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## RESEARCH ARTICLE

# Extravascular fibrinogen in the white matter of Alzheimer's disease and normal aged brains: implications for fibrinogen as a biomarker for Alzheimer's disease

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## Key words

Alzheimer's disease, biomarker, blood–brain barrier, cerebral small vessel disease, fibrinogen, white matter hyperintensities, white matter lesion.

## Abbreviations

WML, white matter lesions; WMH, white matter hyperintensity; MRI, magnetic resonance imaging; SVD, cerebral small vessel disease; AD, Alzheimer's disease; HP $\tau$ , hyperphosphorylated tau; A $\beta$ , amyloid-beta; CAA, cerebral amyloid angiopathy; VaD, vascular dementia; BBB, blood–brain barrier; PVS, perivascular space; LFB, luxol fast blue; IOD, integrated optical density; BiA, Bielschowsky's percentage area; NAWM, normal appearing white matter; IR, immunoreactivity; SI, Sclerotic Index.

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## Abstract

The blood–brain barrier (BBB) regulates cerebrovascular permeability and leakage of blood-derived fibrinogen. Dysfunction of the BBB has been associated with cerebral arteriolosclerosis small vessel disease (SVD) and white matter lesions (WML). Furthermore, BBB dysfunction is associated with the pathogenesis of Alzheimer's disease (AD) with the presence of CSF plasma proteins suggested to be a potential biomarker of AD. We aimed to determine if extravascular fibrinogen in the white matter was associated with the development of AD hallmark pathologies, i.e., hyperphosphorylated tau (HP $\tau$ ) and amyloid- $\beta$  (A $\beta$ ), as well as SVD, cerebral amyloid angiopathy (CAA) and measures of white matter damage. Using human *post-mortem* brains, parietal tissue from 20 AD and 22 non-demented controls was quantitatively assessed for HP $\tau$ , A $\beta$ , white matter damage severity, axonal density, demyelination and the burden of extravascular fibrinogen in both WML and normal appearing white matter (NAWM). SVD severity was determined by calculating sclerotic indices. WML- and NAWM fibrinogen burden was not significantly different between AD and controls nor was it associated with the burden of HP $\tau$  or A $\beta$  pathology, or any measures of white matter damage. Increasing severity of SVD was associated with and a predictor of both higher WML- and NAWM fibrinogen burden (all  $P < 0.05$ ) in controls only. In cases with minimal SVD NAWM fibrinogen burden was significantly higher in the AD cases ( $P < 0.05$ ). BBB dysfunction was present in both non-demented and AD brains and was not associated with the burden of AD-associated cortical pathologies. BBB dysfunction was strongly associated with SVD but only in the non-demented controls. In cases with minimal SVD, BBB dysfunction was significantly worse in AD cases possibly indicating the influence of CAA. In conclusion, extravascular fibrinogen is not associated with AD hallmark pathologies but indicates SVD, suggesting that the presence of fibrinogen in the CSF is not a surrogate marker for AD pathology.

## INTRODUCTION

The blood–brain barrier (BBB) is an essential physical and functional barrier formed by the endothelial cell layer of the *tunica intima* and its associated tight junctions controlling the paracellular flow of water, ions and large molecules into the brain from the blood (8, 52). Together with pericytes, which envelope endothelial cells on the abluminal side

of capillaries and venules, connections with astrocytic foot processes (of which are connected to neurons) and microglia, this comprises the neurovascular unit (1, 8). The BBB protects the central nervous system from blood-born agents, and thereby plays a crucial role in the maintenance of neuronal and glial cell populations. Sporadic type 1 arteriolosclerosis cerebral small vessel disease (SVD) has been

implicated in BBB dysfunction (14, 35) as degenerative changes to white matter artery/arteriole walls as a result of increased cerebral arterial pressure may lead to leakage of blood-derived plasma proteins into the vessel walls and the perivascular space (PVS). The exact neurotoxic effects of plasma proteins are still unclear but it is postulated that plasma protein leakage into the cerebral parenchyma initiates an inflammatory response mediated by the resident microglia and astrocytes (20, 40). Plasma protein leakage into the deep white matter has been shown to be associated with worsening of SVD pathology that may lead to an increase in ischemia-associated white matter damage (36). Histological cerebral white matter lesions (WML) are reflected by white matter hyperintensities (WMH) on *pre-* and *post-mortem* T2-weighted magnetic resonance imaging (MRI) (15) and primarily encompass white matter rarefaction caused by demyelination with or without axonal loss (14). WML are assumed to represent ischemia-associated damage caused by SVD of the white matter arteries and arterioles (37). However, we have shown recently that parietal WML can differ in their pathological and molecular signatures between Alzheimer's disease (AD) and non-demented controls suggesting different etiologies of WML, that is, degenerative axonal and myelin loss in AD and ischemia resulting from SVD (30). Therefore, understanding of all mechanisms involved in WML pathogenesis is important as their presence is included in the clinical criteria for VaD/VCI (13) and mixed AD/VaD (10).

BBB dysfunction and leakage of various plasma proteins have been proposed to play a role in the onset and progression of AD via alterations of the neurovascular unit and secretion of neurotoxic substances that can promote the development of amyloid- $\beta$  ( $A\beta$ ) pathology (11). Neuropathological studies have confirmed a higher prevalence of BBB dysfunction in AD compared to non-demented controls (18, 47, 59), and therefore, the presence of plasma proteins in CSF has been suggested to be a potential biomarker of AD: human *in vivo* studies of BBB dysfunction, as determined by the leakage of plasma protein albumin into the CSF (i.e., albumin:CSF ratio), have revealed increased BBB permeability in demented compared to non-demented patients (12) and plasma fibrinogen  $\gamma$ -chain in the CSF was identified as a specific biomarker for AD over other neurodegenerative disorders (7).

Using *post-mortem* brain tissue from both AD and non-demented individuals we aimed to (i) investigate the relationship of SVD and BBB dysfunction, as determined by extravascular fibrinogen; (ii) investigate the relationship between extravascular fibrinogen with measures of white matter damage; (iii) and to determine if the presence of extravascular fibrinogen was associated with the presence of AD hallmark cortical pathology.

## MATERIALS AND METHODS

### Study cohort

Our study cohort consisted of a 42 serially donated human *post-mortem* brains clinico-pathologically

diagnosed as AD ( $n = 20$ ) or non-cognitively impaired controls ( $n = 22$ ) that all exhibited at least one deep WML in the parietal lobe as determined and assessed for severity by *post-mortem* T2 MRI as previously described (28). Cases that exhibited any large infarcts or cerebral hemorrhages that could account for cognitive impairment were excluded.

During life, all dementia subjects underwent clinical assessments and were diagnosed by board certified Old Age Psychiatrists or Neurologists. Both dementia and control subjects had a review of clinical records after death at Newcastle Brain Tissue Resource (AJT). Control subjects showed no evidence of cognitive impairment and had normal everyday functioning. After autopsy the right hemisphere, brainstem and cerebellum were immersion fixed in 10% buffered aqueous formaldehyde solution for 6 weeks and then subsequently dissected in coronal planes at 7mm intervals and paraffin-embedded. All brains underwent neuropathological assessment according to the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria (33) that included determination of Thal phases of  $A\beta$  deposition (45), Braak staging of hyperphosphorylated tau (HP $\tau$ ) pathology (4) and scoring of neuritic plaques according to the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (32). Additional neuropathological assessment of Lewy body pathology (5, 31), TDP-43 pathology (19) and of the contribution of vascular pathology to cognitive impairment [vascular impairment neuropathological guidelines (VCING) (44), inclusive of cerebral amyloid angiopathy (CAA) scores (25), was performed. Cases did not exhibit any additional pathology, including primary or secondary tumors or age-related tau astrogliaopathy (ARTAG) (22). For a summary of the demographic and neuropathological details please see Table 1.

### Histological and immunohistochemical procedures

Paraffin-embedded serial sections from parietal blocks (Brodmann area 40/22) were cut at 8 $\mu$ m thickness and mounted onto superfrost plus charged glass slides (Thermo Shandon, Cheshire, UK). Sections underwent histochemical staining with Bielschowsky silver stain (cold method) (24) for assessment of axonal density, myelin stain luxol fast blue (LFB) to assess WML area (WMLA: as a measure of WML severity) and myelin pallor (as a measure of demyelination) and hematoxylin and eosin (H&E) for visualization of white matter artery/arteriole walls for assessment of SVD. Immunohistochemistry was performed on all sections for plasma protein fibrinogen (antibody anti-human fibrinogen), HP $\tau$  (antibody AT8) and  $A\beta$  peptide (antibody 4G8) (details on primary antibodies and antigen retrieval protocols for immunohistochemistry are presented in Table 2). Immunopositivity was detected using the Menarini X-Cell-Plus HRP Detection Kit (Menarini Diagnostics, Winnersh-Wokingham, UK) with 3,3 diaminobenzidine (DAB) as a chromagen and hematoxylin as a

**Table 1.** Demographic and neuropathological characteristics of study cohort.

	AD (n = 20)	Control (n = 22)	Statistic <sub>(d.f.)</sub> , P-value
Mean age, years (±SD)	83.40 (6.29)	84.41 (8.57)	$t_{(41)} P = 0.143$
Gender M:F	9:11	11:11	$\chi^2 (1, n = 42) = 0.00,$ $P = 0.988, \text{phi} = 0.05$
Mean PMD, hours (±SD)	54.20 (19.97)	55.38 (24.13)	$t_{(26)} P = 0.209$
Thal Aβ phase (45)	4, n = 2 5, n = 18	0, n = 6 1, n = 7 2, n = 4 3, n = 2 4, n = 2 5, n = 1	–
Braak NFT stage (4)	V, n = 2 VI, n = 18	0, n = 2 I, n = 2 II, n = 10 III, n = 7 IV, n = 1	–
CERAD (32)	C, n = 20	Negative, n = 18 A, n = 3 B, n = 1	–
NIA-AA (33)	High, n = 20	No, n = 6 Low, n = 15 Intermediate, n = 1	–
Braak LB stage (5)	0, n = 17 1, n = 0 2, n = 2 3, n = 1	0, n = 18 1, n = 1 2, n = 1 3, n = 2	–
McKeith criteria (31)	No LBD, n = 17 Brainstem, n = 3	No LBD, n = 18 Brainstem, n = 4	–
TDP-43 in Alzheimer’s disease stage (19)	Absent, n = 12 1, n = 0 2, n = 2 3, n = 1 4, n = 5 5, n = 0	Absent, n = 18 1, n = 0 2, n = 1 3, n = 2 4, n = 1 5, n = 0	–
VCING criteria (44)	Low, n = 18 Medium, n = 2	Low, n = 22	–
Total CAA score* (25)	0, n = 4 1, n = 1 2, n = 1 3, n = 4 4, n = 2 5, n = 3 6, n = 5	0, n = 14 1, n = 4 2, n = 1 3, n = 2 6, n = 1	–
Presence of capillary CAA	Present, n = 2 Absent, n = 18	Present, n = 1 Absent, n = 21	

Abbreviations: AD = Alzheimer’s disease; d.f. = degrees of freedom; t = Independent samples test;  $\chi^2$  = Chi-squared test; F = female; M = Male; U = Mann–Whitney U test; PMD = *post-mortem* delay; Aβ = amyloid-beta; NFT = neurofibrillary tangle; CERAD = Consortium to Establish a Registry for Alzheimer’s Disease; NIA-AA = National Institute on Aging—Alzheimer’s Association criteria for AD neuropathologic change; LB = Lewy body; VCING = vascular cognitive impairment neuropathological guidelines; CAA = cerebral amyloid angiopathy; \*Total CAA score was determined by the accumulation of leptomeningeal (0–3 scale) and cortical CAA (0–3 scale) scores (max score 6; please see reference 25).

counter stain. All histologically and immunohistochemically stained sections were subsequently dehydrated through a series of alcohols, cleared and mounted using DPX (CellPath, Powys, UK).

**Quantitative image analysis**

All image analysis was performed blinded to neuropathological diagnosis using a NIS elements version 3.0

(Nikon, Surrey, UK). Quantitative assessment of WMLA, the percentage area of Bielschowsky staining and the integrated optical density (IOD) in the WML area of LFB stained sections was performed as previously described (30). The mean percentage area of Bielschowsky’s stain in the WML was expressed as WML-BiA (axonal density) and the mean percentage area difference of IOD in the WML was expressed as WML-IOD (demyelination).

**Table 2.** Details of primary antibodies and protocols.

Primary antibody	Source	Target	Species	Primary antibody dilution	Antigen retrieval protocol
Plasma protein fibrinogen	Dako, Denmark	C-terminal	Rabbit polyclonal	1:2000	De-parraffinized sections microwaved in 0.01 mL citrate buffer for 10 min
AT8	Innogenetics, Ghent, Belgium	Phospho-PHF-tau pSer202+Thr205	Mouse monoclonal	1:4000	De-parraffinized sections microwaved in 0.01 mL citrate buffer for 10 min
4G8	Signet Labs, Dedham, MA, USA	Amyloid 17-24	Mouse monoclonal	1:15 000	De-parraffinized sections were immersed in concentrated Formic acid for 1 hour

AT8 and 4G8 immunostained tissue sections were quantified for pathological burden using individual and standardized Red Green Blue (RGB) thresholds as previously described in (29, 30). The percentage areas covered by AT8 and 4G8 immunoreactivity (IR) were measured and the mean values were calculated and are expressed as AT8-IR and 4G8-IR, respectively. Extravascular fibrinogen IR was measured in both the WML and normal appearing white matter (NAWM): the WML area was identified macro- and microscopically on LFB stained sections and delineated by hand using a permanent marker pen to differentiate the WML and NAWM. These sections were subsequently used to identify the corresponding areas in the adjacent fibrinogen stained sections. Separately in both the WML and NAWM areas, five  $3 \times 3$  images at  $100\times$  magnification (combined to yield one large image representing an area of  $1.31 \text{ mm}^2$ ) were captured. Large images were subjected to manual setting of regions of interest to include extravascular fibrinogen-IR only and exclude artery/arteriole vessel lumens (not including capillaries) as well as artifacts and regions of cortex. Standardized RGB thresholds for extravascular fibrinogen-IR were applied to the large images and the area covered by fibrinogen-IR was measured, a percentage of the total measured area calculated and expressed as WML fibrinogen-IR or NAWM fibrinogen-IR (Figure 1).

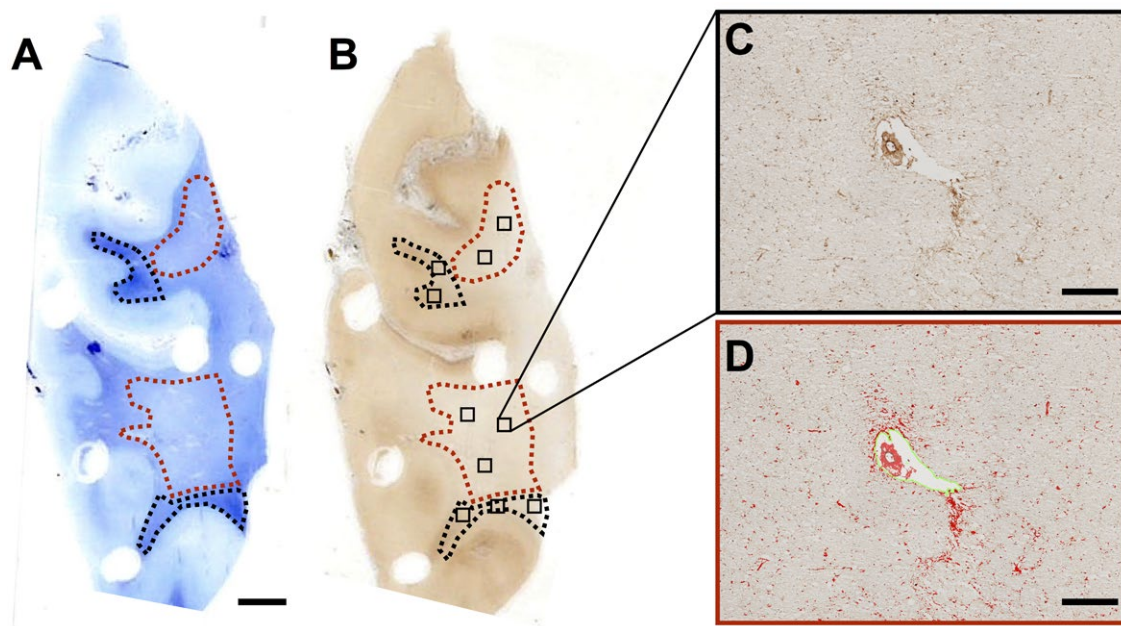
The sclerotic index (SI) calculates vessel wall thickness and is a marker of SVD severity (23) with the SI of normal arteries/arterioles between 0.2 and 0.29, mild SVD is a value of 0.3–0.39, moderate SVD a value of 0.4–0.49 and severe SVD is a value  $\geq 0.5$ . SI measurements were performed on approximately eight white matter arteries/arterioles as seen on H&E stained sections as previously described (30, 55). A mean SI score was calculated for each case.

## Statistics

The Statistical Package for Social Sciences software (SPSS ver. 21) was used for statistical evaluation. Variables were tested for normality using the Shapiro-Wilk test and visual inspection of variable histograms. Age and SI measurements exhibited a normal distribution, therefore, parametric tests were employed; all other variables, that is, *post-mortem* delay, CAA score, AT8-IR, 4G8-IR, WML-BiA, WML-IOD, WML Fibrinogen-IR, NAWM Fibrinogen-IR and WMLA exhibited a non-normal distribution and non-parametric tests were employed. A Chi-square ( $\chi^2$ ) test for independence (with Yates continuity Correction) was employed to explore the relationship between categorical variables. A one-way between-groups multivariate analysis of variance (MANOVA), including preliminary assumption testing (ie, normality, linearity, outliers, homogeneity of variance-covariance matrices and multicollinearity), was performed to investigate differences in measures of white matter and fibrinogen between AD and controls, while controlling for Type I error. Where appropriate, group effects were assessed using either Mann-Whitney U (non-parametric) or independent samples *t*-test procedures (parametric). Spearman's ( $r_s$ ; non-parametric) or Pearson's ( $r$ ; parametric) correlation coefficients were used to assess associations between variables, as well as partial correlations ( $r_p$ ) to control for the effects of age. Linear regression analyses were also conducted to investigate predictors of WML- and NAWM fibrinogen-IR.

## RESULTS

No significant differences were observed in mean age ( $t_{(40)}$   $P = 0.143$ ) or *post-mortem* delay ( $t_{(26)}$   $P = 0.209$ ) between AD and control groups. Clinicopathological diagnosis was not associated with gender ( $\chi^2_{(1)}$   $P = 0.763$ ). Fibrinogen-IR



**Figure 1.** A. WML areas were identified on LFB stained sections of parietal tissue and delineated (red dotted line). This was used to identify the same WML and NAWM (black dotted line) area on serial sections stained with fibrinogen B. B. The fibrinogen stained section of parietal tissue indicating the five randomly selected areas for a 3 × 3 image capture in the WML and NAWM areas (black boxes). C. A 3 × 3 large

image acquisition in WML area. D. Image C again with applied bespoke fibrinogen threshold including region of interest for removal of vessel and PVS (green line). Scale bar, 50 mm, valid for all images in A and B; 100 μm, valid for C and D. WML, white matter lesions; LFB, luxol fast blue; NAWM, normal appearing white matter; PVS, perivascular spaces.

was observed in the vessel walls of small arteries/arterioles of the white matter (Figure 2A), axons (Figure 2B), the endothelia cell layer of white matter capillaries (Figure 2C and Ci) and PVSs (Figure 2D and Di). In addition, fibrinogen positive cells that presented with a hypertrophic astroglial-like morphology were present in the white matter, the cortical-white matter border and PVS (Figure 2E and Ei).

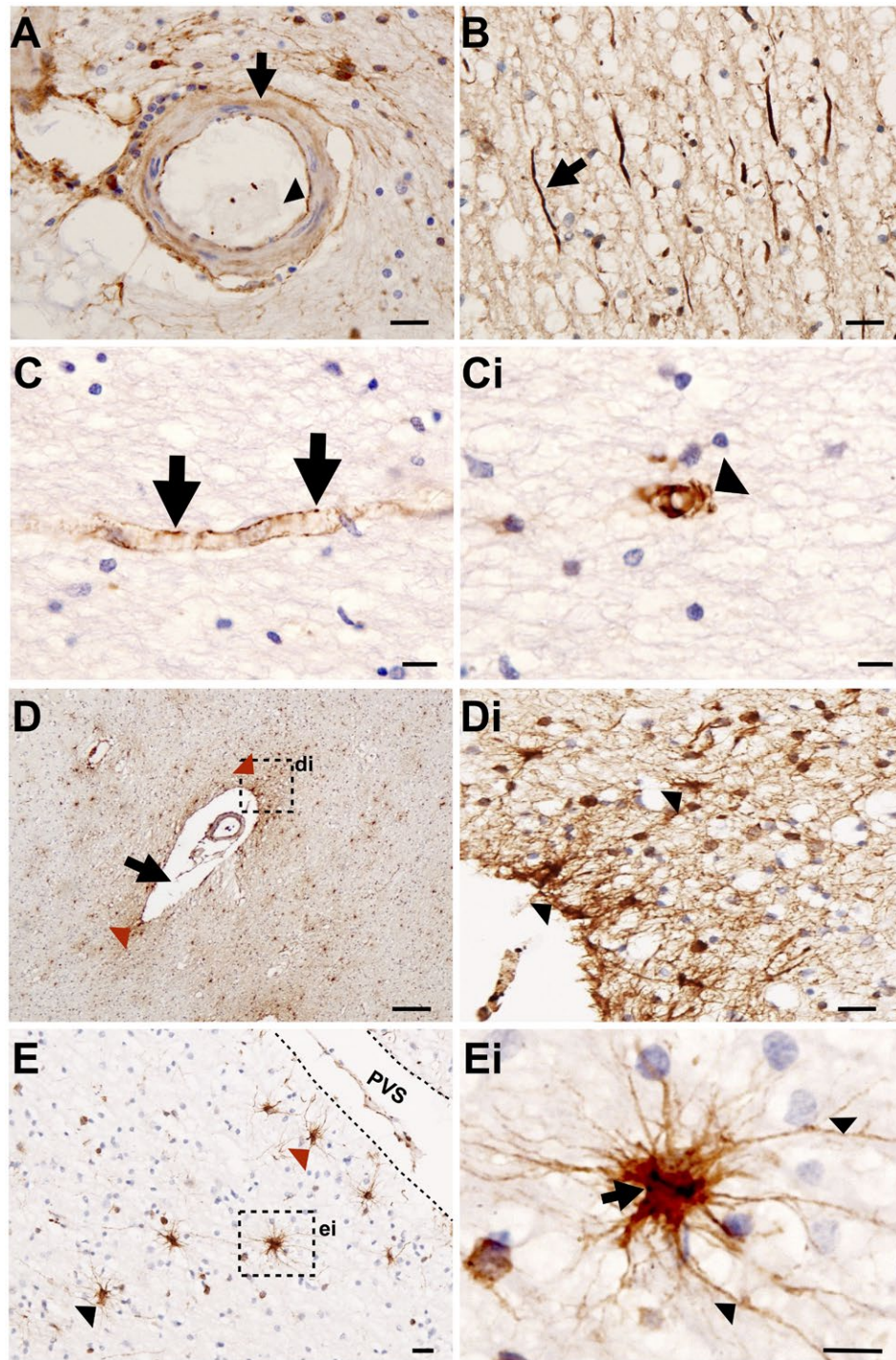
A MANOVA was performed to investigate differences in pathological measures between AD and controls. Seven dependent variables were used: CAA, SI, WMLA, WML-BiA, WML-IOD, WML fibrinogen-IR and NAWM fibrinogen-IR, and clinicopathological diagnosis (i.e. AD or control) as the independent variable, and preliminary assumptions were not violated. A statistically significant difference was observed between AD and controls on the combined dependent variables ( $F = 4.009$ ,  $P = 0.003$ ; Wilks Lambda = 0.525; partial eta squared = 0.375). When considered separately, using a Bonferroni's adjusted level of 0.01, CAA ( $F = 19.889$ ;  $P = 0.0001$ , partial eta squared = 0.350) and WMLA ( $F = 12.187$ ;  $P = 0.001$ , partial eta squared = 0.248) were significantly different and inspection of mean scores indicated that both CAA scores ( $M = 3.471$ ,  $SD = 0.447$ ) and WMLA scores ( $M = 34.616$ ,  $SD = 2.706$ ) were significantly higher in the AD group compared to the controls (CAA,  $M = 0.818$ ,  $SD = 0.394$ ; WMLA,  $M = 22.037$ ,  $SD = 2.379$ ). No difference was seen in means scores of SI ( $P = 0.692$ ),

WML-BiA ( $P = 0.559$ ), WML-IOD ( $P = 0.051$ ), WML fibrinogen-IR ( $P = 0.954$ ) or NAWM fibrinogen-IR ( $P = 0.946$ ).

### Associations between extravascular fibrinogen, CAA, SVD and white matter pathologies

Using correlation coefficient tests, we examined the relationships between NAWM and WML fibrinogen-IR with CAA, SI, WMLA, WML-BiA and WML-IOD (please see Table 3). Briefly, as a whole cohort, SI score both was positively correlated with WML fibrinogen-IR ( $r = 0.263$ ,  $P = 0.048$ ) and NAWM fibrinogen-IR ( $r = 0.310$ ,  $P = 0.026$ ), but when correlations were performed separately based on clinicopathological diagnosis these associations were consistent only in the control group (WML,  $r = 0.487$ ,  $P = 0.011$ ; NAWM,  $r = 0.383$ ,  $P = 0.039$ ) and not in the AD group. NAWM fibrinogen-IR was associated with CAA ( $r_s = 0.291$ ,  $P = 0.033$ ) with regards to the whole cohort.

To investigate whether SI severity independently predicted fibrinogen-IR, linear regression analysis was performed; the dependent variable was WML or NAWM fibrinogen-IR and the independent variable was SI. Regarding the whole cohort, SI was a significant predictor of NAWM fibrinogen-IR (model  $R^2 = 0.195$ ,  $F = 9.18$ ;  $\beta = 0.441$ ,  $P = 0.004$ ), in contrast to WML fibrinogen-IR (model  $R^2 = 0.085$ ,  $F = 3.5$ ;  $\beta = 0.292$ ,  $P = 0.068$ ). Linear regression was again performed dichotomized by



**Figure 2.** Photomicrographs illustrating fibrinogen immunoreactivity in white matter and its vasculature. **A.** A small artery exhibiting fibrinogen in the vessel wall in both the *tunica media* (arrow) and the *tunica intima* (arrowhead) indicating leakage of fibrinogen. **B.** Swollen white matter axons positive for extravascular fibrinogen (arrow). **C.** A capillary exhibiting fibrinogen immunopositivity contained within the endothelial wall in the transverse plane. **Ci.** A capillary exhibiting sustained leakage of fibrinogen into the endothelial wall, basement membrane and surrounding white matter parenchyma (arrowhead). **D.** A moderately dilated PVS (arrow) with accumulation of extraversion of fibrinogen in

the white matter parenchyma (red arrowheads). **Di.** Magnified photomicrograph of extraverted fibrinogen in the surrounding white matter parenchyma (black arrowhead). **E.** Fibrinogen positive cells in the deep white matter (black arrowhead) and lining a dilated PVS (red arrowhead). **Ei.** Magnified photomicrograph of a deep white matter fibrinogen positive cell with the morphology of a hypertrophic fibrous astrocyte; immunoreactivity is present in both the cell soma (arrow) and cellular processes (arrow head). BBB, blood-brain barrier; PVS, perivascular space. Scale bars, 200  $\mu$ m, valid for D and E; 100  $\mu$ m, valid for A and B; 20  $\mu$ m, valid for Di and Ei; 10  $\mu$ m valid for C and Ci.

**Table 3.** Correlation matrix of WML- and NAWM extravascular fibrinogen-IR with SVD and white matter pathology variables.

	Whole cohort	AD	Controls
		<b>WML Fibrinogen-IR</b>	
CAA	$r_s = 0.161, p = 0.157$	$r_s = 0.240, p = 0.161$	$r_s = -0.07, p = 0.371$
SI	$r = 0.263, p = 0.048^*$	$r = 0.073, p = 0.384$	$r = 0.487, p = 0.01^*$
WMLA	$r_s = 0.106, p = 0.252$	$r_s = -0.140, p = 0.279$	$r_s = -0.015, p = 0.474$
WML-BiA	$r_s = -0.083, p = 0.301$	$r_s = -0.171, p = 0.236$	$r_s = -0.003, p = 0.495$
WML-IOD	$r_s = -0.031, p = 0.422$	$r_s = -0.234, p = 0.160$	$r_s = 0.304, p = 0.084$
		<b>NAWM Fibrinogen-IR</b>	
CAA	$r_s = 0.291, p = 0.033^*$	$r_s = 0.149, p = 0.271$	$r_s = 0.174, p = 0.219$
SI	$r = 0.310, p = 0.026^*$	$r = 0.009, p = 0.485$	$r = 0.383, p = 0.039^*$
WMLA	$r_s = 0.191, p = 0.116$	$r_s = 0.061, p = 0.402$	$r_s = -0.172, p = 0.222$
WML-BiA	$r_s = -0.263, p = 0.05$	$r_s = -0.386, p = 0.051$	$r_s = -0.230, p = 0.152$
WML-IOD	$r_s = 0.080, p = 0.310$	$r_s = 0.070, p = 0.388$	$r_s = -0.187, p = 0.202$

Abbreviations: WML = white matter lesion; NAWM = normal appearing white matter; CAA = cerebral amyloid angiopathy; SI = sclerotic index; WMLA = white matter lesion area; WML-BiA = white matter lesion Bielschowsky's measurement for axonal density; WML-IOD = white matter lesion integrated optical density measurement for demyelination; IR = immunoreactivity; AD = Alzheimer's disease;  $r$  = Pearson's correlation coefficient;  $r_s$  = Spearman's correlation coefficient. \* $P < 0.05$ .

clinicopathological diagnosis; SI was found to be a significant predictor of both WML fibrinogen-IR (model  $R^2 = 0.237$ ,  $F = 6.21$ ;  $\beta = 0.487$ ,  $P = 0.022$ ) and NAWM fibrinogen-IR (model  $R^2 = 0.300$ ,  $F = 8.571$ ;  $\beta = 0.548$ ,  $P = 0.008$ ) in the control group but not the AD group (WML, model  $R^2 = 0.067$ ,  $P = 0.301$ ; NAWM, model  $R^2 = 0.022$ ,  $P = 0.557$ ).

### The severity of SVD and the amount of extravascular fibrinogen

To further investigate the role of SVD, cases were divided into normal-mild SVD (SI value  $< 0.39$ ; AD,  $n = 10$ ; controls,  $n = 14$ ) moderate to severe (SI value  $> 0.39$ ; AD,  $n = 9$ ; controls,  $n = 8$ ). Overall, both WML fibrinogen-IR and NAWM fibrinogen-IR were higher in cases with moderate-severe SVD but this did not reach statistical significance (WML,  $P = 0.075$ ; NAWM,  $P = 0.052$ ) (Figure 3A, B). When separated by clinicopathological diagnosis controls had significantly higher WML fibrinogen-IR in cases with moderate-severe SVD ( $P = 0.019$ ; Figure 3C). NAWM fibrinogen-IR was also higher in the moderate-severe SVD cases compared to the normal-mild, but this was not significant ( $P = 0.069$ ; Figure 3D). No differences were seen in either WML or NAWM fibrinogen-IR in AD cases (WML,  $P = 0.823$ ; NAWM,  $P = 0.532$ ; Figure 3C, D). We then investigated the difference in extravascular fibrinogen at different SVD severities across groups: in cases with normal-mild SVD NAWM fibrinogen-IR was significantly higher in AD cases compared to controls ( $P = 0.045$ ; Figure 3D), WML fibrinogen-IR was higher in the AD cases but was not significant ( $P = 0.059$ ; Figure 3C). Regarding cases with moderate-severe SVD no difference in WML or NAWM fibrinogen-IR was seen (WML,  $P = 0.424$ ; NAWM,  $P = 0.752$ ; Figure 3C, D). Linear regression analysis was performed to investigate if a clinicopathological diagnosis of AD was a predictor of WML- and NAWM fibrinogen-IR in cases with normal-mild SVD; no significant association was revealed in for either measure

of fibrinogen-IR (WML, model  $R^2 = 0.368$ ,  $P = 0.076$ ; NAWM, model  $R^2 = 0.224$ ,  $P = 0.294$ ).

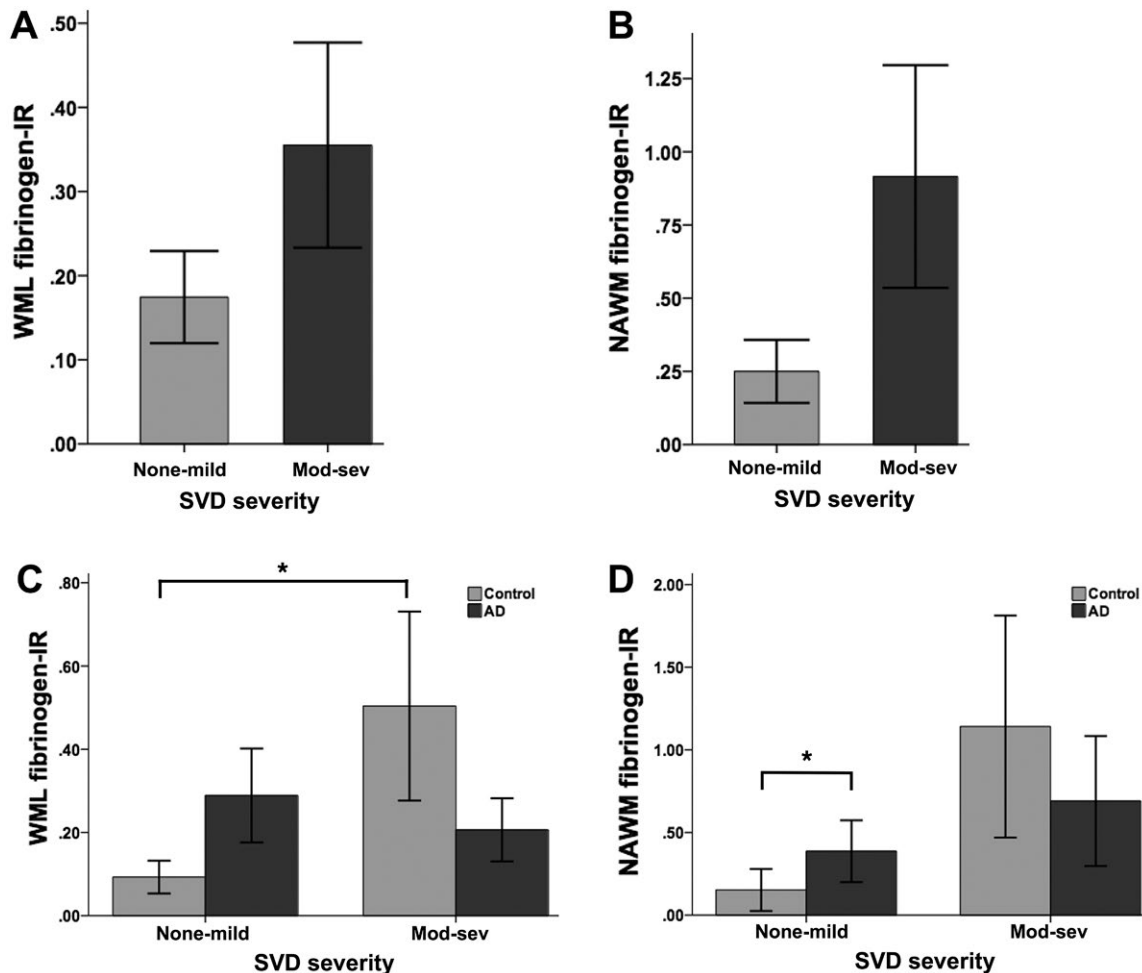
### Relationships between extravascular fibrinogen and the presence of hallmark AD pathology

To determine if increasing extravascular fibrinogen was associated with the development of AD pathology a partial correlations controlled for age were employed to examine the relationships between both whole cohort values of WML and NAWM fibrinogen-IR with AT8-IR, 4G8-IR as well as Braak NFT stage and Thal A $\beta$  Phase. No associations were revealed with either WML or NAWM fibrinogen-IR with AT8-IR (WML,  $r_p = 0.035$ ,  $P = 0.417$ ; NAWM,  $r_p = -0.039$ ,  $P = 0.410$ ), 4G8-IR (WML,  $r_p = 0.086$ ,  $P = 0.306$ ; NAWM,  $r_p = -0.114$ ,  $P = 0.250$ ), Braak NFT stage (WML,  $r_p = 0.03$ ,  $P = 0.493$ ; NAWM  $r = 0.011$ ,  $P = 0.474$ ) or Thal A $\beta$  Phase (WML,  $r_p = 0.088$ ,  $P = 0.301$ ; NAWM,  $r_p = 0.079$ ,  $P = 0.322$ ).

## DISCUSSION

In the cerebral deep white matter, BBB dysfunction and the leakage of "neurotoxic" blood-derived plasma proteins are proposed to initiate inflammatory responses in the surrounding white matter parenchyma, exacerbating the development of WML. In agreement with previous human neuropathological studies (6, 16, 57), we found no associations between extravascular fibrinogen measure in the WML itself or the surrounding NAWM with WML severity or specifically axonal loss or demyelination in either AD or non-demented individuals. This supports the notion that AD related degenerative mechanisms and hypoxia, as a consequence of type 1 arteriolosclerosis SVD, have the strongest impact on the development of WML (30). Other serum components that may leak out of blood vessel not investigated in this study, such as complement and cytokines, have deleterious effects, specifically causing demyelination (43) and should be included in future studies.





**Figure 3.** A, B. Regarding the whole cohort, WML and NAWM fibrinogen-IR was not significantly higher in cases with moderate to severe SVD compared to cases with none-mild SVD. C. WML fibrinogen-IR was significantly higher in control cases with moderate-severe SVD compared to control cases none-mild SVD. D. NAWM fibrinogen-IR was

significantly higher in AD case with non-mild SVD compared to control cases with none-mild SVD. WML, white matter lesion; NAWM, normal appearing white matter; SVD, small vessel disease; mod-sev, moderate to severe; AD, Alzheimer's disease; IR, immunoreactivity. \* $P < 0.05$ .

Although fibrinogen is currently under investigation as a biomarker for AD, we found no association between extravascular fibrinogen with increasing deposition of the AD hallmark cortical pathologies  $HP\tau$  and  $A\beta$ . It has been suggested that BBB impairment induced by cerebrovascular disease can influence the pathogenesis of AD (58) with both human neuropathological studies and murine AD models indicating fibrinogen deposition can contribute to neurovascular damage (18, 41), neuroinflammation (38) and neuronal degeneration (9) that may influence the pathogenesis of AD. However, our findings suggest that fibrinogen does not reflect the pathological burden of AD pathology, and therefore, may not be an accurate diagnostic tool for the staging of AD progression.

A key finding from this study was the relationship between SVD and extravascular fibrinogen that differed between non-demented controls and AD groups. In general,

when cases were dichotomized by the severity of SVD pathology only, a strong trend for higher WML and NAWM extravascular fibrinogen was seen in cases with moderate to severe SVD compared to those without/mild SVD. When stratified by clinicopathological diagnosis, increasing severity of SVD was associated with and a significant predictor of both increasing WML- and NAWM extravascular fibrinogen but only in the controls. Firstly, this data supports the notion that SVD is strongly associated with BBB dysfunction as previously indicated (12), and, therefore, indicates that the presence of fibrinogen is an indicator of underlying SVD. Secondly, a lack of association in the AD group suggests that, as well as SVD, another mechanism maybe impacting the BBB and leading to fibrinogen leakage. In fact, AD cases without or mild SVD had significantly higher fibrinogen in the NAWM compared to controls, and a strong trend was observed with WML fibrinogen,

compared to the controls. In addition, although not significant, linear regression revealed a strong trend of a diagnosis of AD as predictor of WML fibrinogen burden in cases without/mild SVD. This indicates a potential heterogeneity in the mechanism underlying BBB dysfunction that maybe associated to the pathophysiology of AD. A potential influence is CAA; classified as type 2 SVD (36), it is characterized by A $\beta$  deposition (predominately A $\beta$ -40) in the vessel walls of cerebral blood vessels (49) and is prevalent in approximately 80% of AD cases (3). Our data revealed an association between increased severity of CAA and increasing NAWM fibrinogen levels. This is in line with transgenic mouse model studies have indicated that A $\beta$  accumulation contributes to impairment of tight junction proteins expression, therefore, compromising BBB integrity (17, 48). Although CAA rarely affects white matter vessels, CAA impairs vasoreactivity (26) with consequential white matter hypoxia that has been shown to induce BBB disruption as a result of HIF-1 $\alpha$ -mediated alterations of key tight junction proteins (53). In addition, perivascular drainage (PVD), driven by the motive forces of vascular pulsations (51), of interstitial fluid and solutes through the basement membranes of capillary endothelia cells and the *tunic media* in arteries is a key drainage pathway of CNS fluids. As a result of age and CAA, vessels can become stiff (39, 50) leading to a decrease in the amplitude and force of pulsation and, consequently, a reduction in PVD that may lead to reduced clearance of extravascular fibrinogen and contribute to the increased accumulation of fibrinogen observed in the NAWM of AD patients with minimal SVD. Further studies implementing a quantitative assessment of CAA burden are warranted to investigate further the influence of CAA on BBB permeability given its high prevalence in AD.

Numerous immunohistochemical studies have found a positive association between markers of plasma proteins and a dementia diagnosis (16, 18, 34, 47), which has led to the intensive investigation of plasma proteins as a possible biomarker of AD. Increased plasma fibrinogen  $\gamma$ -chain has been shown to be specific for AD (7, 21) as well as a risk factor for conversion of MCI to AD (34, 46, 54, 56). However, our data found no significant differences in the amount of fibrinogen between AD and non-demented subjects in agreement with previous studies (2, 27, 42). Furthermore, it is important to note that SVD is also present in normal aging and our data indicated no significant difference in SVD severity between AD and non-demented controls. Given the strong association with type 1 SVD, and the potential link with CAA, increased levels of extravascular fibrinogen are likely associated with underlying cerebrovascular disease that is not necessarily exclusive to an AD diagnosis. However, the total variance of BBB dysfunction explained by type 1 SVD overall in this study is 19.5% indicating other factors associated with BBB dysfunction and AD pathophysiology remains to be elucidated.

In conclusion, BBB dysfunction was present in both non-demented and AD brains and was not associated with the burden of AD-associated cortical pathologies. BBB dysfunction was strongly associated with SVD but only

in the non-demented controls. In cases with minimal SVD BBB dysfunction was significantly worse in AD cases indicating the influence of other cerebrovascular diseases, most likely CAA, as well as other mechanisms of BBB dysfunction possibly associated with the pathophysiology of AD that are yet to be determined. Therefore, extravascular fibrinogen likely indicates underlying age-associated cerebrovascular disease, specifically SVD that is not necessarily exclusive to an AD diagnosis, and suggests that the presence of fibrinogen in the CSF is not a surrogate marker for AD pathology. The validity of fibrinogen as a biomarker of/risk of AD requires larger longitudinal studies, in conjunction with validated biomarkers of AD and cerebrovascular disease and neuropathological confirmation.

## AUTHOR CONTRIBUTIONS

KEM designed the study and methodologies, performed quantitative assessment for axonal loss, performed the data analysis, wrote the manuscript and produced all figures, tables and graphs. SG performed quantitative assessment for white matter lesion area and myelin loss. EJ performed the fibrinogen assessment. MD performed the Sclerotic Index measurements. MJ provided technical assistance with histological and immunochemical staining. LW, DE, IGM and CD provided critical revisions of the manuscript. AT provided the clinical diagnosis of the cohort and critical revisions of the manuscript. JA provided neuropathological diagnosis of the cohort, neuropathological assistance and assisted with the writing of the manuscript. All authors read and approved the manuscript and declare that they have no competing of interests.

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## REFERENCES

- Abbott NJ, Ronnback L, Hansson E (2006) Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* 7:41–53.

2. Alafuzoff I, Adolfsson R, Grundke-Iqbal I, Winblad B (1987) Blood-brain barrier in Alzheimer dementia and in non-demented elderly. An immunocytochemical study. *Acta Neuropathol* **73**:160–166.
3. Attems J, Jellinger KA (2004) Only cerebral capillary amyloid angiopathy correlates with Alzheimer pathology—a pilot study. *Acta Neuropathol* **107**:83–90.
4. Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K (2006) Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol* **112**:389–404.
5. Braak H, Del Tredici K, Rüb U, de Vos RAI, Jansen Steur ENH *et al* (2003) Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* **24**:197–211.
6. Bridges LR, Andoh J, Lawrence AJ, Khoong CHL, Poon WW, Esiri MM *et al* (2014) Blood-brain barrier dysfunction and cerebral small vessel disease (arteriolosclerosis) in brains of older people. *J Neuropathol Exp Neurol* **73**:1026–1033.
7. Chiam JT, Dobson RJ, Kiddle SJ, Sattlecker M (2015) Are blood-based protein biomarkers for Alzheimer's disease also involved in other brain disorders? A systematic review. *J Alzheimers Dis* **43**:303–314.
8. Chodobski A, Zink BJ, Szmydynger-Chodobska J (2011) Blood-brain barrier pathophysiology in traumatic brain injury. *Transl Stroke Res* **2**:492–516.
9. Cortes-Canteli M, Mattei L, Richards AT, Norris EH, Strickland S (2015) Fibrin deposited in the Alzheimer's disease brain promotes neuronal degeneration. *Neurobiol Aging* **36**:608–617.
10. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K *et al* (2014) Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol* **13**:614–629.
11. Erickson MA, Banks WA (2013) Blood-brain barrier dysfunction as a cause and consequence of Alzheimer's disease. *J Cereb Blood Flow Metab* **33**:1500–1513.
12. Farrall AJ, Wardlaw JM (2009) Blood-brain barrier: ageing and microvascular disease—systematic review and meta-analysis. *Neurobiol Aging* **30**:337–352.
13. Gorelick PB, Scuteri A, Black SE, DeCarli C, Greenberg SM, Iadecola C *et al* (2011) Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke* **42**(9):2672–2713.
14. Grinberg LT, Thal DR (2010) Vascular pathology in the aged human brain. *Acta Neuropathol* **119**:277–290.
15. Hachinski VC, Potter P, Merskey H (1987) Leuko-araiosis. *Arch Neurol* **44**:21–23.
16. Hainsworth AH, Minett T, Andoh J, Forster G, Bhide I, Barrick TR *et al* (2017) Neuropathology of white matter lesions, blood-brain barrier dysfunction, and dementia. *Stroke* **48**:2799–2804.
17. Hartz AM, Bauer B, Soldner EL, Wolf A, Boy S, Backhaus R *et al* (2012) Amyloid-beta contributes to blood-brain barrier leakage in transgenic human amyloid precursor protein mice and in humans with cerebral amyloid angiopathy. *Stroke* **43**:514–523.
18. Hultman K, Strickland S, Norris EH (2013) The APOE varepsilon4/varepsilon4 genotype potentiates vascular fibrin(ogen) deposition in amyloid-laden vessels in the brains of Alzheimer's disease patients. *J Cereb Blood Flow Metab* **33**:1251–1258.
19. Josephs KA, Murray ME, Whitwell JL, Parisi JE, Petrucelli L, Jack CR *et al* (2014) Staging TDP-43 pathology in Alzheimer's disease. *Acta Neuropathol* **127**:441–450.
20. Kemper TL, Blatt GJ, Killiany RJ, Moss MB (2001) Neuropathology of progressive cognitive decline in chronically hypertensive rhesus monkeys. *Acta Neuropathol* **101**:145–153.
21. Kitamura Y, Usami R, Ichihara S, Kida H, Satoh M, Tomimoto H *et al* (2017) Plasma protein profiling for potential biomarkers in the early diagnosis of Alzheimer's disease. *Neurol Res* **39**:231–238.
22. Kovacs GG, Ferrer I, Grinberg LT, Alafuzoff I, Attems J, Budka H *et al* (2016) Aging-related tau astroglialopathy (ARTAG): harmonized evaluation strategy. *Acta Neuropathol* **131**:87–102.
23. Lammie GA, Brannan F, Slattery J, Warlow C (1997) Nonhypertensive cerebral small-vessel disease. An autopsy study. *Stroke* **28**:2222–2229.
24. Litchfield S, Nagy Z (2001) New temperature modification makes the Bielschowsky silver stain reproducible. *Acta Neuropathol* **101**:17–21.
25. Love S, Chalmers K, Ince P, Esiri M, Attems J, Jellinger K *et al* (2014) Development, appraisal, validation and implementation of a consensus protocol for the assessment of cerebral amyloid angiopathy in post-mortem brain tissue. *Am J Neurodegener Dis* **3**:19–32.
26. Di Marco LY, Farkas E, Martin C, Venneri A, Frangi AF (2015) Is vasomotion in cerebral arteries impaired in Alzheimer's disease? *J Alzheimers Dis* **46**:35–53.
27. Mari D, Parnetti L, Coppola R, Bottasso B, Reboldi G, Senin U *et al* (1996) Hemostasis abnormalities in patients with vascular dementia and Alzheimer's disease. *Thromb Haemost* **75**:216–218.
28. McAleese KE, Firkbank M, Hunter D, Sun L, Hall R, Neal JW *et al* (2013) Magnetic resonance imaging of fixed post mortem brains reliably reflects subcortical vascular pathology of frontal, parietal and occipital white matter. *Neuropathol Appl Neurobiol* **39**:485–497.
29. McAleese KE, Firkbank M, Dey M, Colloby SJ, Walker L, Johnson M *et al* (2015) Cortical tau load is associated with white matter hyperintensities. *Acta Neuropathol Commun* **3**:60–71.
30. McAleese KE, Walker L, Graham S, Moya ELJ, Johnson M, Erskine D *et al* (2017) Parietal white matter lesions in Alzheimer's disease are associated with cortical neurodegenerative pathology, but not with small vessel disease. *Acta Neuropathol* **134**:459–473.
31. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H *et al* (2005) Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology* **65**:1863–1872.
32. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM *et al* (1991) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* **41**:479–486.
33. Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW *et al* (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol* **123**:1–11.

34. van Oijen M, Witteman JC, Hofman A, Koudstaal PJ, Breteler MMB (2005) Fibrinogen is associated with an increased risk of Alzheimer disease and vascular dementia. *Stroke* **36**:2637–2641.
35. Pantoni L (2010) Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* **9**:689–701.
36. Pantoni L, Gorelick PB (2014) Cerebral Small Vessel Disease. L Pantoni, PB Gorelick (eds). Cambridge University Press: Cambridge.
37. Pantoni L, Garcia JH, Gutierrez JA (1996) Cerebral white matter is highly vulnerable to ischemia. *Stroke* **27**:1641–1646; discussion 1647.
38. Paul J, Strickland S, Melchor JP (2007) Fibrin deposition accelerates neurovascular damage and neuroinflammation in mouse models of Alzheimer's disease. *J Exp Med* **204**:1999–2008.
39. Revesz T, Ghiso J, Lashley T, Plant G, Rostagno A, Frangione B *et al* (2003) Cerebral amyloid angiopathies: a pathologic, biochemical, and genetic view. *J Neuropathol Exp Neurol* **62**:885–898.
40. Ryu JK, McLarnon JG (2009) A leaky blood-brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. *J Cell Mol Med* **13**:2911–2925.
41. Sengillo JD, Winkler EA, Walker CT, Sullivan JS, Johnson M, Zlokovic BV (2013) Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathol* **23**:303–310.
42. Sevush S, Jy W, Horstman LL, Mao WW, Kolodny L, Ahn YS (1998) Platelet activation in Alzheimer disease. *Arch Neurol* **55**:530–536.
43. Silberberg DH, Manning MC, Schreiber AD (1984) Tissue culture demyelination by normal human serum. *Ann Neurol* **15**:575–580.
44. Skrobot OA, Attems J, Esiri M, Hortobágyi T, Ironside JW, Kalaria RN *et al* (2016) Vascular cognitive impairment neuropathology guidelines (VCING): the contribution of cerebrovascular pathology to cognitive impairment. *Brain* **139**:2957–2969.
45. Thal DR, Rub U, Orantes M, Braak H (2002) Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* **58**:1791–1800.
46. Thambisetty M, Simmons A, Hye A, Campbell J, Westman E, Zhang Y *et al* (2011) Plasma biomarkers of brain atrophy in Alzheimer's disease. *PLoS ONE* **6**:e28527.
47. Tomimoto H, Akiyuchi I, Suenaga T, Nishimura M, Wakita H, Nakamura S *et al* (1996) Alterations of the blood-brain barrier and glial cells in white-matter lesions in cerebrovascular and Alzheimer's disease patients. *Stroke* **27**:2069–2074.
48. del Valle J, Duran-Vilaregut J, Manich G, Pallàs M, Camins A, Vilaplana J *et al* (2011) Cerebral amyloid angiopathy, blood-brain barrier disruption and amyloid accumulation in SAMP8 mice. *Neurodegener Dis* **8**:421–429.
49. Vinters HV (1987) Cerebral amyloid angiopathy. A critical review. *Stroke* **18**:311–324.
50. Weller RO, Boche D, Nicoll JAR (2009) Microvasculature changes and cerebral amyloid angiopathy in Alzheimer's disease and their potential impact on therapy. *Acta Neuropathol* **118**:87–102.
51. Weller RO, Hawkes CA, Kalaria RN, Werring DJ, Carare RO (2015) White matter changes in dementia: role of impaired drainage of interstitial fluid. *Brain Pathol* **25**:63–78.
52. Wilhelm I, Nyul-Toth A, Suciú M, Hermenean A, Krizbai IA (2016) Heterogeneity of the blood-brain barrier. *Tissue Barriers* **4**:e1143544.
53. Witt KA, Mark KS, Hom S, Davis TP (2003) Effects of hypoxia-reoxygenation on rat blood-brain barrier permeability and tight junctional protein expression. *Am J Physiol Heart Circ Physiol* **285**:H2820–H2831.
54. Xu G, Zhang H, Zhang S, Fan X, Liu X (2008) Plasma fibrinogen is associated with cognitive decline and risk for dementia in patients with mild cognitive impairment. *Int J Clin Pract* **62**:1070–1075.
55. Yamamoto Y, Ihara M, Tham C, Low RWC, Slade JY, Moss T *et al* (2009) Neuropathological correlates of temporal pole white matter hyperintensities in CADASIL. *Stroke* **40**:2004–2011.
56. Yang H, Lyutvinskiy Y, Herukka SK, Soininen H, Rutishauser D, Zubarev RA (2014) Prognostic polypeptide blood plasma biomarkers of Alzheimer's disease progression. *J Alzheimers Dis* **40**:659–666.
57. Young VG, Halliday GM, Kril JJ (2008) Neuropathologic correlates of white matter hyperintensities. *Neurology* **71**:804–811.
58. Zhang X, Le W (2010) Pathological role of hypoxia in Alzheimer's disease. *Exp Neurol* **223**:299–303.
59. Zipsper BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Hulette CM *et al* (2007) Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. *Neurobiol Aging* **28**:977–986.