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REGULAR PAPER

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Amyloid β peptide 1–42 highly correlates with capillary cerebral amyloid angiopathy and Alzheimer disease pathology

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Abstract Recent studies reported both positive [Thal et al. (2003) J Neuropathol Exp Neurol 62:1287–1301] and negative [Tian et al. (2003) Neurosci Lett 352:137–140] correlations between cerebral amyloid angiopathy (CAA) and Alzheimer's disease (AD) pathology. We have recently shown high correlations between neuritic AD pathology and amyloid β peptide (Aβ) deposits in the capillary/pericapillary compartment (CapCAA) with only low correlations to general CAA (non-capillary). We have now studied the relationship between CapCAA and AD pathology with respect to the distribution of Aβ40 and 42 in the frontal cortex of 100 human postmortem brains from both male and female, demented and non-demented patients (mean age ± SD 84.3±9.3 years). Using polyclonal antibodies to Aβ40 and 42, capillary and plaques positivity were assessed semiquantiatively on a four-point scale. Aβ42 deposits in capillaries correlated highly with both Aβ42 deposits in plaques and morphological AD criteria (CERAD, Braak stages, and NIA-Reagan-Institute criteria), while only a low correlation with CAA was observed. Aβ40 deposits in capillaries differed morphologically from Aβ42 ones: they were limited to capillary walls, were significantly less frequent in both capillaries and plaques compared to Aβ42 (P<0.01), and showed a low correlation with morphological AD criteria (P<0.05) and general CAA (P<0.01). By contrast, Aβ42 deposits were seen in the glia limitans rather than in capillary walls themselves, and showed high correlation with morphological AD criteria (P<0.01). These data indicate that CapCAA is characterized by Aβ42 deposits in pericapillary spaces or in the glia limitans. A low correlation between CAA and CapCAA, but high correlations between morphological AD criteria and CapCAA suggest different pathomechanisms for both types of CAA, and a close relation between CapCAA and AD pathology (both neuritic and plaque type). These data support the concept of a neuronal origin of Aβ via drainage from interstitial fluid from the central nervous system along basement membranes to capillaries.

Keywords Alzheimer's disease · Cerebral amyloid angiopathy · Capillary cerebral amyloid angiopathy · β-Amyloid peptide 1–40 and 1–42 · Frontal cortex

List of Abbreviations *AD* Alzheimer disease · *Aβ* beta amyloid peptide · *Aβ 40/42 CapS* score of deposits of Aβ 1–40/42 in capillaries · *Aβ 40/42 C* number of Aβ 1–40/42 positive cortical vessels · *Aβ 40/42 PS* score of deposits of Aβ 1–40/42 in plaques · *Aβ 40/42 TS* total score of Aβ 1–40/42 deposits · *Aβ 40/42 Csev* severity of Aβ 1–40/42 affection of cortical vessels · *Aβ 40/42 CS* Aβ 1–40/42 cortical score · *Aβ 40/42 L* percentage of Aβ 40/42 positive leptomeningeal vessels · *Aβ 40/42 Lsev* severity of Aβ 40/42 affection of leptomeningeal vessels · *Aβ 40/42 LS* Aβ 40/42 leptomeningeal score · *AβCTS* Aβ cortical total score · *AβLTS* Aβ leptomeningeal total score · *CAA* cerebral amyloid angiopathy · *CAATS* CAA total score · *CapCAA* capillary CAA · *CERAD* Consortium to Establish a Registry of Alzheimer's Disease · *NFT* neurofibrillary tangle · *NIA* National Institute of Aging · *NIA-RI* National Institute of Aging and Reagan Institute · *NP* neuritic plaque · *SP* senile plaque · *TS* total score

Introduction

The pathological hallmarks of sporadic late onset Alzheimer's disease (AD), the most common cause of dementia in the elderly, include extracellular deposition of β-amyloid peptide (Aβ) in the neuropil (senile plaques, SPs) and in the cerebral vasculature (cerebral amyloid angiopathy, CAA), and neuritic cytoskeletal lesions with deposition of

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hyperphosphorylated microtubule-associated tau protein in neurons and dendrites, as neurofibrillary tangles (NFTs) and neuropil threads (NTs), and around amyloid deposits as neuritic plaques (NPs, [10]). The neuropathological diagnostic criteria for AD is the (semi)quantitative assessment and topographical staging of NPs and NFTs [4, 13, 24].

CAA is defined as deposition of a congophilic material in meningeal and cerebral arteries, arterioles, capillaries and veins, representing deposition of Aβ in the vessel walls [17, 29, 46]. It is a common finding in the brains of elderly demented and non-demented individuals; its incidence and severity increase with age [8, 11, 17, 23, 30, 31, 32, 38, 44], and it is associated with cerebral hemorrhages, infarctions, and white matter lesions [5, 17, 26, 27, 28, 29, 30, 31, 32, 37a, 38] (for review see [31]). The prevalence of CAA in AD patients, according to different studies, varies from 70% to 100% [2, 3, 9, 11, 17, 37, 37a, 38, 39, 40, 45].

By immunohistochemistry, two forms of Aβ can be distinguished. Aβ terminating at amino acid position 40 (Aβ40) and at position 42 or 43 (Aβ42). In CAA, Aβ40 affects vessel walls more frequently and more severely than Aβ42 [7, 12, 14, 15, 16, 18, 20, 22]. In SPs and NPs, however, Aβ42 is predominant and Aβ40 is rarely detected [12, 14, 15, 16, 21, 34].

The relationship between CAA and AD is poorly understood and the origin of Aβ in CAA remains unclear. Basically, three different mechanisms have been proposed (see [31]): (1) derivation of Aβ from blood and/or cerebrospinal fluid [19, 30, 47]; (2) production of Aβ by smooth muscle cells within vessel walls and/or pericytes [1, 25, 30]; or (3) derivation of Aβ from the neuropil (i.e., SP, NP), in the course of its perivascular drainage [6, 29, 33, 41, 42, 43].

Reports focusing on capillary involvement in CAA (CapCAA) showed that Aβ is present as globular deposits on the capillary wall and as linear thin layers in the pericapillary basement membrane, often with dyshoric Aβ deposits in the adjacent neuropil [29, 35, 39, 42]. CapCAA is thought to be related to AD-related Aβ deposition in the brain parenchyma, thus supporting the concept of derivation of Aβ from the neuropil in CAA (see above) [2, 35]. It is still uncertain, however, whether CapCAA represents a distinct type of CAA or is the result of coincidental CAA and AD-related parenchymal β-amyloidosis, or whether it represents an end stage of CAA [35].

Examining 19 different regions from 52 human aged brains, Thal et al. [36] reported that vascular Aβ deposition was accompanied by parenchymal plaques in most instances, and only in a few cases was CAA seen in the absence of Aβ deposits in a given area; only two demented and several non-demented individuals exhibited Aβ plaques in the complete absence of CAA. On the other hand, Tian et al. [37] recently reported a negative association between Aβ plaques and CAA in AD. In line with the latter findings, we recently showed that CAA grading (of non-capillary vasculature) did not correlate with neuritic AD pathology, while the prevalence and severity of CapCAA correlated significantly at a medium degree with the presence of high-grade AD pathology and at a high degree with CERAD, Braak, and NIA-Reagan-Institute (NIA-RI) criteria, respectively, suggesting that CapCAA has pathomechanisms different from those of CAA [2]. In this study, however, we did not discriminate between Aβ40 and Aβ42 distribution in both vessels and plaques.

Consequently, the aim of the present study was to investigate the distribution of Aβ40 and Aβ42 in leptomeningeal and intracortical arteries/arterioles, cortical capillaries and plaques to detect possible differences in the correlation between CAA, CapCAA, and AD plaque pathology with respect to different patterns of Aβ deposits.

Material and methods

We investigated 100 human brains obtained at autopsy from both genders aged 60–100 years (mean age ± SD 84.3±9.3 years), with a clinical diagnosis of dementia in 64 patients. Eight of the 36 non-demented (22.2%) and 42 of demented (65.6%) individuals showed high grade AD pathology (i.e., CERAD B, C; Braak V, VI; NIA-RI high probability [4, 13, 24]).

Our cohort consisted of 50 brains each with high- and low-grade or negative AD pathology; details were described previously [2] (Table 1).

Tissue was fixed in a 8% aqueous solution of formaldehyde, blocks were taken from the cerebral cortex (frontal, temporal, parietal, and occipital lobe), the hippocampus with adjacent entorhinal cortex and amygdala, basal ganglia, brainstem, and cerebellum. Paraffin sections, 4 µm thick, were stained with hematoxylin and eosin, Klüver-Barrera, and cresyl violet. For assessment of AD-related pathology, blocks from frontal and temporal cortex and hippocampus with adjacent entorhinal cortex were stained with modified Bielschowsky silver stain and incubated with commercially available monoclonal mouse anti-human paired helical filament-tau antibody (AT8) according to the manufacturer's directions (Innogenetics, Ghent, Belgium). Neuropathological assessment of AD was performed using CERAD, Braak scores and NIA-RI criteria (see [2]).

For detection of Aβ40 and Aβ42 in cerebral vessels and brain parenchyma, adjacent sections from the frontal lobe were immunostained with commercially available rabbit polyclonal antibody Aβ 1–40, which is specific for the C terminus of Aβ40, and Aβ 1–42, which is specific for the C terminus of Aβ42, respectively, according to the manufacturer's directions (Signet Laboratories, Dedham, MA).

Evaluation of Aβ40 and Aβ42

The total number of leptomeningeal vessels in a given slide differed from case to case; we therefore calculated percentages as follows. All leptomeningeal vessels were counted in each slide. All leptomeningeal vessels positive for Aβ40 were counted, discriminating between total and partial vessel wall positivity. The percentage of leptomeningeal Aβ40-positive vessels per slide was calculated (Aβ40L). To evaluate optimally the severity of Aβ40 deposits, the number of vessels with Aβ in only parts of the vessel wall was multiplied by 0.5 and added to the number of vessels with involvement of the whole vessel wall. The resulting number was used to calculate again the percentage of Aβ40 positivity (Aβ40Lsev). The same procedure was performed with Aβ42-positive leptomeningeal vessels (Aβ42L, Aβ42Lsev).

The total number of cortical vessels in a given slide depends on the size of the specimen, which was approximately the same in every case. Therefore, we counted affected vessels as follows. Cortical arteries/arterioles (not capillaries) positive for Aβ40 were counted in each slide, discriminating between vessels with total and partial

Table 1 Frequency of neuropathological criteria and clinical diagnoses

		Demented patients								Non-demented patients	
		Alzheimer's disease		Senile dementia		Vascular dementia		Total			
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
CERAD	Absent	0	0	12	25.0	2	33.3	14	21.9	26	72.2
	A	0	0	6	12.5	2	33.3	8	12.5	2	5.6
	B	9	90.0	23	47.9	2	33.3	34	53.1	7	19.4
	C	1	10.0	7	14.6	0	0	8	12.5	1	2.8
	Total	10	100.0	48	100.0	6	100.0	64	100.0	36	100.0
Braak staging	0	0	0	3	6.3	0	0	3	4.7	4	11.1
	1	0	0	2	4.2	0	0	2	3.1	2	5.6
	2	0	0	2	4.2	1	16.7	3	4.7	0	0
	3	0	0	3	6.3	0	0	3	4.7	8	22.2
	4	0	0	8	16.7	3	50.0	11	17.2	14	38.9
	5	7	70.0	24	50.0	2	33.3	33	51.6	6	16.7
	6	3	30.0	6	12.5	0	0	9	14.1	2	5.6
	Total	10	100.0	48	100.0	6	100.0	64	100.0	36	100.0
NIA-Reagan-Institute criteria	No probability	0	0	6	12.5	1	16.7	7	10.9	7	19.4
	Low probability	0	0	6	12.5	2	33.3	8	12.5	19	52.8
	Medium probability	0	0	6	12.5	1	16.7	7	10.9	2	5.6
	High probability	10	100.0	30	62.5	2	33.3	42	65.6	8	22.2
	Total	10	100.0	48	100.0	6	100.0	64	100.0	36	100.0

Aβ deposits. The total sum of Aβ40-positive vessels was calculated (Aβ40C), and, to evaluate the severity of Aβ40 affection, the number of vessels with partial vessel wall positivity was multiplied with 0.5 and added to the number of vessels with total vessel wall positivity (Aβ40Csev). The same procedure was performed with Aβ42 positive cortical vessels (Aβ42C, Aβ42Csev).

To combine Aβ40Lsev, Aβ40Csev, Aβ42Lsev, and Aβ42Csev the following gradings were used: Aβ40Lsev and Aβ42Lsev were graded on a four-point scale, with 0, absent; 1, 1–20%; 2, 1–40%; 3, >40%, leading to an Aβ40 leptomeningeal score (Aβ40LS) and an Aβ42 leptomeningeal score (Aβ42LS), respectively. Aβ40Csev and Aβ42Csev were also graded on a four-point scale, with 0, absent; 1, 1–5; 2, 6–20; 3, >20, leading to an Aβ40 cortical score (Aβ40CS) and an Aβ42 cortical score (Aβ42CS), respectively. Mean values of Aβ40LS+Aβ40CS, Aβ42LS+Aβ42CS, Aβ40LS+Aβ42LS, and Aβ40CS+Aβ42SC lead to Aβ40, Aβ42, leptomeningeal, and cortical total scores (Aβ40TS, Aβ42TS, AβLTS, AβCTS). Mean values of Aβ40TS+Aβ42TS and AβLTS+AβCTS were equal and each lead to CAA total score (CAATS).

Aβ40 and Aβ42 capillary positivity were assessed semiquantitatively, by two of the authors independently, by counting affected vessels in ten high-power fields with ×400 magnification (HPF). The average of both counts for each section was calculated, and

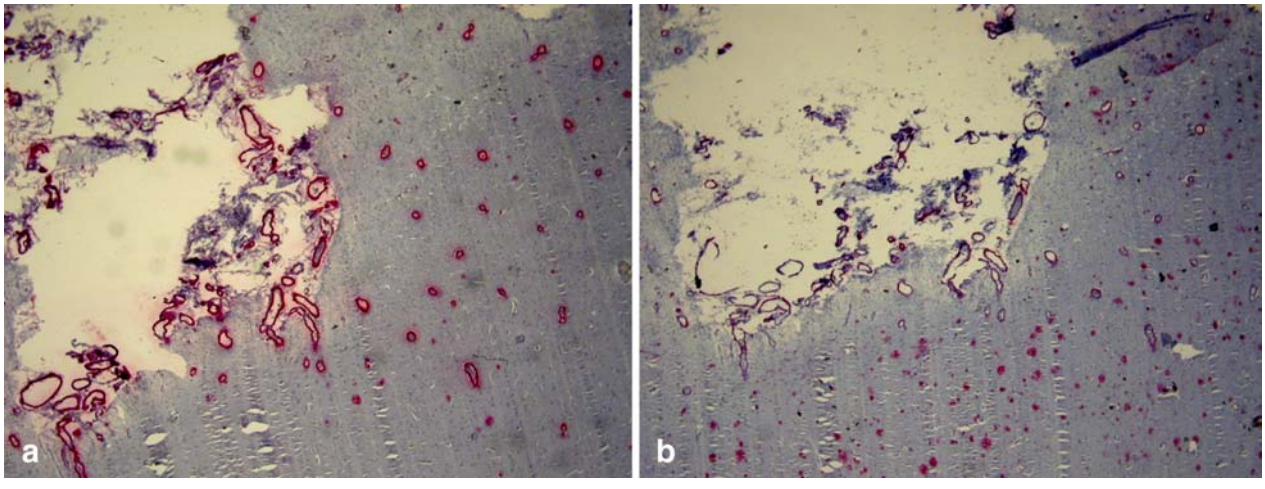

Fig. 1 **a** Aβ40-positive leptomeningeal and cortical arteries; no decoration of plaques. **b** Aβ42-positive meningeal and cortical arteries and multiple plaques. **a**, **b** Frontal cortex, ×100

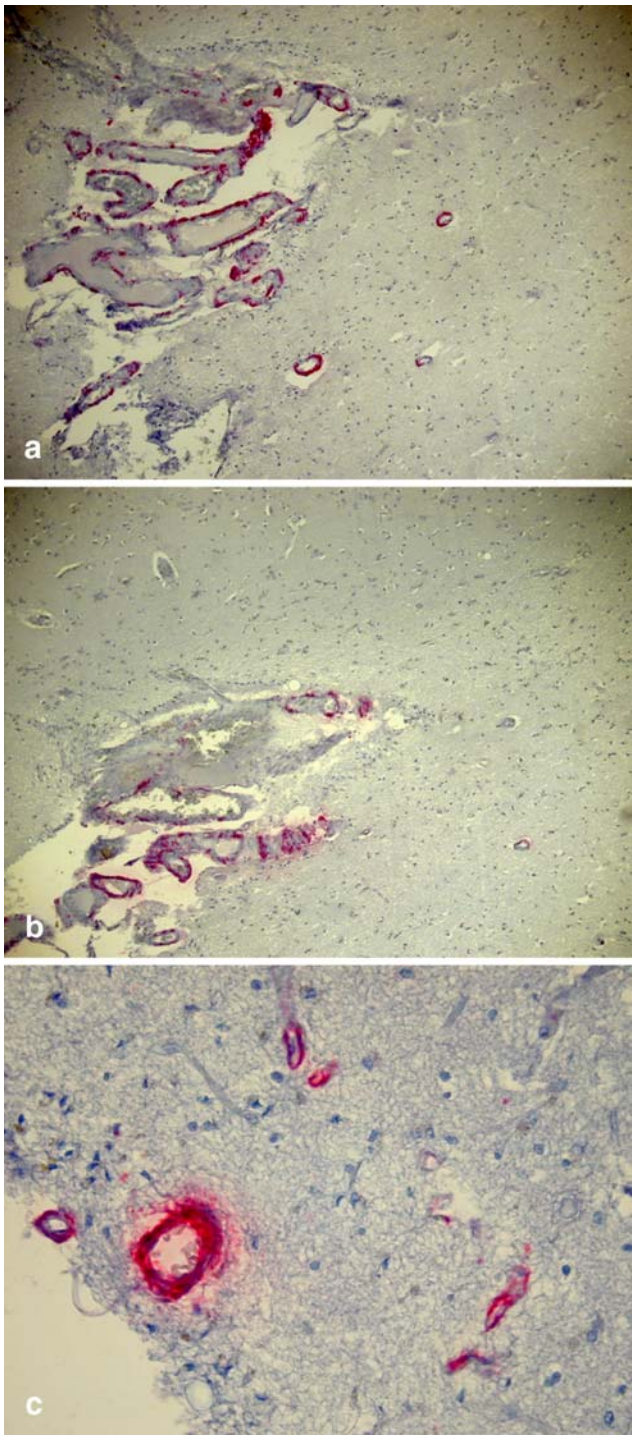

Fig. 2 CAA. **a**, **b** CAA of meningeal and cortical vessels without detectable plaques. **c** Higher magnification of meningeal and cortical CAA. Immunostaining with Aβ against Aβ40 (**a**, **c**) and Aβ42 (**b**) (*CAA* cerebral amyloid angiopathy)

the severity of CapCAA was graded as follows: 0, no affected capillary; 1, less than 1 affected capillary/HPF; 2, one to two affected capillaries; 3, more than two affected capillaries/HPF. This resulted in an Aβ40 and an Aβ42 capillary score (Aβ40CapS, Aβ42CapS).

Aβ40 and Aβ42 plaque positivity were assessed semiquantitatively on a four-point scale: 0, absent; 1, mild; 2, moderate; 3, severe, resulting in an Aβ40 and an Aβ42 plaque score (Aβ40PS, Aβ42PS).

Apolipoprotein E genotyping in this sample is in progress (Attems and Jellinger, in preparation).

Statistics

The Mann-Whitney U test was used to test for differences in the prevalence and severity of each Aβ40 and Aβ42 between cases with high-grade AD pathology/low-grade or no AD pathology, and between clinically demented and non-demented individuals, and the Wilcoxon test for differences between the scores. The Spearman's rank correlation coefficient was calculated for all correlations.

Results

Morphology of Aβ40 and Aβ42 deposits

No qualitative differences in the morphology of either Aβ40 or Aβ42 deposits in leptomeningeal and cortical arterial vessels was observed (Fig. 1a, b), but Aβ40 deposition was much heavier in the vessels. The overall morphology was in concordance with published criteria of CAA morphology [31, 35, 38, 40, 46] (Fig. 2a–c). Severe CAA expressing both Aβ40 and 42 was observed in some brains without any detectable plaques (Fig. 2a, b). Partial vessel wall positivity ranged from only focal to almost total. Therefore, multiplying the number of these vessels with 0.5, to achieve a better evaluation of overall severity seemed appropriate.

In capillaries, Aβ40 was present as strong, continuous deposits in the capillary wall with little or no association with dyshoric changes or plaques, whereas Aβ42 was present as globular deposits and linear thin layers particularly in the glia limitans and in the perivascular basement membrane often with dyshoric changes (Fig. 3a–e).

The morphology of Aβ40 and Aβ42 deposits in plaques was in concordance with published data on the range of Aβ plaque morphology [10] (Fig. 3b, d).

Distribution of Aβ40 and Aβ42 in vessels and plaques

The distribution of Aβ40 and Aβ42 in vessels and plaques is shown in Table 2. To validate the reliability of Aβ40LS, Aβ40CS, Aβ42LS, and Aβ42CS, the corresponding variables Aβ40L/Aβ40Lsev/Aβ40LS, Aβ40C/Aβ40Csev/Aβ40CS, Aβ42L/Aβ42Lsev/Aβ42LS, and Aβ42C/Aβ42Csev/Aβ42CS were correlated, and all showed very high positive correlations ($\rho>0.985$, $P<0.01$). We thus used these scores for all further analysis.

Aβ40LS and Aβ42LS showed no significant difference (mean Aβ40LS 0.91±1.093, mean Aβ42LS 0.71±0.924; $P>0.01$) and highly correlated with each other ($\rho=0.828$, $P<0.01$), Aβ40CS was significantly higher than Aβ42CS (mean Aβ40CS 0.85±1.132, mean Aβ42CS 0.85±0.966; $P<0.01$) and highly correlated with Aβ42CS ($\rho=0.725$, $P<0.01$). No significant difference and high correlations were seen between Aβ40LS and Aβ40CS/Aβ42LS and Aβ42CS ($\rho=0.728$, $\rho=0.712$, $P<0.01$).

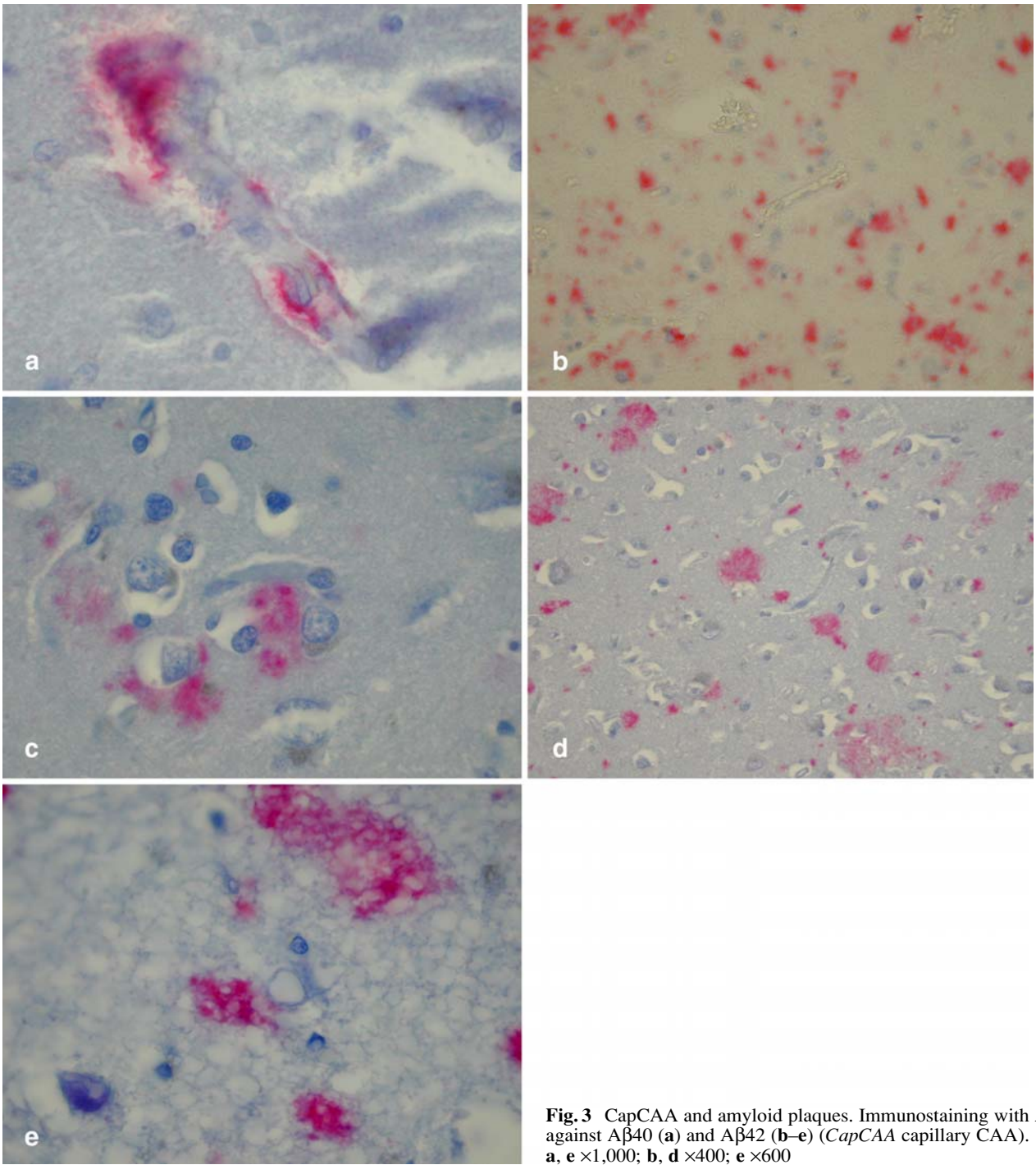

Fig. 3 CapCAA and amyloid plaques. Immunostaining with Aβ against Aβ40 (**a**) and Aβ42 (**b**–**e**) (*CapCAA* capillary CAA). **a**, **e** ×1,000; **b**, **d** ×400; **e** ×600

Aβ40TS was significantly higher than Aβ42TS (mean Aβ40TS 0.88±1.035, mean Aβ42TS 0.65±0.871; $P<0.01$) and highly correlated with Aβ42TS ($\rho=0.819$, $P<0.01$). AβLTS and AβCTS showed no significant differences (mean AβLTS 0.81±0.942, mean AβCTS 0.715±0.975) and highly correlated with each other ($\rho=0.755$, $P<0.01$).

Aβ42CapS and Aβ42PS were significantly higher than Aβ40CapS and Aβ40PS, respectively (mean Aβ40CapS 0.15±359, mean Aβ42CapS 2.02±1.279; $P<0.01$; mean Aβ40PS 0.19±0.456, mean Aβ42PS 2.12±1.241; $P<0.01$). Low correlations were seen between Aβ40CapS and Aβ42CapS/Aβ40PS and Aβ42PS ($\rho=0.260$/$\rho=0.275$, $P<0.01$), a high correlation between Aβ40CapS and Aβ40PS ($\rho=0.855$, $P<0.01$), and a very high correlation between Aβ42CapS and Aβ42PS ($\rho=0.946$, $P<0.01$).

Aβ40CapS, Aβ42CapS, Aβ40PS, and Aβ42PS showed no to low positive correlations with each Aβ40TS and Aβ42TS respectively ($\rho<0.5$, $P<0.05$), with the exception of a medium correlation between Aβ40PS and Aβ40TS ($\rho=0.511$, $P<0.01$).

Table 2 Correlations between cerebral amyloid angiopathy and morphological Alzheimer's disease (*Aβ40/42LS* Aβ 1–40/42 leptomeningeal score, *Aβ40/42CS* Aβ 1–40/42 cortical score, *Aβ40/42CapS* score of deposits of Aβ 1–40/42 in capillaries, *Aβ40/42PS* score of deposits of Aβ 1–40/42 in plaques)

NIA-Reagan-Institute criteria		Aβ40LS	Aβ40CS	Aβ42LS	Aβ42CS	Aβ40CapS	Aβ40PS	Aβ42CapS	Aβ42PS
CERAD									
Absent	Mean	0.60	0.30	0.60	0.48	0.05	0.10	0.68	0.90
	SD	0.928	0.791	0.928	0.960	0.221	0.496	0.971	1.128
A	Mean	0.60	1.10	0.70	0.80	0.10	0.10	2.70	2.70
	SD	0.966	0.994	0.823	0.789	0.316	0.316	0.483	0.483
B	Mean	1.15	1.15	0.71	0.59	0.22	0.22	2.95	2.98
	SD	1.152	1.195	0.873	0.999	0.419	0.419	0.218	0.156
C	Mean	1.56	1.67	1.22	0.78	0.33	0.56	3.00	3.00
	SD	1.236	1.323	1.202	1.093	0.500	0.527	0.000	0.000
Total	Mean	0.91	0.85	0.71	0.58	0.15	0.19	2.02	2.12
	SD	1.093	1.132	0.924	0.966	0.359	0.465	1.279	1.241
Braak stages									
Absent	Mean	0.43	0.00	0.57	0.14	0.00	0.00	0.71	0.86
	SD	0.787	0.000	1.134	0.378	0.000	0.000	0.756	0.900
I0. II	Mean	0.43	0.43	0.29	0.86	0.14	0.43	0.29	0.71
	SD	1.134	1.134	0.756	1.464	0.378	1.134	0.488	1.254
III0. IV	Mean	0.67	0.56	0.69	0.56	0.06	0.06	1.31	1.44
	SD	0.926	0.909	0.889	0.877	0.232	0.232	1.305	1.297
V0. VI	Mean	1.22	1.24	0.80	0.62	0.24	0.28	2.96	2.98
	SD	1.166	1.222	0.948	1.008	0.431	0.454	0.198	0.141
Total	Mean	0.91	0.85	0.71	0.58	0.15	0.19	2.02	2.12
	SD	1.093	1.132	0.924	0.966	0.359	0.465	1.279	1.241
No probability	Mean	0.43	0.21	0.43	0.50	0.07	0.21	0.50	0.79
	SD	0.938	0.802	0.938	1.092	0.267	0.802	0.650	1.051
Low probability	Mean	0.70	0.41	0.70	0.48	0.04	0.04	0.85	1.04
	SD	0.912	0.797	0.912	0.893	0.192	0.192	1.167	1.224
Medium probability	Mean	0.56	1.00	0.67	0.78	0.11	0.11	2.67	2.67
	SD	1.014	1.118	0.866	0.833	0.333	0.333	0.500	0.500
High probability	Mean	1.22	1.24	0.80	0.62	0.24	0.28	2.96	2.98
	SD	1.166	1.222	0.948	1.008	0.431	0.454	0.198	0.141
Total	Mean	0.91	0.85	0.71	0.58	0.15	0.19	2.02	2.12
	SD	1.093	1.132	0.924	0.966	0.359	0.465	1.279	1.241

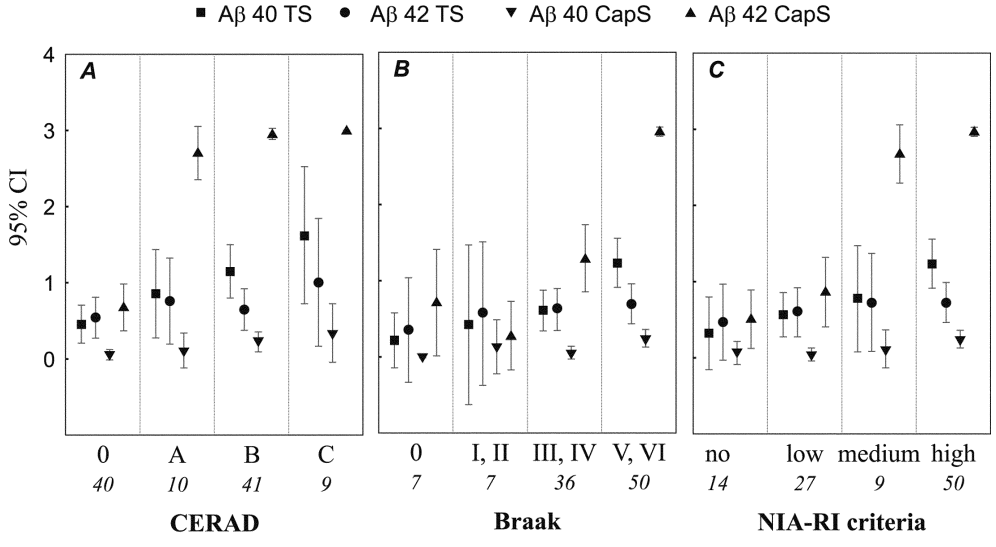

Fig. 4 Correlations between CAA and CapCAA with CERAD and Braak scores, and NIA-RI criteria for Aβ. *Italic figures*: number of cases

Comparison of Aβ40 and Aβ42 with AD pathology and clinical data

Aβ40 deposits were present in leptomeningeal vessels/cortical arteries-arterioles/capillaries/plaques in 32 (64.0%)/30 (60.0%)/12 (24%)/14 (28%) of cases with high-grade AD pathology, and in 32 (50.0%)/31 (48.4%)/12 (18.8%)/15 (23.4%) individuals with clinically overt dementia, with prevalence of Aβ40 deposits in leptomeningeal vessels and cortical arteries/arterioles (Fig. 2c). The number of plaques was significantly higher in cases with high-grade AD pathology compared to those with no or low-grade AD pathology ($P<0.01$).

Aβ42 deposits were present in leptomeningeal vessels/cortical arteries-arterioles/capillaries/plaques in 26 (52.0%)/17 (34.0%)/50 (100%)/50 (100%) of cases with high-grade neuritic AD pathology and in 27 (42.2%)/19 (29.7%)/56 (87%)/57 (89%) individuals with clinically overt dementia. There was a prevalence of Aβ42 deposits in capillaries and plaques, being significantly higher in both cases with high-grade AD pathology than in those with no or low-grade AD lesions, and in subjects with clinically overt dementia compared to non-demented ones ($P<0.01$). The presence of Aβ42 in/at capillaries and in plaques showed medium positive correlations with high-grade neuritic AD pathology ($\rho=0.531/\rho=0.562$, $P<0.01$).

Aβ40LS, Aβ40CS, Aβ40TS, Aβ40PS, Aβ42CapS, and Aβ42PS were significantly higher in cases with high-grade AD pathology compared to those with no or low-grade AD lesions ($P<0.01$). Aβ42CapS and Aβ42PS were significantly higher in individuals with clinically overt dementia compared to non-demented subjects ($P<0.01$).

Correlation with CERAD, Braak stages, and NIA-RI criteria

We correlated all CAA/CapCAA scores with CERAD, Braak stages, and NIA-RI criteria (Fig. 4a–c); high positive correlations were seen between each Aβ42CapS and Aβ42PS and each of the morphological AD criteria ($\rho>0.76$, $P<0.01$), and low positive correlations between each Aβ40LS, Aβ40CS, Aβ40TS, AβLTS, AβCTS, CAATS, Aβ40CapS, and Aβ40PS and all three AD criteria ($0.2<\rho<0.5$, $P<0.05$). When controlling the positive correlation between each AβLTS, AβCTS, and CAATS and the three morphological AD criteria for Aβ40LS and Aβ40CS, no positive correlation was seen, reflecting the influence of Aβ40LS and Aβ40CS on each AβLTS, AβCTS, and CAATS. This was also true for Aβ40TS as it represents the mean value of Aβ40LS+Aβ40CS.

Discussion

The present study clearly indicates that the severity of Aβ42 deposits in/at capillaries (i.e., Aβ42CapS) significantly correlates with the severity of neuritic AD pathology using CERAD, Braak stages, and NIA-RI criteria, and to a very high degree with the severity of Aβ42 deposits in plaques (i.e., Aβ42PS). The severity of Aβ40 in both leptomeningeal and cortical arterial vessels (i.e., Aβ40LS, Aβ40CS, Aβ40TS) increased significantly with increasing CERAD, Braak stages, and NIA-RI criteria; however, only low positive correlations between non-capillary CAA and AD pathology were observed. The very high correlation between Aβ42CapS and Aβ42PS in the present study, supports the concept of perivascular drainage of Aβ, with Aβ entering the perivascular pathways at the level of capillaries, leading to (peri)capillary Aβ deposition and consequently to CapCAA [2, 29, 41, 42, 43]. Although the flow of interstitial fluid within the perivascular space occurs in the opposite direction to that of the arterial blood flow, it may be enhanced by the pulsatile arterial distension. A failure of this propulsive mechanism has been proposed to explain the association of capillary CAA with thrombosis of overlying cortical arteries. It is widely accepted, however, that CapCAA only occurs in the presence of CAA, and thus may be a part of CAA [6, 35]. This assumption, however, is contrasted by our findings, since Aβ42CapS showed no correlation with Aβ42TS and only

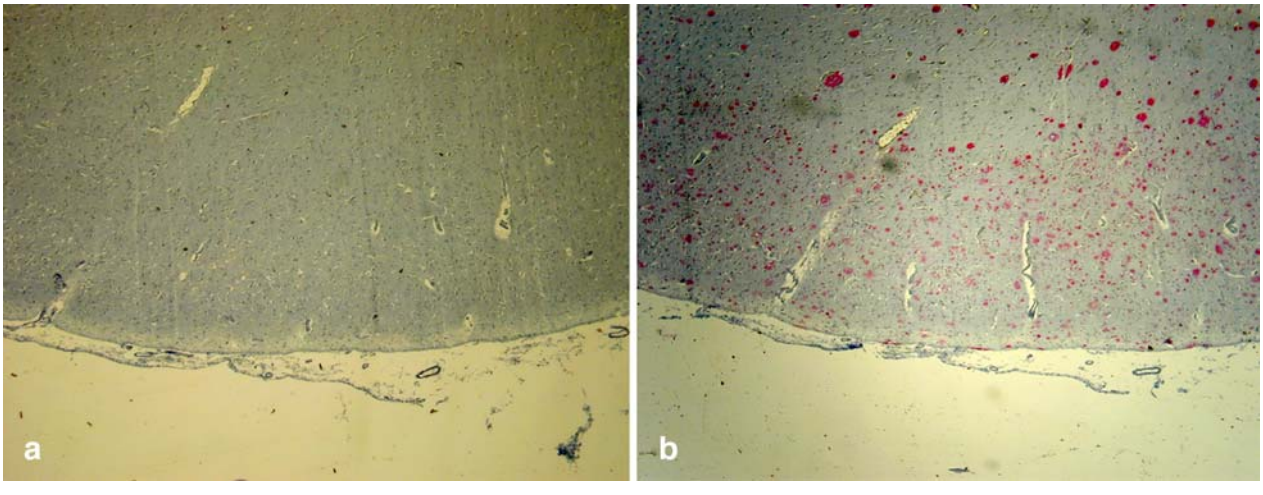

Fig. 5 Cortical plaques without detectable CAA in meningeal and cortical vessels. Immunostaining with Aβ against Aβ40 (**a**) and Aβ42 (**b**). **a, b** ×100

low correlation with Aβ40TS. This is reflected by the fact that in 17 cases of the present cohort with high Aβ42CapS, no Aβ deposits were detected in leptomeningeal and cortical arterial vessels (Fig. 5a, b), whereas severe CAA was present in a few brains without any plaques detectable by either Aβ40 or 42 immunohistochemistry (Fig. 2a, b). The low correlation of Aβ42PS with each Aβ40TS and Aβ42LS, and the lack of a correlation with Aβ42CS indicates that general CAA is *not* a result of perivascular draining of Aβ. This is in line with a previously observed inverse relation between the overall severity of CAA and parenchymal Aβ load in patients with moderate to severe AD [41].

Aβ40 deposits in capillary walls were rarely detected and morphologically differed from capillary Aβ42 deposits (see results, Fig. 3), and, albeit low correlation with Aβ40TS (ρ=0.490, P<0.01), was almost exclusively seen in cases with high Aβ40TS. These findings indicate that Aβ40 deposits in capillaries are a sign of extensive CAA and are not associated with Aβ42CapCAA.

It was previously reported that Aβ40 is more frequent and more severe than Aβ42 in CAA [7, 12, 14, 15, 16, 20, 22]. This is in concordance with our findings. In addition, we observed high correlations between Aβ40TS and Aβ42TS, suggesting that both Aβ40 and Aβ42 contribute to Aβ deposition in CAA.

Our findings of low/no correlations between Aβ40TS/Aβ42TS and neuritic AD pathology argue against the possibility of one common pathomechanism for both AD pathology and non-capillary CAA. Since we only used sections from to frontal cortex to evaluate CAA, this could have led to the lower prevalence and severity of CAA in our cohort, thus biasing the results. On the other hand, we observed high CAATS in the complete absence of AD pathology, suggesting different pathomechanisms for AD and CAA. The presence of CAA could, under yet unknown additional influences, have a promoting effect on AD pathology, which is reflected by the significant increase of Aβ40TS with increasing neuritic AD lesions in our cohort and the significant association between CAA and AD pathology in previous studies [35, 36]. Support for this comes from the occasional finding of tau immunopositive neurites clustered around larger arteries with dyshoric CAA (angiopathy in which amyloid extends from the affected blood vessels into the surrounding brain parenchyma) [18].

In conclusion our results suggest that:

- CAA is characterized by Aβ40/Aβ42 deposits in leptomeningeal and cortical arterial vessels, with Aβ40 being more frequent and more severe. Involvement of capillaries is very rare and is considered to represent an indicator of high-grade CAA. CAA (together with other influences) possibly promotes AD pathology.
- By contrast, CapCAA is characterized by globular Aβ42 deposits entrapped in the glia limitans of cortical capillaries and in pericapillary compartments, often in conjunction with parenchymal Aβ42 deposits. It is presumably a result of Aβ42 drainage from SPs and NPs along basement membranes, i.e. perivascular drainage, and, thus, closely related to both plaques and neurofibrillary AD pathology, but not to general (non-capillary) CAA.

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