VACUOLAR MYOPATHY

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ABSTRACT

We present a male patient aged 46 years, with gait difficulties and predominant proximal weakness in the lower limbs with insidious onset and slowly progression. EMG indicated an active chronic myopathy, NCV and serum CK levels were in normal limits.
The main deltoid muscle biopsy data: numerous muscle fibers presenting 1-2 vacuoles empty or containing a fine granular material. Rare vacuoles contained minimal PAS-positive material. Vacuoles were bounded by a membrane positive for dystrophin, alpha- and beta-sarcoglycans and merosin.
The differential diagnosis of this case is discussed, and the general data concerning vacuolar myopathies are presented.
Key words: vacuolar myopathy

ABREVIATIONS:

EMG: electromyography
NCV: nerve conduction velocity
CK: creatine kinase
XMEA: X-linked myopathe with excessive autophagy

Vacuolar myopathy is the morphological expression of some muscular diseases or of some general diseases with muscle involvement, characterized by the presence of single or multiple vacuoles in a variable number of muscle fibers in a muscle sample.

Vacuolar myopathies are a heterogeneous group of diseases with different pathogenies, clinical phenotypes and morphological particularities (1,2).

The most known vacuolar myopathies are:

- periodic paralyses whose vacuoles are clear and derive from T-tubular system
- sarcotubular myopathy which presents multiple vacuoles arising from the sarcoplasmic reticulum
- lysosomal diseases like acid maltase deficiency, some cases of Batten's disease, GM1 gangliosidosis, sialidosis, etc. present vacuoles which may be autophagic ones or resulted from removal of a stored material. Lysosomal vacuoles are also the main morphological sign in X-linked vacuolar myopathy (Danon disease) and in X-linked myopathy with excessive autophagy (XMEA) – described by Kalimo et al (3). Acid phosphatase activity is usually present in lysosomal vacuoles.
- peroxisomal diseases like Lafora's disease present vacuoles which are better seen in resin sections as multiple clear vacuoles.
- defects of fatty acid metabolism like mitochondriopathies, carnitine or carnitine palmitoyltransferase deficiency, etc. present the so called lipid vacuoles
- toxic myopathies: vincristine, chloroquine, alcohol, etc.
- occasional cases – example: denervation atrophy.

Different types of vacuoles can appear in muscle samples:

- empty vacuoles membrane bounded, the membrane being better observed in electron microscopy. Example: vacuoles in periodic paralyses.
- vacuoles containing a fine granular material; they can appear in different vacuolar myopathies.
- vacuoles usually clear resulted after removal of a stored material in the muscle fiber like glycogen in Pompe disease (acid maltase deficiency)
- autophagic vacuoles containing cellular debris and acid phosphatase are present in different lysosomal diseases.
- rimmed vacuoles bounded by dark blue granules after hematoxiline-eosine staining and reactions for oxidative enzymes, and red after Gomori trichrome. They are present in oculopharingeal dystrophy, inclusion body myopathy, some neurogenic lesions of the muscle, desminopathies, etc.

In some lysosomal diseases like Danon disease or XMEA vacuoles are bounded by a membrane sharing properties with plasma membrane such sarcolemma-specific proteins as dystrophin, merosin, sarcoglycans. They can also contain basal lamina layers, aspects certifying the continuity of the vacuole membrane with the sarcolemma (4, 5), as well as subsarcolemmal small calcium deposits and complement C5b9 (6).

The differential diagnosis of vacuolar myopathies is not always easy, genetic tests being sometimes necessary.

We present a male patient aged 46 years, who, 15-20 years before presentation in our hospital, observed the insidious appearance and then slowly progression of gait difficulties with predominant proximal weakness in the lower limbs. Motor and sensory nerve conduction velocities were normal, EMG was suggestive for an active chronic myopathy, and the seric creatine kinase and lactic dehydrogenase had normal levels.

Right deltoid muscle biopsy showed a muscular fragment with normal architecture, but containing numerous muscle fibers with 1-2 vacuoles, some of them empty (fig.1), others containing a pale or fine granular material (fig.2). Rare vacuoles contained minimal PAS-positive deposits (fig.3). Vacuoles were bounded by a membrane positive for dystrophin (fig.4), alpha-sarcoglycan (fig.5) and beta-sarcoglycan (fig.6) and merosin (fig.7). Muscle fibers without vacuoles had normal internal structure (fig.8). Some other nonspecific changes were observed too: a moderate variability of the fiber size without a specific topography and without changes in fiber shape excepting two fibers with a more or less angular contour, some fibers with 1-2 internal nuclei, a splitting fiber. Vacuoles were present in both histoenzymatical types of muscle fibers. The proportion of type 2 fibers was severely reduced (fig.9).

The personal history of our patient helped us to eliminate from the beginning periodic paralyses and the toxic myopathies from the differential diagnosis of our patient. His age and clinical data as well as the morphological aspect of his muscle biopsy eliminate also the myopathy due to the carnitine deficiency. Most of the lysosomal myopathies are excluded too by the absence of any stored material in the muscle fibers. The minimal glycogen deposits present in some vacuoles in our case were too small and in a too small number of fibers that is why they can not be considered as a sign of a glycogenosis.

Muscle fibers containing multiple vacuoles characterizing sarcotubular myopathy have a different aspect than those of our case, as well as peroxisomal vacuoles in Lafora disease which can be recognized on cryostat sections as multiple basophilic dense dots, only the largest ones looking like vacuoles.

The presence of only 1-2 vacuoles in a muscle fiber, their pale or fine granular content, as well as their limiting membrane having sarcolemmal immunohistochemical characteristics observed in the muscle biopsy of our patient imposes a differential diagnosis between X-linked myopathy with excessive autophagy (XMEA) described by Kalimo (3) and the X-linked vacuolar myopathy (Danon disease). From pathogenic point of view for both diseases two theories are under discussion: a) the myofiber injury based theory: vacuoles are autophagosomes clearing up sarcoplasmic debris after sublethal injury to the myofiber; (b) the membrane attack complex theory: the injury is induced by deposition of the complement C5b9 ("membrane attack complex") and of calcium on myofibers with secondary invagination and/or endocytosis.

The Danon disease is determined by the deficit in LAMP-2 (lysosomal associated membrane-2 protein), one of the glycoproteins coating the inner side of the lysosomal membrane. Its gene is located on Xq24, while the genetic defect of the XMEA is located on Xq28, its protein being till now unknown.

Molecular genetic tests could not be performed for our patient, that is why for the differential diagnosis we had to consider only his clinical and paraclinical data.

From the clinical point of view, both diseases – XMEA and Danon disease – share some common aspects: all patients are males (although recently two twin girls have been reported as manifesting carriers of XMEA, or alternatively, a new form of disease having autosomal recessive inheritance (7)); in both diseases the myopathy is usually mild and slowly progressive, the weakness and amyotrophy are predominantly proximal, but in Danon disease weakness and amyotrophy affect predominantly the shoulder girdle, while in XMEA, like in our case, the lower limbs are especially involved.

In both diseases serum creatine kinase levels are 5-15 times above upper normal values in contrast with the normal values of our patient.

The main clinical differences between XMEA and Danon disease are represented by the fact that in XMEA only skeletal muscles are affected, like in our case, while in Danon disease cardiomyopathy is a major phenomenon, around 60% of

the patients have a mild mental retardation and some patients have their liver primarly affected. All these particularities of Danon disease were absent in our case.

In contrast with Danon disease, the evolution of XMEA may be worse in the 6th-7th decades of life, the patients becaming then wheelchair bound. The motor deficit of our patient at his age of 46 was relatively mild.

The clinical phenotype of our patient was more suggestive for XMEA, but his electrophysiological data showing only an active chronic myopathy was different from the known data in XMEA where abundant myotonic and high frequency discharges without clinical myotonia are characteristic.

Morphologically, the presence in many muscle fibers of one or two vacuoles bounded by a dystrophin positive membrane and containing a fine granular or a hyaline material is suggestive for XMEA. In Danon disease muscle fibers contain multiple vacuoles which on hematoxilin-eosin staining often appear like solid basophilic granules and can be easily overlooked (4); only some vacuoles look like true vacuoles bounded by a dystrophin positive membrane. Multiple basophilic granules were totally absent in our patient.

We consider our patient to be an XMEA case, his electrophysiological and seric particularities representing the confirmation of the principle that each patient has his particular disease.

REFERENCES

1. **Carpenter S, Karpati G** – Vacuoles. In: Pathology of Skeletal Muscle. 2nd edition, Oxford University Press, (2001): 297-311
2. **Weller RO, Cummings WJR, Mahon M, Ellisson DW** – Primary muscle disease: myopathy. In: Greenfield's Neuropathology. 7th edition. ID Graham and PL Lantos (eds). Arnold, London-New York-New Delhi, (2002): 731-748
3. **Kalimo H, Savontaus ML, Lang H** – X-linked myopathy with excessive autophagy: a new hereditary muscle disease. *Ann Neurol* (1988),23: 258-265
4. **Nishino I, Hirano M, DiMauro S** – LAMP-2 deficiency. In: Structural and Molecular Basis of Skeletal Muscle Diseases. G.Karpati (ed). ISN Neuropath Press, Basel, (2002): 142-144
5. **Minassian B, Levy N, Kalimo H** – X-linked myopathy with excessive autophagy. In: Structural and Molecular Basis of Skeletal Muscle Diseases. G.Karpati (ed), ISN Neuropath Press, Basel, (2002): 145-147
6. **NeurLearnNeuroHelp Muscle** – X-linked vacuolar myopathy with excessive autophagic vacuoles (2008)
7. **Holton JL, Beesley C, Kackson M** – Autophagic vacuolar myopathy in twin girls. *Neuropathol Appl Neurobiol* (2006), 32: 253-259

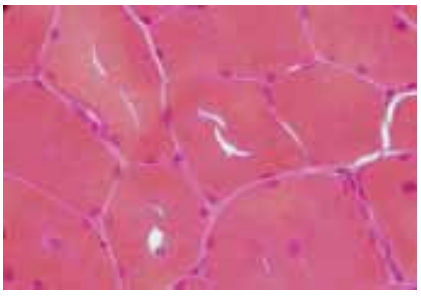

Figure 1. Empty vacuoles in muscle fibers. HE, ob.40.

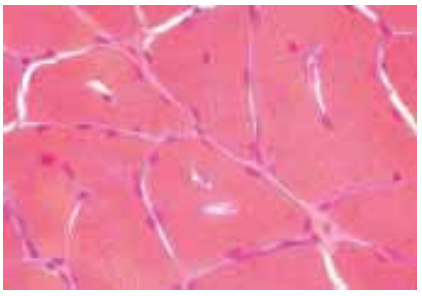

Figure 2. Empty vacuole and a vacuole containing a fine granular material. HE, ob.40.

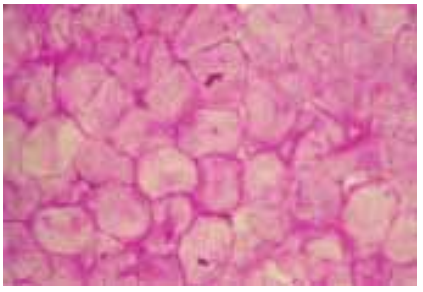

Figure 3. Minimal PAS-positive material in 2 muscle fibers. PAS, ob.20.

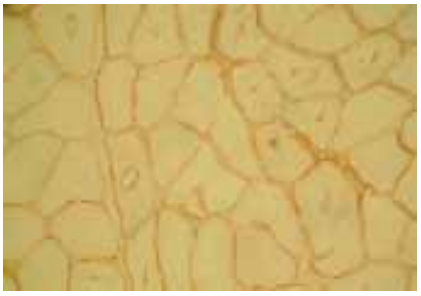

Figure 4. Dystrophin – positive membrane bounding vacuoles. NCL-DYS2 antibody, ob.20.

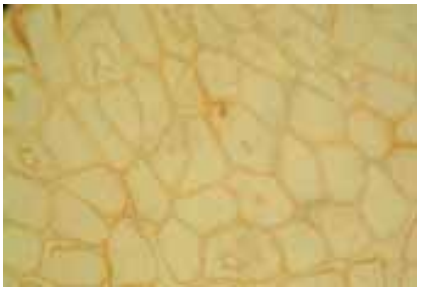

Figure 5. Alpha-sarcoglycan positive membrane bounding vacuoles. NCL-a-SARC, ob.20

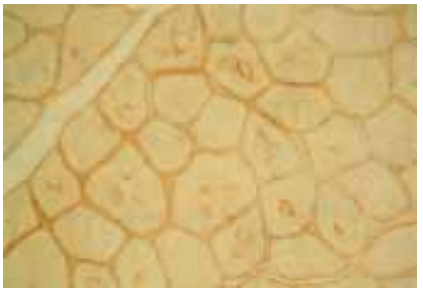

Figure 6. Beta-sarcoglycan positive membrane bounding vacuoles.NCL-b-SARC, ob.20.

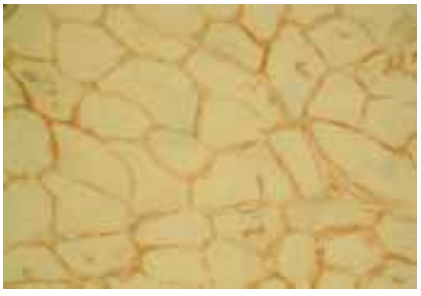

Figure 7. Merosin – positive membrane bounding vacuoles. NCL-MEROSIN, ob.20.

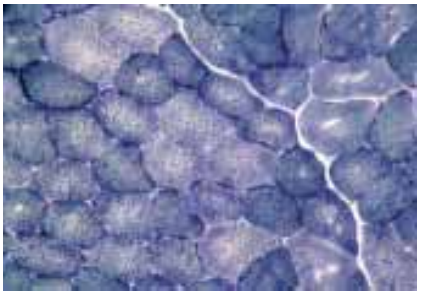

Figure 8. Normal internal structure of the majority of muscle fibers. NADH, ob 20.

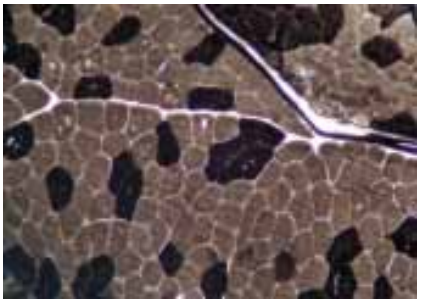

Figure 9. Decreased number of type 2 muscle fibers. Vacuoles are present in both types of muscle fibers. ATP-ase pH 9,4,ob.20.