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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, whilst the role of MHC-II staining is not clearly defined. We investigated the sensitivity and specificity of MHC-I and MHC-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

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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 (Tables 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 to 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 (Fig. 1, Tables 3 and 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