

Kufs' disease: diagnostic difficulties in the examination of extracerebral biopsies

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Abstract

Kufs' disease or NCL4 (neuronal ceroid lipofuscinosis type 4) is a rare and poorly characterized, adult-onset form of NCL. The mutation in gene CLN, underlying Kufs' disease, still remains unknown. The diagnosis of this disease is difficult because it is based only on clinical and ultrastructural examinations.

We report the case of a 45-year-old woman referred to the Neurological Department with suspicion of Creutzfeldt-Jakob disease (CJD). CJD as well as infectious, autoimmune and some lysosomal diseases were excluded. Since clinical symptoms, i.e. psychotic, auditory and visual hallucinations as well as behavioural disturbances, still suggested metabolic or neurodegenerative disease, a skin and muscle biopsy was performed.

On ultrastructural examination the muscle biopsy revealed the subsarcolemmal accumulation of lipofuscin, lipofuscin-like and granular osmiophilic deposits (GRODs). The most unique fingerprint deposits (FP) and curvilinear profiles (CP) for diagnosis of Kufs' disease were located in vascular smooth muscle cells (VSMCs). In these cells lipofuscin-like deposits and GRODs were also visible.

The fact that FP and CP were found exclusively in VSMCs jointly with clinical and laboratory data allows us to diagnose Kufs' disease in our patient.

Key words: NCL, fingerprint deposits, curvilinear profiles, vascular smooth muscle cells, ultrastructure.

Introduction

The neuronal ceroid lipofuscinoses (NCLs) represent the most common group of inherited neurodegenerative lysosomal storage disorders in childhood [5,16,26]. To date, eight forms of NCLs have been distinguished on the basis of age of onset, clinical course, and morphological features [14]; for some of them causative genes are also known [19,27]. All

forms of NCL are manifested by lysosomal storage granules of autofluorescent lipopigments in the cytoplasm of neurons in the central nervous system (CNS) and extraneuronal cells [12,13,16]. The pigment chemically is characterised as ceroid [22]. At the ultrastructural level 5 types of storage material could be distinguished: fingerprint deposits (FP), curvilinear profiles (CP) and granular osmiophilic deposits (GRODs), as well as typical lipofuscin deposits and

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microtubular aggregates [3,8,11]. It has been postulated that the type of storage material roughly correlates with the age of onset of NCLs [8,10]. The NCL forms result from mutations in genes *CLN₁* to *CLN₈*, which encode lysosomal hydrolytic enzymes and lysosomal transmembranous proteins [19,27]. NCLs have an autosomal recessive pattern of inheritance except for adult-onset type (ANCL), which occurs in autosomal dominant, autosomal recessive and sporadic fashions [2,4,9,13,18].

The adult form or Kufs' disease is the rarest form of NCLs. Clinical symptoms usually appear about 30 years of age, but can also be visible at an earlier age [25]. Two types (A and B) of Kufs' disease are distinguished depending on the dominant clinical symptoms [1,4,18]. Onset of disease is usually connected with myoclonic epilepsy, dysarthria, ataxia in type A or dementia, extrapyramidal and cerebellar signs, and frequently with personality and behavioural changes, including psychosis in type B [4,18].

The diagnosis of Kufs' disease is based on clinical and pathological data because *CLN₄* genes as well as gene products are still unknown [1,2,6,9,25]. ANCL identification is based on the presence of fluorescent material (lipopigments) in histological data, as well as on fingerprint, curvilinear deposits and GRODs at the ultrastructural level [3,16,25].

Lipopigment inclusions, most frequently described in neuron cytoplasm, have been found in extraneural tissues, including vascular smooth muscle cells (VSMCs), Schwann cells, and eccrine sweat gland epithelial cells [13,15,16]; however, only CNS demonstrates the most severe damage with neuronal loss. The potentiality of ultrastructure diagnosis of NCLs, based on extracerebral biopsies, is very important for the diagnosis of Kufs' disease, in which the use of genetic and enzymatic analysis is not possible.

Therefore, we report clinical and ultrastructural findings from examinations of the muscle and skin biopsy indicating Kufs' disease.

Case report

A 45-year-old female patient was admitted to the Neurological Department, Institute of Psychiatry and Neurology, Warsaw with suspected Creutzfeldt-Jakob disease. The first symptoms of the disease started 18 months before admission. Progressive dementia and psychotic symptoms as well as auditory and visual hallucinations and delusions emerged,

causing anxiety. Behavioural disturbances, including aggression, personal and social awareness, and lack of criticism were also observed. Additionally, speech disturbances developed gradually. The patient had also great problems with insomnia. Gait disturbances and urinary incontinence developed.

The first brain MRI (18 months earlier) showed mild cerebral atrophy and hyperintense signal (in T2 and FLAIR) within the cortex of frontal, parietal and temporal lobes as well as in basal ganglia. The examination of cerebrospinal fluid was normal; however, the test for 14-3-3 protein suggestive of neuronal damage was positive.

On neurological examination the patient showed generalized increase in muscle tone and the extensor type of planar response on the left side as well as positive left Babinski sign. Involuntary movements were also observed.

Laboratory parameters, including full blood count, urea, electrolytes, liver and thyroid tests, serum vitamin B₁₂ and ceruloplasmin level were normal except CRP and ESR, which were increased (non-specific marker of inflammation). The level of fibrinogen was raised.

VDRL test was negative. No HIV infection was found; anti-toxoplasma Gondi, anti-CMV, and anti-borrelia burgdorferi antibody were also normal. Anti-thyroid microsomal antibody, anti-paraneoplastic antibody, and anti-cardiolipin antibody were also normal. Antinuclear antibodies in serum were repeated twice. The first examination showed a several-fold elevation of ANA, but the other one revealed normal ANA and ANCA.

The lysosomal diseases as metachromatic leukodystrophy, alpha-mannosidosis, and gangliosidoses GM1 and GM2 were excluded. No changes were identified in the tau gene, preseniline-1 gene and preseniline-2 gene.

The second brain MRI was repeated 18 months after disease onset (Fig. 1). It revealed, apart from cerebral atrophy and basal ganglia involvement, chronic subdural haematoma in the left hemisphere. The haematoma was evacuated in the Department of Neurosurgery but the psychological and neurological state of the patient remained unchanged. The EEG examination showed a general slowing of background activity. Doppler ultrasound of carotid artery was normal. Cerebrospinal fluid was investigated repeatedly. White cells, glucose, protein and protein electrophoresis were normal. However, the test for 14-3-3 protein in the cerebrospinal fluid was positive

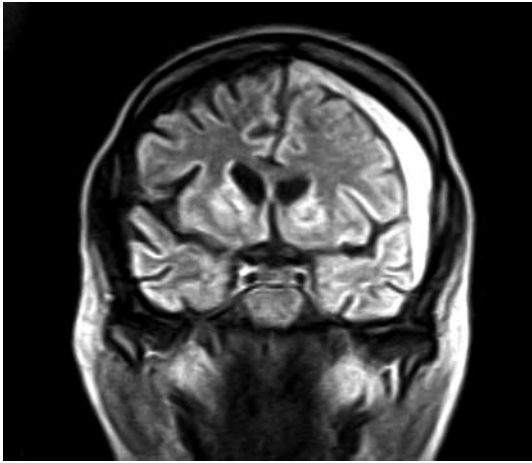


Fig. 1. MRI of the patient's brain showing cortical atrophy and basal ganglia involvement as well as left side subdural haematoma. FLAIR.

again. A tonsillar biopsy was performed. The lymphoreticular tissue in the tonsils did not stain for abnormal prion protein. Skin and muscle biopsies were performed for ultrastructural examinations.

Material and Methods

For the ultrastructural studies, small samples of skin and skeletal muscle were fixed in 2.5% glutaral-

dehyde with post-fixation in osmium tetroxide, and routinely processed into epoxy resin. The ultrathin sections were contrasted with uranyl acetate and lead citrate, and examined with a transmission electron microscope (Opton DPS 109).

Results

On electron microscopic examination the majority of muscle fibres showed normal structure. However, most of them showed non-specific subsarcolemmal lipofuscin granules and large osmiophilic and heterogeneous deposits usually located in the neighbourhood of nuclei (Fig. 2). In addition, granular osmiophilic deposits were dispersed in muscle fibres (Fig. 3). Numerous muscle fibres contained an array of tubules of unknown origin beneath the sarcolemma (Fig. 4). In the samples of muscle biopsy, numerous blood vessels were visible. Most of them were capillaries with narrow endothelial cells, showing lipopigment deposits in the cytoplasm (Fig. 5), whereas in vascular smooth muscle cells of small arterioles abundant and extensive inclusions were seen (Fig. 6A-C). They demonstrated fingerprint profiles (Fig. 7A), curvilinear profiles, granular osmiophilic deposits (Fig. 7B) and lipofuscin-like material. Usually CP and FP were present in the same inclusions (Fig. 8A-B). FP were

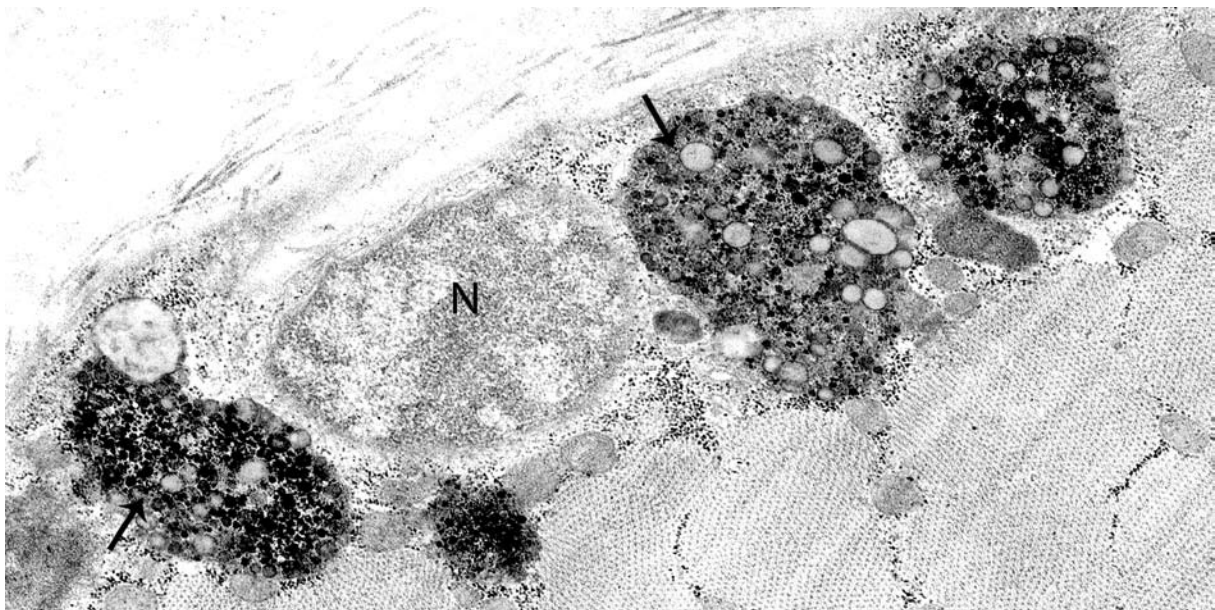


Fig. 2. Muscle fibre with osmiophilic and heterogeneous deposits (arrows) located near nuclei (N). Orig. magnific. $\times 7000$.

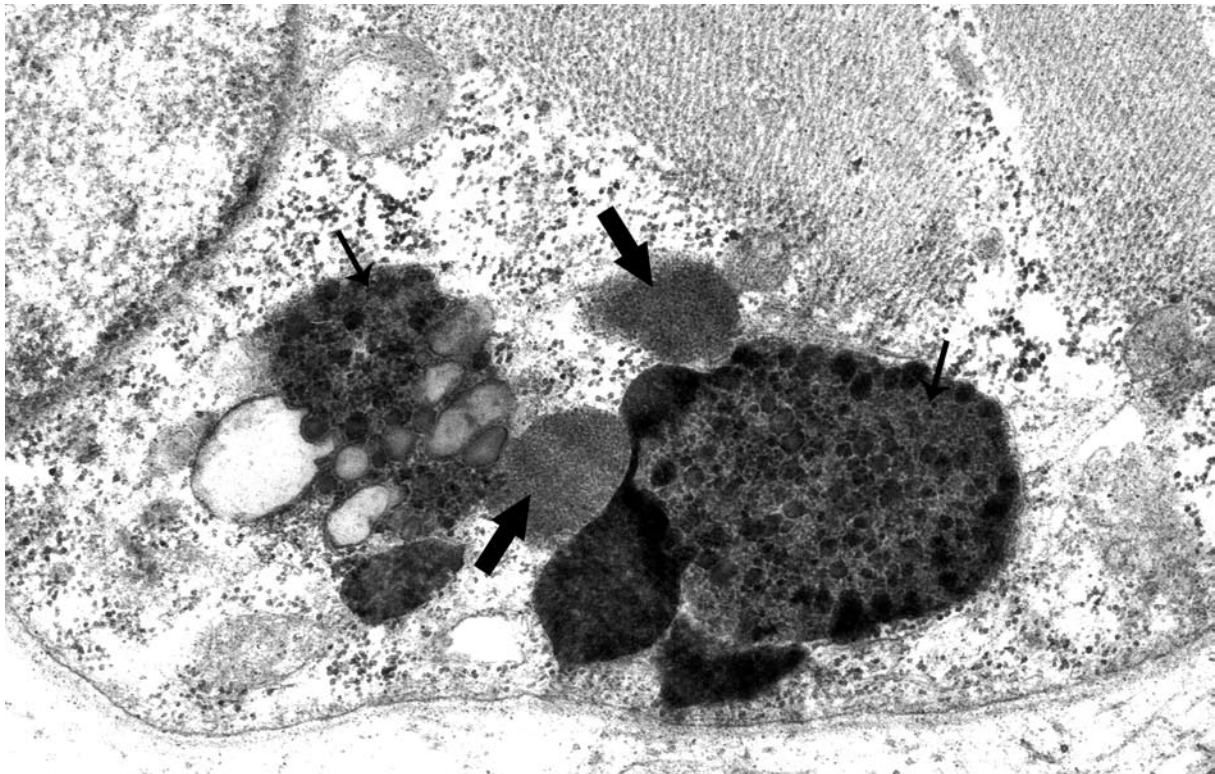


Fig. 3. Fragment of muscle fibre containing GRODs (bold arrows) and lipofuscin-like material (thin arrows). Orig. magnific. $\times 12\,000$.

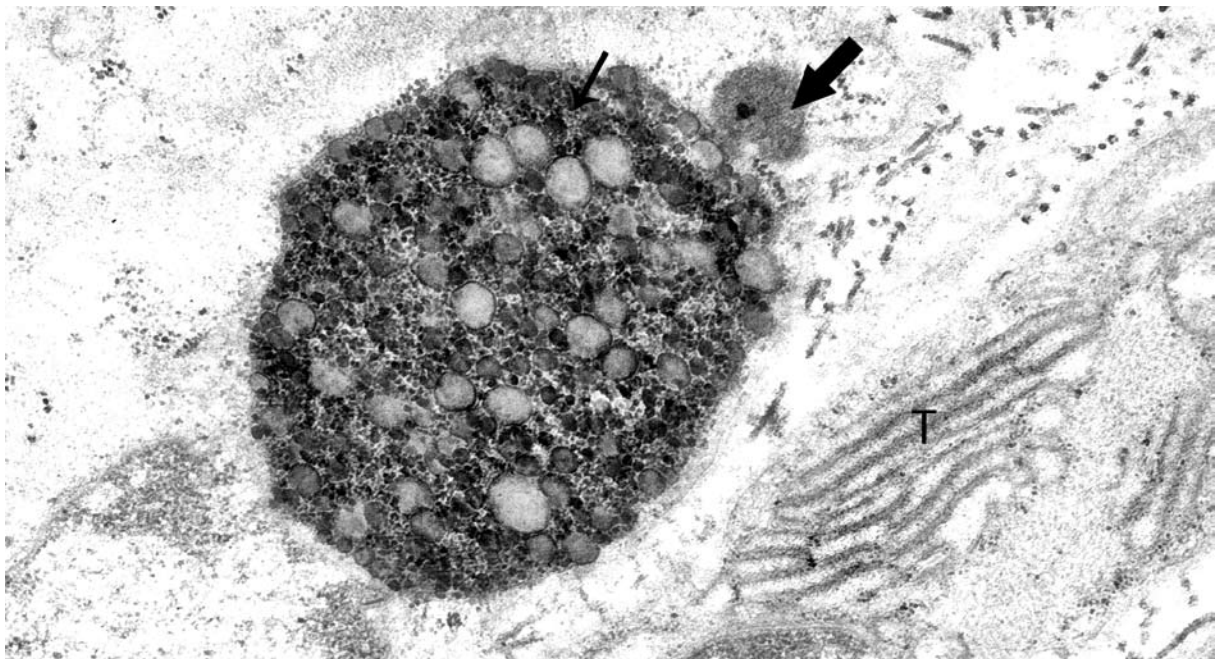


Fig. 4. Muscle fibres with lipofuscin-like material (thin arrow), GROD (bold arrow) and tubules (T) located under sarcolemma. Orig. magnific. $\times 7000$.

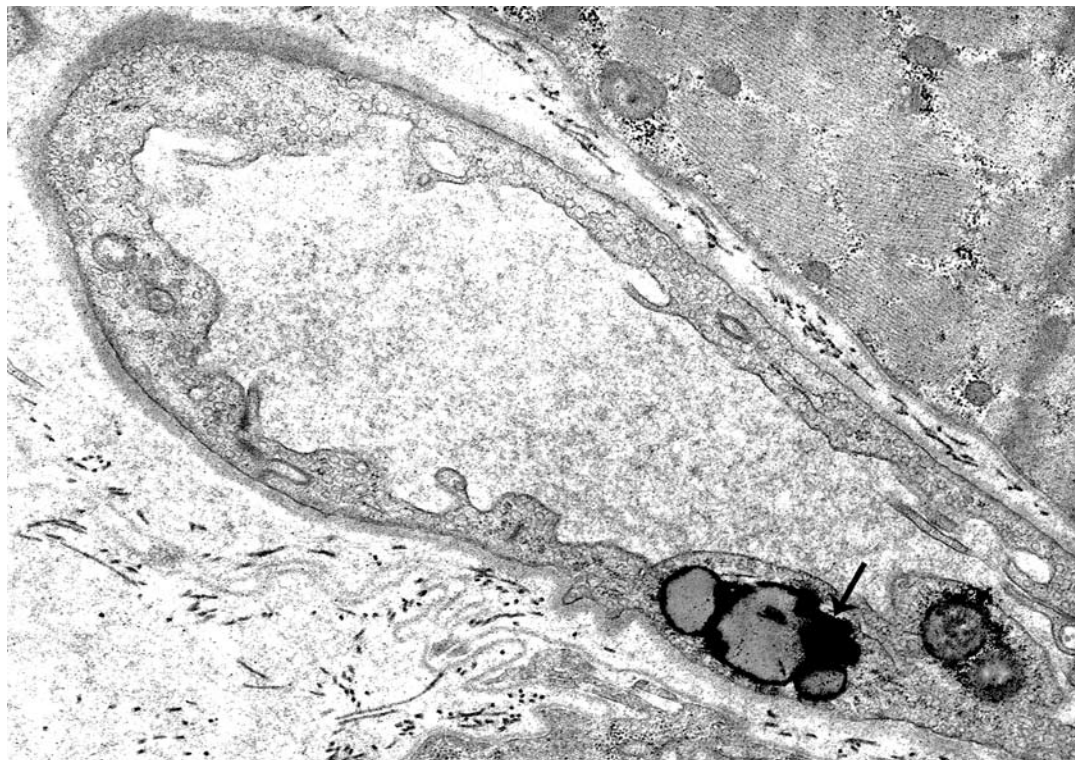


Fig. 5. Capillary with lipopigment deposit (arrow) located in endothelial cell. Orig. magnific. $\times 7000$.

sometimes intermixed with globules and/or granular material. FP exhibited varied size and number in one muscle cell and some of them were membrane bound. The wall of these vessels was thickened; in particular the basement membrane between endothelial and vascular smooth muscle cells contained numerous clusters of collagen fibres. Junctions between VSMCs were frequently detached, but muscle cells showed abundant cytoplasm with agglomeration of the mitochondria usually near fingerprint inclusions. Some of the mitochondria were swollen and showed a changed shape (Fig. 6C). Narrow endothelial cells with cytoplasmic protrusions penetrating to VSMCs contained lipopigment but without FP (Fig. 9). The skin biopsy samples did not contain typical diagnostic elements, e.g., eccrine sweat gland and nerve elements, which can accumulate specific lipopigment deposits.

Discussion

We present the ultrastructural findings of the skin and muscle biopsies from a patient with progressive encephalopathy showing clinical symptoms similar

to phenotype B of Kufs' disease, such as dementia and behavioural abnormalities. In our patient, initial clinical signs occurred about the age of 42 years. Due to the fact that the ANCL gene was not identified, the final diagnosis is based on clinical and pathological data only [6,25].

It is commonly acknowledged that diagnosis of ceroid lipofuscinosis is based on the accumulation of abnormal intracellular lipopigment and ultrastructural hallmarks of Kufs' disease, whereas FP are required for neuropathological diagnosis [1,15,16,24]. Cases with numerous GRODs found in muscles, eccrine glands and brain tissue, but without FP and CP, have also been diagnosed as Kufs' disease; however, this disease is usually associated with a mixed type of inclusions (GRODs, FP and CV) [4,18].

Our careful ultrastructural study of biopsy samples in the patient revealed numerous GRODs and lipofuscin-like material in numerous skeletal muscle fibres. The latter were usually large, located close to the nucleus, whereas GRODs were smaller and located under the sarcolemma and between sarcomeres. GRODs were also visible in vascular smooth muscle cells in both muscle and skin vessels.

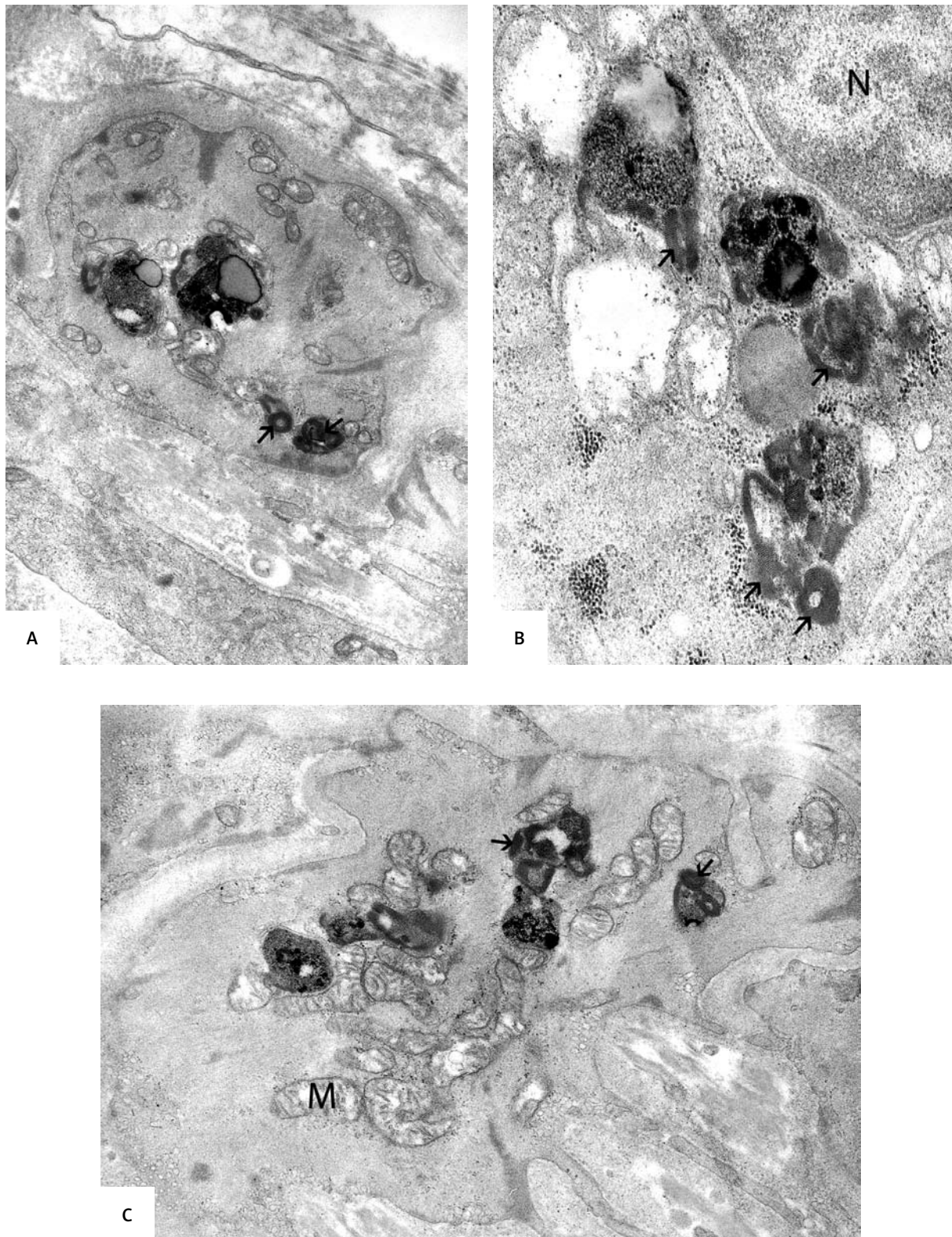


Fig. 6A-C. Vascular smooth muscle cells with numerous inclusions – lipofuscin, GRODs and FP (arrows). N – nuclei, M – swollen mitochondria with changed shape. Orig. magnific.: 6A \times 7000, 6B \times 12 000, 6C \times 7000.

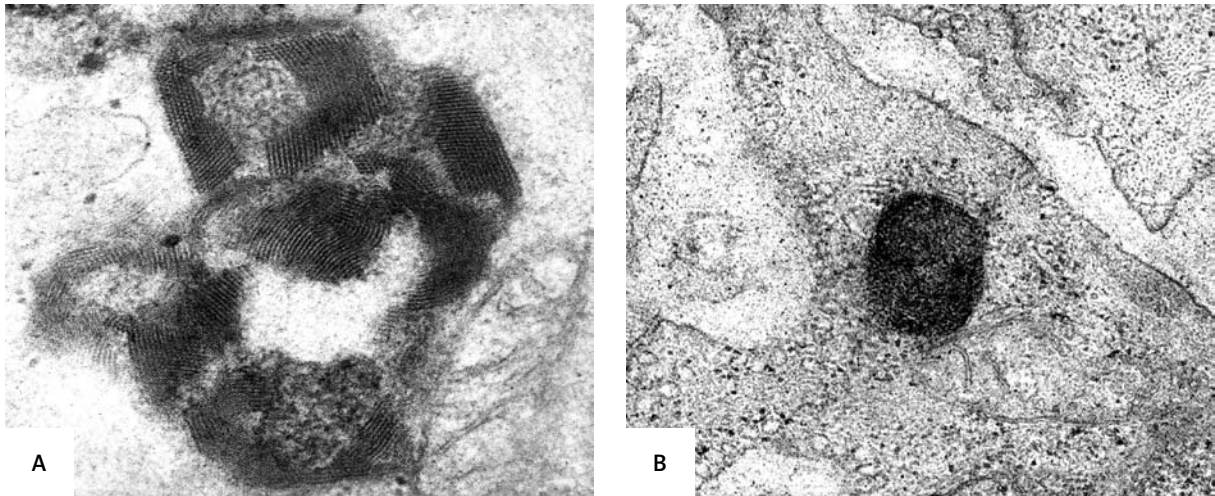


Fig. 7A-B. Vascular smooth muscle cells with fingerprint deposits (7A) and granular osmiophilic deposit (7B). Orig. magnific. 7A $\times 50\,000$, 7B $\times 12\,000$.

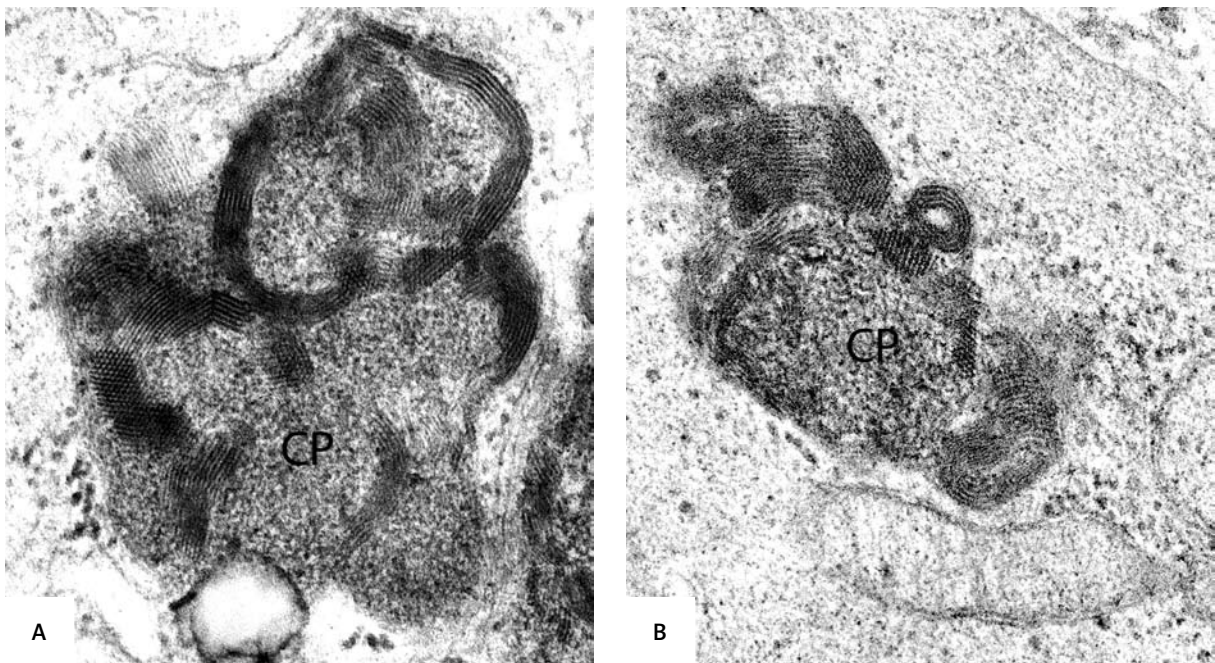


Fig. 8A-B. Fingerprint deposits and curvilinear profiles (CP) located in vascular smooth muscle cells. Orig. magnific. 8A $\times 20\,000$, 8B $\times 30\,000$.

In contrast, fingerprint deposits were found exclusively within vascular smooth muscle cells in all observed arterioles of the skeletal muscle biopsy. The majority of FP deposits comprised a mixed complex with lipopigment of varied morphology or curvilinear profiles. FP showed paracrystalline or parallel orientated lamellae, depending on the cutting plane. Their

number and size in one cell also varied and sometimes they filled a significant part of the cell. The presence of FP exclusively in VSMCs in rectal, skin and muscle biopsy specimens have been previously described by Pasquinelli et al. 2004 and Gelot et al. 1998 [15,20] in a patient with clinical picture consistent with Kufs' disease. Although the presence of typical FP only in

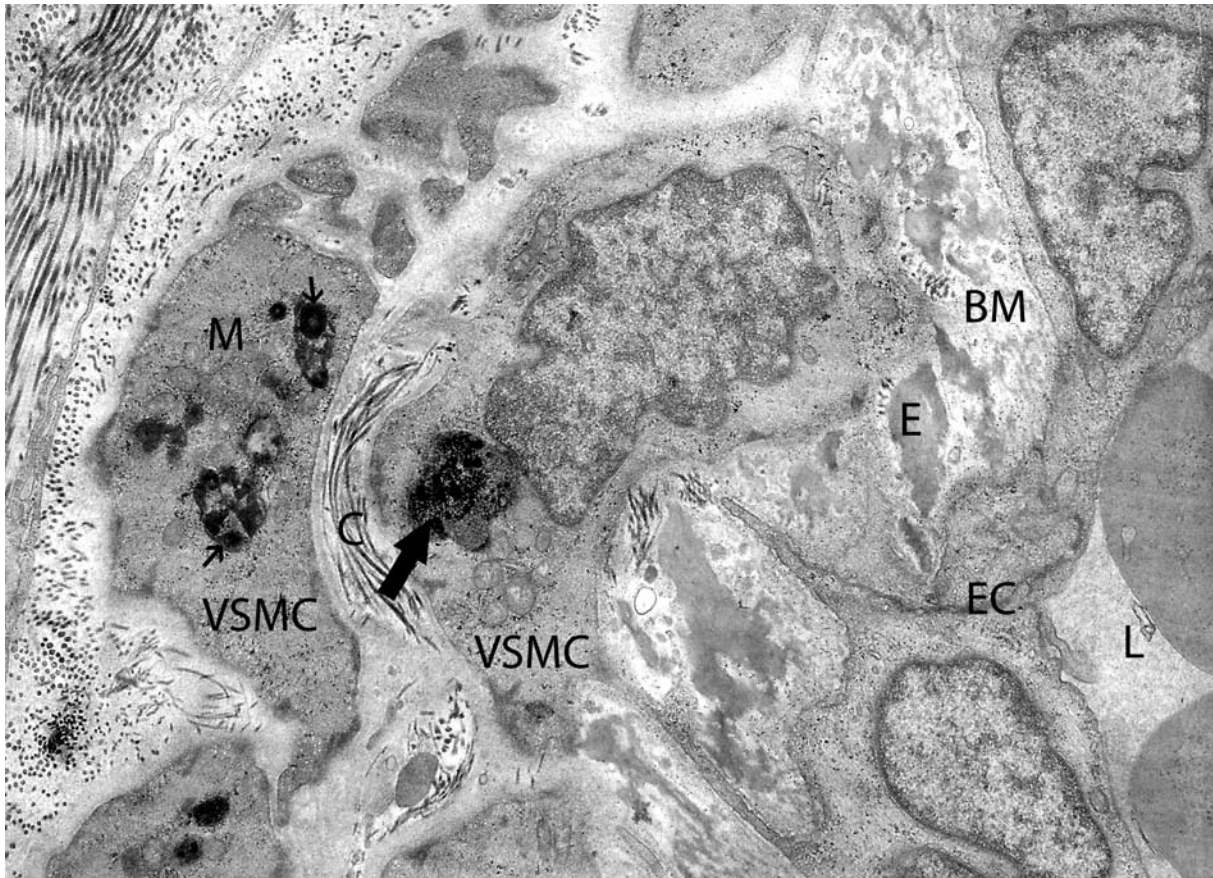


Fig. 9. Thickened wall of the arteriole with clusters of collagen (C) and elastin (E) in basement membrane (BM). Vascular smooth muscle cells (VSMCs) containing mitochondria (M), FP (thin arrows) and lipofuscin-like material (bold arrow). L – lumen, EC – endothelial cell. Orig. magnific. $\times 4400$.

VSMCs is consistent with the diagnosis of Kufs' disease, the authors consider whether the presence of specific inclusions in only one cytotype is enough for a well-established diagnosis [20].

In our patient, both FP and CP were found in the VSMCs, but were absent in the skeletal muscle fibres and other cells. The presence of FP only in smooth muscles, especially in vascular smooth muscle cells, was reported by Gelot et al. 1998 [15] and Pasquinelli et al. 2004 [20]. In our patient, as in the case reported by Gelot et al. 1998 [15], to confirm the diagnosis, only findings from the peripheral biopsy specimens were taken in the absence of cerebral tissue. The presence of a specific inclusion for Kufs' disease only in VSMCs suggested that vascular smooth muscle cells may be peripheral targets in Kufs' disease [15].

In the majority of ANCL cases reported earlier, the diagnosis was based on the examination of ce-

rebral tissue obtained either by biopsy or at autopsy [1,6,23], and in a few patients only extracerebral biopsies were additionally examined [1,4,7,17,20]. It is worth mentioning that in a very few cases of Kufs' disease reported to date, the diagnosis has exclusively been based on the examination of extracerebral biopsies, e.g., sural nerve, muscle, skin, conjunctiva and rectum [15,18,21]. It should be emphasized that the sensitivity and specificity of the diagnosis of neuronal ceroid lipofuscinosis based on the electron microscopic examination of extracerebral tissues is extremely important in ANCL, in which the mutation of gene *CLN* has not as yet been identified [4]. In our opinion, deposits with a specific ultrastructural picture, such as FP and CP, which can be easily differentiated from lipopigments accumulated in cells with aging, are very useful in supporting clinical diagnosis.

To sum up, additional and numerous electron microscopic examinations of different extracerebral biopsies from patients with progressive encephalopathy and also typical clinical features suggestive of Kufs' disease can only help to develop a "golden diagnostic procedure" for non-neuronal tissues indispensable to make a well-established diagnosis of Kufs' disease. The development of such a procedure would facilitate further investigations concluded with accurate diagnoses for the benefit of patients.

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