Acta Neuropathol (1992) 84: 674 – 679

Acta Neuropathologica

Morphology of cerebral plaque-like lesions in hereditary cerebral hemorrhage with amyloidosis (Dutch)

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Received February 26, 1992/Revised, accepted May 27, 1992

Summary. We studied the presence and morphology of plaque-like lesions in the frontal cortex of six patients, aged 40 to 76 years, with hereditary cerebral hemorrhage with amyloidosis – Dutch type (HCHWA-D), using β/A4 immuno-, silver, Congo red and thioflavin S staining. Two types of β/A4 immunoreactive and Congo red-negative plaques were detected. The first type was composed of argyrophilic fibrous material in periodic acid-methenamine silver (PAM) and modified Bielschowsky staining and lacked silver-stained degenerating neurites. Therefore, this type of plaque has the same staining properties as the diffuse plaque described in Alzheimer's disease, Down's syndrome and non-demented elderly. The second type of plaque, occurring only in the three oldest patients and numerically increasing with age, consisted of a spherical non-argyrophilic area of granular texture with a rim of PAM-positive material. The PAM-positive fibrous material of both types of plaques was mingled with coarser and compact, irregular-shaped argyrophilic structures in the oldest patient. The described plaques did not show bright fluorescence with thioflavin S staining. These results indicate, that the morphology of plaques, encountered in HCHWA-D, is diverse and changes with age.

Key words: Hereditary cerebral hemorrhage with amyloidosis-Dutch type – Amyloid β/A4 protein – Diffuse plaques – Immunohistochemistry

Hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) is an autosomal dominant disease, manifesting in the fifth or sixth decade with hemorrhagic strokes [11, 16]. Even in the absence of clinical or radiological evidence of strokes, dementia may develop [12]. Amyloid angiopathy of leptomeningeal and cerebral cortical bloodvessels is the main pathological feature in HCHWA-D [16]. The amyloid is composed of a 39-residue peptide, named β/A4 protein, which is also the main constituent of amyloid present in Alzheimer's disease (AD), Down's syndrome (DS), sporadic cerebral amyloid angiopathy and normal aging [3, 9, 10, 28]. In HCHWA-D, a point mutation has been found in the gene on chromosome 21, which encodes for the amyloid precursor protein (APP) [15, 27]. This mutation segregates with the disease [1].

Beside the amyloid angiopathy, cortical silver-stained, non-congophilic plaque-like structures have been described in HCHWA-D [28]. These structures appeared to stain with anti-SP28, an antibody to the first 28 amino acids of synthetic β/A4. Therefore, they have been compared to a putative early form of senile plaque in AD and DS [28]. Anti-SP28 immunostaining combined with thioflavin S staining revealed cortical amyloid as well as preamyloid deposits in patients with HCHWA-D [26]. In contrast to AD and DS, dystrophic neurites or neurofibrillary tangles proved to be consistently absent [3, 24, 26, 28]. The aim of this histological and immunohistochemical study is to define the morphology of the plaque-like lesions in HCHWA-D in further detail. To detect any age-related changes, patients of increasing age at death were studied.

Material and methods

The frontal and frontobasal cortices were investigated of six HCHWA-D patients (four males) between the ages of 40 and 76 (mean 55) years. The patients were numbered 1–6 according to increasing age at death. The diagnosis was based on family history, a history of recurrent hemorrhagic strokes, the presence of amyloid angiopathy and of the point mutation. Post-mortem delay did not exceed 24 h. Formalin-fixed, paraffin-embedded blocks were cut into serial 6-μm-thick sections, which were either stained with hematoxylin and eosin, Luxol fast blue (Klüver and Barrera), alkaline Congo red and thioflavin S or incubated with one of the following anti-sera: anti-SP1-10, a rabbit polyclonal antiserum to residues 1–10 of β/A4 protein, and antiserum Angela (antiserum A), a rabbit polyclonal antiserum raised against native HPLC-purified β/A4 protein [22]. As second antibody swine

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(No reprints available)

Table 1. Staining properties of plaques in hereditary cerebral hemorrhage with amyloidosis – Dutch type (HCHWA-D) compared with those of putative early plaques in AD, DS and non-demented elderly (formalin-fixed, paraffin-embedded material)

		Anti-β/A4	Co r	Thio S	PAM	Mod BB	Holmes	Bodian	Selected references	Subjects
HCHWA-D										
Plaque type I		+	–	●	+	+	–	n	Present study	HCHWA-D
Diffuse plaque		+	–	n	+	+	n	–	[13,14,31,32]	AD ND
Preamyloid deposit		+	–	–	n	n	n	–	[8,25]	AD DS ND
Amorphous plaque		+	–	n	n	n	n	–	[21]	AD DS ND
HCHWA-D										
plaque type II	p	+	–	●	+	+	±	n	present study	HCHWA-D
	c	+	–	●●	●●	●●	●●	n		
Plaque A		+	–	●●	?	n	●●	n	[19,20]	AD ND

AD, Alzheimer's disease; DS, Down's syndrome; n, not performed; Co r, Congo red; Thio S, thioflavin S; PAM, periodic acid-methenamine silver; Mod BB, modified Bielschowsky; c, center; p, periphery; ND, non-demented; ●, pale yellowish but not brightly fluorescent; ●●, detectable as an area of increased density

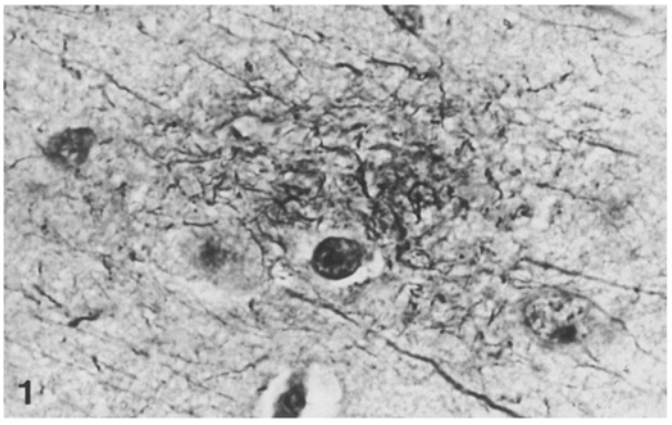
anti-rabbit antiserum (DAKO-immunoglobulins a/s) was used, which was visualized with 3,3-diaminobenzidine and 0.015% H_2O_2. The sections were treated with 85% formic acid for 30 min before immunostaining. Negative control sections were prepared by incubation with TRIS-buffered saline as the first step reagent. From patient 6 additional sections were incubated with Alz50, a monoclonal antibody (Abbot Laboratories) that recognizes a 68-kDa protein in brains from AD patients and immunoreacts with paired helical filaments [30]. Serial 10-μm-thick sections were stained with periodic acid-methenamine silver (PAM) according to Jones [2] and with silver stains according to Palmgren, Holmes and modified Bielschowsky. Furthermore, modified Bielschowsky and PAM stain were used alternatively with and without pretreatment with 98%–100% formic acid to stain serial 4-μm-thick sections in patient 1. Finally 4-μm-thick sections were double-stained with PAM and anti-SP1-10. Separate blocks were silver-impregnated according to Bielschowsky and cut into 10-μm-thick sections.

Results

All patients showed plaques with PAM and modified Bielschowsky staining. The density of the plaques ranged between 10 to 30/mm^2. Two types of plaques were distinguished. Their respective staining properties are summarized in Table 1.

Plaque type 1 consisted of a cluster of argyrophilic fibrous material in PAM and modified Bielschowsky staining (Fig. 1). The fibers were often curved and seemed to branch. Their thickness varied slightly. Plaque type 2 was composed of a rounded, non-argyrophilic, granular center, in part or entirely surrounded by PAM-positive material (Fig. 2). Shadows of nuclei were sometimes discernable in this center. In addition to PAM-positive fibrous material both types of plaques contained coarser and compact, irregular-shaped argyrophilic structures in the oldest patient (Fig. 3). Plaque type 2 was only occasionally found in patients 4 and 5, but it locally made up almost half of the total number of plaques per mm^2 in patient 6. Serial sections of plaque type 1, performed in the youngest patient, showed this plaque to consist entirely of PAM-positive fibrous material. The center of plaque type 2 was detectable with Holmes and Palmgren staining because of its granular, dense texture and virtual lack of stained cell processes in comparison with the background. These stains did not evidence plaque type 1 in patients 1–5 and gave variable results as to plaque type 1 and the rim of plaque type 2 in patient 6. However, staining of plaque type 1 as well as the rim of plaque type 2 was observed in this patient with Bielschowsky's block impregnation. Occasionally a plaque type 1 was stained with this method in patients 1–5. Both plaque types were β/A4 immunoreactive. Although plaque type 1 was rather weakly β/A4 immunostained, its at least partly fibrous structure showed up in some of the sections (Fig. 4). The center of plaque type 2 was evenly β/A4 immunostained, while its edge, where silver staining revealed the PAM-positive fibrous material and structures, stained unevenly. PAM and β/A4 double staining showed plaque type 2 as an immunoreactive center with argyrophilic periphery (Fig. 5). The two types of plaques did not show green birefringence after Congo red nor bright fluorescence with thioflavin S staining. With the latter method plaque type 1 (Fig. 6) and the rim of plaque type 2 contrasted pale yellowish with the background. After pretreatment with 98%–100% formic acid of sections stained with modified Bielschowsky plaque type 1 was no longer discernable.

Notably Palmgren staining revealed degenerating neurites in association with some of the congophilic blood vessels in all six patients. In patients 2, 5 and 6 neurites, visualized by Holmes, Palmgren, modified Bielschowsky staining or in Bielschowsky's block impregnation were occasionally encountered around compact amyloid deposits (Fig. 7a). Such deposits, showing bright yellow fluorescence on thioflavin S staining (Fig. 7b), were mostly found in direct association with congophilic blood vessels. Neurites were not demonstrable with Alz50 immunostaining, performed in patient 6.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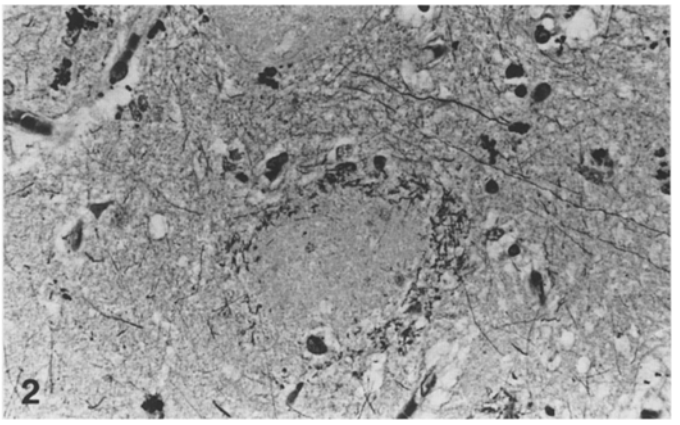
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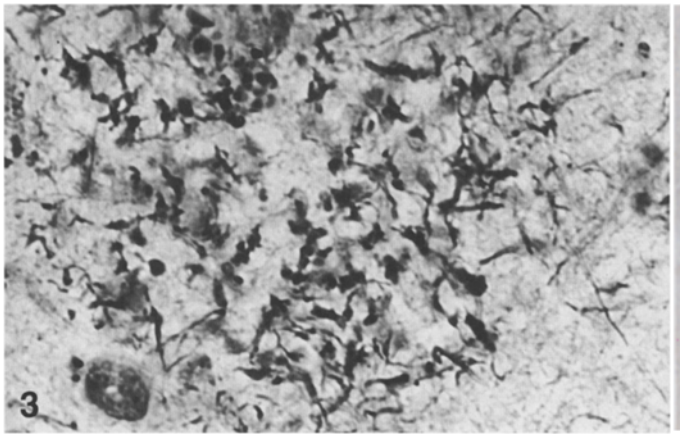
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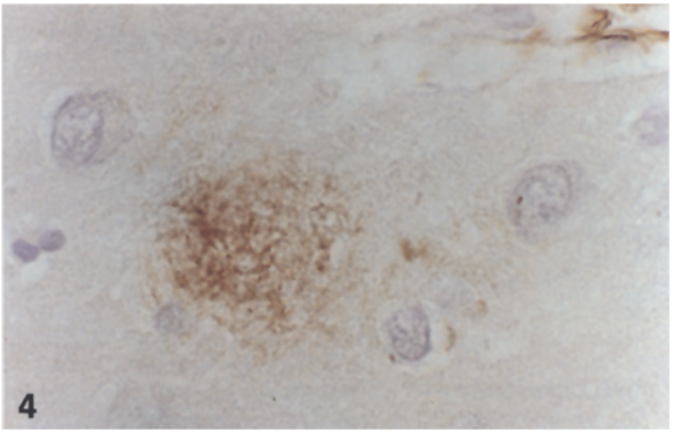
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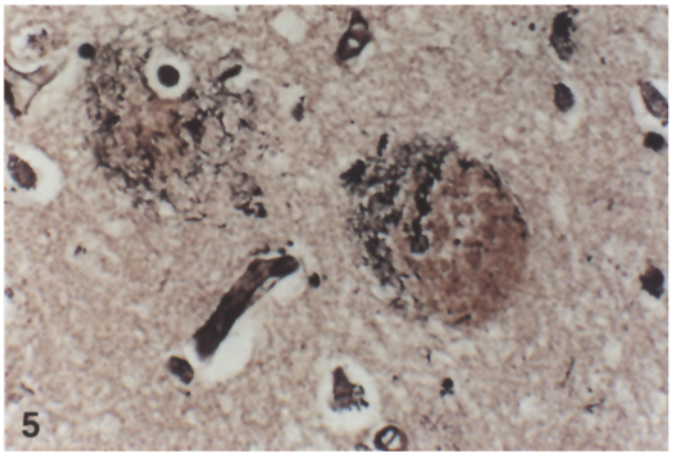
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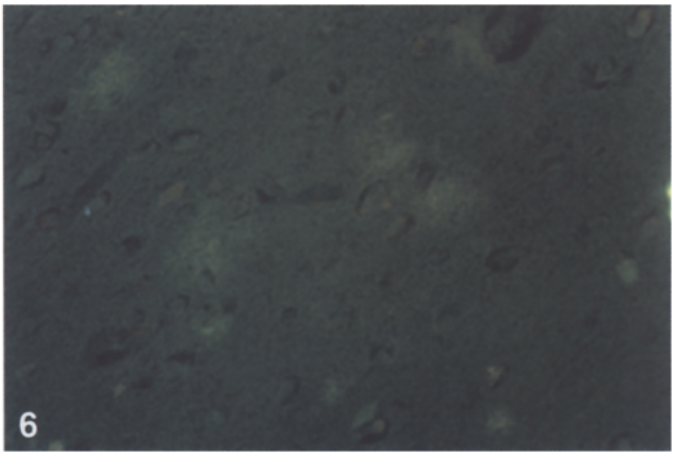
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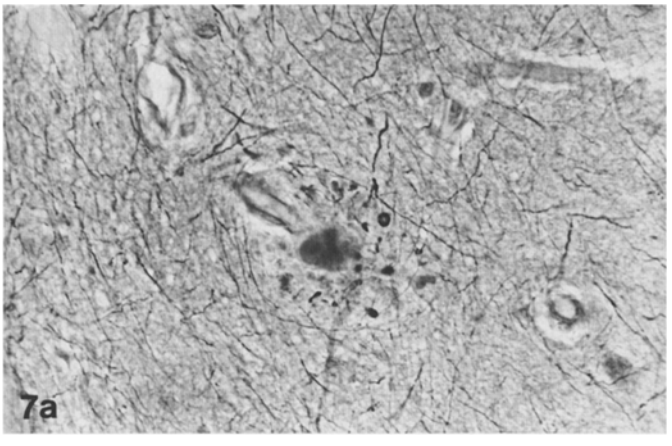
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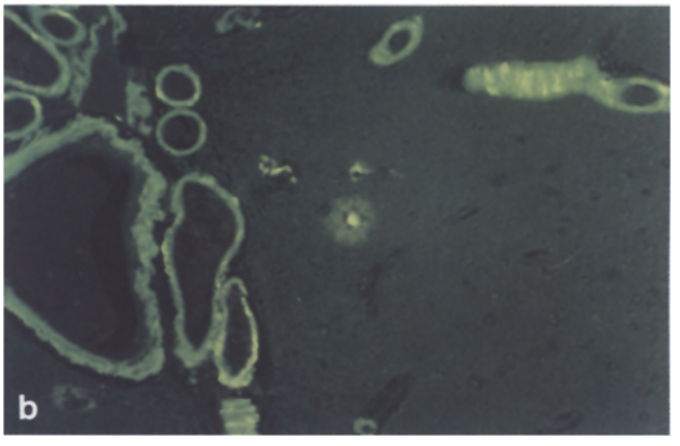
b

Discussion

Putative early forms of senile plaques, called "diffuse plaques", "amorphous plaques" or "preamyloid deposits" have been observed in AD, DS and in small numbers also in non-demented, mostly old subjects [8, 21, 25, 31, 32]. The variously reported staining properties of these plaques together with the selected references are summarized in Table 1. Accordingly diffuse or amorphous plaques are β/A4 immunoreactive, Congo red negative and devoid of silver-stained degenerating neurites. Preamyloid deposits are further defined by their thioflavin S negativity. PAM and modified Bielschowsky staining proved to be as sensitive as β/A4 immunostaining for detecting diffuse plaques [31]. At the ultrastructural level these presumably early plaques appeared to contain extracellular β/A4-immunoreactive and PAM- or methenamine silver-positive electron-dense amorphous material, presumably representing "preamyloid". Amyloid fibrils were not or rarely encountered or present in scattered bundles [13, 14, 29, 33, 35]. Some of the cell processes present in these plaques were recently identified as dendritic or astrocytic in origin [35]. Yamaguchi et al. [34] reported the appearance of degenerating neurites and astroglial processes concomitant with increasing amounts of amyloid fibrils in diffuse plaques, which led them to distinguish initial diffuse plaques and advanced diffuse plaques, the advanced plaque still being Bodian negative light microscopically.

It is evident from Table 1, that the staining properties of plaque type 1, as encountered in HCHWA-D in previous studies [16, 28] and in patients 1–5 in this study, correspond to those of the diffuse or amorphous plaque. In view of its appearance in thioflavin S staining it is tempting to compare plaque type 1 to the advanced type of diffuse plaque described by Yamaguchi et al. [34]. However, comparative study with AD and DS patients has to determine whether plaque type 1 and the diffuse plaque are indeed morphologically similar. Furthermore, quantitative analysis is needed to confirm the presence of β/A4-immunoreactive preamyloid deposits, not detectable with thioflavin S staining, as noted in HCHWA-D by Timmers et al. [26]. PAM or modified Bielschowsky staining was not performed in that study, rendering comparison of the results with those of our study difficult.

Another type of putative early plaque, "plaque A", has been described by Probst [19, 20]. "Plaque A" is sharply delimited and spherical in shape. Its staining properties are shown in Table 1. By its shape and staining properties the center of plaque type 2 in HCHWA-D resembles this "plaque A". However, plaque type 2 differs from "plaque A" by its silver-stained periphery. The appearance of plaque type 2 in the oldest three patients suggests that its occurrence is age-related. Whether plaque type 2 develops independently or arises from type 1 remains to be clarified. Furthermore, the virtual non-argyrophilia of its center is enigmatic in view of its βA4 immunoreactivity. As suggested by the presence of shadows of nuclei, the center might result from a degenerative process starting within plaque type 1. This may indicate a development of plaque type 1 different from that of the diffuse plaque. The appearance of coarse irregular-shaped argyrophylic structures in both types of plaques in the oldest patient, may also be an age-related phenomenon. In view of the results with Bielschowsky's block impregnation their nature could be neuritic. The variable results obtained with this method in staining plaque type 1 in the five younger patients do not allow assumption as to the nature of the components of this plaque.

Silver-stained degenerating neurites were found in association with a number of congophilic blood vessels in all our patients. A similar observation with immunohistochemical staining methods was done with respect to congophilic blood vessels in AD [4, 18]. The neuritic reaction appeared to be the result of exudation of amyloid fibrils from the blood vessels into the parenchyma, i.e., dyshoric angiopathy [18]. This phenomenon may account for the occasional presence of a classical plaque-like structure in some of our patients. Some of the thioflavin S-positive amyloid deposits described by Timmers et al. [26] in two patients with HCHWA-D may represent such lesions. The Alz50 negativity of the silver-stained degenerating neurites as observed in patient 6 may be comparable to the absence of immunoreactivity with antibodies to tau protein and paired helical filaments in degenerating neurites in non-demented elderly, in diffuse Lewy body disease and in subsets of degenerating neurites in AD and DS [5–7, 17, 23]. On the other hand prolonged formalin fixation may have compromised possible immunoreactivity in this patient.

Our study showed the occurrence of morphologically different plaque-like lesions in HCHWA-D. We suggest,

Fig. 1. Paraffin section of the cerebral cortex of patient 1, aged 40 years. Plaque type 1, composed of argyrophilic fibrous material. Modified Bielschowsky stain, × 300

Fig. 2. Paraffin section of the cerebral cortex of patient 6, aged 76 years. Plaque type 2, composed of a non-argyrophilic center, surrounded by an argyrophilic rim. Modified Bielschowsky stain, × 150

Fig. 3. Paraffin section of the cerebral cortex of patient 6, aged 76 years. Part of plaque type 1, composed of PAM-positive fibrous material and coarser irregular-shaped structures. PAM stain, × 300

Fig. 4. Paraffin section of the cerebral cortex of patient 5, aged 59 years. Plaque type 1, revealing its fibrous nature in β/A4 immunostaining, × 180

Fig. 5. Paraffin section of the cerebral cortex of patient 6, aged 76 years. Plaque type 2, composed of a β/A4-immunoreactive center, partly surrounded by an argyrophilic rim. β/A4 immuno- and PAM double staining, × 120

Fig. 6. Paraffin section of the cerebral cortex of patient 2, aged 48 years. Plaques type 1 are discernable but do not show bright fluorescence with thioflavin S, × 60

Fig. 7a,b. Paraffin sections of the cerebral cortex of patient 2. **a** Classical plaque-like deposit in close association with a blood vessel and surrounded by delicate silver-stained degenerating neurites. Palmgren stain, × 120. **b** Similar deposit in thioflavin S fluorescence microscopy, × 30

that the changes in the morphology are related to age. With the described methods it proved to be difficult to identify the components of the plaque-like lesions in HCHWA-D. Thus, it remains to be elucidated whether the structure and pathogenesis of plaque type 1 in HCHWA-D and the diffuse plaque of AD and DS, though they have a number of staining properties in common, are identical. Their development with time may at least be different as accounted for by the appearance of plaque type 2 in the older patients. Obviously, the nature of the point mutation causing HCHWA-D may determine differences between the plaque-like lesions in HCHWA-D and the plaques of AD and DS. Ultrastructural and further immunohistochemical investigations are needed to solve these issues.

Acknowledgement. We thank Dr. D. J. Selkoe of the Center for Neurologic Diseases, Brigham and Women's Hospital, Boston, Massachussetts, USA, for his gift of antiserum Angela and Dr. B. H. Anderton of the department of Neuroscience, the Bethlehem Royal Hospital and the Maudsley Hospital, London, England, for his gift of antiserum anti-SP1-10.

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