I and MHC-II in the idiopathic inflammatory myopathies group (IIM) and non-inflammatory myopathies group (NIM).
Footnote: MHC-I + (blue); MHC-II + (green); MHC-I -, MHC-II - (light green)

Figure 3:

Title: Proportions of cases with positive and negative MHC-I and MHC-II staining in the idiopathic inflammatory myopathies group (IIM) and in the different subgroups of non-inflammatory myopathies (NIM).
Footnote: MHC-I + (blue); MHC-II + (green); MHC-I -, MHC-II - (light green).

Figure 4:

Title: Proportions of cases with positive and negative MHC-I and MHC-II staining in the different subgroups of the inflammatory myopathies.
Footnote: Sporadic inclusion body myositis (s-IBM); polymyositis (PM); overlap syndromes (OS); dermatomyositis (DM); immune-mediated necrotising myopathies (IMNM); focal myositis (FM); unclassified myositis (UM). MHC-I + (blue); MHC-II + (green); MHC-I - and MHC-II - (light green)

Figure 5:

Title: Receiver operating characteristic (ROC) curves for positive MHC-I and MHC-II staining

Footnote: MHC-I is shown in blue (0.530, SE: 0.28; p=0.215) and MHC-II in green (0.754, SE: 0.025; p<0.001). The diagonal line in grey colour represents diagnosis by chance. The closer a ROC curve approaches the upper left corner of the diagram, the more discriminatory is the test.

Figure 1: Diagnostic categories and numbers of cases included in the study.

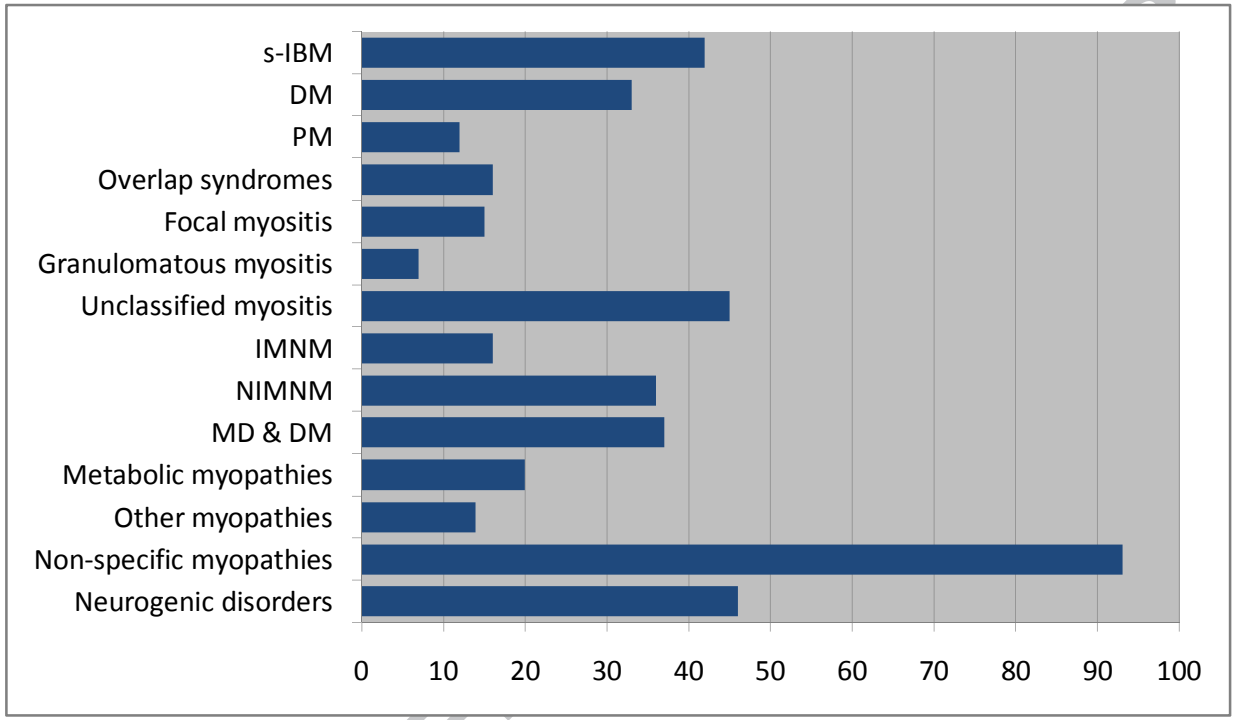

Figure 2: Proportions of cases with positive and negative staining for MHC-I and MHC-II in the idiopathic inflammatory myopathies group (IIM) and non-inflammatory myopathies group (NIM).

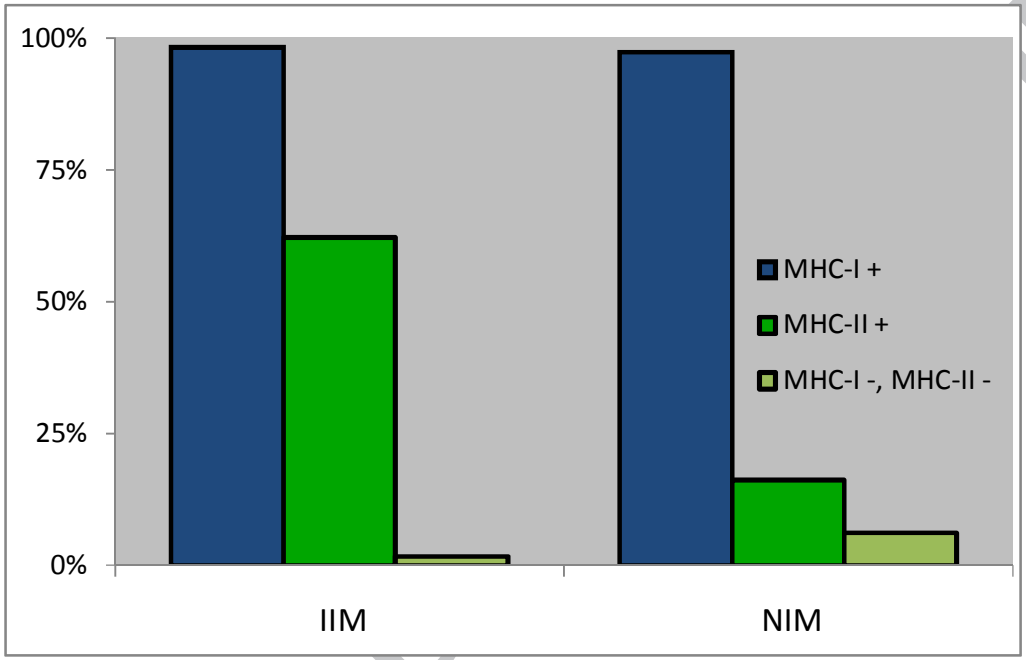

Figure 3: Proportions of cases with positive and negative MHC-I and MHC-II staining in the idiopathic inflammatory myopathies group (IIM) and in the different subgroups of non-inflammatory myopathies (NIM).

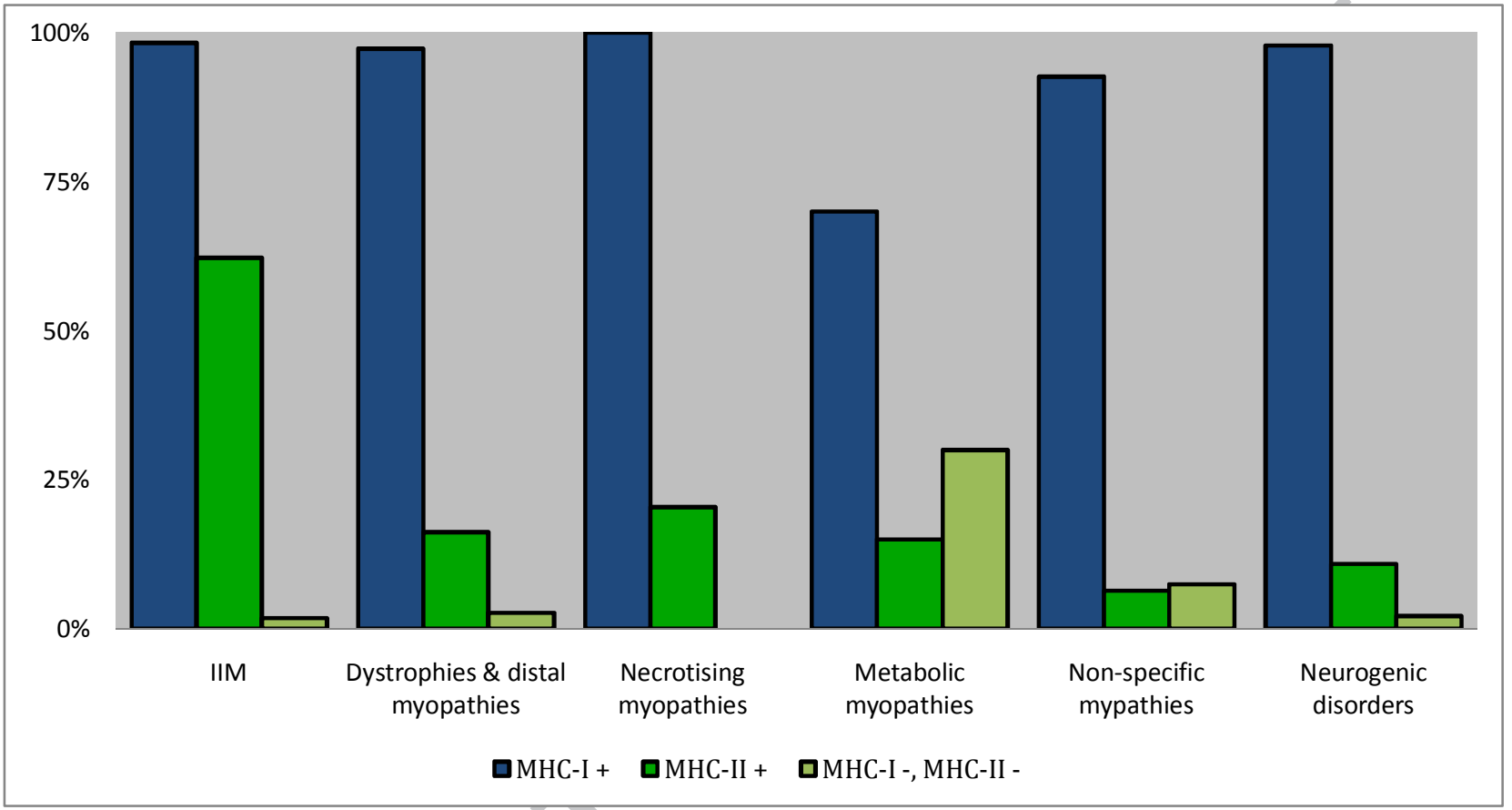

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Figure 4: Proportions of cases with positive and negative MHC-I and MHC-II staining in the different subgroups of the inflammatory myopathies.

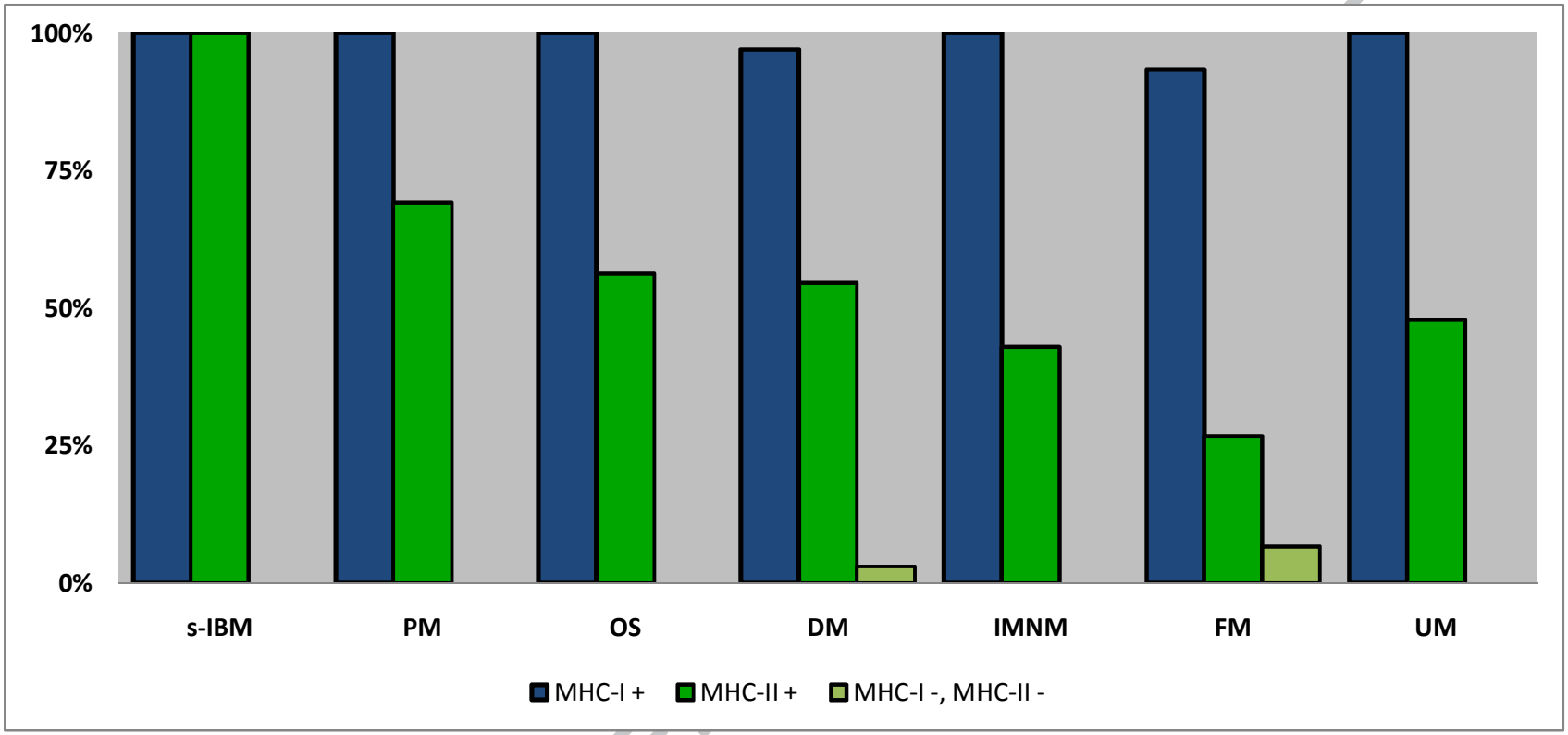

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Figure 5: Receiver operating characteristic (ROC) curves for positive MHC-I and MHC-II staining

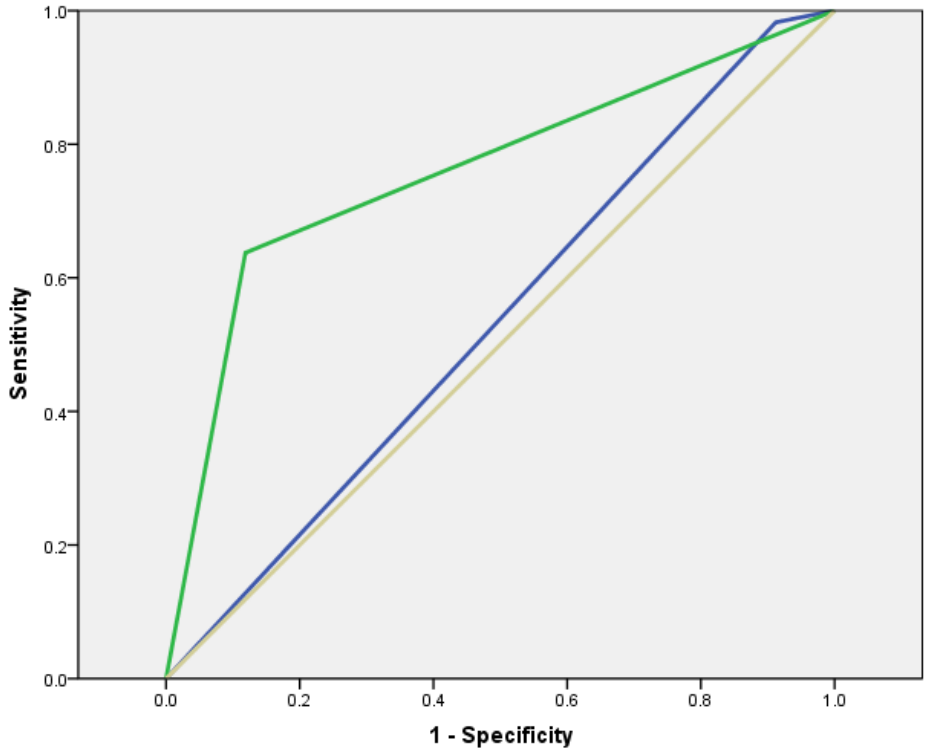

Table 1: Summary of previous MHC immunohistochemical studies in idiopathic inflammatory myopathies.

Ref	Year	n	Conditions	MHC-I +	MHC-II +	MHC-I antibody, dilution	MHC–II antibody, dilution
[7]	2013	120	61PM, 14DM, 45IBM	100%	100% IBM, 87% PM, 93% DM	W6/32 (Dako, Glostrup, Denmark), NR	CR3/43 (Dako), NR
[8]	2012	18	14PM, 4 DM	94%-100%	-	W6/32 (Dako Cytomation, Milan, Italy), 1:300	-
[52]	2012	56	28DM, 28jDM	96% DM, 50% jDM	53.6% DM, 14.3% jDM	Dakopatts (Glostrup, Denmark), 1:100	Dakopatts (Glostrup, Denmark), 1:100
[33]	2010	13	5PM, 4IBM, 4DM	100%	100%	W6/32 (Dako), NR	CR3/43 (Dako), NR
[37]	2007	62	20DM, 38PM, 3 IBM, 1 sarcoidosis	100% DM, 81.6% PM, 100 % IBM, 100% sarcoidosis	20% DM, 23.7% PM, 66.6 % IBM, 100% sarcoidosis	W6/32 (Dako), 1:50	TAL.1B5 (Dako), 1:40
[54]	2008	15	7DM, 6PM, 2sIBM	100%	-	W6/32-HL (Novocastra, Newcastle, UK), 1:100	-
[17]	2007	8	IMNM	100%	25%	W6/32 (Dako, Glostrup, Denmark), 1:600	L243 (Becton Dickinson, San Jose, CA), 1:100
[50]	2006	11	8PM, 3DM	100% PM, 33% DM	87.5% PM, 0% DM	W6/32 (Dako, Glostrup, Denmark), 1:3500	L243 (Becton Dickinson, San Jose, CA), 1:320
[9]	2004	61	9DM, 23PM, 29 IBM	67% DM, 61% PM, 96% IBM	-	W6/32 (Dako, Carputeira, California, USA), 1:20	-
[55]	2003	22	22DM, 5IBM, 1PM	100%	-	B9.12.1 (Immunotech, Marseille, France), 1:200	-
[56]	2003	15	15PM	100%	20%	(Dako), NR	(Dako), NR
[49]	2001	32	12DM, 20PM	100% DM, 85% PM	66% DM, 65% PM	W6/32 (DAKO, Glostrup, Denmark), 1:3500	L243 (Becton Dickinson, San Jose, CA), 1:640
[46]	1994	18	9PM, 7DM, 2IBM	100%	33% PM, 29% DM, 100% IBM	B9.12.1 (Immunotech, Marseille, France), NR	L243 (Becton Dickinson, San Jose, CA), NR
[57]	1989	19	6PM, 7IBM, 6DM	100%	-	W6/32 (Seralab, Crawley Down, Sussex, UK), 45-0.018 µg/mL	-
[16]	1989	33	2DM, 23jDM 5PM, 3IBM	100%	0%	W6/32 (Professor H. Festenstein, The London Hospital), NR	L227/CA2 (Professor H. Festenstein, The London Hospital), NR
[47]	1988	29	13DM, 2jDM, 7PM, 7IBM	62% DM, 0% jDM, 100% PM, 100% IBM	0%	PHM4 (Cedarlene Laboratories, Hornby, Ontario), 1:20	CMD1 (Cedarlene Laboratories, Hornby, Ontario), 1:20
[58]	1988	15	15PM	-	100%	-	M704 (Dako), 1:20
[45]	1985	3	2DM, 1 acute myositis	100%	-	W6/32, 1:1 or undiluted	-
[59]	1985	13	8PM, 2DM, 1 MCTD	-	100%	-	α-HLA-DR (Beckton Dickinson, Sunnyvale, CA), NR
[60]	1983	13	12PM, 1DM	100%	92%	2A1, NR	DA2, NR

Footnote: DM: dermatomyositis; IBM: inclusion body myositis; jDM: juvenile dermatomyositis; MCTD: mixed connective tissue disease; MHC: major histocompatibility complex; NR: not reported; PM: polymyositis.

Table 2: Summary of previous MHC immunohistochemical studies of other muscle conditions.

Ref	Year	n	Conditions	MHC-I +	MHC-II +	MHC-I antibody, dilution	MHC–II antibody, dilution
[9]	2013	27	6N, 7MD, 8MC, 2CM, 2NS	44.4%	11.1%	W6/32 (Dako, Glostrup, Denmark), NR	CR3/43 (Dako), NR
[10]	2012	46	7 MC, 6MB, 5MD, 6HC, 22MX	43.5% approx.	-	W6/32 (Dako Cytomation, Milan, Italy), 1:300	-
[52]	2012	56	4MD, 5MB	25% MD, 0% MB	0%	Dakopatts (Glostrup, Denmark), 1:100	Dakopatts (Glostrup, Denmark), 1:100
[32]	2010	13	4N, 10MG	70% MG, 0% N	30% MG, 0% N	W6/32 (Dako), NR	CR3/43 (Dako), NR
[36]	2007	64	45MD, 5N, 3MB, 3CM, 8HC	11%	0%	W6/32 (Dako), 1:50	TAL.1B5 (Dako), 1:40
[54]	2008	15	20MD	0%	-	W6/32-HL (Novocastra, Newcastle, UK), 1:100	-
[11]	2004	163	63MD, 6DM, 14MB, 6MC, 11CM, 19N, 24MX, 20HC	11% MD, 4% MX	-	W6/32 (Dako, Carputeira, California, USA), 1:20	-
[56]	2003	18	18MD (10DYS, 8DMD)	70% DYS, 0% DMD	20% DYS, 0% DMD	(Dako), NR	(Dako), NR
[49]	2001	10	5 N, 5 MD	60% N, 60% MD	0% N, 0% MD	W6/32 (DAKO, Glostrup, Denmark), 1:3500	L243 (Becton Dickinson, San Jose, CA), 1:640
[57]	1989	16	6 MD, 6 HC	100% MD	-	W6/32 (Seralab, Crawley Down, Sussex, UK), 45-0.018 µg/mL	-
[18]	1989	33	88MD, 22N	MD (+++, ++, +/++, +), N (-/+)	0%	W6/32 (Professor H. Festenstein, The London Hospital), NR	L227/CA2 (Professor H. Festenstein, The London Hospital), NR
[47]	1988	29	7MD, 6N, 2MC, 1CM, 1MX	0%	0%	PHM4 (Cedarlene Laboratories, Hornby, Ontario), 1:20	CMD1 (Cedarlene Laboratories, Hornby, Ontario), 1:20
[58]	1988	7	6MD, 1N	-	0%	-	M704 (Dako), 1:20
[45]	1985	26	21MD, 5N	MD (++, +/++, -), N (-/+)	-	W6/32, 1:1 or undiluted	-
[60]	1983	7	7MD	100%	100%	2A1, NR	DA2, NR

CM: congenital myopathy; DMD: Duchenne muscular dystrophy; DM: distal myopathy; DYS: dysferlinopathy; HC: healthy control; MB: metabolic myopathy; MC: mitochondrial myopathy; MD: muscular dystrophy; MG: myasthenia gravis; MHC: major histocompatibility complex; MX: miscellaneous neuromuscular disorder; N: neurogenic disorder; NR: not reported; NS: non-specific myopathy.

Table 3: Details of the immune-mediated necrotising myopathies (IMNM), non-immune mediated necrotising myopathies (NIMNM), and muscular dystrophy / distal myopathy (MD & DM) subgroups and numbers of cases.

Group	Number of cases
Immune-mediated necrotising myopathies (IMNM)	
Anti-HMGCR antibody positive	10
Anti-SRP antibody positive	2
Anti-Jo-1 antibody positive	1
Anti-PM/Scl 75 antibody	1
Anti-Ro52 antibody positive	1
Paraneoplastic necrotising myopathy	1
Non-immune mediated necrotising myopathies (NIMNM)	
Statin-induced necrotising myopathy	23
Post-viral / post-infectious myopathy	3
Indeterminate necrotising myopathy	10
Muscular dystrophies and distal myopathies (MD & DM)	
Dystrophinopathy	13
Facioscapulohumeral dystrophy (type I)	2
Dysferlinopathy	8
Calpainopathy	5
Myotonic dystrophy (type I)	1
Congenital dystrophy with rigid spine (SEPN1)	1
Oculopharyngeal muscular dystrophy	1
Infantile-onset *LMNA*-associated myopathy	1
Indeterminate muscular dystrophy	5

Table 4: Mean ages, standard deviations (SD) and age ranges for the different subgroups of cases included in the study

Group	Number of cases	Mean age ± SD (years)	Age range (years)
s-IBM	42	66.6 ± 10.9	35-89
DM	33	53.4 ± 15.2	21-75
PM	12	62.5 ± 17.2	23-77
Overlap syndrome	16	50.5 ± 14.5	28-77
IMNM	16	64.6 ± 17.0	39-84
Focal myositis	15	54.7 ± 11.9	37-82
Granulomatous myositis	7	51.1 ± 16.7	27-66
Unclassified myositis	45	58.9 ± 18.5	7-85
Dystrophy/Distal myopathy	37	34.5 ± 23.6	1-76
Metabolic myopathies	20	40.2 ± 25.8	1-89
NIMNM	36	63.7 ± 16.1	6-87
Neurogenic disorders	46	54.9 ± 22.2	1-86
Non-specific myopathies	93	56.9 ± 15.4	16-86
Other myopathies	14	39.1 ± 18.5	5-66
Total	432	55.0 ± 19.7	1-89

Footnote: S-IBM (sporadic inclusion body myositis); DM (dermatomyositis); PM (polymyositis); IMNM (immune-mediated necrotising myopathies); NIMNM (non-immune mediated necrotising myopathies). The 'Other myopathies' group included two cases of myofibrillar myopathy, which showed patchy low level MHC-I staining, but negative MHC-II staining.

Table 5: Sensitivity, specificity and predictive values of MHC-I and MHC-II for the diagnosis of IIM.

	MHC-I	SE	CI (95%)	MHC-II	SE	CI (95%)
Sensitivity	0.984	0.009	0.983 ± 0.018	0.605	0.031	0.605 ± 0.061
Specificity	0.071	0.016	0.057 ± 0.032	0.908	0.020	0.891 ± 0.039
PPV	0.441	0.025	0.423 ± 0.048	0.818	0.033	0.781 ± 0.065
NPV	0.864	0.073	0.833 ± 0.143	0.756	0.025	0.769 ± 0.049

Footnote: SE: standard error; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value

Table 6: Proportion of MHC-I and MHC-II positive and negative cases in muscle biopsies with and without inflammatory infiltrates in the idiopathic inflammatory myopathies (IIM) and non-inflammatory myopathies (NIM) groups.

Group	IIM		NIM	
Inflammatory Infiltrates	Yes	No	Yes	No
Number of cases	147	39	43	203
MHC-I +	145 (98.6%)	38 (97.4%)	41 (95.3%)	186 (91.6%)
MHC-II +	96 (65.3%)	16 (41.0%)	8 (18.6%)	17 (8.4%)
MHC-I - / MHC-II -	2 (1.4%)	1 (2.6%)	2 (4.7%)	17 (8.4%)
Total	186		246	

Table 7: Patterns of MHC-I and MHC-II staining in the idiopathic inflammatory myopathies (IIM) and non-inflammatory myopathies (NIM) groups.

	Staining	IIM			NIM			χ^2	p-value
MHC-I	Sarcolemmal	Widespread	Focal	Negative	Widespread	Focal	Negative		
		89.0%	9.3%	1.7%	53.8%	38.9%	7.3%	58.9	0.000
		Complete	Incomplete	Negative	Complete	Incomplete	Negative		
		81.4%	16.9%	1.7%	63.3%	29.4%	7.3%	20.4	0.000
	Sarcoplasmic	Widespread	Focal	Negative	Widespread	Focal	Negative		
		73.9%	8.7%	17.4%	41.0%	9.5%	49.5%	76.9	0.000
MHC-II	Sarcolemmal	Widespread	Focal	Negative	Widespread	Focal	Negative		
		25.7%	36.0%	38.3%	0.8%	9.3%	89.9%	148.0	0.000
		Complete	Incomplete	Negative	Complete	Incomplete	Negative		
		45.6%	15.8%	38.6%	4.8%	3.8%	91.4%	146.1	0.000
	Sarcoplasmic	Widespread	Focal	Negative	Widespread	Focal	Negative		
		29.1%	14.0%	56.9%	2.9%	1.4%	95.7%	99.8	0.000

Footnote: Percentages indicate the proportions of cases with a particular staining pattern. Staining was classified as 'focal' if only single fibres or small groups of fibres were positively stained and 'widespread' if staining was present in most or all the fibres in the biopsy. Sarcolemmal staining was classified as being 'complete' or 'incomplete', depending on whether it involved the entire surface of muscle fibres or was patchy in distribution. Differences between the IIM and NIM groups were tested using the chi-square test for independence.

Table 8: Patterns of MHC-I and MHC-II staining in the different groups of inflammatory myopathy.

		MHC-I staining				MHC-II staining			
Group	**Number of cases**	**Positivity**	**Sarcolemmal**		**Sarcoplasmic**	**Positivity**	**Sarcolemmal**		**Sarcoplasmic**
			Widespread	Complete	Widespread		Widespread	Complete	Widespread
s-IBM	42	100%	95.2%	83.3%	95.2%	100%	52.4%	69.0%	52.4%
DM	33	97.0%	81.8%	87.9%	78.8%	54.5%	21.2%	45.5%	33.3%
PM	12	100%	84.6%	61.5%	58.3%	69.2%	23.1%	58.3%	46.2%
OS	16	100%	100%	87.5%	62.5%	56.3%	25.0%	43.8%	18.8%
IMNM	16	100%	68.7%	75.0%	87.5%	43.7%	6.2%	21.4%	18.8%
UM	45	100%	95.7%	87.0%	73.9%	47.8%	10.9%	34.8%	13.0%

Footnote: Percentages refer to the proportion of cases with a particular staining pattern. s-IBM: sporadic inclusion body myositis; DM: dermatomyositis; PM: polymyositis; OS: overlap syndrome; IMNM: immune mediated necrotising myopathies; UM: unclassified myositis.

References 41

- Positive MHC-I immunostaining has high sensitivity but low specificity
- MHC-II positivity has greater specificity for the diagnosis of inflammatory myopathy
- Positive MHC-II staining in biopsies with inflammation suggests immune-mediated myopathy
- Both MHC-I and MHC-II staining should be included in the evaluation of inflammatory myopathy