Dystrophin-Significance for Diagnostics of Neuromuscular Diseases

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Abstract

Immunoperoxidase reaction for dystrophin was performed using monoclonal antibodies to central rod domain and C-terminus. It was confirmed that the diagnosis of Duchenne muscular dystrophy can be already established immunohistochemically, based on the absence of immunoreactivity with antibodies to C-terminus. In eight male patients where Becker muscular dystrophy (BMD) was considered in the differential diagnosis, immunolabelling with antibodies to rod domain was indistinguishable from normal. Only in one patient, where discontinuous pattern of immunolabelling of dystrophin was observed, the diagnosis of BMD was established immunohistochemically. Our results show, that immunohistochemical analysis alone cannot always diagnose BMD and are in agreement with some observations from the literature.

Key words: dystrophin, dystrophinopathies, immunohistochemical analysis, differential diagnosis.

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Dystrophin is a protein coded by a gene at position Xp21 on the short arm of the X chromosome [13]. It is 3685 amino acids long with a predicted molecular weight of 427 kd [18] and is about 0.002% of total muscle protein [13]. These are serious obstacles to isolate native protein in order to study its structure, localisation and abundance. Therefore monoclonal antibodies have been raised to different domains (N-terminus, rod domain, C-terminus) of dystrophin using sintethic peptides or fusion proteins as immunogens. Immunohistochemical studies have localised dystrophin at the periphery of muscle fibres [6], while electron microscopy localizes dystrophin to the cytoplasmic face of muscle fibre membrane [30]. By immunoblot dystrophin can be detected in normal skeletal muscles as a doublet of approximately 400 kd [22]. Recent studies of membrane organisation of dystrophin [11] have shown, that dystrophin is associated with a glycoprotein membrane complex and also closely linked to actin filaments [24]. The precise function of dystrophin is unknown. It is however speculated that dystrophin contributes to plasma membrane structural integrity [30].

Myopathies due to mutation at Xp21 (Xp21 myopathies, dystrophinopathies) result in dystrophin abnormalities. Duchenne muscular dystrophy (DMD), the severe form of dystrophinopathies, where patients are wheel chair dependent before the age of 12 years and die about 20 years, is characterised by marked dystrophin deficiency [14,16]. The allelic, less severe form of Becker muscular dystrophy (BMD) where patients are wheel chair bound after 12 years and have possible normal life expectancy, is characterised by qualitative and/or quantitative dystrophin abnormalities [14,16]. It has been established that abnormal dystrophin in BMD has preserved C-terminus of dystrophin molecule [3]. The exception to these rules is the recently described patient [17] who had particularly severe DMD but biochemically his muscle contained substantial amounts of Becker-like dystrophin. However, his abnormal dystrophin was missing the C-terminus [17]. Symptomatic carriers of DMD show immunohistochemical and biochemical abnormalities of dystrophin [19]. In a variety of other neuromuscular disorders [6,14] dystrophin analysis yielded normal results. In a recent report [26] it has been documented that secondary changes in the expression of dystrophin can occur in polymyositis and dermatomyositis.

The purpose of our work was to introduce the method for immunohistochemical demonstration of dystrophin in Slovenia and to describe the immunohistochemical pattern

of dystrophin staining in patients in whom the possibility of dystrophinopathies was raised in differential diagnosis.

Patients and Methods

Standard diagnostic methods (level of creatine kinase-CK in serum, electromyography-EMG, and muscle biopsy) were used to establish the diagnosis of myopathy in 13 patients (12 male, one female), aged from 2 to 59 years. In two patients, who lost the ability to walk before the age of 12 years, clinical diagnosis of DMD was established. The diagnosis of DMD was suspected also in a 2 year old boy with about 150 times elevated level of CK and myopathic EMG. In eight sporadic male patients with proximal distribution of muscle weakness it was not possible to differentiate on clinical grounds between BMD and limb-girdle muscle dystrophy. In one male patient BMD was suggested because of the presence of a similar affected male patients (uncle of the propositus and brother) in the family. In a sporadic case of muscular dystrophy in a female patient differential diagnosis between symptomatic carrier of DMD and limb-girdle muscular dystrophy was raised.

Monoclonal antibodies against central rod domain and C-terminus characterised previously [9, 21] were a gift of Mr. Glenn Morris from North-East Walles Institute, London. Unfixed frozen sections were mounted on microscopic slides coated with L-lysine. Primary antibody was diluted 1:100 in PBS. The sections were incubated for three hours at room temperature and washed in PBS (3 times 10 minutes). Peroxidase conjugated rabbit anti mouse immunoglobulins (P260, Dakopatts) were diluted 1:100 in PBS containing 1% horse or bovine serum albumin and applied to sections for one hour, at room temperature. After washing in PBS (3 times 10 minutes), peroxidase label was visualised with 0.05% 3.3'-diaminobenzidine tetrahydrocloride and 0.01% hydrogen peroxide in 50 mM acetate buffer pH 5.0-5.2. Parallel sections were lightly counterstained with hematoxylin. On the same slide, sections from a patient and from a control muscle (positive control) obtained from orthopaedic surgery, were processed as described. Concomitantly negative controls were incubated without primary antibody. All sections were dehydrated and covered with DPX.

In patients with possible DMD and in a patient being a possible DMD carrier both types of antibodies were tested for immunoreactivity. In patients with possible BMD only immunoreactivity with antibodies to rod domain was investigated.

Results

In two patients with clinical diagnosis of DMD and in two year old boy where DMD was suspected clinically muscle fibres exhibited no immunoreactivity with antibodies to C terminus and rod domain (Figure 1). Only exceptionally occasional fibres exhibited immunoreactivity as "reverted fibres".

In eight male patients, including a patient with positive family history, where the possibility of BMD was considered in differential diagnosis, immunohistochemical pattern of dystrophin was normal with antibodies to the rod domain (Figure 2).

Only in one patient with possible diagnosis of BMD a discontinuous pattern of immunoreactivity was observed with antibodies to the rod domain (Figure 3).

In a female patient where differential diagnosis between limb girdle muscle dystrophy and manifest carrier of DMD was raised the immunohistochemical pattern of dystrophin labelling was normal with both types of antibodies.

Discussion

In DMD an immunohistochemical absence of dystrophin labelling [23] is described in the majority of muscle fibres. Isolated dystrophin positive fibres [23] or even larger part (up to 25%) of dystrophin positive fibres [29] are compatible with diagnosis of DMD and most probably represent somatic mutation in myonuclei [16]. Diagnostic for DMD is absence of immunolabelling with antibodies to C-terminus [3]. Immunohistochemically, in BMD a greater inter and intra fibre variability in the intensity of labelling as well as normal patterns of immunolabelling are described by some authors [23, 27, 1] while others report of discontinuous pattern of immunoreactivity in BMD [2]. Patients with BMD have preserved C-terminus of dystrophin [3]. In manifest carriers of DMD a mosaic pattern of dystrophin positive and negative fibres is observed [19].

Our results are in agreement with previous reports [3] and indicate that the diagnosis of DMD can be already established immunohistochemically based on the absence of immunoreactivity with antibodies to C-terminus. It seems, however that for diagnosis of BMD immunohistochemical reactions are not always diagnostic. Only in one case discontinuous pattern of immunolabelling of muscle fibres has pointed towards the diagnosis of BMD in nine our patients, where BMD was considered in differential diagnosis. In eight of our patients immunohistochemical analysis did not yield definite results. It is possible that patients with normal immunohistochemical pattern of dystrophin immunoreactivity have in fact limb-girdle muscle dystrophy, but as normal pattern of immunoreactivity for dystrophin is observed in some BMD patients [23, 27, 1], BMD can not be excluded. Diagnostic for BMD would be immunoblot analysis [1, 15]. In a female patient where all muscle fibres were dystrophin positive, the diagnosis of limb girdle muscle dystrophy was established.

Contemporary diagnostics of dystrophinopathies resides on DNA analysis and in dystrophin testing (immunohistochemical and immunoblot analysis) [16]. Only 65% of gene mutations can be detected by DNA analysis, but nearly all can be detected by dystrophin testing [16]. In addition DMD and BMD patients can have similar gene mutations [16]. Therefore dystrophin testing is also required for differential diagnosis between DMD and BMD especially when milestones of diseases are not reached.

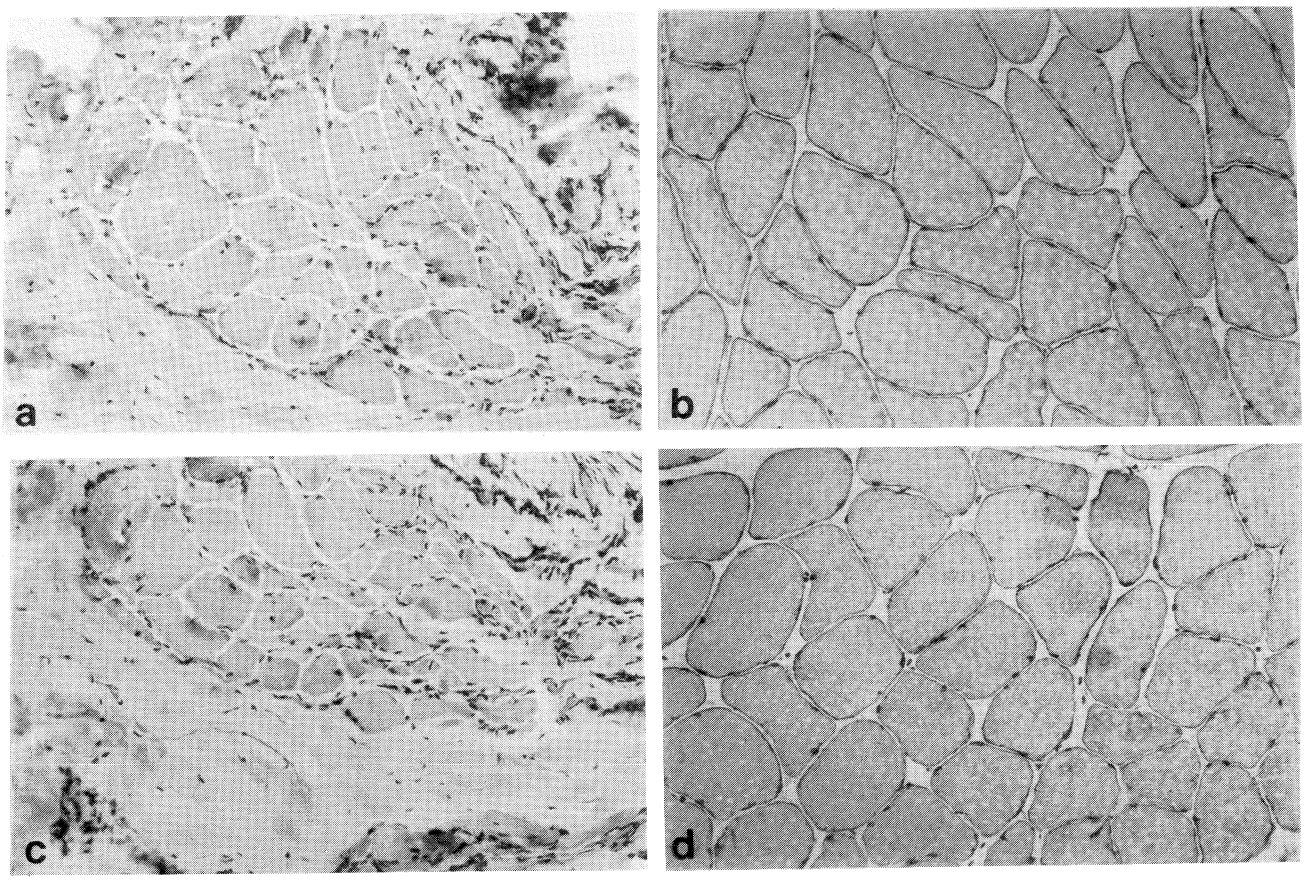

Figure 1. Immunohistochemical pattern of dystrophin in a 12year old boy with DMD. Sections were counterstained with hematoxylin.
1a, no immunoreactivity with antibodies to rod domain
1c, no immunoreactivity with antibodies to C terminus
1b, 1d, control muscle where periphery of muscle fibres is labelled: 1b, antibodies to rod domain, 1d, antibodies to C terminus.

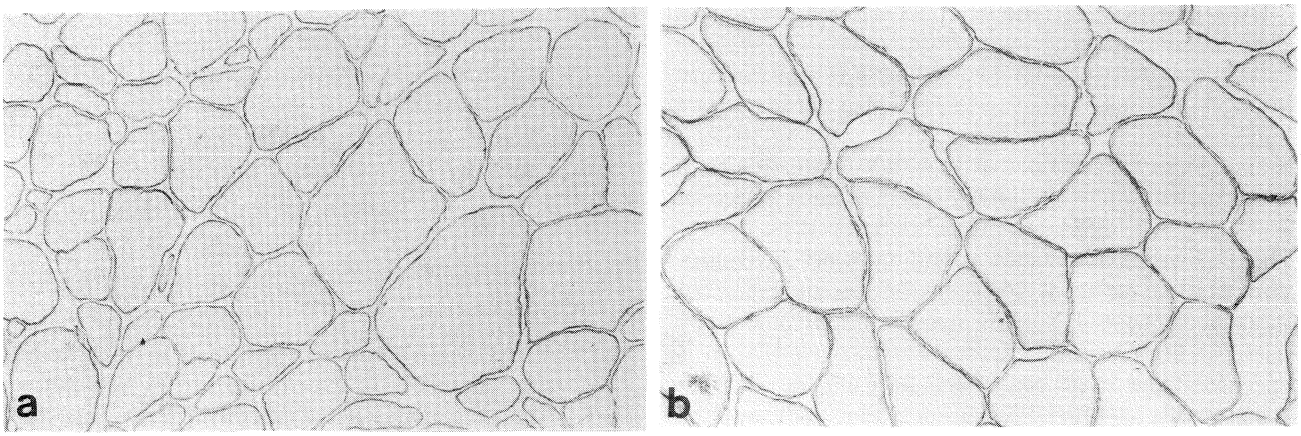

Figure 2. Normal immunohistochemical pattern of dystrophin immunoreactivity in a 16 year old patient where BMD was considered in differential diagnosis. Immunoreactivity for dystrophin with antibodies to rod domain in a patient (2a) is undistinguishable from immunoreactivity for dystrophin of control muscle (2b).

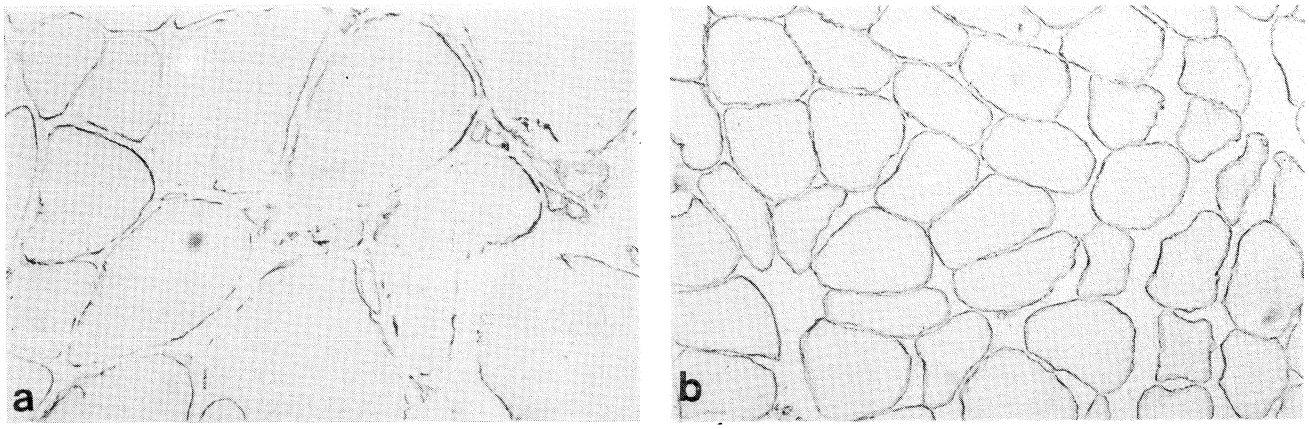

Figure 3. Discontinuous pattern of immunoreactivity for dystrophin in a 29 year old boy with possible BMD.
3a, discontinuous pattern of immunoreactivity with antibodies to rod domain
3b, control muscle where continuous pattern of immunoreactivity is observed with antibodies to rod domain.

Sporadic cases of BMD and manifest carriers of DMD are clinically and with standard investigative methods (values of CK, EMG, muscle biopsy) indistinguishable from limb-girdle syndromes [4]. The differentiation is very important for genetic counselling, since dystrophinopathies are X linked disorders and limb-girdle muscle dystrophy is autosomal recessive. It is recommended that all sporadic male and female patients with proximal distribution of muscle weakness are evaluated with dystrophin analysis for accurate diagnosis. For example, by dystrophin testing BMD was discovered among patients with quadriceps myopathy [28] and also among patients with mild proximal muscle weakness [10]. Dystrophin analysis is also important to distinguish between DMD and congenital muscular dystrophy where dystrophin analysis is normal [5]. In Fukuyama congenital muscular dystrophy high percentage (about 25 to 28%) of fibres exhibited abnormal staining patterns both to dystrophin and spectrin and it is postulated that other factors produce abnormalities of the plasma membrane in Fukuyama congenital muscular dystrophy [5].

Dystrophin analysis is however not sensitive enough for the detection of adult asimptomatic carriers of DMD/BMD [20]. It may be useful for establishing a carrier status in younger girls (less than five years of age) according to one single report [7]. Dystrophin analysis on in utero fetal muscle biopsy is described in 19 weeks of gestation [12]. Most probably it is of limited value in early phase of gestation, since the largest myotubes first express dystrophin in 9 weeks of gestation and all myotubes are dystrophin positive in about 26 weeks of gestation [8]. For prenatal diagnosis dystrophin analysis may be potentially useful in MyoD convertants from amniocytes [25]. It is speculated that MyoD convertants from amniocytes from foetuses affected with DMD can not synthetize dystrophin.

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