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Amyloid angiopathy in idiopathic Parkinson's disease. Immunohistochemical and ultrastructural study

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Abstract

The prevalence of cerebral amyloid angiopathy (CAA) and its association with intellectual decline in idiopathic Parkinson's disease (iPD) remain unclear. To identify the role of CAA in iPD dementia the prevalence and severity of CAA were investigated, with particular respect to changes in vessel wall structure. Twenty-eight autopsy Parkinsonian brains and fourteen age-matched controls, post-mortem revised histopathologically for the presence of α-synuclein and Alzheimer's disease (AD)-type pathology, using standardized clinico-neuropathological criteria, underwent further investigation. Histological, immunohistochemical staining methods with antibodies to amyloid β-peptide, α-actin, collagen III, collagen IV and CD34 as well as ultrastructural methods were used. The findings showed that the prevalence of CAA in the iPD cohort was higher (53%) than in controls (28%). CAA occurred more frequently in the iPD+AD (70%) sub-set than in the iPD-AD (44%) one. The progression of CAA was differentiated, with predominance of mild stage. Diminished smooth muscle actin and collagen IV expression in the vascular media with concomitant collagen III positive immunoreactivity in the intima were observed only in very severe CAA. Ultrastructural assay revealed degenerative changes in vessel smooth muscle cells and thickening of their basement membrane with the focal accumulation of amyloid fibres and fibrillar collagen in both iPD –AD and iPD+AD cases, but the most severe CAA-type changes were visible in the iPD+AD sub-set. The same type of immunoreactivity (Aβ42 positive and Aβ40 positive) of arterial CAA and parenchymal neuritic plaques, as well as capillary CAA and diffuse plaques (Aβ42 positive and Aβ40 negative), may indicate pathogenic similarities and differences between both types of degenerative changes on the one hand and time-different changes or local different processing of amyloid precursor protein on the other.

Key words: *idiopathic Parkinson's disease, Alzheimer's disease-type pathology, cerebral amyloid angiopathy, immunohistochemistry, ultrastructure.*

Introduction

Sporadic cerebral amyloid angiopathy (CAA) is a progressive degenerative process of leptomeningeal and cortical vessels caused by the accumulation of amyloid β-peptide (Aβ) in their walls [31]. CAA primarily affects medium and small leptomeningeal arteries and cortical arterioles, less frequently veins and capillaries, and occasionally subcortical vessels [36]. CAA may be responsible for cerebral haemorrhage [32], pale infarcts [8,28] and

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probably for progressive intellectual decline leading to dementia [29,46]. In view of its morphological and biochemical differences [3,21], two types of CAA are distinguished, arterial CAA, involving larger and medium vessels, and capillary CAA (CapCAA), involving pre-capillary and capillary vessels, known as 'dyshoric' angiopathy of Morel or 'drusige entartung' of Scholtz [26,27,35,40]. Sporadic CAA is primarily observed in Alzheimer's disease (AD) [3,34] and also in the elderly over sixty years of age and is strongly age-related [9]. The literature data show that in the elderly without evident intellectual deterioration, the prevalence of CAA does not usually exceed 50% on average [18,46] and it appears to be higher in dementia-affected populations [3,16], reaching up to 90% in AD [20,29,31]. Nowadays, it is also accepted that CAA is an early and integral part of sporadic AD pathogenesis [27,42,43]. It has been suggested that CAA may be an independent pathogenic factor contributing to cognitive decline and dementia, even without coexistent AD-type pathology, pale infarcts or haemorrhages [4]. The prevalence of CAA and its relationship with intellectual disturbances observed in idiopathic Parkinson's disease (iPD) are not known. The number of publications on this subject is very limited [19,22]. The aim of our study was to assess (1) structural changes in CAA-affected vessels; (2) CAA severity; (3) CAA prevalence; and (4) the prevalence of AD-type pathology (neurofibrillary tangles and senile plaques) in iPD cases in the cerebral regions responsible for intellectual efficiency.

Material and Methods

The study was performed on 28 autopsy brains derived from patients (14 women and 14 men; mean 77.46±7.43 years; range 59-91 years) who died because of iPD, selected from 45 clinically diagnosed iPD cases. The diagnosis was made according to generally accepted criteria, and confirmed by a routine neuropathological examination. The controls, selected from 21 deceased persons, consisted of 14 age-matched cases (7 women and 7 men, mean 74.96±9.80 years, range 66-89 years), free of neurodegenerative diseases and psychic disorders or symptoms of dementia. A preliminary screening of material was carried out semiquantitatively, using immunohistochemical (IHC) methods, according to: (1) Newcastle neuropathological criteria [24], modified by Harding and Halliday [14], to exclude from further studies cases fulfilling the clinico-neuropathological criteria for dementia with Lewy bodies (DLB); (2) CERAD (Consortium to Establish a Registry for Alzheimer's Disease) criteria [23] and the Braak tangle staging protocol [7] modified by Harding et al. [13], to exclude from further studies cases fulfilling the clinico-neuropathological criteria for possible AD. All the iPD cases which met the criteria for possible coexistence of AD, or the criteria for DLB, and controls meeting the clinico-neuropathological criteria for possible, probable and definite AD, as well as the criteria for DLB or a subclinical form of iPD, were excluded from further studies. A precise description of the material selection methodology was published elsewhere [5].

To assess the amyloid-dependent degenerative vascular and cerebral parenchymal changes four tissue blocks were taken in each case, as recommended by McKeith et al. [24] and Mirra et al. [25], from the following regions:

1) the substantia nigra (SN)-containing mesencephalon,
2) frontal cortex at the plane of the cross-section through the genu of the corpus callosum with the anterior cingulate gyrus (BA 24),
3) the middle and superior frontal gyrus (BA 8/9),
4) the hippocampus with entorhinal cortex and temporal lobe (BA 29) at the plane of the cross-section through the lateral geniculate body.

Formalin-fixed, paraffin-embedded samples were cut into 8-µm-thick sections and stained using histological haematoxylin and eosin (H&E) and Klüver-Barrera methods to perform a topographic assessment of the structure under study, Congo red to preliminarily assess the presence of CAA, and modified Bielschowsky silver impregnation method to test for the presence of AD-type pathology.

Immunohistochemical methods, selection of cases, and group subdivision

Immunohistochemical staining methods and a grading system were used to select the study cohort and for the group subdivision as previously published [5]. Paraffin-embedded sections were pretreated with formic acid and stained with primary antibodies as follows:

Specificity	Cat. no.	Type/Dilution	Company
α-Synuclein	NCL-ASYN	Monoclonal, mouse/1:30	Novocastra
Tau	A 0024	Polyclonal, rabbit/1:300	Dako
β-amyloid8-17	NCL-β-Amyl	Monoclonal, mouse/1:100	Novocastra
β-amyloid40	AHP 676	Polyclonal, rabbit/1:250	Serotec
β-amyloid42	AHP 677	Polyclonal, rabbit/1:250	Serotec
Cystatin C	AO451	Polyclonal, rabbit/1:500	Dako
α-Actin	M0851	Monoclonal, mouse/1:300	Dako
Collagen III	AF-5850	Monoclonal, mouse/1:200	Medicorp
Collagen IV	M 0785	Monoclonal, mouse/1:35	Dako
CD34	NCL-END	Monoclonal, mouse/1:25	Novocastra

The primary antibodies were detected with a biotinylated secondary antibody and the ABC complex, then visualised with diaminobenzidine as chromogen. Sections were counterstained with H&E.

Assessment of CAA severity

Semi-quantitative assessment of CAA severity was carried out in the middle frontal gyrus along the superior frontal sulcus (BA 8/9), and in the middle temporal gyrus along the superior temporal sulcus (BA21) as regions representative of the neocortex, and in the entorhinal cortex as a region representative of the limbic system, separately in adjacent subarachnoid space, in ten consecutive fields (×200), in IHC-staining for Aβ42, using a five-score intensity scale of changes: 1 = trace to scattered Aβ deposits (mild changes), 2 = at least some vessels with circumferential Aβ deposits (moderate changes), 3 = many vessels with circumferential Aβ deposits (severe changes), 4 = similarly severe changes and double barrelling, microaneurysms or fibrinoid necrosis (very severe changes). A single CAA severity score for each case was calculated by averaging across brain regions [28].

Electron microscopic procedures

Electron microscopic (EM) procedures were performed on the material embedded in paraffin after formalin fixation. After deparaffination, the material derived from the region of the highest incidence of vascular amyloid lesions was fixed in 2.5% glutaraldehyde and postfixed in 2% OsO_4 and routinely processed to Spurr resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under an Opton DPS 109 electron microscope.

Results

Light microscopic IHC study

In the sub-set of iPD-AD cases (n=18; 7 women and 11 men; mean 74.2±7.5 years; range 59-91 years) without severe AD-type changes, previously screened for the presence of neurodegenerative diseases, occasional cortical Lewy bodies (LBs) in the middle temporal gyrus were observed in one case only. LBs showed strong immunoreaction to α-syn and manifested as cytoplasmic inclusions, frequently spherical, with fibrillar structure, frequently pushing the cellular nucleus off to the cytoplasmic periphery (Fig. 1). Degenerative AD-type changes, senile plaques (SPs) and/or neurofibrillary tangles (NFTs) were observed in all the iPD-AD cases. Only SPs were present in two cases (11%) and NFTs only in eight cases (44%). Diffuse plaques (DPs) in IHC staining for Aβ had neither an amyloid core nor Aβ fibrillar fascicles. They were tau-negative and characterized

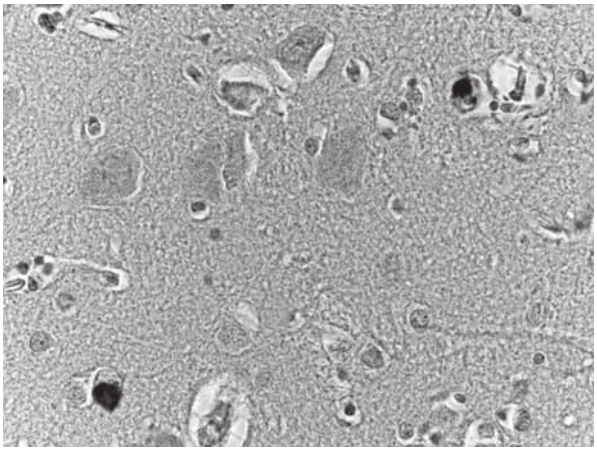

Fig. 1. Lewy bodies in the frontal cortex in the case of iPD. Anti-α-syn, orig. magn. ×630

by a significantly diversified size and blurred borders due to gradual dispersion in the surrounding of still less dense Aβ depositions. Their shape was close to spherical or irregular, their structure was granular or fibrillar and resembled cotton bits (Fig. 2). They frequently contained normally appearing neurons. They were Aβ42 positive and Aβ40 negative (Fig. 3A-B) and did not show tau-protein expression. Neuritic plaques (NPs) were smaller than DPs, almost spherically shaped and well separated from the surrounding. In IHC staining for Aβ8-17, Aβ42, and Aβ40 they were characterized by the presence of thick fibrillar amyloid fascicles and frequent presence of amyloid core (Fig. 2). They were tau protein positive.

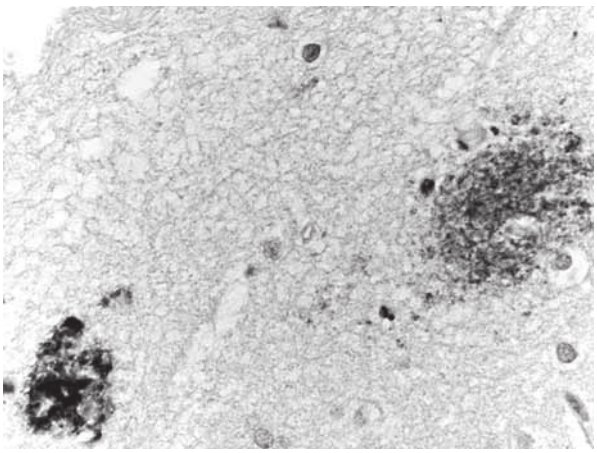

Fig. 2. Diffuse (right) and neuritic (left) plaques. Anti-Aβ42, orig. magn. ×400

Senile plaques, the vast majority of DPs, were present in neocortical regions (middle frontal gyrus and middle temporal gyrus) and in the limbic entorhinal cortex, showing a laminar arrangement, starting from the second cortical layer, and sometimes also plane deposits in the molecular layer. In all iPD-AD cases, NFTs were present in limbic structures, usually in the transentorhinal and entorhinal cortex (Fig. 4A). Sometimes NFTs were noted occasionally in the CA1 hippocampal region. In addition, in one case a few NFTs were visible in the inferior temporal gyrus. There were no NFTs in the cortex of the middle frontal and temporal gyrus.

Arterial CAA was observed in eight (44%) iPD-AD cases, including only one (12.5%) case in which leptomeningeal vessels were affected and seven (87.5%) cases in which both leptomeningeal and cortical vessels were affected. In the iPD-AD case with arterial CAA present only in leptomeningeal vessels, the lesions were mild and characterized by the presence of focal, spotted or laminar Aβ deposits at the junction of the vascular media and adventitia (stage 1) (Fig. 6A-B), whereas in iPD-AD cases, in which arterial CAA was present in both leptomeningeal and cortical vessels, the degree of lesion advancement varied in particular regions, starting with mild through moderate and ending with very severe stage, but regional and final average arterial CAA score indicated mild stage (Table I). In the moderate stage, arterial CAA manifested as circumferential infiltration of the vessel wall (Fig. 6C). In the severe stage, circumferential overloading of the vascular wall was visible

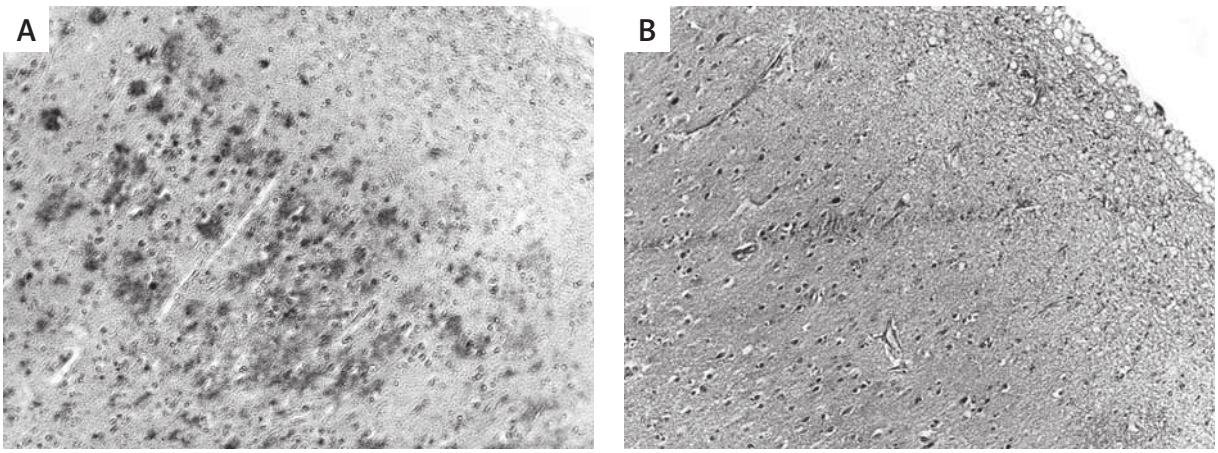

Fig. 3. Immunoreactivity of diffuse plaques in the same region in iPD-AD case. A. Positive expression of Aβ; B. Negative expression of Aβ peptide. A. Anti-Aβ42; B. Anti-Aβ 40. A-B. Orig. magn. ×100

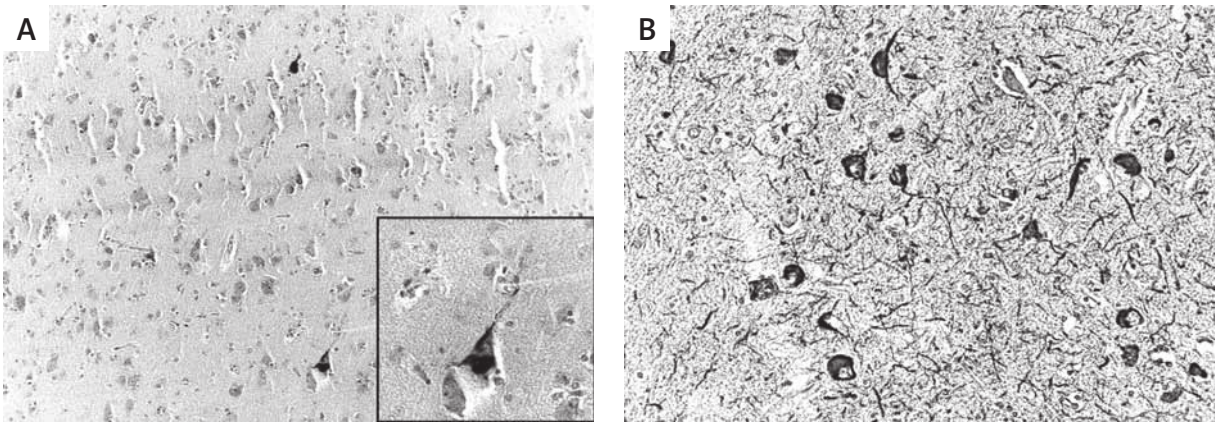

Fig. 4. Neurofibrillary tangles (NFTs). A. Non-numerous NFTs in the entorhinal cortex in iPD-AD case; B. Numerous NFTs and neuropil threads in the frontal cortex in PD+AD case. A-B. Anti-tau. A. Orig. magn. ×100; B. Orig. magn. ×200. Insert A.: NFT in higher magn. Anti-tau, orig. magn ×200

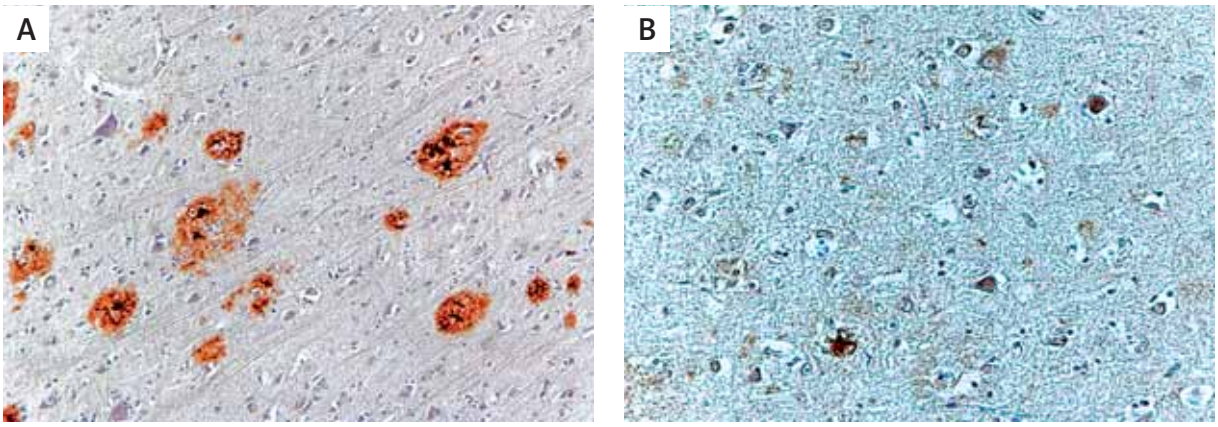

Fig. 5. Immunoreactivity of senile plaques in iPD+AD case. A. Positive reaction of diffuse and neuritic plaques to Aβ42 in the frontal cortex; B. Negative reaction of diffuse plaques and positive reaction of neuritic plaques to Aβ40 in the same region. A-B. Orig. magn. ×100

Table I. Progression of arterial CAA in iPD-AD cases (n=8)

Case No.	Frontal region		Temporal region		Entorhinal region		Final aver. score	
	Menin.	Cortex	Menin.	Cortex	Menin.	Cortex	Menin.	Cortex
2	-	-	1	1	1	-	0.7	0.3
3	4	3	2	-	1	-	2.3	1.0
4	-	-	3	3	1	-	1.3	1.0
11	2	1	1	-	-	-	1.0	0.3
13	1	-	1	1	-	-	0.7	0.3
15	2	2	3	2	2	2	2.3	2.0
16	2	2	-	-	-	-	0.7	0.7
17	1	-	1	-	1	-	1.0	-
Final aver. score	1.5	1.0	1.5	0.9	0.8	0.25	1.2	0.7

1 – trace to scattered Aβ deposits (mild changes); 2 – at least some vessels had circumferential Aβ deposits (moderate changes); 3 – many vessels had circumferential Aβ deposits (severe changes); 4 – similarly severe changes and double barrelling, microaneurysms or fibrinoid necrosis (very severe changes).

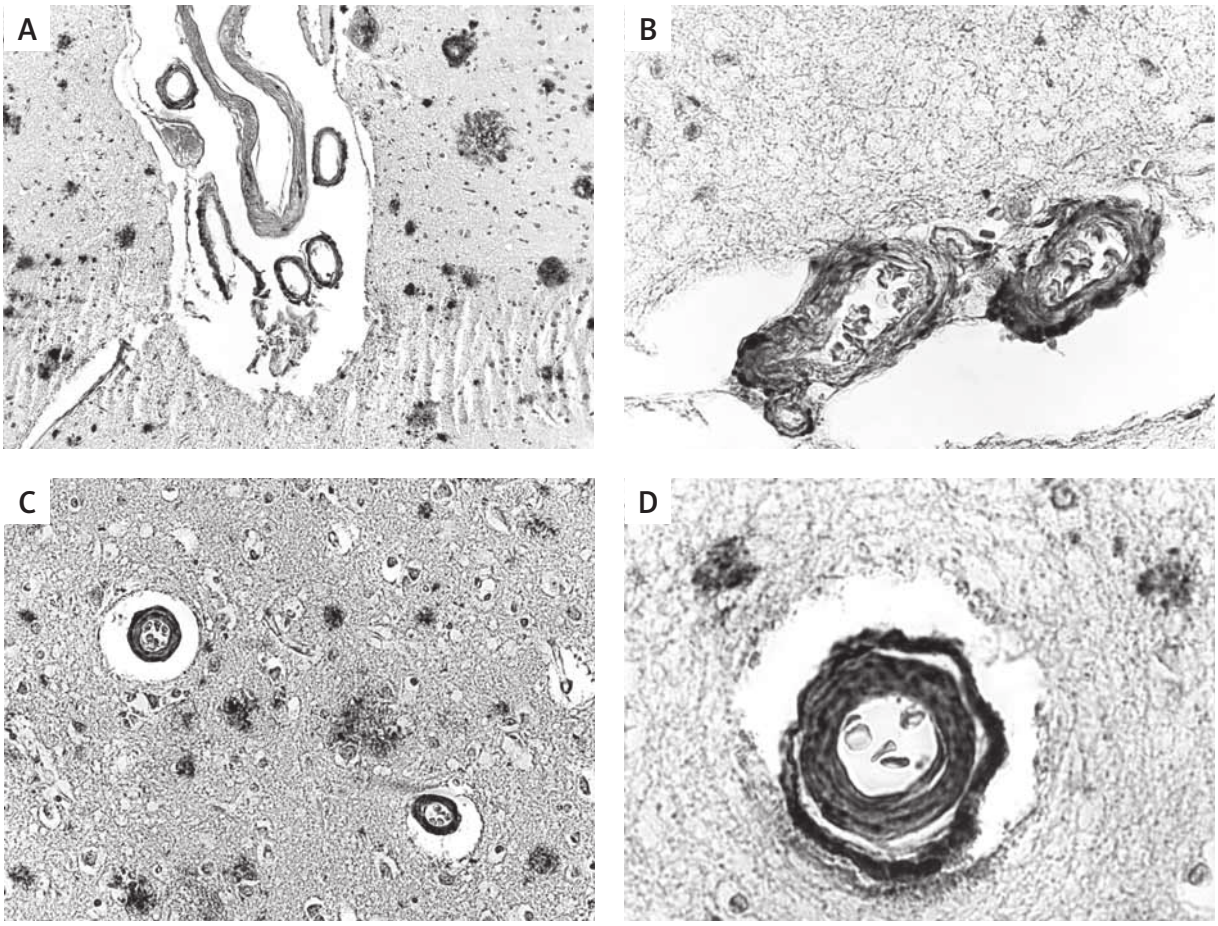

Fig. 6. Various stages of arterial CAA severity. A. Focal positive expression of Aβ in numerous meningeal vessels in the depth of cortical sulcus in iPD case; B. Positive Aβ focal reaction in meningeal vessels in higher magnification; C. Rim-like Aβ loading of cortical vessels; D. Aβ-loaded cortical vessel shows degenerative changes, so-called vessel within vessel appearance, typical of very severe CAA. Anti-Aβ42, orig. magn. A, C. ×5; B. ×400; D. ×630

with its thickening and narrowing of the lumen. The very severe arterial CAA stage was observed only in one (12.5%) iPD-AD case in the subarachnoid region (Table I). Only in this single case, some leptomeningeal arterioles were characterised by limited positive immunoreactivity of non-fibrillar collagen IV. The main vascular basement membrane component, present as a rim-like expression in the inner part of the vessel, close to the vascular lumen, limited positive immunoreactivity of smooth muscle actin (SMA) corresponding to the location of collagen IV, as well as by fibrosis of the inner part of the vascular wall, probably the intima, visible in IHC staining for collagen III (Fig. 7A-D). Microaneurysms of some Aβ overloaded leptomeningeal vessels were visible (Fig. 8A-B).Overloaded Aβ meningeal vessels were Aβ8-17, Aβ42 and Aβ40 positive (Fig. 9A-B). In one (12.5%) iPD-AD case, besides moderate arterial CAA, CapCAA was found in the frontal, temporal and entorhinal cortex. CapCAA involved cortical, capillary and precapillary microvessels, and was characterized by segmental Aβ infiltration of a thin-walled vessel and the presence of spotted, perivascular Aβ infiltrates frequently forming compact nodules (drusen), tightly linked with the capillary wall (Fig. 10A-B). Aβ-loaded cortical microvessels showed positive Aβ immunoreactivity with use of antibodies against Aβ8-17 and Aβ42, as well as negative immunoreactivity with use of antibodies against Aβ40 (Fig. 11A-B). Positive immunoreactivity of fibrillar collagen III was also observed (Fig. 12).

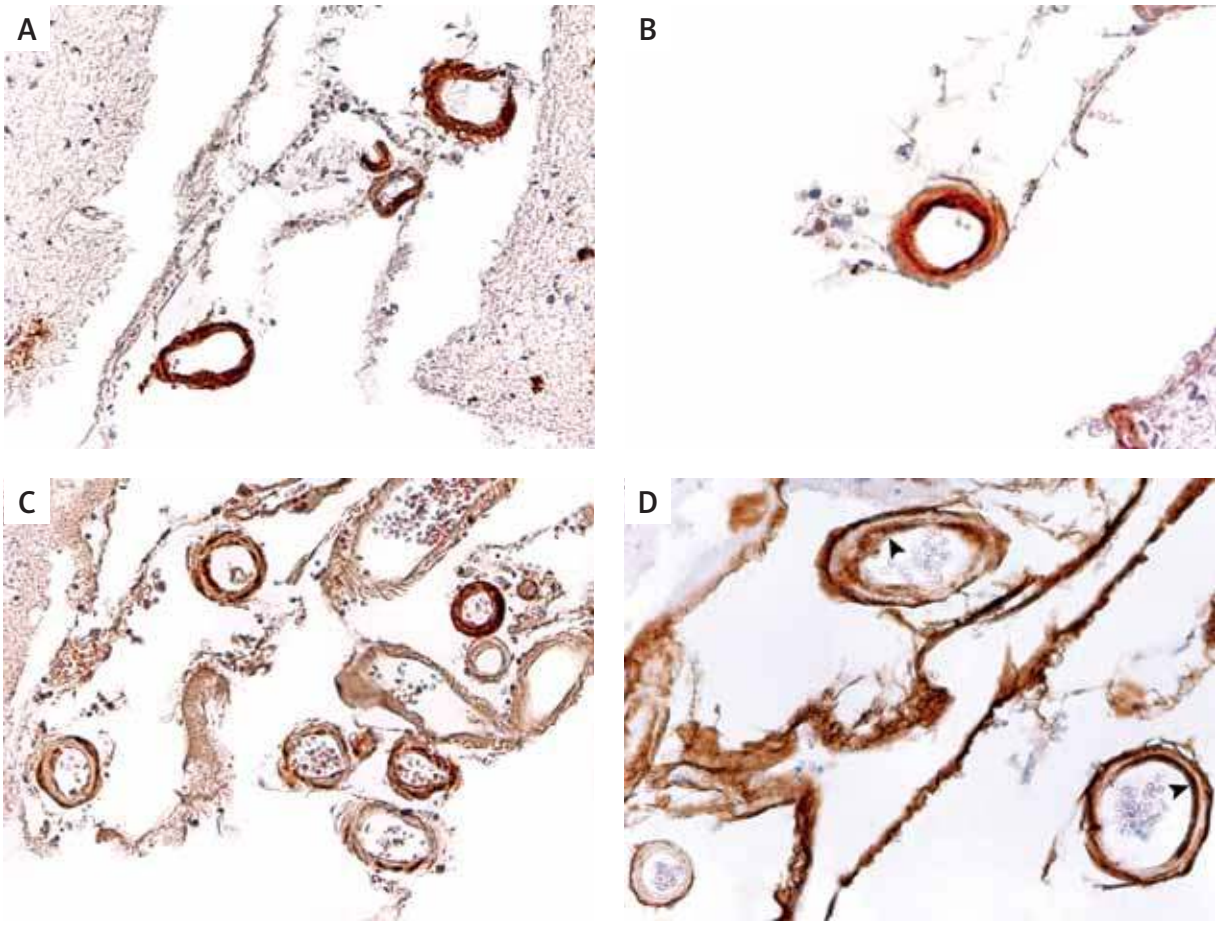

Fig. 7. Immunoreactivity of meningeal vessels in PD-AD case with very severe CAA. A. Circumferential accumulation of Aβ in many meningeal vessel walls; B. Limited rim-like expression of collagen IV in the inner part of the meningeal vessel wall; C. Partial or total lack of α-actin expression in many meningeal vessels; D. Positive expression of collagen III in the inner part of some meningeal vessel walls (arrow heads). A, C, D. Orig. magn. ×200; B. Orig. magn. ×400

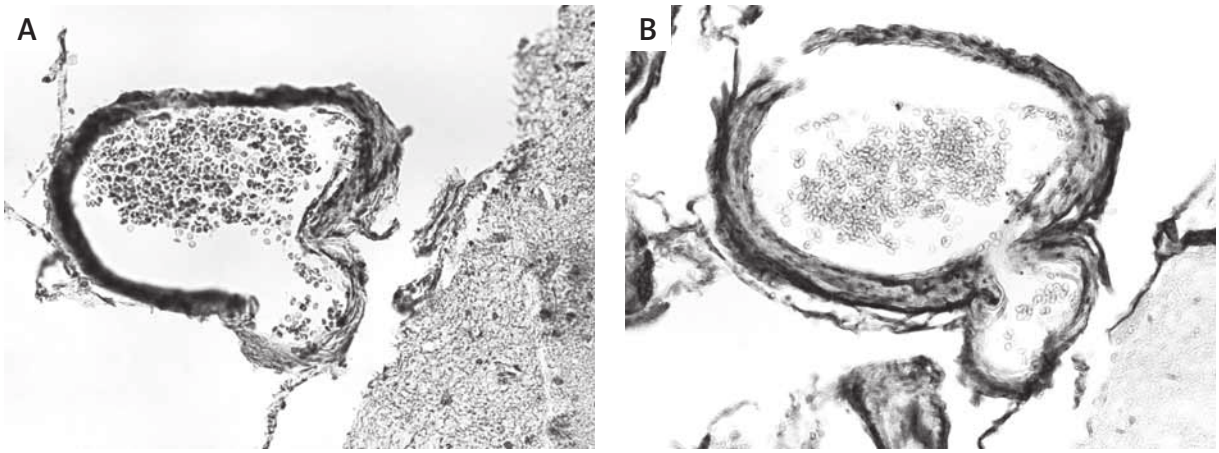

Fig. 8. Microaneurysm of meningeal vessel in immunochemical staining. A. Nearly total loss of α-actin reactivity; B. Expression of collagen III better preserved. A-B. Orig. magn. ×200

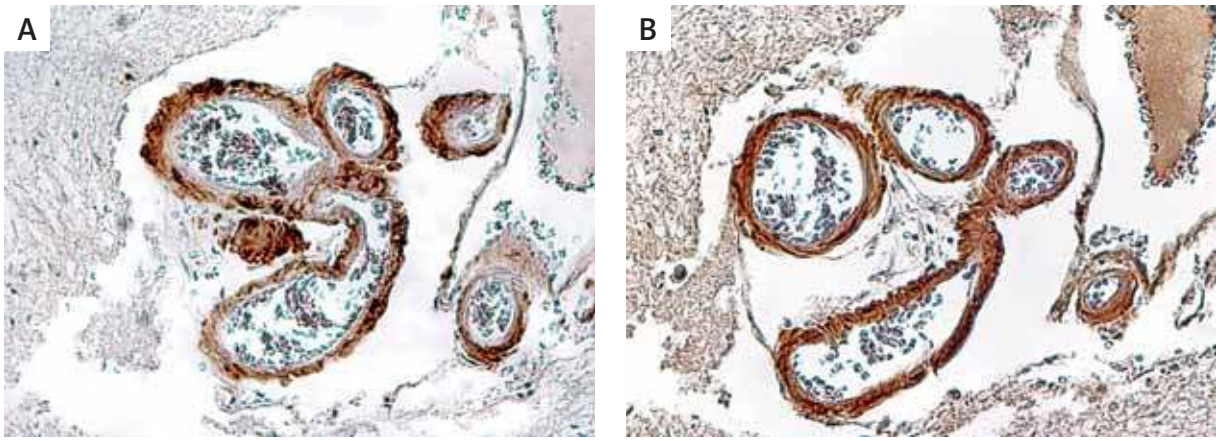

Fig. 9. Immunoreactivity of meningeal vessels in arterial CAA. A. Aβ42 positive focal Aβ deposits in meningeal vessels in iPD-AD case. B. Aβ40 positive focal Aβ deposits in the same group of meningeal vessels. A-B. Orig. magn. ×200

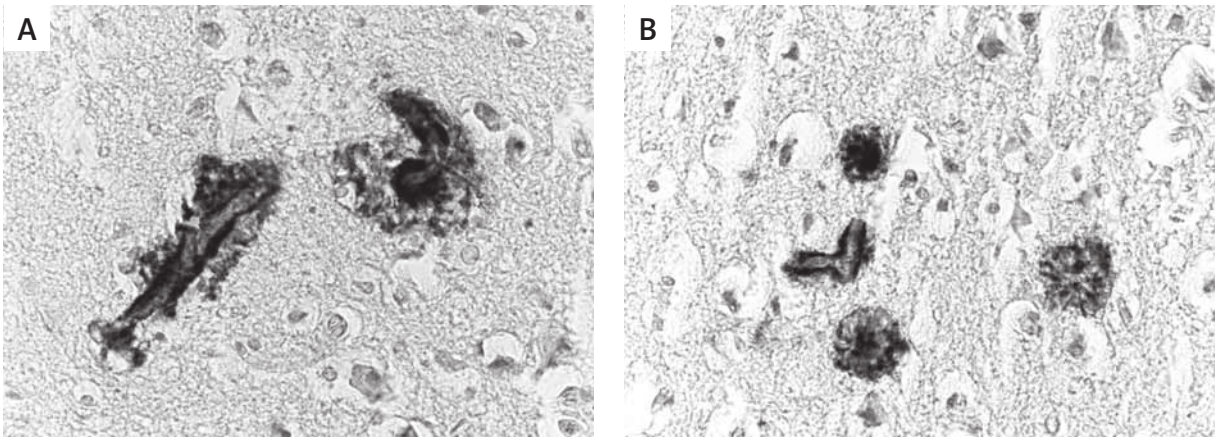

Fig. 10. Aβ loaded capillaries in CapCAA. A. Aβ42 positive reaction to the capillary walls and their closest vicinity. B. The same Aβ42 positive reaction to the neuritic plaques. A-B. Orig. magn. ×400

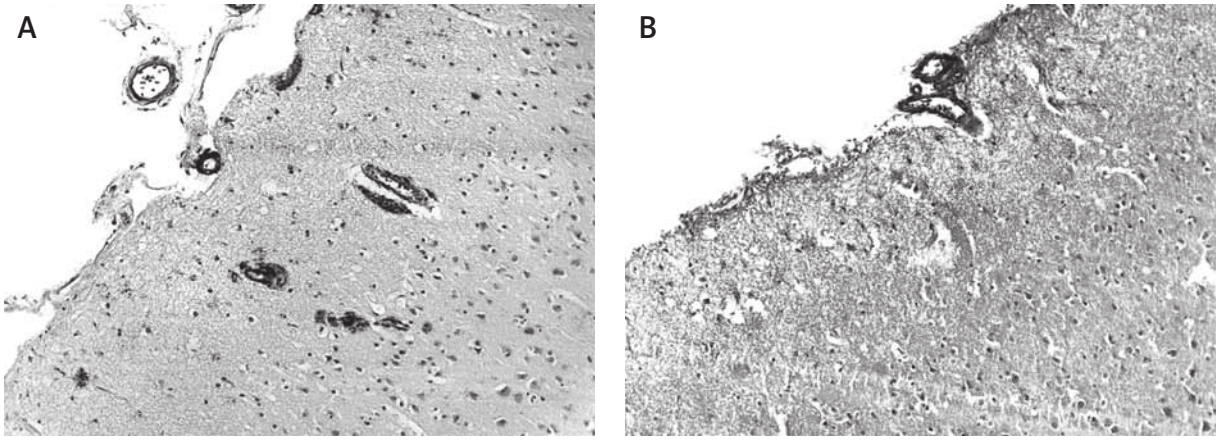

Fig. 11. Immunoreactivity of Aβ loaded capillaries in CapCAA. A. Positive expression of Aβ42; B. Negative expression of Aβ40 in the capillaries of the same cortical region. A-B. Orig. magn. ×100

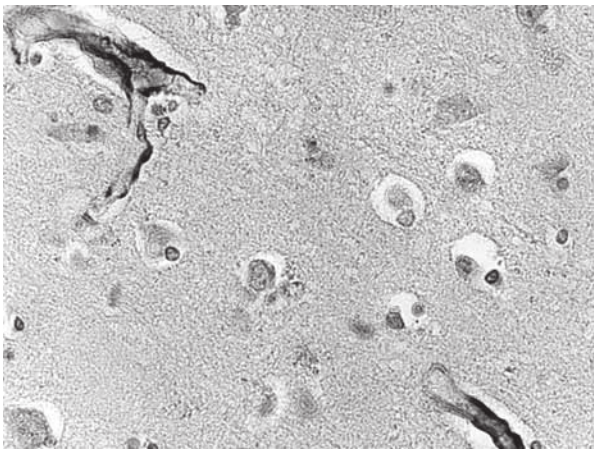

Fig. 12. Positive expression of collagen III in capillary walls in iPD case with CapCAA. Orig. magn. ×400

In the iPD+AD sub-set (n=10; 7 women and 3 men; mean 68.2±7.1 years, range 66-85 years) with concomitant severe AD-type pathology, selected from eighteen cases with clinical diagnosis of iPD, four (40%) cases showed cortical LBs in the middle and deep layers (III – VI) of the investigated cortical regions. They were not numerous in the frontal and temporal cortex, their number was slightly larger in the entorhinal cortex and quite large in one case. Degenerative changes of AD-type, SPs and NFTs, were seen in all cases. In all these cases, there were flame-like shaped NFTs and neuropil threads in both the neo- and limbic cortex with the majority of lesions in deep cortical layers (Fig. 4B). Two types of amyloid plaques, DPs and NPs, were observed. Their immunoreactivity was the same as in the iPD-AD sub-set (Fig. 5A-B). They occurred in all cortical layers (II-VI). DPs predominated in superficial and NPs in deep layers of the cortex or they were scattered within the whole cortical structure. Plane amyloid deposits, present in the molecular layer of the cortex, were rather rare. In only one (10%) case, SPs were not numerous.

Aβ infiltrated vessels were observed in seven (70%) PD+AD cases, of which four showed affected leptomeningeal vessels (in three cases only occasionally) and three overloaded leptomeningeal and cortical vessels. In the later iPD+AD cases, arterial CAA severity varied in particular regions from mild to very severe, but the average regional and final CAA score indicated mild or moderate vascular changes. Additionally, the final average score in each studied region indicated that arterial CAA severity was more advanced in the meningeal than in cortical vessels (Table II). Very severe arterial CAA was observed only in one iPD+AD case in frontal and temporal cortical regions. Only in this case, limited immunoreactivity of collagen IV and SMA in the media and positive expression of collagen III in the inner part of the vascular wall were observed in some cortical vessels (Fig. 13A-C). In addition, so-called double barrelling of a few cortical arterioles was visible, with the presence of empty space between their thin intima and degenerating media. Severely changed arterioles were observed in the areas of some cortical pale microinfarcts (Fig. 14A-B). Microaneurysms were not found. Overloaded Aβ vessels were Aβ8-17, Aβ42 and Aβ40 positive (Fig. 15A-B). CapCAA was found in two (20%) PD+AD cases. In one case, only CapCAA without arterial CAA presence was observed in the cortical regions, whereas in the other case, both CAA and CapCAA were present. Microvessels representing morphological features of CapCAA were Aβ42 positive and Aβ40 negative. They also showed positive immunoreactivity of fibrillar collagen III. CAA-related cerebral haemorrhage was not visible. Nor were de-

Table II. Progression of arterial CAA in iPD+AD cases (n=4)

Case No.	Frontal region		Temporal region		Entorhinal region		Final aver. score	
	Menin.	Cortex	Menin.	Cortex	Menin.	Cortex	Menin.	Cortex
1	2	-	3	3	3	-	1.3	1.0
6	1	4	3	4	3	2	2.3	3.3
8	2	-	2	-	-	-	1.0	-
10	1	-	1	-	1	-	1.0	-
Final aver. score	1.5	1.0	2.25	1.75	1.75	0.5	1.4	1.1

1 – trace to scattered Aβ deposits (mild changes); 2 – at least some vessels had circumferential Aβ deposits (moderate changes); 3 – many vessels had circumferential Aβ deposits (severe changes); 4 – similarly severe changes and double barrelling, microaneurysms or fibrinoid necrosis (very severe changes).

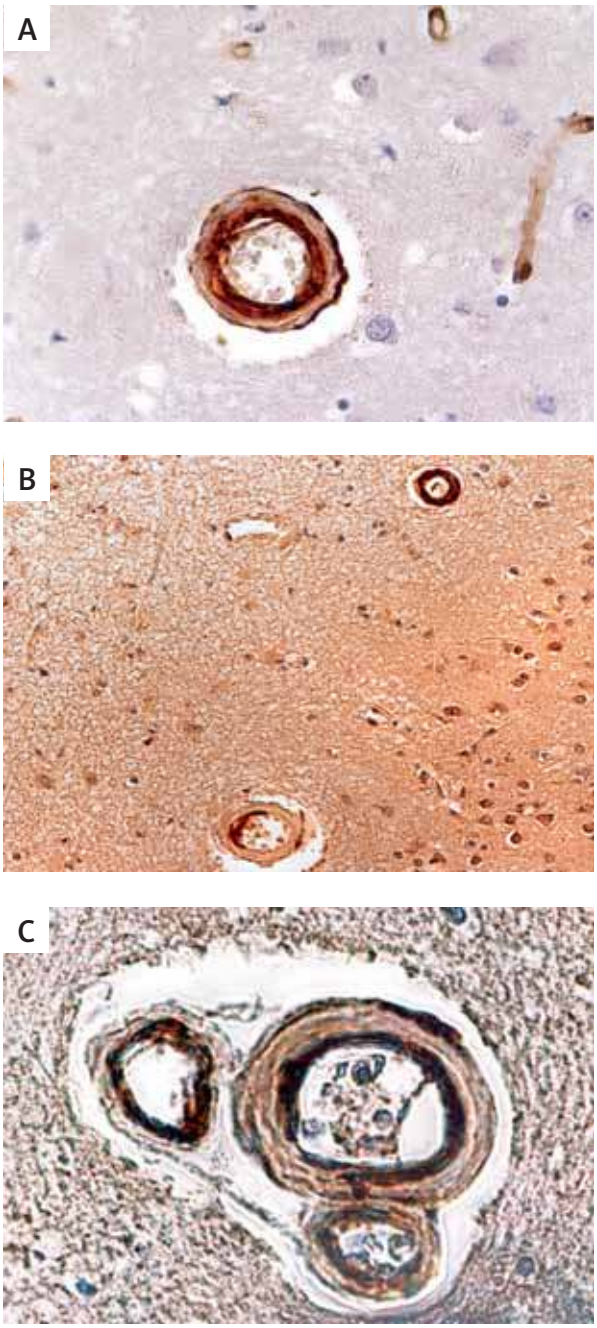

Fig. 13. Immunoreactivity of cortical vessels in PD+AD case with very severe arterial CAA. A. Rim-like expression of collagen IV in the inner part of the cortical arteriole wall; B. Narrow focal expression of α-actin in the closest vicinity of the vessel lumen. For comparison, a better preserved vessel wall visible next to it. ×200; C. Thin circumferential expression of α-actin in the inner part of the vessel wall directly adjoining its lumen. A. Anti-collagen IV, orig. magn. ×400; B-C. Anti-actin; B. Orig. magn. ×200; C. Orig. magn. ×630

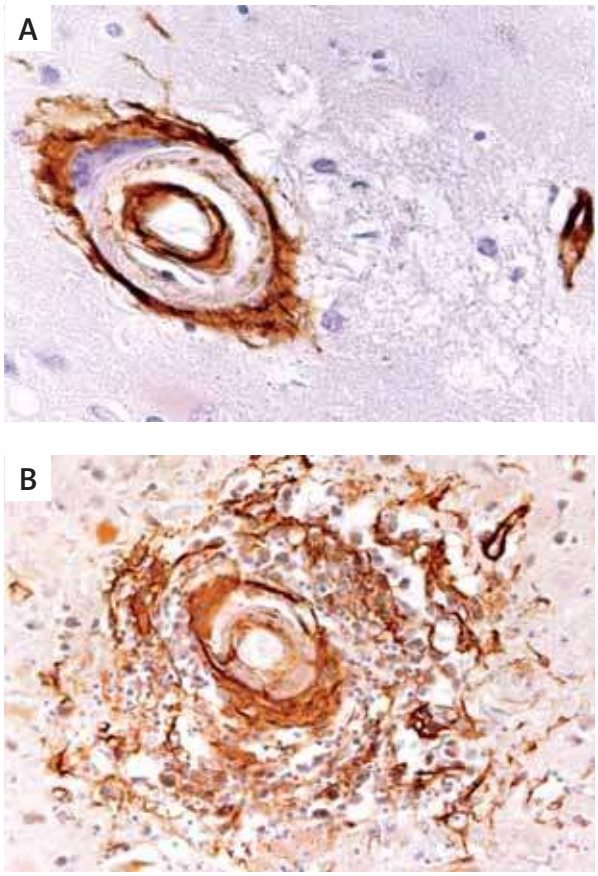

Fig. 14. Reaction to collagen III in very severe arterial CAA in the areas of pale microinfarcts. A. Positive expression of collagen III in the vascular intima; B. Enhanced expression of collagen III in the vascular adventitia. A. Orig. magn. ×200; B. Orig. magn. ×400

posits of cystatin C found. In none of the cases was attenuation of CD43 antigen immunoreactivity seen.

The controls comprised 14 age-matched cases selected from 21 deceased patients without clinically observed dementia or symptoms of degenerative diseases or psychic disorders. There was no evidence for AD presence according to CERAD criteria (category 0) and Braak staging (I-II stage). Degenerative AD-type changes were manifested in nine (64%) controls as non-numerous SPs and/or NFTs of limited topographical distribution. DPs were observed only in two (14%) cases, whereas NFTs were observed in seven (50%) cases, mostly in persons aged over 75 years, and they were almost always confined to the entorhinal cortex. In five controls, there were no AD-

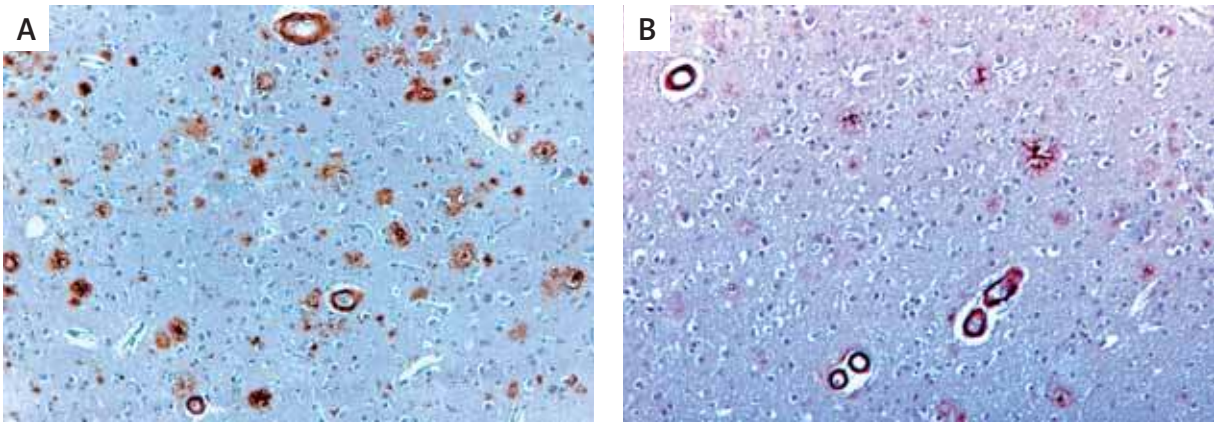

Fig. 15. Immunoreactivity of cortical vessels and amyloid plaques in arterial CAA. A. Aβ42 positive reaction of arterioles as well as diffuse and neuritic plaques in iPD+AD case with severe CAA; B. Aβ40 positive reaction of small cortical vessels and only neuritic plaques in the same cortical region. A-B. Orig. magn. ×100

type changes, SPs or NFTs, in the investigated cortical regions. CAA was observed in four (28%) controls, including two cases with its location in meningeal vessels only and two cases in which CAA was found in both meningeal and cortical vessels. CAA severity varied in individual cases. The average regional and final CAA score indicated mild or moderate stage, with their slight predominance in the subarachnoid region over those in the cortex (Table III). None of the controls showed the presence of CapCAA. In controls without CAA presence positive immunoreactivity of collagen IV was observed in the media and collagen III in the adventitia of leptomeningeal arteries. Furthermore, weak positive immunoreactivity of collagen IV in cortical arterioles and capillaries as well as positive reaction to collagen III only in superficial cortical arterioles, but not in capillaries, were visible.

Electron microscopic study

The ultrastructural analysis was carried out on two iPD-AD cases, no. 3 with arterial CAA and CapCAA, and no. 15 with arterial CAA only in the cortical regions (Table I); two iPD+AD cases, no. 6 with arterial CAA and no. 10 with CapCAA only (Table II); and two controls, no. 11 without amyloid angiopathy and no. 14 with arterial CAA (Table III). In control cases with CAA some arterioles showed damaged vascular smooth muscle cells (VSMCs) separated from each by thick BM containing focal deposits of amyloid and collagen fibres (Fig. 16). In all the iPD-AD cases with concomitant CAA, arterioles exhibited usually thickened basement membrane between (VSMCs). The accumulation of numerous amyloid fibres in arteriole basement membranes sometimes was accompanied by collagen fibres (Figs. 17, 18). A different degree of changes in

Table III. Progression of arterial CAA in control cases (n=4)

Case No.	Frontal region		Temporal region		Entorhinal region		Final aver. score	
	Menin.	Cortex	Menin.	Cortex	Menin.	Cortex	Menin.	Cortex
7	-	-	1	-	-	-	0.3	-
9	3	2	3	2	-	-	2.0	1.3
10	-	-	1	-	2	-	1.0	-
14	2	2	3	2	3	-	2.7	1.4
Final aver. score	1.25	1.0	2.0	1.0	1.25	-	1.5	0.7

1 – trace to scattered Aβ deposits (mild changes); 2 – at least some vessels had circumferential Aβ deposits (moderate changes); 3 – many vessels had circumferential Aβ deposits (severe changes); 4 – similarly severe changes and double barrelling, microaneurysms or fibrinoid necrosis (very severe changes).

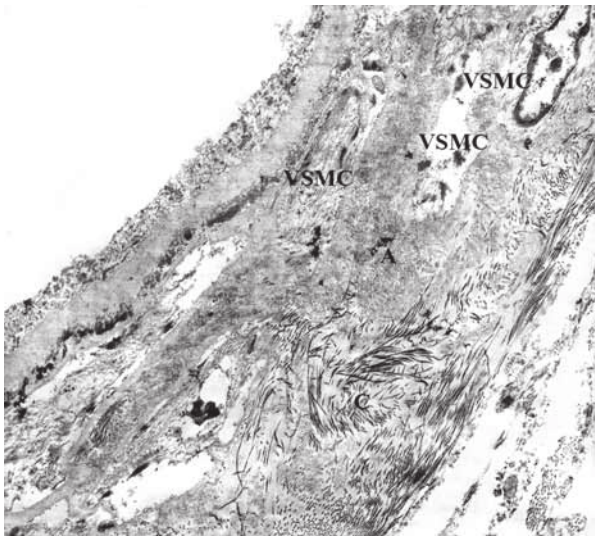

Fig. 16. Deposits of amyloid (A) and collagen (C) fibres in small arteriole with thickened basement membrane (BM) in control case with moderate CAA. Orig. magn. ×4400

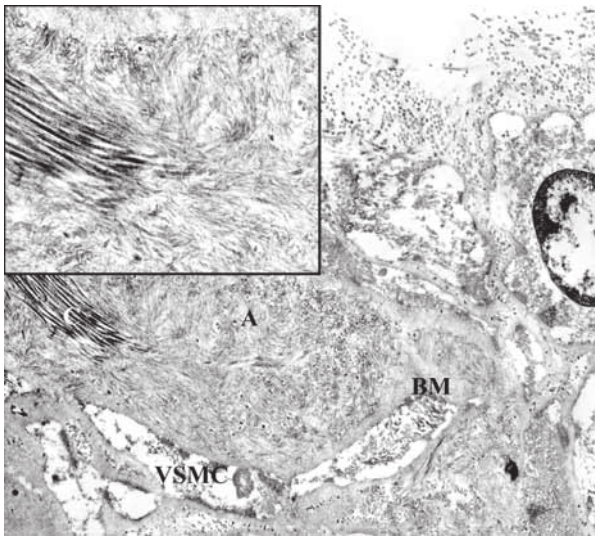

Fig. 17. Focal deposits of amyloid (A) and collagen (C) fibres in small arteriole with thickened basement membrane (BM) and degenerating vascular smooth muscle cells (VSMCs) in the iPD-AD case with moderate CAA. Orig. magn. ×7000. Insert: collagen and amyloid fibres in higher magn. Orig. magn. × 15 000

the structure of smooth muscles was observed with increasing amounts of amyloid fibres in thickened basement membranes (Fig. 19). iPD+AD arterioles, usually severely affected, showed loss of VSMCs and their replacement by amyloid and collagen fibres and in such vessels clusters of vacuoles among amyloid fibres were visible (Fig. 20). In both iPD-AD and iPD+AD cases deposits of collagen fibres were often present in

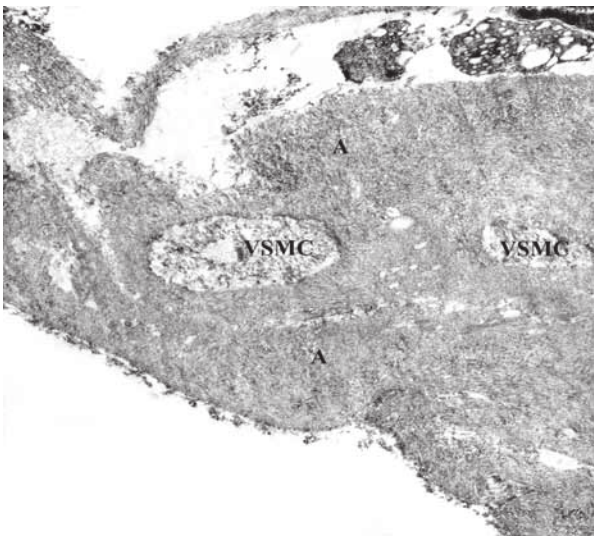

Fig. 18. Small arteriole with very thickened basement membrane overloaded by amyloid (A) fibres. Enhanced degenerative changes in vascular smooth muscle cells (VSMCs) in iPD-AD case with moderate CAA. Orig. magn. ×7000

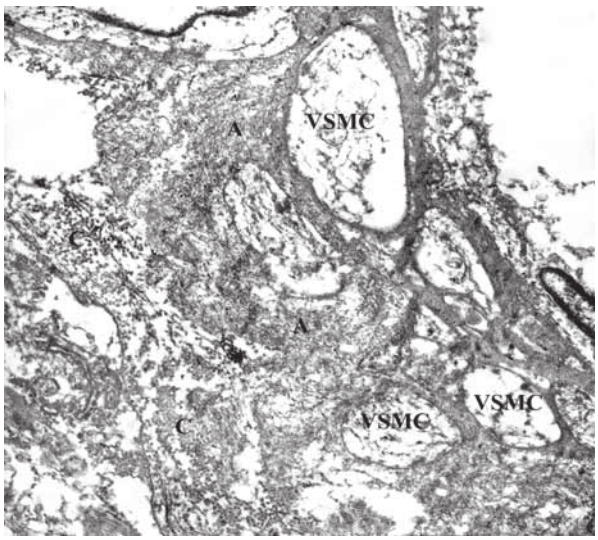

Fig. 19. Amyloid (A) deposits between vascular smooth muscle cells (VSMCs) and collagen (C) fibres in the periphery of small arteriole wall. VSMCs show residual myofilaments in iPD+AD case with severe CAA. Orig. magn. ×7000

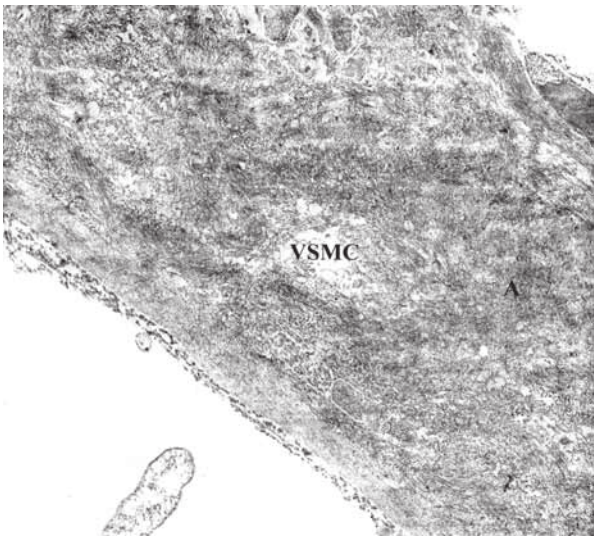

Fig. 20. Arteriole wall completely overloaded by amyloid (A) fibres. Only residual vascular smooth muscle cells (VSMCs) visible in iPD+AD case with very severe CAA. Orig. magn. ×7000

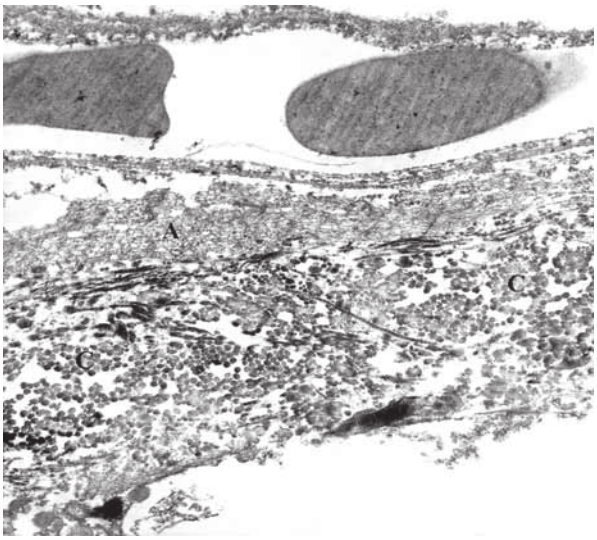

Fig. 21. Capillary with laminar deposits of amyloid (A) and numerous collagen (C) fibres in iPD+AD case with capillary CAA (CapCAA). Orig. magn. ×7000

the peripheral part of the vessel wall (Figs. 19, 21). The capillaries of iPD-AD and iPD+AD cases were also affected. Capillary basement membrane thickening and collagen as well as amyloid accumulation were characteristic pathological changes. The amyloid fibres were visible between endothelial cells and the collagen layer (Fig. 21). Endothelial cells demonstrated features of damage, i.e. they were elongated and thin.

Discussion

Our results show that in the response to amyloid loading the vessel media degenerates with concomitant atrophy of collagen IV fibres and deposition of fibrillar collagen III. In the very severe arterial CAA collagen IV and III was visible only in the inner part of the vessel wall, near the vessel lumen, probably in the intima. Progressive degeneration of the cytoplasm in VSMCs, most probably due to gradually increasing volume of the VSMC basement membranes infiltrated by still accumulated Aβ and collagen III deposits, were observed under electron microscopy (EM). Myofilaments of VSMCs seemed to be a structural element that was most resistant to progressing damage of the vessel wall. A complete loss of VSMC fibres did not occur at least in the moderate stage of CAA, which was evidenced by retained expression of SMA and the presence of myofilaments.

Atrophy of VSMCs with concomitant lack of immunoreactivity to collagen IV in the outer part of the vascular media and positive expression of fibrillar collagen III in the inner part of the vessel wall, probably in the intima, were observed exclusively in the very severe arterial CAA in iPD cases. The same type of vascular changes recently observed by us in familial Alzheimer's disease cases with very early onset [37] and in a case of dementia with Lewy bodies (DLB) with very severe CAA [6] suggest lack of specificity of vascular damage to a particular type of dementia. Selective preservation of collagen IV in the inner part of the vascular wall may be explained by very recently observed lack of amyloid deposits in the endothelial basement membranes in arteries affected by CAA [43]. Preservation of the arterial endothelium basement membranes in arterial CAA may mean that the structure of endothelial cells was in essence retained, which may be evidenced by preservation of their immunoreactivity. However, degenerative changes of endothelium were seen in our EM study. Our ultrastructural examination suggests, in agreement with Farkas et al. [10], that degenerative changes of pericytes in control and pathological groups appeared at a similar frequency.

Both processes of Aβ and fibrillar collagen III accumulation and the thickening of vascular basement membranes may play an essential role in progressing

VSMCs damage. These complex vessel wall changes may lead to diminution of endurance of the vascular wall and impair its contractility, leading finally to haemodynamic disorders and hampered neuronal metabolism with cognitive decline in consequence [10].

Two types of CAA have recently been distinguished, arterial CAA and capillary, i.e. CapCAA [2,30,40]. In arterial CAA Aβ deposits are present in the leptomeningeal medium and small arteries as well as in small cortical arterioles, whereas in CapCAA they are present in capillaries. In our material, we found three CapCAA cases. Only CapCAA is suggested to correlate with the count of NPs and progression of AD-type pathology [2,18,39]. Microvessel Aβ deposition leading to vascular occlusion presumably contributes to degeneration of neurons in iPD by disturbance of cerebral blood flow [38]. Furthermore, morphological capillary abnormalities, i.e. BM thickening and Aβ and fibrillar collagen accumulation, without occlusion of the vessel lumen, can lead to severe haemodynamic and metabolic disturbances [10].

The origin of vascular amyloid lesions in CAA still remains unclear despite numerous studies carried out for many years. It is assumed that there are three basic sources of vascular Aβ: (1) blood and/or cerebrospinal fluid [35]; (2) vascular wall and/or perivascular pericytes [43]; and (3) interstitial perivascular drainage pathways of amyloid peptides from the brain [42,43]. According to literature data CapCAA should be characterized by the accumulation of Aβ42 and not Aβ40 deposits [3] in the capillary basement membrane and in its closest vicinity, which finds its confirmation in our IHC study.

We demonstrated that arterial CAA is characterized by the accumulation of both Aβ42 and Aβ40 positive deposits in line with earlier studies carried out by Roher et al. [33], who using biochemical methods have evidenced that vascular Aβ deposits in CAA in AD are composed of Aβ42 and Aβ40 with the predominance of Aβ42. Recent experimental studies curried out by McGowan et al. [22] seem to provide evidence that Aβ42 is the initiating molecule and appears to be necessary for both parenchyma and vascular amyloid deposition. It is most likely that a high Aβ40:Aβ42 ratio is necessary to evoke the advanced stage of CAA [15]. Human studies by Alonzo et al. [1] seem to provide evidence that arterial CAA progresses through the accumulation of Aβ40 deposits on already existing Aβ42 aggregates in the wall of the affected vessel and finally Aβ40 deposits outnumber Aβ42 ones with progressing CAA. Immunoreactivity of arterial CAA (Aβ42 and Aβ40 positive), different from that of CapCAA (Aβ42 positive and Aβ40 negative), may suggest different pathogenesis of both types of vascular amyloid lesions or time differences in Aβ deposition. However, local differences in amyloid precursor protein (APP) processing and differences in Aβ clearance or enzyme-mediated Aβ degradation are not excluded. Immunoreactivity of the same type (Aβ42 positive and Aβ40 negative) of CapCAA and DPs may argue for a common, probably neurogenic pathogenesis, mediated by cerebral ISF, or a similar pathogenesis of both types of lesions. However, Aβ produced by VSMCs may contribute to or even initiate deposition of Aβ in the perivascular drainage pathways [11,27]. Immunoreactivity of the same type (Aβ42 and Aβ40 positive) of arterial CAA and NPs also may suggest a common or similar pathogenesis of both types of amyloid changes. Arterial CAA predominance in the subarachnoid space, a frequent predominance observed in our study and reported in other studies [3], may be explained by the most recent hypothesis on CAA and sporadic form of AD pathogenesis, according to which Aβ is first deposited in leptomeningeal, then in smaller cortical vessels and finally in parenchyma in the vicinity of vessels due to disorders progressing with age in the drainage and outflow of cerebral interstitial fluid (ISF) via perivascular routes towards meninges [42,43].

The prevalence of CAA in iPD still remains unknown. We have managed to find only two publications [19,22] with the author's statement that CAA occurs frequently in iPD, especially in iPD cases with concomitant dementia. The results of our study are in agreement with those previously published and suggest that CAA in iPD is not rare (43%). CAA may be more frequent than in elderly populations free of neurodegenerative processes (28%). Our results show that CAA may occur more frequently in iPD+AD (70%) than in iPD-AD (40%) cases. Nevertheless the obtained results should be regarded with caution in view of the relative small study group. We found severe AD-type lesions in about 36% of iPD cases verified neuropathologically, which is within the limit of the prevalence of AD-type lesions in iPD according to the literature data [12,17].

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