Case report

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) – literature review apropos an autopsy case

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a non-atherosclerotic, non-amyloid cerebral angiopathy involving small arteries and arterioles. This entity presents vascular changes in the form of smooth muscle degeneration with swollen myocytes and PAS-positive granular deposits, together with vascular fibrosis and hyalinization. In parallel, diffuse white matter destruction with infarcts, tissue rarefaction, spongiosis, lacunes and demyelination are characteristic. Ultrastructurally, vascular granular osmiophilic material (GOM) is pathognomonic for this hereditary disease caused by *NOTCH3* mutation.
We diagnosed CADASIL in the autopsy examination of a 53-year-old woman with a 9-year history of a progressive neurological disease with complex motor and cognitive deficits, accompanied by non-specific diffuse white matter changes on neuroimaging. Despite several multicentre hospitalizations, the precise diagnosis was not established until the post-mortem examination of the brain was made.
CADASIL is a rare entity, but it should be considered by a pathologist in a differential diagnosis of vascular diseases of the brain, especially in cases with atypical clinical presentation and familial history. The prompt diagnosis depends on the quality of the brain autopsy and proper sampling. The *post mortem* examination, where "*Morituri vivos docent*", is still significant.

Key words: CADASIL, brain, arteriopathy, stroke, autopsy.

Introduction

Small vessel diseases (SVDs) of the brain are increasingly common in parallel to the general population aging. These diseases are mostly sporadic, rarely hereditary, while hallmark features of both categories encompass vascular wall sclerosis and secondary white matter changes. However, their pathogenesis is different and they present diversity in morphology, as well as intensity and topography of the lesions [1, 2, 3, 4]. The parenchymal changes in SVD include subcortical infarcts, lacunes, widened perivascular spaces, cerebral microbleeds, and leukoencephalopathy. There are several clinical and neuropathological classifications of SVDs [2, 3, 5]. The sporadic group has several forms, and is multifactorial in origin, having a close relation to hypertension, atherosclerosis and aging. One of its examples is Binswanger's type encephalopathy, caused essentially by arterio-, arteriolosclerosis and lipohyalinosis. The next representative of SVD is the cerebral amyloid angiopathy group (more prone to hemorrhages), often coexisting

with Alzheimer's disease [1, 2, 3]. Moreover, several rare monogenic hereditary SVDs have been discovered and genotyped in the last 20 years (Table I). The most common hereditary SVD is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) [1, 2, 4, 6].

CADASIL is a non-atherosclerotic, non-amyloid angiopathy, with autosomal dominant inheritance [2, 6]. The clinical features in CADASIL comprise recurrent strokes and/or silent ischemic episodes, migraine with aura, motor deficits, pseudobulbar palsy, mood disturbances and subcortical dementia in relatively young patients [7, 8]. This disease, caused by mutation of the *NOTCH3* gene, leads to degeneration and loss of vascular smooth muscle cells in the tunica media of small and medium size arteries [6, 8, 9, 10]. The arteries affected in CADASIL reveal progressive smooth muscle cell degeneration with wall thickening due to fibrosis and hyalinization. These changes give rise to permeability disturbances and reduce the blood flow with multifocal metachronous ischemic injury of the brain [1, 2, 3, 11]. CADASIL usually occurs in the absence of typical cardiovascular risk factors. The wide variability of the clinical picture of this arteriopathy means that, like in the presented case, the final answer cannot be given until autopsy [6, 8, 9].

Case study

A 45-year-old woman with an uneventful medical history was hospitalized for the first time in 2004, due to dizziness, speech processing difficulties, and memory and gait disturbances for several months. The patient's family history disclosed that her father died bedridden with dementia in the age of 56 years due to an unknown neurological disease lasting for several years. Magnetic resonance imaging (MRI) scans showed many irregular areas of increased signal on T2-weighted imaging in the white matter of the brain especially in the periventricular area, also within the pons and brain pedunculi, with no contrast enhancement. The ventricular system and cerebral cortex were normal. The differential diagnosis comprised leukodystrophy or a demyelinating process. Subsequent CT examination confirmed disseminated non-specific changes of the white matter, with the suggestion of vasogenic changes or an inflammatory process.

In the following years the patient was periodically hospitalized in reference neurological departments due to potentiation of symptoms, progressive dementia and pyramid-cerebellar syndrome. A wide spectrum of biochemical blood and cerebrospinal fluid tests were irrelevant. Repeated neuroimaging revealed multiple small lacunar foci in the deep white matter. Single-photon emission computed tomography (SPECT) revealed deficits of hemispheric perfusion, EEG showed diffuse changes in frontal and temporal areas, and nerve conduction examination revealed features of axonal neuropathy. Multiple sclerosis, neurodegenerative, metabolic and mitochondrial diseases, intoxications and infections were consecutively excluded. In the last two years of life, the patient developed tetraparesis and mutism. She died in 2012 at the age of 53 years, due to pneumonia, and because of the unestablished final neurological diagnosis, the *post mortem* examination was performed.

Macroscopic appearance

General autopsy confirmed purulent bronchopneumonia as the cause of death. Mild atherosclerotic changes were detected in the main arteries. The sections for all organs were taken for the microscopic examination. The whole brain and spinal cord were formalin fixed for 3 weeks for the neuropathological examination. Slight cerebral atrophy was evident,

Table I. Hereditary small vessel diseases of the brain [1, 2]

1. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)
2. Some forms of cerebral amyloid angiopathy
3. Hereditary endotheliopathy with retinopathy, nephropathy, and stroke (HERNS)
4. Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL)
5. Retinal vasculopathy with cerebral leukodystrophies (RVCL)
6. Pontine autosomal dominant microangiopathy and leukoencephalopathy (PADMAL)
7. Portuguese-French type familial small vessel disease of the brain
8. Swedish type hereditary multi-infarct dementia
9. COL4-related disorder (stroke syndrome)
10. Hereditary systemic angiopathy (HSA)
11. Hereditary diffuse leukoencephalopathy with axonal spheroids (HLDS)

the meninges presented congestion, and mild focal atherosclerotic changes of the circle of Willis arteries were found. On the surface of the left parietal lobe an excavation 3 / 2 cm was noticed. The whole brain cut sections disclosed multifocal decay in the form of irregular yellowish-rusty partially cystic foci within the white matter 0.5-5 cm, especially in parietal and temporal lobes (Fig. 1A, B). Moreover, discoloration, foci of spongy degeneration and lacunae of frontal white matter, and *status cribrosus* of the pons, basal ganglia, cerebellum, and the spinal cord were found (Fig. 1C, D). A few bigger cystic foci with scarring were also present. The ventricular system showed dilatation. Single linear cortical softening up to 1.5 cm in the left temporal region was observed.

Many topographically oriented samples were taken from the brain and the spinal cord for histopathology according to neuropathological recommendations [12]. All sections were stained with HE, and further the selected sections were stained with histochemical methods – PAS, Masson trichrome, van Gieson, Gomori, Spielmayer, and Congo red. Immunohistochemistry was performed with a routine procedure using anti-SMA and GFAP antibodies (anti Smooth muscle actin, anti GFAP; flex, DAKO) according to the producer's description, with PT-linker for antigen retrieval.

Microscopic findings

Histological examination of the central nervous system (CNS) showed multifocal metachronous vasogenic changes (Fig. 1E–H). They were located primarily in the white matter of the whole CNS, in the form of tissue rarefaction, as well as old and subacute ischemic infarcts of different size with foamy macrophages surrounded with the astroglial reaction. Perivascular spaces were in general enlarged, containing focal hemosiderin deposits. Status cribrosus and lacunaris, a few foci of recent cerebral softening, as well as confluent postapoplectic cysts were the next prominent findings. The relatively spared white matter showed demyelination with U-fibers sparing, intensive in periventricular regions. The arterioles and arteries of small and medium size, within the cerebrum, meninges, cerebellum, spinal cord, and the nerve roots exhibited specific changes (Fig. 2A). The arteriolar wall was markedly thickened with a conse-

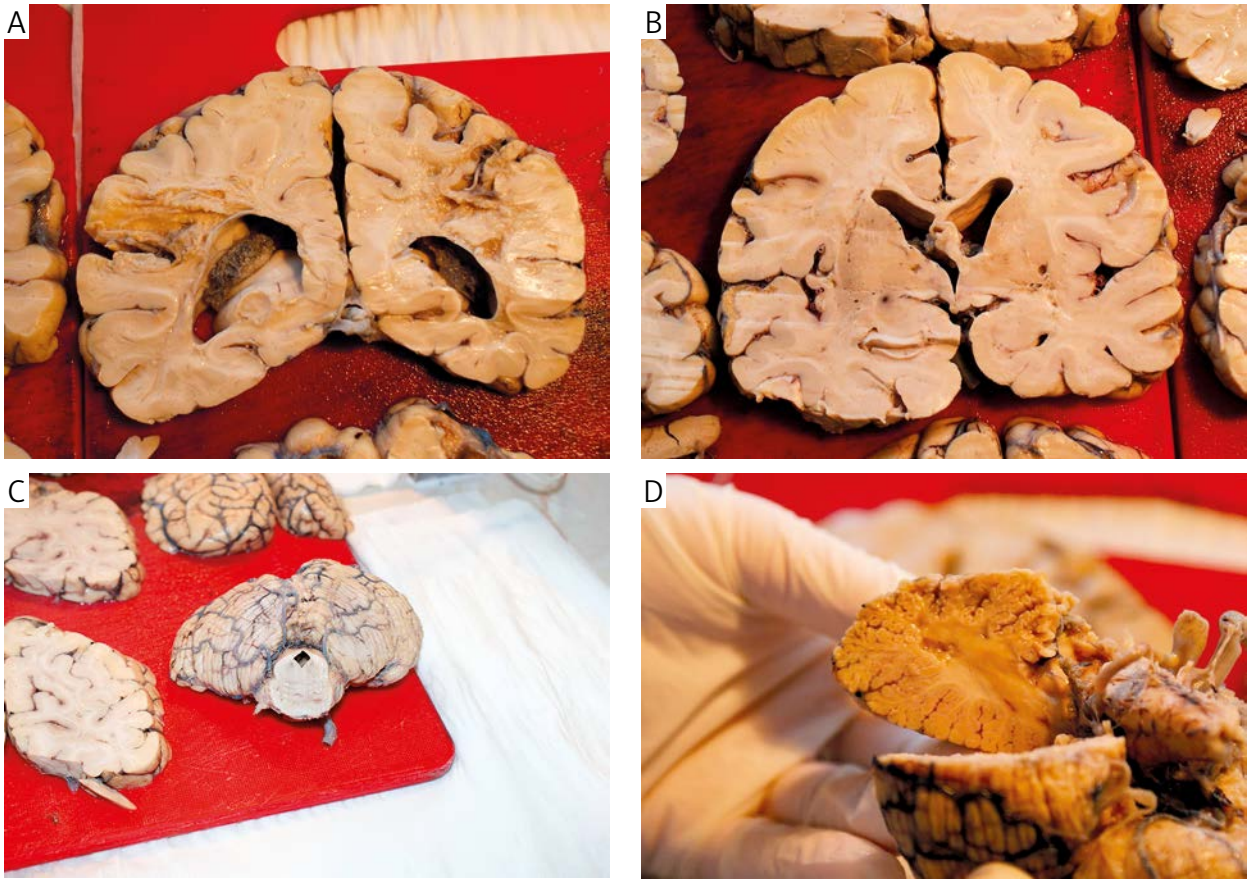

Fig. 1. Gross and histological features of described case. **A)** Diffuse multifocal destruction of the white matter with yellowish necrotic cavitations (gross image). **B)** White matter changes with status cribrosus and small subcortical and basal ganglia lacunes. Linear softening with yellowish discoloration of superior temporal lobe cortex (gross image). **C)** Multiple lacunes within the pons (gross image). **D)** Complete old infarction in cerebellar white matter and subcortical decay (gross image)

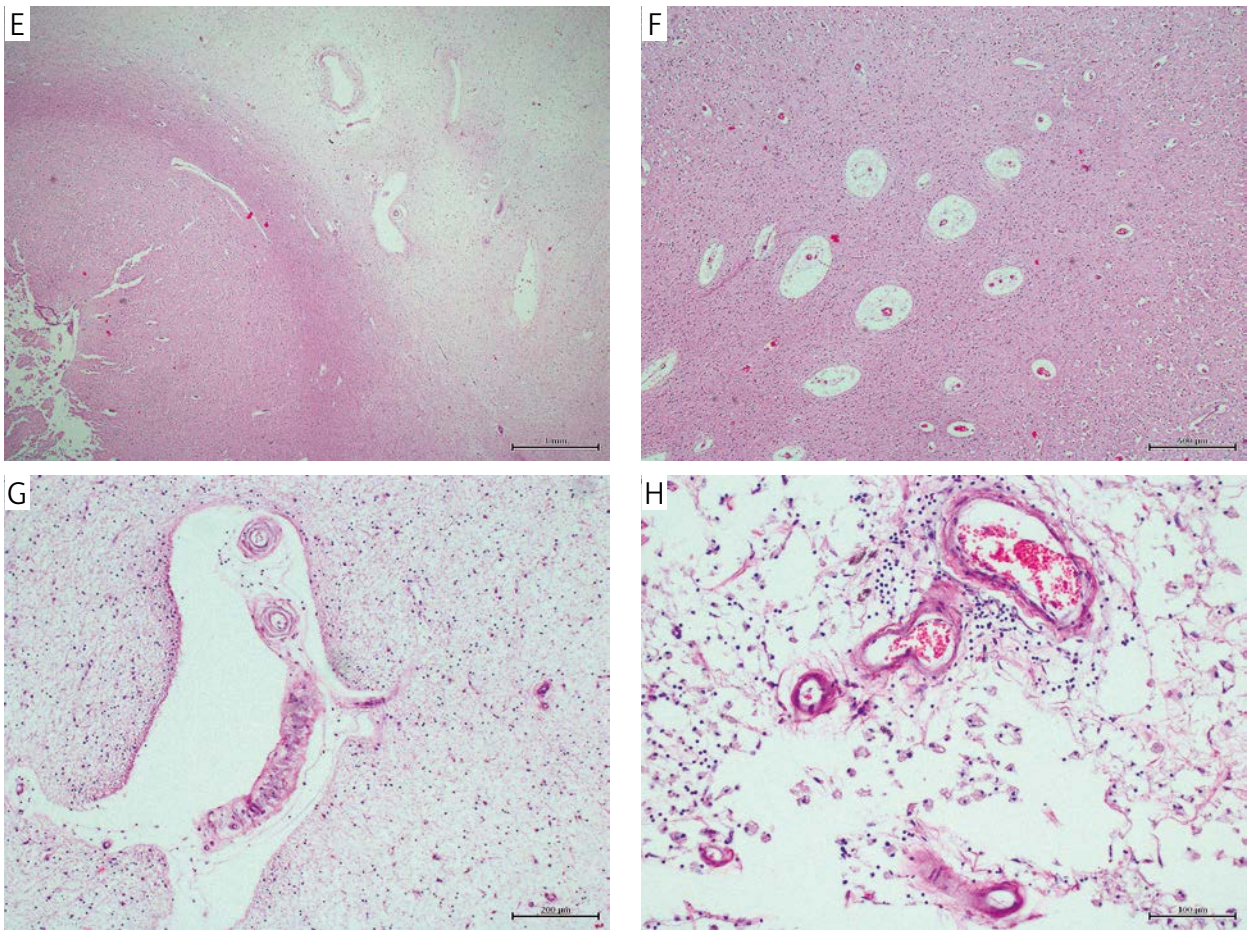

Fig. 1. Gross and histological features of described case. **E)** Zonal distribution of process: white matter with demyelination, rarefaction, perivascular widening; spared myelinated U-fibers; quasi normal cortex (HE, 20×). **F)** Frontal subcortical white matter showing generalized perivascular space widening with sclerosed blood vessels inside (HE, 40×). **G)** Leukoencephalopathy: paucicellular demyelinated, rarefaction of white matter with slight astrogliosis, perivascular lacunes with thick-walled bluish arterioles inside (HE, 200×). **H)** Focus of subacute infarction with foamy cells, small hemosiderin deposits and sparse lymphocytes (HE, 200×)

quent vascular lumen narrowing. Hypertrophic and degenerated, swollen myocytes with distinct hyperchromatic nuclei were seen within the media of many vessels. Moreover, many vessels presented medial bluish discoloration, with accumulation of slightly brownish granular deposits (Fig. 2B). Consecutive fibrosis and hyalinization progressing from the external part of the vascular wall was seen in different stages up to completely glazed vessels. Masson staining emphasized this phenomenon well (Fig. 2E). PAS staining visualized granular basophilic material within the vascular media (Fig. 2C, D). SMA immunohistochemical reaction revealed degeneration of medial smooth muscle cells (Fig. 2F) along with depletion and thinning of this layer. The Congo-red stain was negative. Ultrastructurally, GOM presence was confirmed in the form of round granular deposits located in smooth muscle cell/pericyte membrane infoldings (Fig. 2G).

The analysis of the systemic organ sections revealed that the small arteries in the liver, kidneys, fat tissue, and spleen presented analogous abnormalities, although of a lesser intensity (Fig. 2H). All above findings supported the diagnosis of CADASIL. The members of the patient's family were informed about the diagnosis and hereditary character of the disease, but they refused to undergo further genetic consultation.

Discussion

In the general population, CADASIL is a rare neurological disorder, but the most frequent non-atherosclerotic monogenic hereditary degenerative vascular cerebral disease [1, 2, 6]. It was described for the first time by van Bogaert and colleagues as a familial type of Binswanger's disease in 1955 [8]. In 1997 *NOTCH3* was discovered as responsible for the disease. Human *NOTCH3* is composed of 33 exons and its product is a 2321 amino acid transmembrane receptor widely expressed on smooth muscle cells and pericytes [13, 14]. Notch3 functions in cellular sig-

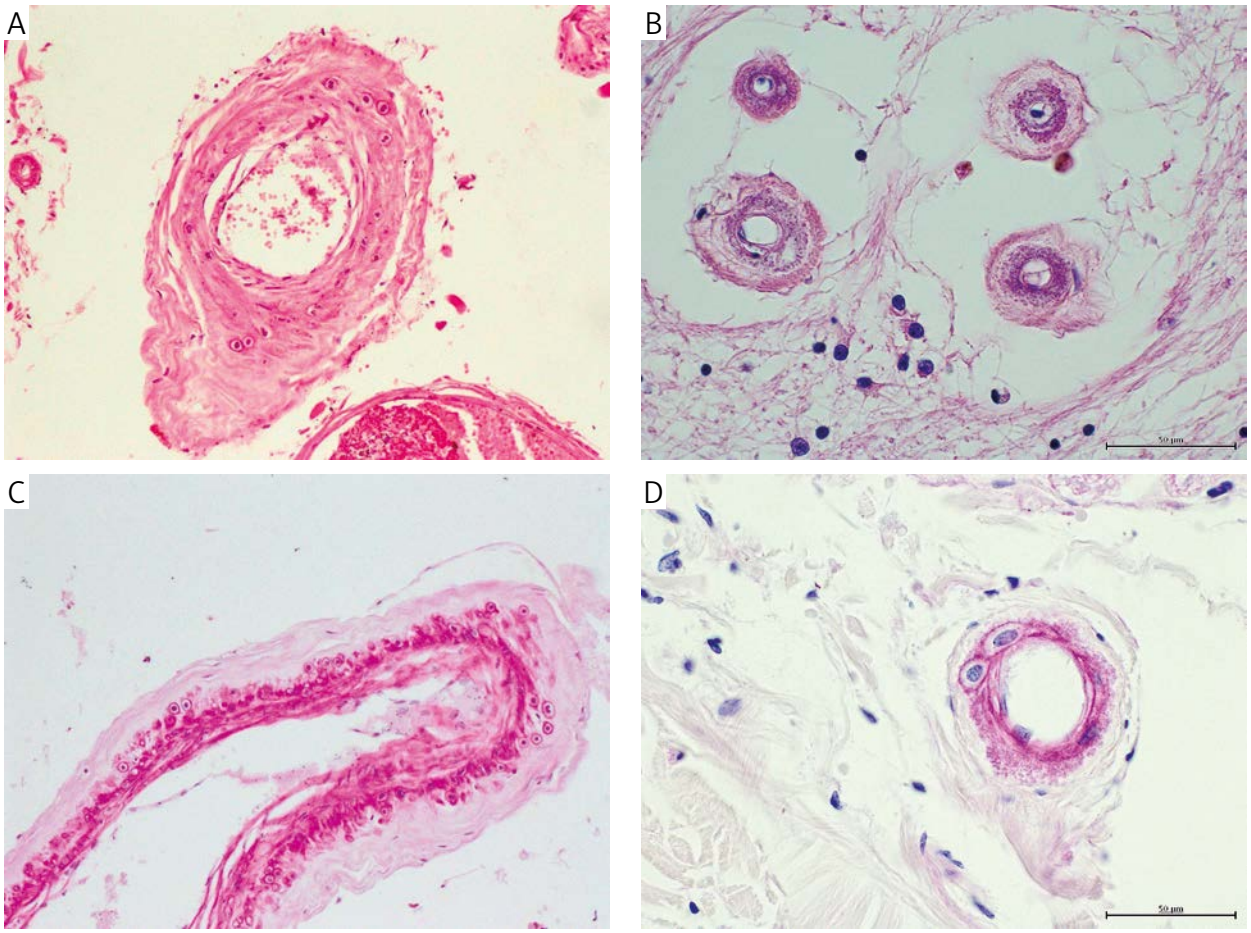

Fig. 2. Histology of arteriopathy in CADASIL. **A)** Small artery and arteriole showing thickening of the vascular wall, degeneration of muscular layer and presence of swollen myocytes (HE, 200×). **B)** Arterioles with profound degeneration and dense granular deposits (HE, 200×). **C, D)** PAS staining disclosing muscular degeneration with granular basophilic material, and homogenization ad externo (PAS 200×)

naling, regulating apoptosis, cytoskeleton function and cellular adhesion. Large Notch3 extracellular domain (N3-ECD) is a heterodimer which has 34 epidermal growth factor-like repeats, each of them including six cysteine residues. So far about 170 mutations of *NOTCH3* located in exons 2-24 have been described, and all refer to *EGFR* repeats, changing the number of cysteine residues in Notch3 protein [4, 11, 14]. Probably, the mutation prevents Notch3 proteolysis and produces a toxic effect due to an increased tendency to form aggregates and its accumulation in the vascular media. Another hypothesis explaining smooth muscle injury is Notch3 signaling pathway malfunction as a cell receptor [2, 6, 11]. About 500 families with *NOTCH3* mutations have been described worldwide [6, 8].

The first clinical symptoms of CADASIL present typically in the late third decade of life, including transient ischemic attacks (TIA)/strokes, mood and cognitive disturbances, migraine with aura and apathy [9, 10]. Progression of the disease leads to dementia, neurological dysfunctions such as dysarthria, hemiparesis, pseudobulbar palsy, and finally wasting and death [5, 6, 7]. There is a lack of classic vascular risk factors. The patients die usually 15-25 years after the first symptoms. The treatment of CADASIL is the supporting therapy and the reduction of stroke risk factors such as arterial hypertension or hyperlipidemia, the modification of lifestyle and psychological assistance [6, 8]. The clinical course of CADASIL varies even in families with the same mutation, so the coexisting genetic and epigenetic factors play a role in disease evolution [7, 14]. Radiologically, at the beginning of the disease, symmetrical focuses of increased signal on T2-weighted MRI images in semioval center and periventricular areas appear. Next they become prominent in the temporo-polar region and capsula externa, remain confluent and can involve all the brain white matter, sparing the cortex and U-fibers [2, 7, 15]. These are complemented by periventricular, initially spotty leukoaraiosis. Due to different patterns, CADASIL can be misdiagnosed as multiple sclerosis or even a brain tumor [2, 15]. Clinically, in a suspicion of CADASIL, the diagnostic gold standard is a histological examination of a deep skin biopsy with PAS staining and electron microscopy

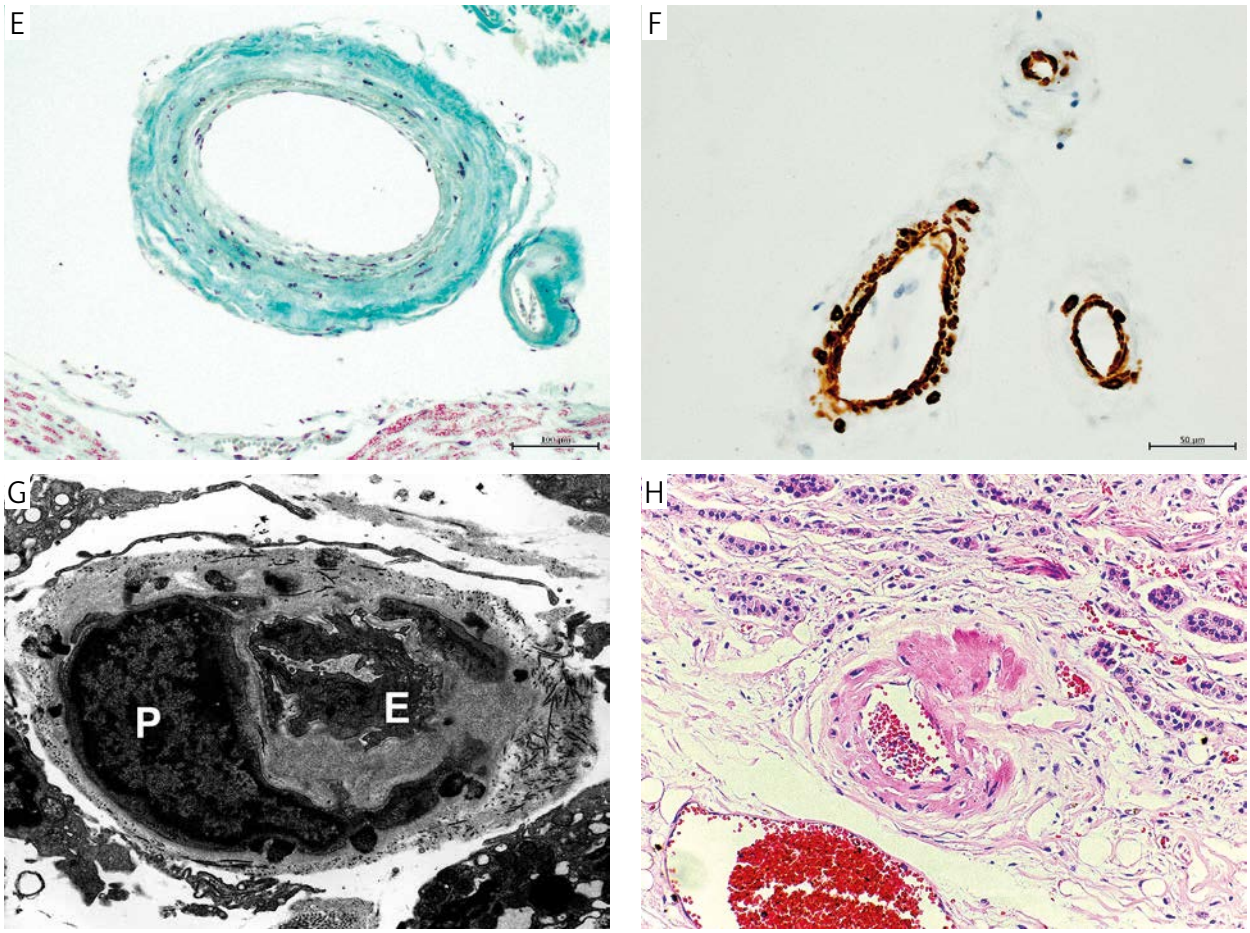

Fig. 2. Histology of arteriopathy in CADASIL. **E)** Fibrosis and hyalinization of the vascular wall (Masson, 200×). **F)** Plumpy swollen decreased in number myocytes (SMA, 200×). **G)** Numerous GOM deposits of different shapes localized near pericyte body and within thickened basal lamina at the circumference of the capillary vessel. Visible degenerative changes in endothelium, and narrow misshapen lumen of the capillary (ultrastructural examination; P – pericyte, E – endothelium). **H)** Vasculopathy features within the small artery from the perisuprarenal fat (HE, 200×)

analysis [6, 16]. At the ultrastructural level, presence of pathognomonic granular osmiophilic material (GOM) deposits confirms the diagnosis with 100% specificity, even without genetic results [10, 14, 16, 17]. Typical GOM, containing Notch3 extracellular domain, is a round granular deposit located in vascular smooth muscle cells (VSMC)/pericyte membrane infoldings. GOM also occurs within the thickened basement membrane, forming round to oval to irregular-shaped structures with different density of granules [16, 17, 18, 19]. Another diagnostic possibility is immunohistochemical staining with antibody against the extracellular domain of Notch3 receptor [6, 7, 8]. Finally, the diagnosis of CADASIL may be correctly established based on the sequencing of the *NOTCH3* gene, which is the most definitive method, but should be properly interpreted [7, 20].

Our case falls within the classical morphological spectrum found in CADASIL. Small arteries and arterioles displayed degeneration of the muscular layer with presence of swollen myocytes accumulating a brownish-bluish granular substance and consecutive fibrosis and hyalinization. Typically, the narrowing of the thick-walled hyalinized long penetrating arterioles and their branches supplying subcortical structures conforms to increased ischemic damage in the white matter. The alterations include complete and incomplete infarction, lacunar strokes, perivascular spacing, cavitations with or without macrophages and areas of reactive astrogliosis. The additional important elements of leukoencephalopathy comprise tissue rarefaction, pallor or swelling of myelin, demyelination, spongiosis, loss of oligodendrocytes and myelin fibers, as well as axonal degeneration and loss [2, 3, 5, 7]. The U-fibers and cortex are usually spared; however, our case showed focal unusual cortical destruction. In parallel, disseminated lacunar infarcts affect the basal ganglia, brainstem and spinal cord [3, 6, 7]. It is interesting why CADASIL, being a systemic arteriopathy, affects symptomatically almost exclusively the brain. We observed vascular changes also in other organs, but without features of ischemia or hypoperfusion. One explanation is that, because of the specificity of autoregulation and

blood-brain barrier functions, cerebral vessels have fewer and a distinct type of VSMC and pericytes compared to systemic vessels. Changes within the capillaries also occur, especially within the brain [6, 19]. Probably cerebral vessels have an increased susceptibility to injury. In addition, limited capacity for regeneration of CNS tissue and insufficient collateral circulation predispose to strokes, especially in view of the lower density of vascularization in the white matter [5, 6, 16].

CADASIL is a rare entity, but it should be considered by a pathologist in a differential diagnosis of vascular diseases of the brain, especially in cases with atypical clinical presentation and familial history. We present our case to show that during *post mortem* examination, the pathologist may make the final diagnosis of a rare neurologic disorder. The basic condition is a clinicopathological correlation of findings obtained with a standard methodical approach to the brain autopsy. It is important to perform the brain examination carefully with a sufficient number of topographically matched slides, while also bearing in mind the several forms of SVDs [1, 3, 12]. Nowadays pathology can still be used to ascertain the diagnosis if the final neurological recognition is lacking, reliable genetic screening is not readily available or if genetic screening gives an equivocal, negative or previously unknown result. Furthermore, the correct autopsy performance in cases like this has great importance not only for clinicians, but also for the deceased patient's family.

The authors declare no conflict of interest.

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