Vascular factors in dementia: prevention and pathology
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Chapter 5

Capillary cerebral amyloid angiopathy: neuropathological and clinical characteristics

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Abstract

Background: Cerebral amyloid angiopathy (CAA) affects parenchymal and leptomeningeal arteries and arterioles. Capillary CAA (capCAA) occurs in a minority of the cases, with amyloid-β (Aβ) depositions spreading into the neuropil, known as dyshoric changes. The APOE-ε4 allele has been implicated as a risk factor for capCAA, whereas it’s not for CAA. The relation between capCAA and Aβ plaques, neurofibrillary changes and inflammation is currently unclear. Whether capCAA has a clinical correlate is not known. The aim of this study was to investigate the pathological characteristics of capCAA, the association with neurofibrillary changes and inflammation and the clinical symptomatology.

Methods: Twentytwo patients with abundant dyshoric capillary CAA were selected and clinical data were collected. Using immunohistochemical techniques the neuropathological changes in and around Aβ-affected capillaries were characterized, and comparisons with larger vessel CAA and amyloid plaques were made based on immunoreactivity with antibodies against Aβ 1-17, 1-40 and 1-42, hyperphosphorylated tau, ubiquitin, LN3, GFAP and APOE-ε4.

Results: Aβ1-40 was the main component of the dyshoric changes, whereas Aβ1-42 formed dense bulb-like deposits adjacent to the capillary wall. An inverse correlation between local plaque load severity and capCAA (Spearman’s rho -0.52, p = 0.01) was observed. A high APOE-ε4 allele frequency (54%) was found and 43% was homozygous for the ε4-allele. The severity of capCAA increased with the number of ε4-alleles, as did the presence of APOE in the capillaries. Deposits of hyperphosphorylated tau and ubiquitin and clusters of activated microglia, closely resembling the changes around Aβ plaques, were found around capCAA while absent around larger Aβ-laden vessels. CapCAA occurred in patients with different neurodegenerative diseases and was not found to be associated with a specific clinical symptom.

Discussion: Our findings show that capCAA is neuropathologically distinct from larger vessel CAA on several aspects. The inverse correlation between capCAA and plaque load suggests transport of Aβ from the neuropil towards the circulation, where it can accumulate in and around the capillaries. The co-localization of APOE with the capillaries and the high APOE-ε4 allele frequency stress the role of APOE in capCAA, distinguishing it from larger vessel CAA. The finding of deposits of tau, ubiquitin and clusters of activated microglia closely resembling the changes around Aβ-plaques, make it conceivable that these perivascular changes contribute to the cognitive decline in these subjects. We speculate that the parenchymal Aβ and concomitant neuroinflammatory reaction and tau-pathology surrounding the capCAA might contribute to cognitive decline.
Introduction

Cerebral amyloid angiopathy (CAA) is a neuropathological finding characterized by deposits of β-amyloid (Aβ) in the meningeal and parenchymal arteries, arterioles and, to a lesser extent, capillaries of the brain. The sporadic form of CAA is a common finding at autopsy in elderly subjects increasing with age and it is a very common finding in Alzheimer’s disease (AD), in up to 70-100% of the cases. CAA can occur in any region of the brain, and spreads in a characteristic pattern starting in the neocortex and finally involving other brain areas including the diencephalon, striatum and cerebellum. In the neocortex the occipital lobe is a predilection site for these deposits.

Sporadic CAA can be classified into CAA-type 1, involving cortical capillaries in addition to leptomeningeal and cortical arteries and arterioles, and CAA-type 2, not involving the cortical capillaries. Capillary CAA (capCAA) can occur in any stage of CAA-type 1 and correlates with severity of AD pathology as scored using Braak-, NIA-Reagan- or CERAD-criteria, whereas CAA-type 2 does not. A high APOE-ε4 allele frequency (46.7%) has been found in subjects with capCAA, whereas CAA-type 2 is not clearly associated to the APOE-ε4 genotype. Severe capCAA has been described in a limited number of APOE-ε4 homozygous subjects.

In capCAA-affected capillaries flamelike amyloid depositions sometimes extend beyond the vessel wall and radiate into the neuropil. This was first described by Scholz in 1938, who named it ‘drusige entartung’ and was later referred to as dyshoric angiopathy. The occurrence of capCAA can be very prominent, with only limited Aβ in leptomeningeal and parenchymal arterioles and arteries.

The distribution and occurrence of distinct Aβ isoforms in capCAA is different from CAA-type 2. CapCAA contains mainly Aβ1-42, whereas in CAA-type 2 Aβ1-40 is the predominant isoform. A limited amount of Aβ1-40 can be found in the vessel wall in capCAA as well, and is then positively correlated to the amount of Aβ1-40 in plaques. As for the correlation between plaque Aβ1-42 and capillary 1-42 results are conflicting; a positive correlation has been found by some, whereas this correlation was negative in another study. Neurofibrillary changes, such as tau-positive dystrophic neurites have been observed around Aβ-laden arteries and arterioles in CAA. Interestingly, the presence of tau-positive structures is correlated with perivascular Aβ deposits, but not with Aβ in the vessel wall, suggesting that parenchymal Aβ might trigger the tau pathology, similar to tau around plaques.

The presence of activated glial cells and colocalization of inflammatory proteins (cytokines, complement factors) around classical plaques indicates the occurrence of an ongoing neuroinflammatory response, which is absent around CAA-type 2 vessels. In contrast with CAA-type 2, the parenchymal Aβ in dyshoric capCAA is accompanied by perivascular tau deposits and could elicit an inflammatory reaction similar to that
observed around plaques. Whether the presence of such a neuroinflammatory reaction might contribute to cognitive decline remains to be investigated. Although a low correlation between capCAA severity and dementia has been shown, the clinical significance of capCAA both in AD and in non-demented subjects has not been elucidated yet. The clinical significance of type 2 CAA is unclear, as it is a common finding at autopsy in both demented and non-demented subjects and it is not associated with dementia. This study aims to further investigate the differences between dyshoric capCAA and CAA-type 2, with respect to the distribution of different Aβ-isoforms, the relationship with plaques and the surrounding neurofibrillary changes and inflammation. The previously reported correlation with APOE genotype is evaluated in a larger unselected series of patients. In addition the clinical symptoms and signs of the subjects are studied in an attempt to delineate a clinical correlate of capCAA.

Materials and methods

Subjects and clinical data

Patient selection was based on neuropathological findings at autopsy and collection of clinical data was performed retrospectively. All subjects with extensive capCAA and dyshoric changes were collected from four neuropathological databases, concerning autopsies between 2000 and 2007. The Netherlands Brain Bank contains tissue from subjects with different types of dementia and elderly controls without neurological disease. The tissue from the University Medical Centre in Utrecht, The Netherlands concerns patients who were clinically suspected of having Creutzfeldt-Jakob disease (CJD). The tissue from the Laboratory for Neuropathology East Netherlands, Enschede, The Netherlands contains mainly autopsy material of patients with Parkinson's disease and related disorders. The tissue from the VU University Medical Center, Amsterdam concerns subjects with both AD and subjects who died from a variety of causes without any neurological disease.

Subjects were included if there was extensive capCAA; subjects with just an occasional Aβ-positive capillary, were not included. Four age-matched controls without CAA and without any neurological disease were collected from the Netherlands brain bank.

Information about clinical diagnosis, clinical symptoms, disease course, medical history, medication use and cause of death was available from the databases and additional information was collected from patient records at the departments where the patients were followed, or through the family physician. All subjects or their legal representative had signed an informed consent for use of clinical data and tissue for scientific purposes, before the information was added to one of the databases.
**Neuropathological assessment**

Human brain specimens were all obtained at autopsy with a short postmortem interval. All neuropathological evaluations were performed on formalin-fixed, paraffin-embedded tissue from occipital pole cortex (Brodmann area 18/19). Staging of neurofibrillary changes was evaluated according to Braak and Braak. In order to determine CAA stage for descriptive purposes, temporal pole cortex, hippocampus (essentially CA1 and entorhinal area of the parahippocampal gyrus), cerebellum (vermis) and striatum (pallidum and caudatum) were analyzed as described before.

**Immunohistochemistry**

Formalin-fixed (4%, 24 h) paraffin-embedded tissue was used. Sections (5 µm thick) were mounted on superfrost plus tissue slides (Menzel-Gläser, Germany) and deparaffinized. To quench endogenous peroxidase activity, sections were treated with 0.3% H$_2$O$_2$ in methanol for 30 min, and antigen retrieval was performed in either 10 mM pH 6.0 sodium citrate buffer heated by microwave for 10 min and cooled down at room temperature or formic acid for 15 min at room temperature, and subsequently rinsed in water and phosphate buffered saline (PBS). Sections were stained using the avidin–biotin–peroxidase complex (ABC) method, EnVision method or Power Vision method. (Table 1)

Sections stained with ABC method, were pre-incubated for 10 min with normal rabbit serum (1:50 dilution; DAKO, Glostrup, Denmark) and incubated overnight with the appropriate primary antibodies (table 1).

**Table 1.** Primary antibodies used in this study.

<table>
<thead>
<tr>
<th>primary Antibody</th>
<th>antigen species raised in</th>
<th>dilution</th>
<th>method</th>
<th>ARS</th>
<th>source</th>
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<td>1:200</td>
<td>ABC</td>
<td>Na-citrate</td>
<td>Innogenetics</td>
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<td>Dako</td>
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<td>ABC</td>
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<tr>
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<td>Ubiquitin mouse</td>
<td>1:25600</td>
<td>PV</td>
<td>Formic acid</td>
<td>Chemicon (Millipore)</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glial Fibrillary Acidic Protein</td>
<td>1:100</td>
<td>EV</td>
<td>Na-citrate</td>
<td>Monosan</td>
</tr>
<tr>
<td>LN3</td>
<td>HLA-DR mouse</td>
<td>1:200</td>
<td>EV</td>
<td>Na-citrate</td>
<td>VUMC, gift Dr. Hilgers</td>
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<td>EV</td>
<td>Formic acid</td>
<td>MBL</td>
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</table>

After washing with PBS, slides were incubated with a biotin-conjugated secondary antibody (for ABC method, RaM, 1:500 dilution, DAKO, Glostrup, Denmark) or with EnVision Kit horseradish peroxidase-labeled Goat anti-Mouse/Rabbit (undiluted, DAKO, Glostrup, Denmark) for 30 minutes at room temperature. Sections stained with ABC method were further incubated with streptavidin-biotin horseradish peroxidase complex (Strept ABComplex/HRP, 1:200 dilution, DakoCytomation, Glostrup, Denmark) for 60 minutes at room temperature.

Sections stained with the PowerVision method, after overnight incubation with primary antibody, were incubated with post antibody blocking for PowerVision plus (undiluted, Immunologic, Duiven, The Netherlands) for 15 minutes. Subsequently sections were incubated with Poly- HRP-Goat anti-Mouse/Rabbit/Rat IgG (undiluted, Immunologic, Duiven, The Netherlands) for 30 minutes.

3,3’-diaminobenzidine (EnVision Detection system/HRP, 1:50 dilution, DakoCytomation, Glostrup, Denmark, 10 minutes) was used as chromogen. Between incubation steps, sections were thoroughly washed with PBS. After a short rinse in tap water sections were incubated with haematoxylin for 2 minutes and extensively washed with tap water for 10 minutes. Sections were then dehydrated with ethanol followed by xylene and mounted with Depex mounting medium (BDH Laboratories Supplies). Normal serum and antibodies were dissolved in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA Factor V, Roche, Germany).

The primary antibodies, dilutions and manufacturers of the antibodies used in this study are listed in table 1. The sections immunohistochemically stained for phosphorylated tau (AT8), ubiquitin, glial fibrillary acidic protein (GFAP) and HLA-DR (LN3) were co-stained with Congo Red to better visualize the relationship between these changes and the capCAA.

Immunofluorescent doublestaining with Aβ1-40 (mouse IgG2b) and Aβ1-42 (mouse IgG1) was performed by means of isotype specific secondary antibodies to visualize the distribution of the different isoforms around the capillaries. Sections were deparaffinized as described above and preincubated in normal goat serum (1:10 dilution; DAKO, Glostrup, Denmark) and incubated overnight with a mixture of primary antibodies (see table for concentrations). After washing with PBS, slides were incubated with goat anti-mouse isotype specific secondary antibodies (IgG₁HPR and IgG₂bBIO, 1:100 dilution, diluted in PBS containing 1% BSA, 10% normal goat serum and 10% human pooled serum) for 60 minutes at room temperature. Sections were then washed extensively with PBS, incubated with Streptavidin-ALEXA488 (1:750) for one hour to obtain the green fluorescence, and washed again with PBS. In order to detect the red fluorescent signal sections have been reacted with rhodamine-tyramide (1:3000) in presence of 0.01% of H₂O₂ for 5 minutes. After washing with PBS, autofluorescence of the tissue has been blocked with short incubation (2 minutes) in 0.3% Sudan Black solution. Slices were mounted with Glycerol/Tris-HCl buffer (4:1).
Morphological analysis and quantification

Morphological analysis of capCAA and CAA-type 2 was done with antibodies against Aβ1-17, Aβ1-40 and Aβ1-42. Vessels smaller than 10 µm were considered as capillaries. Four microscopic fields (capillaries: magnification 10x, larger vessels: magnification 2.5x) were analyzed and categorized as follows: 0, none; 1, occasional positive vessel; 2, several positive vessel scattered throughout the field; 3, almost every vessel affected. Aβ plaques were quantified in the same manner as the Aβ-positive larger vessels. Immunohistochemical stainings against AT8 and Ubiquitin were scored according to severity as follows: 0, none; 1, mild; 2, moderate and 3, severe. All scoring has been done in consensus by two observers blinded to the clinical diagnosis or any patient information (E.R. and A.C.). Doublestainings with the primary antibodies and congo-red were evaluated in a qualitative way, as well as doublestainings with Aβ1-40/1-42. Adjacent sections were stained with anti-APOE-ε4 and Aβ1-17 for evaluation of co-localization.

Statistical analysis

Considering the relatively small number of subjects in our study and the use of ordinal scales to grade the neuropathological changes, non-parametric test were used for all analyses. Spearman’s rank correlation coefficients were calculated. When using dichotomized variables Mann-Whitney test was used.

Results

Subjects and Clinical symptoms

A total of 22 patients with capCAA were extracted from four different databases, based on description of capCAA in the neuropathological reports. Six cases were found out of 189 subjects from the CJD-suspect database (89 (48 %) did not have CJD; none of the capCAA subjects had CJD), ten cases in the series from the Netherlands Brain Bank (containing 380 subjects), of which eight AD cases and 2 controls, two cases from the VUMC database (containing 1045 subjects) and four cases out of 110 cases in the PD and related disorders database.

Information about the clinical diagnosis of all patients is summarized in table 2. Six had rapidly progressive dementia and were clinically suspected of having CJD, nine had clinically AD, four had Parkinson’s disease (PD) of which one was rapidly progressive, and three did not have any neurological symptoms or signs.

Virtually all symptoms and signs that can be seen in neurodegenerative disease occurred, but with a large variety of occurring symptoms between the capCAA patients. The pattern of symptoms was, as expected, mostly consistent with the clinical diagnosis (table 2); the CJD-suspected cases with capCAA all had clinical symptoms compatible with this -erroneous- clinical diagnosis, including rapid progression of cognitive...
Table 2. Overview of patients and clinical symptoms.

<table>
<thead>
<tr>
<th>case</th>
<th>age</th>
<th>sex</th>
<th>clinical diagnosis</th>
<th>NP diagn</th>
<th>CAA Stage</th>
<th>Braak T</th>
<th>ApoE</th>
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<tbody>
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<td>1</td>
<td>71</td>
<td>f</td>
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<td>AD</td>
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<td>4</td>
<td>44</td>
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<tr>
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<td>86</td>
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<td>AD</td>
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<td>6</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>f</td>
<td>CJD susp</td>
<td>AD</td>
<td>2</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>4</td>
<td>76</td>
<td>m</td>
<td>CJD susp</td>
<td>AD</td>
<td>2</td>
<td>4</td>
<td>n.d.</td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>f</td>
<td>CJD susp</td>
<td>AD changes</td>
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<td>3</td>
<td>n.d.</td>
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<tr>
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<td>2</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>25</td>
<td>84</td>
<td>m</td>
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<td>n.a.</td>
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<tr>
<td>26</td>
<td>93</td>
<td>f</td>
<td>Control</td>
<td>n.a.</td>
<td>0</td>
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</tr>
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</table>

NP: neuropathological diagnosis; Braak T: Braak tangles; CJD: Creutzfeldt-Jakob Disease; AD: Alzheimer’s disease; PD: Parkinson’s disease; LBD-NT: Lewy body disease-neocortical type; ApoE: apolipoprotein E genotype. CAA stage: 0 = none, 1 = mild, 2 = moderate, 3 = severe. CD: cognitive decline, Cort: other
decline, myoclonus and akinetic mutism. The AD cases with capCAA had a prolonged
cognitive decline varying from 3-10 years, with multiple cortical deficits, sometimes
extrapyramidal or pyramidal signs and sometimes seizures. The PD cases with capCAA
all had signs of Parkinsonism, and in addition had neuropsychiatric disturbances, but
no pyramidal signs except for one case.
Neuropathological findings

All clinically diagnosed AD patients and four of the six CJD-suspected cases fulfilled neuropathological criteria for AD concerning the tau pathology with Braak tangle stage of four or higher; all clinically diagnosed PD cases had severe Lewy-body pathology at autopsy. The three non-demented cases had Braak tangle scores of 1-3.


Neuropathological findings

All clinically diagnosed AD patients and four of the six CJD-suspected cases fulfilled neuropathological criteria for AD concerning the tau pathology with Braak tangle stage of four or higher; all clinically diagnosed PD cases had severe Lewy-body pathology at autopsy. The three non-demented cases had Braak tangle scores of 1-3.
Dyshoric changes were mainly observed around the capillaries, and only rarely around larger vessels. Both Aβ 1-40 and Aβ 1-42 were detected around the capillaries and they were highly correlated (Spearman’s rho 0.855, p < 0.000), but the distribution showed remarkable differences. Aβ1-40 was the main component of the dyshoric changes, completely surrounding the capillary with also extensive spread into the neuropil, as well as the main component in the vessel wall. Aβ1-42 on the other hand was mainly present in dense bulb-like deposits directly adjacent to the capillary wall, but virtually absent in the flamelike depositions radiating into the neuropil. (Fig 1) This is the opposite of the distribution in plaques with a dense core consisting of Aβ 1-40 and a
Figure 3 A-O. Immunoreactivity in capCAA (left column of panels), CAA (middle column of panels) and plaques (right column of panels). All stainings are double stainings with congo-red, except panels a,b,c. (A,B,C) Aβ 1-17 staining; the dyshoric changes are found around the capillaries, and not the larger vessels. (D,E,F) Microglial activation is seen around the capillaries, especially when there are dyshoric changes. (G,H,I) Activated astrocytes are seen around both capillaries and larger vessels, mostly when a dyshoric component is present. (J,K,L) Hyperphosphorylated tau is seen only around the Aβ-laden capillaries, and hardly around the larger vessels. (M,N,O) ubiquitin is found around the Aβ-laden capillaries, mainly when there are dyshoric changes as well, and not around the larger vessels.
diffuse spread around consisting of mainly Aβ1-42. (Fig 1) In the four control brains (nr 23-26, table 2) no Aβ immunoreactivity was detected. The severity of capCAA was correlated with the severity of CAA type 2 (Spearman’s rho 0.71, p < 0.000); CapCAA occurred in any stage of CAA and no subjects had capCAA without any larger vessel CAA. We observed a significant inverse correlation between capCAA severity and plaque density with relatively few plaques in subjects with the most extensive capCAA (Spearman’s rho -0.52, p = 0.013, fig 2). When separately analyzed for Aβ1-40 and Aβ1-42 this correlation was the same for both isoforms (Spearmans rho -0.59, p = 0.004 vs. -0.53, p = 0.011). No significant correlation was found between CAA type 2 and plaque load (Spearman’s rho – 0.39, p = 0.076).

** Tau and ubiquitin**

Few or no neurofibrillary tangles were observed in the occipital cortex of any of the subjects, although present in other regions of the brain, especially in the AD-subjects (table 2, Braak tangle score). AT8 immunoreactivity was observed surrounding Congo red positive dyschoric capillaries and was absent around the larger Congo red positive vessels. (fig. 3j,k) Similarly, ubiquitin-immunoreactive neuritic dystrophy was found around capCAA, but not around larger congo-red positive vessels (fig 3 m,n).

The severity of AT8-immunoreactivity was correlated with the severity of ubiquitin (UB) reactivity (Spearman’s rho 0.527, p = 0.03). The cases with little UB (score ≤ 2) had significantly less AT8 immunoreactivity than the cases with abundant UB (> 2), 0.8 vs 2.4, p = 0.001 (Mann-Whitney U) and no cases demonstrated tau pathology in the absence of ubiquitin immunoreactivity, whereas in two cases ubiquitin immunoreactivity without any tau pathology was observed around the dyschoric capCAA-affected vessels. Both tau and ubiquitin deposits were more severe in the demented subjects than the non-demented subjects (1.4 vs. 1.0 and 2.2 vs. 1.3 respectively), but this did not reach significance, probably due to the small number of non-demented subjects (n=3). No tau and ubiquitin immunoreactivity was detected in control brains.

**Glia activation**

The presence of astrocytes was assessed using GFAP immunolabeling; doublestaining with GFAP and Congo-red demonstrated the presence of astrocytes around virtually all Aβ-laden vessels, albeit strongest around capillaries, in particular in the presence of dyschoric changes, which were surrounded by clusters of GFAP immunoreactive astrocytes. (fig. 3g,h) Clusters of LN3-positive microglia were strongly associated with Aβ laden capillaries with dyschoric changes, but were only sporadically observed around larger vessels harbouring CAA. (fig. 3d,e) Clusters of activated microglia and astrocytes were, as expected, found around the classical plaques in the same region. In the control subjects some GFAP-immunoreactivity was present, but no LN3-positive microglia were seen.
**APOE**

APOE-genotype was determined for 14 of the 22 cases. The APOE-ε4 allele frequency in this cohort was 54%. Six of the fourteen patients (43%) were homozygous for the APOE ε4 allele. When stratified for APOE genotype, subjects with at least one APOE-ε4 allele had higher scores for capillary Aβ1-17 (2.4 vs. 2.0), Aβ1-40 (2.1 vs. 1.4) and Aβ1-42 (2.3 vs. 2.0) than subjects without an ε4 allele. In these small groups, none of these differences reached significance, but a trend for more severe capCAA depending on the number of ε4 alleles is clearly illustrated in figure 4. Subjects heterozygous for the ε4 allele had the strongest association with capillary Aβ, as shown on adjacent sections.

**Figure 4 A-C.** Capillary cerebral amyloid angiopathy and plaque severity stratified for APOE genotype, analyzed in the Aβ1-17 (A), Aβ1-40 (B) and Aβ1-42 (C) stainings shows a correlation between capCAA severity and APOE genotype. Plotted values are means with SEM.

**Figure 5 A-F.** Adjacent sections (10X) stained for Aβ1-17 (left, panels A,C,E) and APOE4 (right, panels B,D,F) in a patient with no ε4 allele (A,B), ε4 heterozygous (C,D) and ε4 homozygous (E,F). The dyshoric capillary cerebral angiopathy severity is low the in ε4 negative subject, intermediate in the heterozygous, and high in the homozygous patient.
stained for APOE-ε4 and Aβ1-17, and a clear increase of capillary Aβ with the number of ε4 alleles can be seen in figure 4 and 5.

Discussion

This series of 22 cases of dyshoric capCAA is the largest series specifically studying the neuropathological changes accompanying the parenchymal Aβ surrounding the capCAA. In order to minimize selection bias, which is often a problem in retrospective studies using neuropathological databases, we selected cases with extensive capCAA with dyshoric changes from four distinct databases containing different patient populations.

The neuropathological abnormalities in most of our capCAA patients showed that they were suffering from a neurodegenerative disease compatible with the clinical diagnoses during life. However, interpretation of the causes of the wide range of symptoms in this group of patients with capCAA is far from straightforward. Most patients suffered from combinations of symptoms that could largely be attributed to their neurodegenerative disease other than the capCAA: e.g. all PD patients had extrapyramidal symptoms and most had hallucinations, whereas many AD patients had multiple higher cortical disturbances like apraxia and aphasia. CapCAA with dyshoric changes by itself probably does not lead to cognitive decline, considering the fact that some cognitively intact elderly have these changes. We can speculate however, that when capCAA and the associated neuroinflammatory response occur in addition to extensive neurofibrillary tangles and in the absence of neuritic plaques, that this could possibly lead to a rapid progression of dementia, since we observed capCAA in some subjects clinically suspected of suffering from CJD, based on rapid clinical progression, while having extensive neurofibrillary tangles with very few plaques.

Several clear neuropathological differences between capCAA and type 2 CAA have been found in this study. In line with previous reports, we demonstrated that in capCAA-affected vessels Aβ1-42 is present within the walls of Aβ-laden capillaries and in dense bulb-like deposits adjacent to the capillary wall. In these previous studies, Aβ1-42 was observed to be the main isoform in capCAA as opposed to Aβ1-40 in CAA type 2. In contrast with these findings, we found a distinct presence of Aβ1-40 in capCAA. The less soluble Aβ1-42 we observed in our series of capCAA was mainly as dense, bulb-like deposits adjacent to the capillary wall. A possible explanation for this difference with previous reports could be that few patients in previous series had abundant dyshoric changes (in which Aβ1-40 is the most prominent isoform), and therefore Aβ1-42 was described as the main isoform in capCAA.

The high APOE ε4 allele frequency (54%) in our patient group is comparable to what Thal and colleagues found in their series of capCAA (46.7%). This occurrence of 54% is a lot higher than in the general population (14%) and than in late onset sporadic AD
The rate of ε4/ε4 homozygous subjects of 43% is extraordinary high compared to the population (3%) and AD-subjects (13%), and a lot higher than in the series of Thal, in which 3 out of 15 (20%) genotyped type 1 CAA subjects had ε4/ε4 genotype. Two other cases homozygous for the ε4 allele with severe capCAA with dysorphic changes were published before. Our findings in combination with the previous reports indicate that this specific genotype might represent a strong risk factor for the occurrence of capCAA specifically with concomitant dysorphic changes. Our very high percentage of ε4/ε4 genotype compared to the previous series might be explained by the fact that the subjects in our study were selected on the occurrence of widespread capCAA, probably including more severe cases. The co-localization of APOE ε4 with capillary Aβ and the increasing severity of capCAA with increasing number of ε4 alleles, stress the role of this risk allele. Apparently APOE ε4 genotype is a strong genetic risk factor for dysorphic capCAA, like it is for AD, but the presence of an ε4 allele is not required to develop these changes, since five out of fourteen of our subjects (36%) had a genotype without ε4 allele. In previous studies an association was found between capCAA and AD pathology, assessed according to CERAD, Braak and NIA-Reagan-Institute criteria, as well as between tau and CAA. We observed tau-pathology and ubiquitin immunoreactivity around the capCAA-affected vessels in the occipital lobe, an area where few tangles are found, even in advanced AD. This supports the hypothesis that the tau pathology is secondary to the Aβ depositions around the capillaries, as suggested before. The presence of ubiquitin and tau close to the dysorphic changes resembles closely the changes around classical plaques in AD. In our series some cases exhibit ubiquitin without any tau, but no cases showed tau without any ubiquitin, suggesting a sequence of events similar to plaques, where ubiquitin immunoreactivity can be found before tau. Microglial activation around the capillaries with dysorphic changes was markedly increased compared to Aβ-laden vessels without dysorphic changes, indicating a strong inflammatory response, again similar to what is found around Aβ plaques in AD. The inflammatory reaction associated with Aβ plaques has been implicated to play a role in the pathogenesis of AD and likely contributes to the symptoms of cognitive decline. In the same manner the Aβ accumulation around capCAA can induce an inflammatory reaction with activated microglia as opposed to CAA in the larger vessels, which is not associated with microglial activation. It is probably the parenchymal Aβ in dysorphic capCAA that evokes the neuroinflammatory response, while the Aβ in CAA type 2 is mainly present in the vessel wall where it does not lead to an inflammatory response. This is another argument that the occurrence of capCAA is a distinctly different process from larger vessel CAA and that based on neuropathological characteristics, capCAA more closely resembles plaques than large vessel CAA. Whereas type 2 CAA is generally considered to be a process not contributing to the development of cognitive decline, the neuroinflammatory response and depositions of hyperphosphorylated tau surrounding the parenchymal Aβ in dysorphic capCAA suggest that it might play a role in the cognitive decline in the same manner as plaques do.
The inverse correlation between capCAA severity and plaque-density around the capillaries is striking. Results from previous studies on this aspect are contradicting, but this might be explained by different definitions of capCAA, and by the fact that no clear distinction was made between capCAA with or without dyshoric changes in previous studies.\textsuperscript{6,13,14} In this study we specifically looked at the parenchymal (dyshoric) Aβ surrounding the capCAA. The inverse correlation between plaques and capCAA is compatible with the hypothesis of Aβ flow from the neuropil towards the circulation, thus decreasing plaque-load while increasing Aβ deposits in and around the capillaries. This is in concordance with the findings in a recent Aβ-vaccination trial in AD patients, which has shown that a decrease in plaque load is accompanied by an increase in CAA-severity.\textsuperscript{28} Subsequently CAA severity decreases again, suggesting that Aβ removal from plaques and clearance via the vascular system can take place and is a dynamic process.\textsuperscript{28} Several possible mechanisms of this clearance have been hypothesized. Clearance of Aβ via receptor-mediated transport across the blood-brain barrier (BBB) has been shown to take place.\textsuperscript{29-31} Another possible route of Aβ elimination is the perivascular drainage of Aβ.\textsuperscript{32} Impaired clearance along this route could explain the increasing amount of Aβ-depositions in the brain and finally an increase of the symptoms of cognitive decline.\textsuperscript{32} Our findings could be compatible with such a faulty BBB clearance mechanism resulting in accumulating depositions in and around the capillaries, resulting in dyshoric angiopathy. Our findings could also be compatible with clogging of the perivascular route and thus accumulating Aβ as CAA and finally capCAA. However, capCAA also occurs with relatively little non-capCAA, suggesting that the problem does not necessarily start downstream from the capillaries, but rather with insufficient clearance at the BBB in the capillaries.

Taken together, the pathological hallmarks of type 1 CAA with concomitant dyshoric changes clearly differ from type 2 CAA underscoring the fact that CAA type 1 and 2 might represent distinct disease entities. We observed a characteristic distribution of Aβ\textsubscript{1-40} and Aβ\textsubscript{1-42} in dyshoric capCAA that was not described before. The strong correlation with APOE genotype and the presence of APOE in the capillaries that we found stresses the difference with larger vessel CAA. The deposits of hyperphosphorylated tau and ubiquitin and clusters of activated microglia surrounding the capillaries closely resemble those around plaques, and are practically absent around larger CAA-laden vessels. Considering the parenchymal changes, dyshoric capCAA could possibly contribute to cognitive decline; however, capCAA does not seem to be associated with a specific clinical syndrome. The strong negative correlation between dyshoric capCAA severity and the local plaque load that we found, suggests failure of Aβ transport directed from the parenchyma towards the capillary system as a possible explanation for accumulation of Aβ in the capillary wall and surrounding neuropil as dyshoric changes. Future studies on expression of proteins involved in transendothelial Aβ-transport in subjects with capCAA with dyshoric changes could possibly help clarifying the underlying mechanisms.
Reference List


