

Value of immunohistochemical investigation in the diagnosis of neuromuscular diseases in children

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Abstract

Neuromuscular diseases represent an important group in pediatric pathology. Immunohistochemistry together with clinical examination and morphologic exam are very important in the diagnosis of neuromuscular disorders. *Patients and Methods:* One hundred children diagnosed with neuromuscular disorders were included in a prospective-retrospective study in 25 years. *Results:* There were 58 neurogenic diseases and 42 muscular dystrophies. In positive and differential diagnosis very important were the morphologic and immunohistochemical investigations. Seventy-three percent had positive family history and 27% of dystrophic patients had "de novo" mutations. The most part of the neurogenic disorders were spinal motor atrophies, 91.38%. *Conclusions:* Neurogenic muscular diseases were the most common neuromuscular diseases in our patient group. The immunohistochemical investigation was very useful in diagnosing some of these cases.

Keywords: immunohistochemistry, neuromuscular disorders.

□ Introduction

The most important progress in the field of muscular diseases started in the 6th decade of the XX-th century when histochemical and histoenzymology techniques were applied to the muscular biopsy. Electronic microscopy, immunohistochemistry, and Western blotting followed the introduction of these techniques.

For every diagnosis of muscular disease, in the present, it is necessary to corroborate the clinical data with the electrophysiological ones (electromyography and nervous conduction data), with the biochemical ones (creatinkinase), with complex morphological data and genetic studies.

It is well known that most of the myopathies – defining the diseases of the skeletal muscles – as well as many muscular diseases of neurogenic origin are hereditary monogenic diseases. In the group of myopathies, the most affected tissue is the skeletal muscles, but other organs also may be involved.

Regardless of their type, we were interested of the primitive muscular diseases as well as those of neurogenic origin in a group of Romanian children.

Objective

Our objective was to obtain data regarding the

neuromuscular pathology of children in Romanian population.

□ Patients and Methods

Our material included children with neuromuscular diseases admitted to our Institute in the period 1976–2000. Some of them were retrospectively studied (1976–1993), 48 subjects, but for others 52 subjects there was a prospectively study (1993–2000).

The patients were clinically examined and then multiple investigations followed – electrophysiologic, biochemical and morphological through muscular biopsy (histological, histochemical, histoenzymatic, immunohistochemical). Western blotting, electronic microscopy and genetic tests followed in cases with special indication. We included also electrocardiographic, electroencephalographic, radiologic investigations and computer tomography evaluation if indications existed. Anamnesis was an important tool to obtain information about the other members of the family.

□ Results

Of the total 100 cases of neuromuscular diseases studied for more than 25 years, retrospectively and

prospectively, 58 were neurogenic diseases and 42 were dystrophy cases (Figure 1).

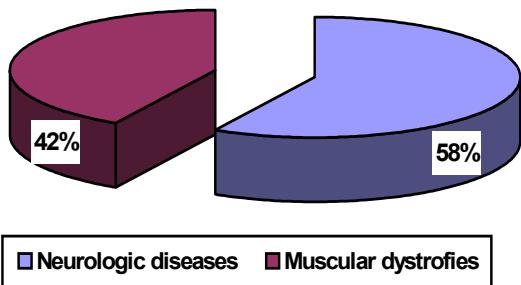


Figure 1 – Muscular dystrophy and neurogenic diseases in the studied group.

Among the studied cases, 73% had a positive family history, but in 27% of the mutations were “*de novo*”.

In the group of neurogenic diseases, children having spinal muscular atrophies (SMA) represented 91.38% (54 cases), Charcot–Marie–Tooth disease 6.90% (four cases) (Figures 2 and 3) and Friedreich’s ataxia 1.72% (one case).



Figure 2 – Typical aspects in Charcot–Marie–Tooth disease (a, b).



Figure 3 – Typical aspect in a family with Charcot–Marie–Tooth disease.

Among the forms of children’s SMA, most were type I – 67.9% (Figure 4) and type II – 22.4% (Figure 5). The type III was diagnosed in 9.4% of cases. In most of neurogenic cases, the onset of symptoms were present in the first three months of life, 41.5% of neonates and 26.4% between one and three months of life.



Figure 4 – Typical aspect in SMA I.



Figure 5 – Typical aspect in SMA II.

In our group of patients, each type of muscular dystrophies had typical clinical manifestations (Figures 6 and 7) and morphological changes: fiber size variability, presence of fibers in hypercontraction, necrobiotic fibers, internal nuclei, connective tissue proliferation, etc as well as absence or abnormal aspects of dystrophin in immunohistochemical tests (Figures 8–13). In rare cases when electron microscopy was performed interruptions of sarcolemma were observed (Figure 14). Cardiomyopathy was present in 16% of cases and motor delays in 13% of cases. At the age of 11–13 years most patients had lost the ability to walk (42%), before 11-year-old only 26% and after the age of 13 years another 19% had lost it. It is worth mentioning that 13% have maintained the ability to walk. An important tool in the positive and differential diagnosis was the morphological and especially immunohistochemical investigations.



Figure 6 – Clinical aspect of Duchenne’s MD.

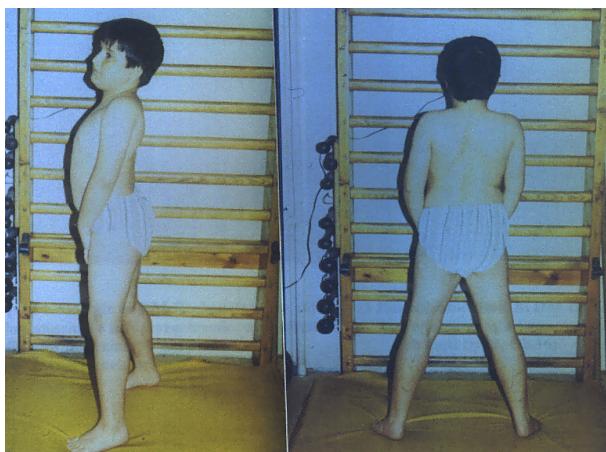


Figure 7 – Clinical aspect of Becker's MD.

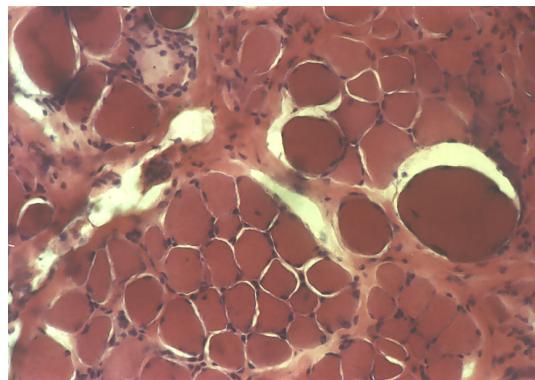


Figure 8 – Histological aspect in MD (HE stain, ob. x16).

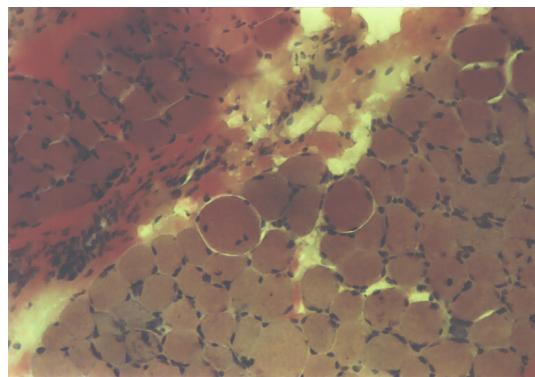


Figure 9 – Muscular fibers in hypercontraction, variability in size of muscular fibers MD (HE stain, ob. x16).

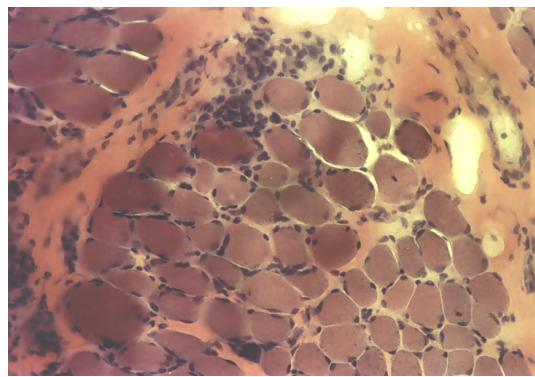


Figure 10 – Variable sized fibers, fiber in hypercontraction, interstitial cell reaction, collagen proliferation in endo- and perimisium (HE stain, ob. x16).

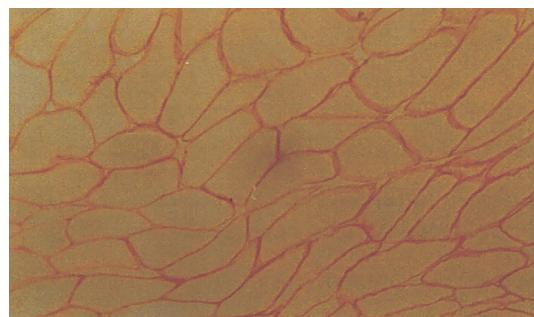


Figure 11 – Immunohistochemistry. Normal expression of dystrophin 2 in a case of limb girdle muscular dystrophy (anti-Dys 2, ob. x16).

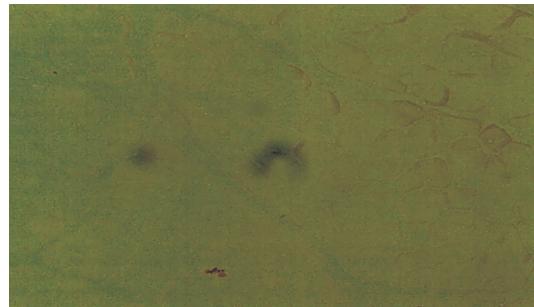


Figure 12 – Immunohistochemistry in Duchenne's MD: dystrophin absence (anti-Dys1, ob. x16).

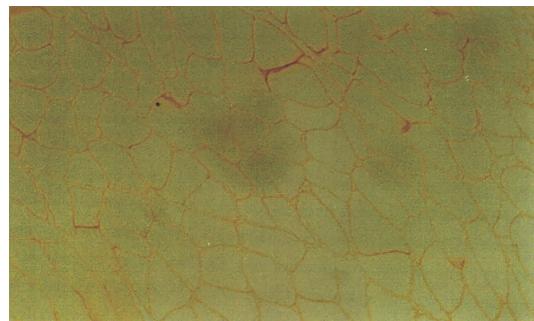


Figure 13 – Immunohistochemistry in Becker's MD: low mark of dystrophin (anti-Dys1, ob. x16).

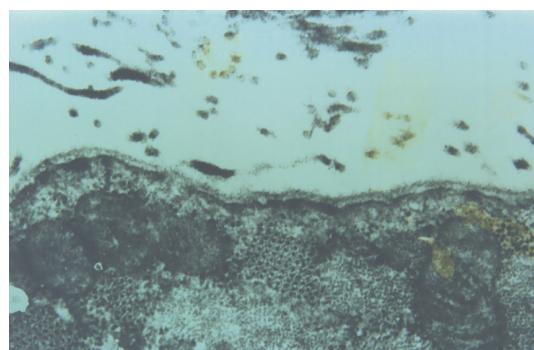


Figure 14 – Electron microscopy examination: DMD – many interruptions of sarcolemma; agglomeration of subsarcolemmal mitochondrial (x18 000).

□ Discussion

Medical literature as well as our experience showed that muscular biopsy is an important instrument for positive and differential diagnosis of myopathies. Modern techniques – histochemical, histoenzymatic, immunohistochemical, along with the classical histolo-

gical ones, and, eventually electronic microscopy were used for preparation of muscular biopsies.

Using the histochemical techniques, we can recognize some myopathies generated by deposition of abnormal quantities of normal substances, like glycogen or lipids.

Muscular fibers can be differentiated by evaluating their histoenzymatic types. Every modification of the normal proportion for each muscle and normal distribution of these types represent important clues for the diagnosis of muscular diseases. Some diseases like central core disease could be diagnosed by histoenzymology studies only. The information regarding the internal structure of the muscular fibers brought by histoenzymology techniques was completed by electron microscopy.

Electron microscopy is not used in current practice. There are some indications for it, suggested by light microscopy, like confirmation of nemalinic myopathy or mitochondrial myopathy, etc. Electron microscopy brings some data regarding the pathogeny of the muscular diseases, like the presence of interruption of the muscular cell membrane in the initiation of the necrobiosis of the muscular fibers in Duchenne's muscular dystrophy.

Immunohistochemistry permits differentiation of many forms of muscular dystrophy – dystrophinopathies, sarcoglycanopathies, caveolinopathies, etc. indicating the absence of some specific muscular protein [1, 2]. In addition, it permits differentiation between Duchenne's muscular dystrophy (DMD) and Becker's muscular dystrophy (BMD) when dystrophin is missing, quantitatively reduced, or abnormally structured [3].

The percentage of 73% cases with positive family history and 27% cases with "de novo" mutations in the studied group is identical with others in literature [4].

Most of the myopathies (90%) are recessive X-linked transmitted – DMD and BMD. In the remaining 10%, the transmission may be autosomal recessive or dominant (facioscapulohumeral MD, myotonic MD, etc.). DMD has an incidence of 1:3600 live born infant boys [5] and 1:1400 births in families of patients. These are the most common genetic diseases in children after cystic fibrosis [5, 6]. The science progresses in elucidating the etiopathogeny of these diseases: identification of the importance of locus p21 on the chromosome X for muscular diseases, the discovery of dystrophin, and the discovery of other cytoskeletal or sarcolemmal proteins (utrophin, sarcoglycans, dystroglycans, laminin, etc.) deficient in other myopathies [7].

In one-third of dystrophinopathies the gene mutations cannot be identified, and this may have some implications in prenatal diagnosis [8]. There is no correlation in carriers of DMD/BMD between dystrophin abnormalities and variability of clinical manifestations [9]. In order to maintain a normal function it is necessary at least 30% of normal quantity of dystrophin to be present [10]. The linkage between sarcolemma cytoskeleton and extracellular matrix is affected by dystrophin deficiency [11].

Muscular neurogenic diseases are monogenic genetic diseases with autosomal recessive (AR) or dominant

(AD) transmission. The most common forms in children in our group were spinal muscular atrophies (SMA) Werdnig–Hoffmann and Kugelberg–Welander types [12].

Infantile SMA represents the secondary frequent category of neuromuscular diseases in children. Clinical classification of SMA cases is based on the age of clinical onset and maximum function achieved at that age [13]: type I SMA (Werdnig–Hoffmann's disease) with earliest onset, the chronic intermediate type is SMA II, and the chronic mild type is SMA III (Kugelberg–Welander's disease) [14]. Spinal muscular atrophy is caused by mutations in the survival motor neuron genes SMN I and SMN II located on the chromosome 5q11.3–13.3 [14]. The result of gene mutations is the deficiency of the protein implicated in the survival of motor neurons (SMN) which will degenerate.

Charcot–Marie–Tooth disease (peroneal muscular atrophy or hereditary sensorimotor neuropathy) is a peripheral nervous system disorder with autosomal dominant transmission in most cases, rarely X-linked. Its prevalence is 1:2500. Numerous gene anomalies determine the different types of the disease.

Cardiomyopathy and eventually mental retardation are probably the expression of the deficiency of an isoform of dystrophin with cardiac or cerebral localization.

Conclusions

Neurogenic muscular diseases were the most common (58%) neuromuscular diseases in our patient group, followed by muscular dystrophies (42%). In dystrophinopathies (DMD and DMB) group, 27% of cases were "de novo" suggesting the existence of a high rate of mutations in Romanian population. Morphological and especially immunohistochemical investigations are essential for the neuromuscular diseases, indicating the deficient protein in muscular diseases. A good collaboration between pediatrician, neurologist, electrophysiologist and pathologist is mandatory for a correct diagnosis and an optimal therapeutic strategy.

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