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Neuropathology of white matter lesions, blood-brain barrier dysfunction and dementia

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Abstract

Background and Purpose—We tested whether blood-brain barrier dysfunction in subcortical white matter is associated with white matter abnormalities, or risk of clinical dementia, in older people (n=126; mean age 86.4, SD: 7.7 years) in the MRC Cognitive Function and Ageing Study.

Methods—Using digital pathology we quantified blood-brain barrier dysfunction (defined by immunohistochemical labelling for the plasma marker fibrinogen). This was assessed within subcortical white matter tissue samples harvested from post mortem T₂ MRI-detected white matter hyperintensities, from normal-appearing white matter (distant from co-existent MRI-defined hyperintensities) and from equivalent areas in MRI-normal brains. Histopathological lesions were defined using a marker for phagocytic microglia (CD68, clone PGM1).

Results—Extent of fibrinogen labelling was not significantly associated with white matter abnormalities defined either by MRI (OR 0.90; 95% CI 0.79, 1.03; p=0.130) or by histopathology (OR 0.93; 95% CI 0.77, 1.12; p=0.452). Among participants with normal MRI (no detectable

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white matter hyperintensities) increased fibrinogen was significantly related to decreased risk of clinical dementia (OR 0.74; 95% CI 0.58, 0.94; $p=0.013$). Among participants with histological lesions, increased fibrinogen was related to increased risk of dementia (OR 2.26; 95% CI 1.25, 4.08; $p=0.007$).

Conclusions—Our data suggest that some degree of BBB dysfunction is common in older people and that this may be related to clinical dementia risk, additional to standard MRI biomarkers.

Keywords

vascular cognitive impairment; dementia; white matter lesion; leukoaraiosis; neuropathology

Introduction

Diffuse white matter hyperintensities (WMH) are frequently seen on T2-weighted and Fluid-attenuated inversion recovery (FLAIR) MRI scans of older people. WMH are associated with increased risk of lacunar stroke, vascular cognitive impairment (VCI) and Alzheimer's disease (AD)¹.

The blood-brain barrier (BBB) is a property of cerebral blood vessels, resulting from endothelial expression of tight junction proteins and molecular transporters. BBB dysfunction has been proposed as a cause of WMH, VCI and dementia^{2, 3}. MRI data suggest that leakage of intra-vascular contrast agent, a marker of BBB dysfunction, is associated with WMH and VCI^{4–8}. Association of white matter pathology with BBB dysfunction, inferred from detection of extra-vascular plasma proteins (such as fibrinogen, albumin or IgG) is supported by some histological studies^{9, 10} though not others^{11, 12}.

We tested the hypotheses that BBB dysfunction (measured using fibrinogen labelling) is associated i) with risk of white matter abnormalities (defined either by MRI or by histology) and ii) with risk of in-life dementia diagnosis. Throughout, we will maintain the distinction between radiologically-defined WMH and histological white matter lesions (WML) defined on the basis of phagocytic microglia.

We used systematic MRI-guided tissue sampling in subcortical white matter from participants in a large population of donated human brains, the MRC-CFAS Neuropathology study^{13–15}.

Materials and Methods

Human tissue

The MRC-CFAS Neuropathology study is a prospective longitudinal population-based study of cognitive impairment and frailty in the elderly^{13–15}. Here we sampled frontal and parietal subcortical white matter tissue blocks from donated brains. The 126 brains sampled represent all the donated brains from 3 (out of the 6) MRC-CFAS centres, Newcastle, Nottingham and Cambridge, for which: 1) formalin exposure was <10 years (median formalin time 5 y, range 3-7 y) and 2) luminal fibrinogen immunoreactivity was evident.

All cases reported (n=126) were over the age of 65 years (Table 1). Post mortem tissue was obtained from subjects who signed a Declaration of Intent to donate their brain after death. This study had approval from the UK National Research Ethics Service.

Diagnosis of dementia

Dementia status at death was determined from cognitive assessments during the last years of life (based on the full GMS AGE-CAT diagnostic algorithm, see Supplementary Methods) combined with detailed retrospective interviews with a knowledgeable informant, and death certificates^{13, 14, 16}. Clinical dementia status was recorded in 116 participants. Among these 69 (55%) had a clinical diagnosis of dementia during life (Table 1).

Post mortem MRI definition of WMH

MRI scans of fixed brain hemispheres were used to guide histological sampling of white matter tissue blocks, as detailed in previous reports^{15, 17}. Briefly, fixed hemispheres were cut into 10 mm thick coronal slices (“thick slices”, Supplementary Figure I) for MRI. Scans were rated by consensus by three blinded observers using a modified Scheltens rating scale, as described previously^{15, 17}. See Supplementary Methods. WMH were classified as “deep subcortical” or “periventricular”. In thick slices containing subcortical WMH, tissue blocks were cut from the thick slice in order to sample the MRI-defined WMH (these blocks inevitably also contained surrounding tissue). See Supplementary Figure I. In addition, tissue blocks were cut from the same brain, from MRI WMH-free locations (“normal appearing white matter”, NAWM) after WMH blocks had been sampled. NAWM blocks were anatomically remote from WMH and from periventricular areas. Equivalent white matter areas were sampled from “MRI normal” control brains, in which no WMH had been detected. All blocks sampled were approximately 20 mm x 20 mm x 5 mm in size. All blocks were taken from deep subcortical white matter of frontal or parietal cortex, avoiding the corpus callosum and internal capsule. Periventricular WMH and periventricular white matter were avoided.

Histopathology and immunohistochemical labelling

Paraffin wax embedded sections (6 µm thickness) were processed for immunohistochemistry as in our previous studies¹¹. Primary antibodies were to human fibrinogen (rabbit polyclonal A-0080)¹¹, human IgG (rabbit polyclonal A-0423)¹¹, human CD68 (mouse monoclonal, clone PGM1)¹⁵ and smooth muscle actin (mouse monoclonal, 1A4) all from Dako-Cytomation, Ely, UK; CD34 (mouse monoclonal, QBEnd10) and human IgG (mouse monoclonal, Clone RWP49)¹¹ were from Novocastra-Leica Microsystems, Newcastle-upon-Tyne, UK.

Definition of histopathological lesions

CD68-labelled sections from all tissue blocks were viewed by a registered senior neuropathologist (LRB) blind to MRI and clinical data. The CD68-PGM1 antibody is selective for phagocytic microglia and macrophages. CD68 positive cells with amoeboid microglial morphology in clusters of greatest diameter at least 1.1 mm, were defined as “histopathological lesion” (Figure 1A, B, Supplementary Figure II). This definition showed

good inter-rater agreement when assessment of all sections was repeated by two independent, blinded cytopathologists (KE, JJ; Cohen's $\kappa = 0.97$).

Histological image analysis

All sections were digitally scanned under a 20 x objective lens (Leica Slidescanner, UCL-Advanced Diagnostics, http://www.uclad.com/digital_pathology/). Scanned sections in SCN file format were viewed using Leica Slidepath Gateway free software (www.leicabiosystems.com/). Digital image analysis with NIH ImageJ free software (<http://imagej.nih.gov/ij>) was used to estimate the extent (percent area fraction) of labelling with fibrinogen. See Supplementary Methods, Supplementary Figures II and III.

Statistical analysis

All histochemical labelling and image analyses were performed blind to clinical and MRI data. Sample size of 120 was estimated to detect a 20% difference in fibrinogen labelled area fraction ($\alpha < 0.05$, $\beta > 0.8$) based on previous studies using similar material 11, 17.

Statistical analyses were performed using STATA, version 12. Analyses were performed by participant or by tissue block, as some participants had more than one brain region studied. As some participants contributed more than one tissue block, and each tissue block cannot be considered independent within the same brain, we used weighted analyses. All histological sections used to measure fibrinogen area fraction were used in the analyses and received a weight inversely proportional to the number of sections. For the comparison of categorical data, Fisher's exact test was used as Cochran's restrictions were present.

To test whether BBB dysfunction was a risk factor for MRI-defined WMH, we conducted weighted ordinal logistic regression analyses with WMH status as dependent variable (MRI-normal, NAWM, WMH) and fibrinogen area fraction as independent variable. These analyses were repeated with histopathologically-defined WML as dependent variable (none or present) and fibrinogen area fraction as independent variable. In addition we conducted weighted linear regression analyses with MRI classification (MRI normal, NAWM, WMH) as independent variable and fibrinogen area fraction as dependent variable. The same analyses were repeated with histologically-defined WML (none, present) as independent variable and also with combined MRI/histological methods (Supplementary Table I). In these analyses, control group (MRI normal or WML-free) was set as the reference.

To test whether BBB dysfunction was a risk factor for dementia we conducted weighted logistic regression analysis, with clinical dementia status (dementia, without dementia) as the dependent variable, and fibrinogen area fraction as independent variable.

All analyses were controlled for sex, age at death and formalin time (time during which post mortem tissue was stored in formalin). All tests were two-tailed, $p < 0.05$ considered significant.

Results

We report a sample of older people ($n=126$; mean age 86.4 (SD: 7.7) years, Table 1) including 98 with MRI-detected WMH and 28 WMH-free (MRI-normal) individuals. Neuropathological data are reported for 151 tissue blocks. These included 28 blocks from MRI-normal donors, 82 blocks from MRI-defined WMH and 41 from normal appearing white matter (NAWM) areas anatomically distinct from any WMH. In 25 cases the WMH and NAWM areas came from the same donor. Histological white matter lesions detected on blind inspection of CD68-labeled sections by a registered neuropathologist (LRB) were reported in 17 out of 82 WMH-containing tissue blocks (20.7%). Histological WML were also reported in NAWM-derived tissue blocks (6/41 blocks, 14.6%) and in blocks from MRI normal brains (2/28 blocks, 7.1%).

Extravascular fibrinogen was frequently observed within parenchymal cells and axons, and also as a diffuse extracellular pattern, showing accentuation around vessels (Figure 1C, D; further examples in Supplementary Figures II, III and IV). Parenchymal cell labelling with fibrinogen was recorded as a histological feature in many tissue blocks (81/151, 54% of blocks examined). The fibrinogen labelling pattern was confirmed in neighbouring sections treated with another plasma marker, human IgG (polyclonal and monoclonal antibodies, Supplementary Figure IV) and was absent in neighbouring sections treated with an irrelevant primary antibody (rabbit anti-sheep IgG, not shown). Fibrinogen labelled cells and axons were seen around small arteries (example in Figure 2) and around small veins (example in Supplementary Figure IV). We did not attempt to quantify the prevalence of fibrinogen labelling around a particular vessel type.

The mean extent of fibrinogen labelling was 1.03 % (linearised SE 0.04 %) across tissue blocks. There was no significant difference between tissue groups defined by MRI or histopathology in terms of fibrinogen area fraction ($F(3, 648)=0.21$; $p=0.892$). We tested whether BBB dysfunction was a risk factor for white matter abnormalities. Fibrinogen labelling was not significantly related to white matter abnormalities, either as MRI-defined WMH (OR 0.90; 95% CI 0.79, 1.03; $p = 0.130$) or as CD68-defined histological lesions (OR 0.93; 95% CI 0.77, 1.12; $p = 0.452$). According to linear regression analyses, the presence of a white matter abnormality identified either by MRI, histology or combined methods was not significantly related to fibrinogen area fraction (Supplementary Table I).

We tested whether BBB dysfunction was associated with risk of clinical dementia. Across the cohort as a whole fibrinogen-labelled area fraction was not significantly associated with dementia status (OR 0.93; 95% CI (OR) 0.80, 1.09; $p = 0.378$). We repeated the analyses among MRI-defined subgroups and histology-defined subgroups (Table 2). Considering only participants who had no MRI-detected WMH, increased fibrinogen was significantly related to decreased risk of clinical dementia (OR 0.74; 95% CI 0.58, 0.94; $p = 0.013$, Table 2). Among participants where no CD68-defined WML was detected, fibrinogen was negatively associated with decreased dementia risk (OR 0.83; CI(OR) 0.70, 0.98, $p=0.032$, Table 2). Among participants where at least one CD68-defined WML was detected (irrespective of MRI-defined tissue categories) a greater extent of fibrinogen labelling was related to increased risk of dementia (OR 2.26; 95% CI 1.25, 4.08; $p = 0.007$, Table 2).

Discussion

Fibrinogen immunolabelling of parenchymal cells and tissue was a frequent finding in this population-based study of older persons. We have interpreted this as indicative of BBB dysfunction, though clearly the degree of labelling is also dependent on tissue clearance pathways and cellular uptake and metabolism. In common with prior neuropathology studies^{10, 11, 18, 19} we routinely observed cellular and axonal labelling, suggesting an active uptake process. The concept that some degree of BBB permeability is common in older people is supported by extravasation of contrast agent in MRI studies^{4–8, 20} and biochemical detection of plasma markers in cerebrospinal fluid^{5, 20, 21}. The concept is further supported by observations that brain amyloid can be depleted by systemic immunotherapy²², implying BBB penetration by circulating antibodies. Elevated BBB permeability may occur transiently, possibly in a regional mosaic, becoming more common with increasing age^{23, 24}. Recent studies in experimental mice suggest that the healthy BBB exhibits some (small) degree of permeability to most solutes, across a wide range of molecular size (MW 86 – 150,000)^{25, 26}. Efflux may be via defective tight junctions, or alternatively may be via a trans-cellular pathway²⁷.

In our data, BBB dysfunction in brains harbouring histologically-defined white matter lesions was associated with greater risk of clinical dementia. This finding may be explained by toxic effects of plasma-derived fluid within areas of tissue damage (where transmembrane ionic gradients and transport pathways are likely to be disrupted). Our data agree with previous neuropathology cohort studies where markers of plasma extravasation were positively associated with dementia diagnosis^{10, 11, 18} or specifically with AD^{3, 19, 28, 29}. Experiments in animals demonstrate cytotoxic effects of plasma-derived molecules such as fibrinogen on axons, myelin and other white matter components^{30, 31}. Taken together, these findings suggest that BBB dysfunction may exacerbate the cognitive impact of active white matter lesions¹.

On post hoc subgroup analysis, we found a significant negative association of BBB dysfunction with dementia risk among participants without detectable WMH (“MRI normal” brains, Table 2). This finding was at borderline significance ($p = 0.013$) and may represent a false-positive, due to multiple hypothesis testing. If correct, a negative association implies a possible “pre-conditioning” action of plasma extravasation. Brain tissue expresses a complex array of molecular mechanisms for degradation and clearance of parenchymal debris²⁴. We speculate that in older persons without WMH, these clearance pathways may be augmented by modest plasma-derived fluid efflux.

Contrary to our hypothesis, the extent of fibrinogen labelling was not a risk factor for white matter abnormalities. Fibrinogen labelling within blocks cut from WMH, or within blocks containing CD68-defined histological lesions, did not significantly differ from other tissue types. Thus, our data do not support the hypothesis that BBB dysfunction is associated with WMH, or with white matter lesions. This contrasts with previous neuropathology studies in small cohorts^{9, 10}. Unlike the present study, these were not targeted to subcortical white matter. In accord with the present findings, previous well-powered neuropathology studies found no relation of histological BBB markers with WMH^{11, 12}. These findings resemble

those in white matter of multiple sclerosis patients, where clusters of activated microglia were not significantly associated with heightened BBB dysfunction³².

Our data conflict with some previous MRI studies, where enhanced contrast agent extravasation was seen in patients with cerebrovascular disease^{4–7 8}. Size-selective changes in BBB permeability may reconcile neuropathology data from our study and others^{11, 12} with these MRI findings. Size-selective BBB opening is observed in experimental mice subjected to chronic systemic treatment with low dose angiotensin II²⁶, or where the tight junction protein claudin-5 was genetically deleted³³. In the brains of these mice, efflux of water and small solutes (including MRI contrast agents, MW<1000) was evident, while large plasma protein molecules were retained^{11, 26, 33}. Extravasation of small solutes may be associated with WMH while larger solutes (including proteins such as fibrinogen) are not.

The present study has several limitations. First, MRI scans were carried out post mortem and with low field strength (1.0 Tesla). Nevertheless, recent quantitative comparisons show reasonable correlation of post mortem T2-weighted images with ante mortem MRI³⁴. Second, we report on just one, late-stage time-point for each participant. By the nature of a neuropathology study, we lack information on the longitudinal development of tissue lesions.

Third, the post hoc analyses of fibrinogen data raise the possibility of false-positive associations and should be viewed with caution. In addition, we did not include potential confounding covariates (such as APOE genotype).

Conclusions

Our data confirm that some degree of BBB dysfunction is common in older people. For the histological marker of BBB dysfunction that we used, we detected post hoc significant associations with dementia risk. No direct association with white matter changes was detected. If confirmed, these data raise the possibility that BBB dysfunction may be an independent marker for clinical dementia risk, additional to standard MRI biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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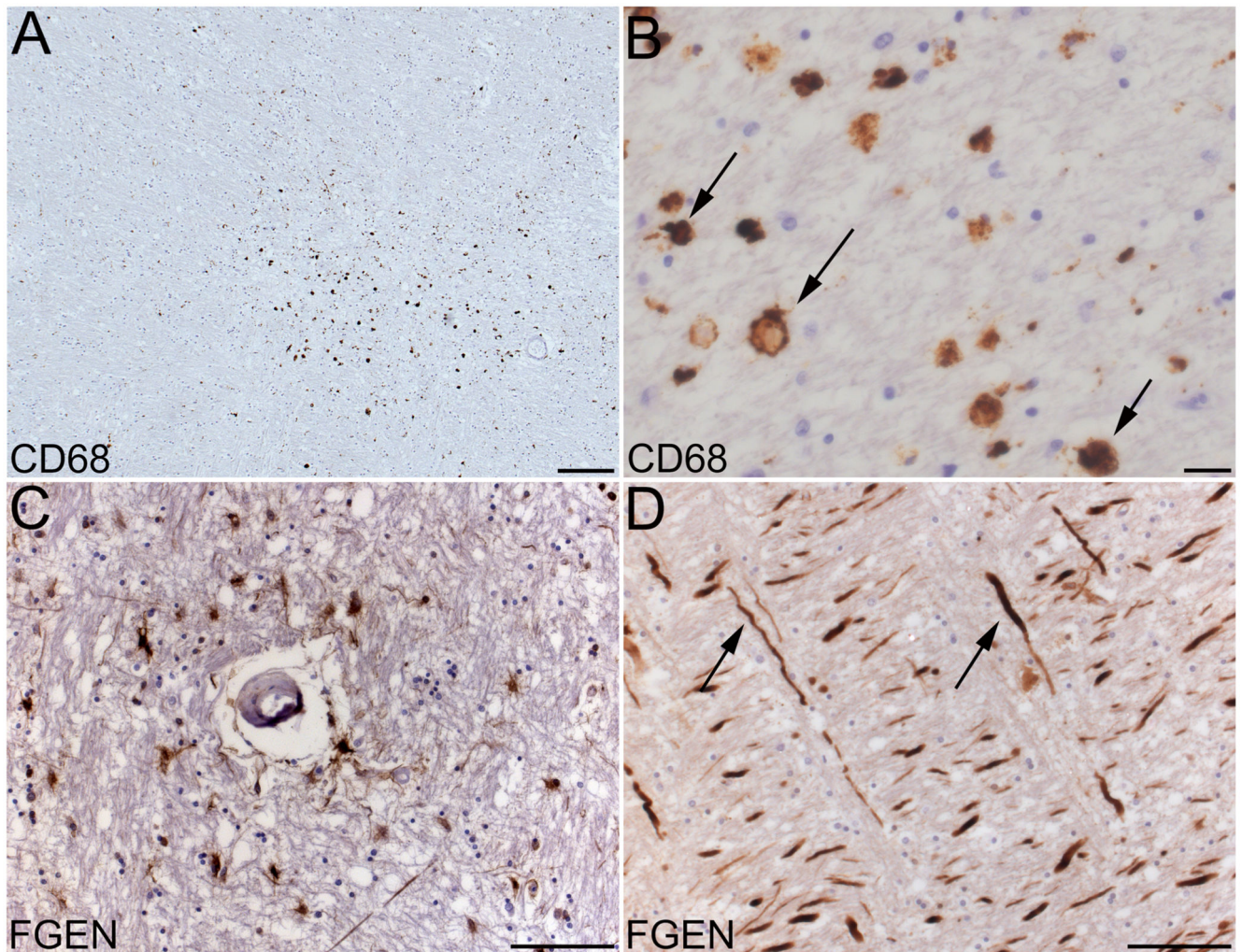


Figure 1. Histopathology of white matter lesions and BBB dysfunction in subcortical white matter. A, CD68-PGM1 immunolabelled section shows a histopathological white matter lesion, defined by a cluster of amoeboid CD68 positive cells. B, CD68 positive amoeboid cells, assumed to be phagocytic microglia (arrows show examples) at higher magnification. C, fibrinogen positive cells and axons, around a small blood vessel. D, nerve axons strongly positive for fibrinogen (arrows show examples). Scale bars 200 μm (A), 20 μm (B) or 100 μm (C, D).

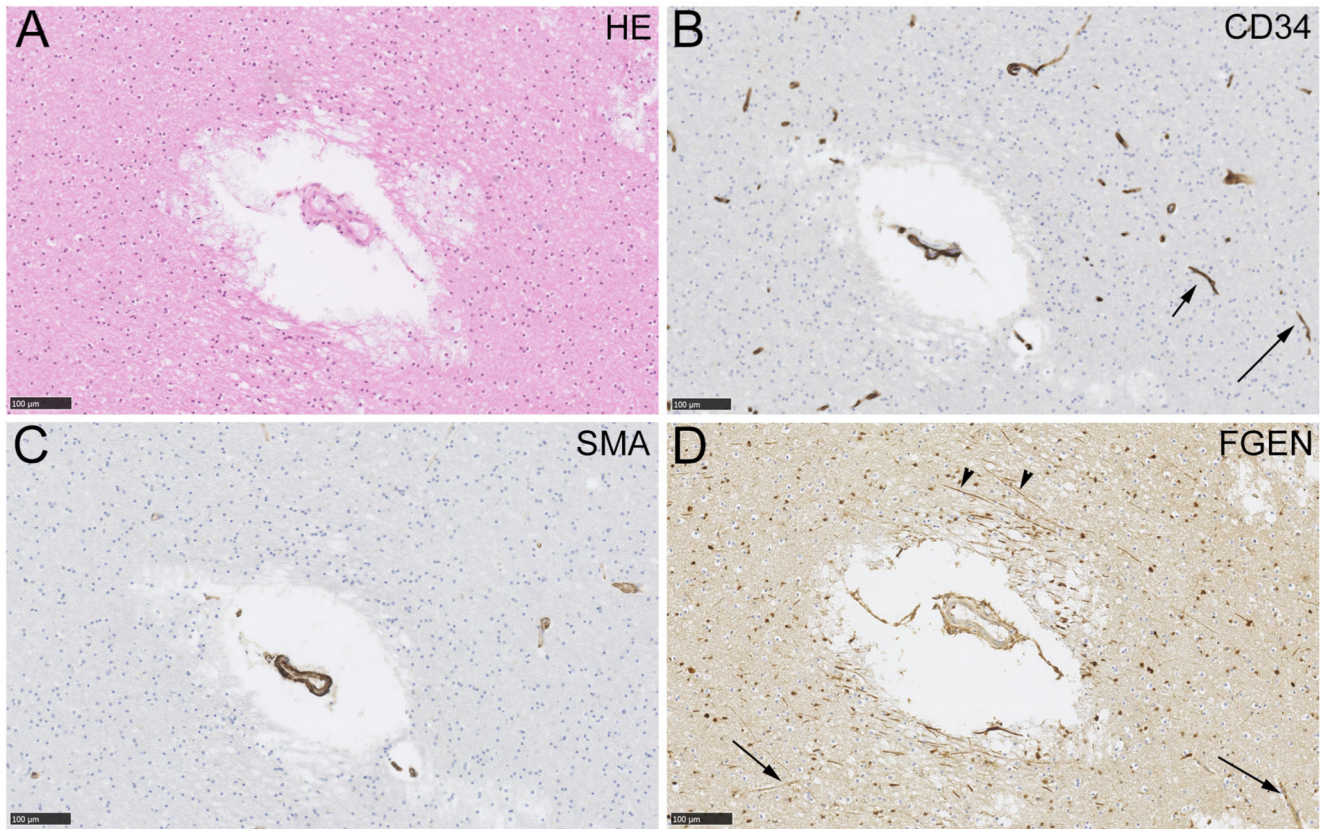


Figure 2.

Fibrinogen immunolabelling around a small artery in deep subcortical white matter. Neighbouring sections were labelled with haematoxylin and eosin (HE, panel A), an endothelial marker (CD34, panel B), a myocyte marker (smooth muscle action, SMA, panel C) or fibrinogen (FGEN, panel D). Capillaries are evident in the CD34-labelled section (arrows, B) and not in the SMA-labelled section. In panel D, fibrinogen labelling is evident around the small artery in cells and in axons (arrowheads). In this example, enhanced perivascular fibrinogen labelling is not seen around capillaries (arrows). Scale bars 100 µm.

Table 1

Demographic characteristics and neuropathological findings, according to clinical dementia status.

	No dementia	Dementia	Not Determined
n	47	69	10
Number female (%)	21 (44)	47 (68)	3 (30)
Age, median (inter-quartile range)	82 (76-90)	89 (85-93)	84 (77-87)
AD pathology present	8 (17)	39 (56)	2 (20)
<i>APOEε4</i> present	10	23	0
Braak stage I/II	23 (49)	15 (22)	4 (40)
Braak stage III/IV	12 (26)	16 (23)	2 (20)
Braak stage V/VI	1 (2)	21 (30)	0 (0)
Moderate-severe parenchymal CAA severity	2 (4)	10 (14)	0 (0)
Moderate-severe meningeal CAA severity	5 (11)	16 (23)	0 (0)

Unless otherwise stated, data shown are number of participants (%). AD: Alzheimer's disease, CAA: cerebral amyloid angiopathy. *APOE* genotype, data available for n=108 participants. Braak stage and CAA severity are defined in Supplementary Methods, *Neuropathological Assessment*.

Table 2

Relationship between extent of fibrinogen immunolabelling and risk (Odds Ratio, OR) of clinical dementia.

	OR	95% CI (OR)	p-value
<i>MRI-defined WMH</i>			
MRI normal	0.74	(0.58; 0.94)	0.013
MRI NAWM	0.96	(0.56; 1.65)	0.887
MRI WMH	1.27	(0.92; 1.75)	0.139
<i>Histopathology-defined white matter lesions</i>			
Histology non-lesion	0.83	(0.70; 0.98)	0.032
Histological lesion	2.26	(1.25; 4.08)	0.007
<i>Combined MRI and histopathology</i>			
MRI normal, histology non-lesion	0.72	(0.56; 0.93)	0.010
MRI NAWM, histology non-lesion	0.77	(0.42; 1.40)	0.391
MRI WMH, histology non-lesion	1.00	(0.72; 1.38)	0.988
MRI WMH, histological lesion	1.82	(0.81; 4.09)	0.146

To test BBB dysfunction as a risk factor for dementia we conducted weighted logistic regression analysis, with clinical dementia status (dementia, without dementia) as dependent variable and fibrinogen area fraction as independent variable. $P < 0.05$ considered significant. Abbreviations: WMH: white matter hyperintensity. NAWM: normal appearing white matter.