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Vasodilator Dysfunction and Oligodendrocyte Dysmaturation in Aging White Matter

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Abstract

Objective—Microvascular brain injury (mVBI) is a common pathologic correlate of vascular contributions to cognitive impairment and dementia (VCID) that leads to white matter injury (WMI). VCID appears to arise from chronic recurrent white matter ischemia that triggers oxidative stress and an increase in total oligodendrocyte lineage cells. We hypothesized that mVBI involves vasodilator dysfunction of white matter penetrating arterioles and aberrant oligodendrocyte progenitor cell (OPC) responses to WMI.

Methods—We analyzed cases of mVBI with low Alzheimer's disease neuropathologic change in prefrontal cortex WM from rapid autopsies in a population-based cohort where VCID frequently occurs. Arteriolar vasodilator function was quantified by videomicroscopy. OPC maturation was quantified using lineage specific markers.

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Author Contributions

S.A.B., Z.B., C.D.K., E.B.L., T.N. and T.J.M. were responsible for conception and design of the studies. C.D.K. acquired and analyzed all human pathology data. Z.B., H.D., and D.J.F. acquired and analyzed all vascular reactivity studies. D.B., D.M., X.G. and P.L. acquired and analyzed all OL maturational data. TN performed all statistical analyses for OL maturational studies. S.A.B., Z.B. D.B., C.D.K. and T.J.M. contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

None

Results—ACh-mediated arteriolar dilation in mVBI was significantly reduced in WM penetrators relative to pial arterioles. Astrogliosis-defined WMI was positively associated with increased OPCs and was negatively associated with decreased mature oligodendrocytes.

Interpretation—Selectively impaired vasodilator function of WM penetrating arterioles in mVBI occurs in association with aberrant differentiation of OPCs in WMI, which supports that myelination disturbances in VCID are related to disrupted maturation of myelinating oligodendrocytes.

Vascular contributions to cognitive impairment and dementia (VCID) are highly prevalent in the elderly and detected in over 85% of cognitively normal individuals 75 years of age or older.^{1, 2} VCID is accompanied by microvascular brain injury (mVBI), which comprises a spectrum of subcortical small vessel disease that contributes to progressive periventricular and frontal WMI^{3, 4} and impairment in cognitive and executive function.^{5–7} WMI appears related to microvascular disturbances that reduce cerebral perfusion. However, postmortem human and murine physiological studies were limited mostly to the middle cerebral artery^{8–11} and pial microvessels.¹² Consistent with microvascular dysfunction in VCID, we identified significant oxidative damage to myelin and axons in mVBI independent of Alzheimer's disease (AD) pathology.¹³ Oxidative damage coincided with extracellular matrix disruption and increased oligodendrocyte (OL) lineage cells. While strong associations between oxidative stress and WMI in chronic hypertension and diabetes support a microvascular etiology in VCID,^{1, 14} the role of microvascular dysfunction in myelination failure in VCID remains undefined.

We hypothesized that vasomotor dysfunction of WM penetrating arterioles triggers WMI that results in a functionally dysmature injury response where OPCs proliferate but fail to terminally differentiate to myelinating OLs. We restricted our study to a unique cohort of relatively uncommon cases of mVBI with low AD pathology and exclusion of other commonly co-morbid pathologic changes associated with brain aging. We analyzed frontal WM of older adults, obtained by rapid autopsy from a population-based cohort. In contrast to pial arterioles, vasodilation of WM penetrating arterioles was selectively impaired in mVBI cases with WMI. WMI involved a significant increase in OPCs and a reduction in mature myelinating OLs. Thus, selective vasodilator dysfunction of WM penetrating arterioles appears to contribute to remyelination failure that involves arrested maturation of OPCs that fail to generate myelinating OLs in chronic WMI.

Materials and Methods

Subjects and Tissue Handling

All specimens were donated by participants in the Adult Changes in Thought (ACT) study, an ongoing population-based study of aging and incident dementia among men and women in Seattle, WA.¹³ For vascular reactivity studies, between 2013 and 2016, we prospectively collected 38 human WM samples at the level of prefrontal cortex (PFOC) by rapid autopsy in the University of Washington Neuropathology Core.¹³ From the 38 prospective cases, we identified five No VBI cases and eight with mVBI. For OL maturational studies, we retrospectively analyzed 36 WM samples from PFOC¹³ of cases with or without mVBI

collected between 2007 and 2013.¹³ Demographic/autopsy data for these cohorts is indicated in tables 1 and 2.

Neuropathologic examination of autopsied brains was performed as described with postmortem interval <8 hours.^{13, 15} Cases were identified with mVBI and excluded other commonly co-morbid pathologic changes in the aging brain including Lewy body disease and gross infarcts or hemorrhages. Inclusion criteria for vascular reactivity studies took into consideration the impact of vascular and parenchymal amyloid and employed the NIA-AA (National Institute on Aging-Alzheimer's Association) criteria and scoring for cerebral amyloid angiopathy (CAA) by evaluation of amyloid β immunohistochemistry in leptomeningeal and parenchymal vessels. Cases with intermediate or high NIA-AA scores for amyloid β plaques were excluded. Since the OL maturational studies included many cases that preceded the NIA-AA criteria, we employed Braak scoring of neurofibrillary degeneration as employed in our prior studies.^{13, 16} For both cohorts, cases with cortical, white matter, basal ganglia or thalamic microinfarcts were stratified into the mVBI group. Cases that lacked microinfarcts were assigned to the No VBI group. For vascular reactivity studies, PFOC samples were immersed in ice-cold artificial cerebrospinal fluid (aCSF) and transferred overnight to Georgia. For OL maturation studies, tissue was immersion fixed in buffered paraformaldehyde.¹³

Videomicroscopic Assessment of Cerebral Arterioles

Pial surface and WM penetrating arterioles were dissected from PFOC samples and maintained at 37°C in Krebs solution (mmol/L): 110 NaCl, 5.0 KCl, 1.25 CaCl₂, 1.0 MgSO₄, 1.0 KH₂PO₄, 5.0 glucose and 24.0 NaHCO₃ equilibrated with 21% O₂/5% CO₂, pH 7.4. Microvessels were cannulated on both ends and mounted on glass micropipettes connected to a pressure servo controller. Internal arteriolar diameter at the midpoint of the arteriolar segment was measured by videomicroscopy with a microangiometer (Living Systems Instrumentation).^{17, 18} After 1-h equilibration, internal arteriolar diameter (ID) was measured in response to step increases in intraluminal pressure from 20 to 120 mmHg (20 mmHg pressure increments) in the presence of normal Ca²⁺ (1.25 mM) and absence of extracellular Ca²⁺ concentrations (Ca²⁺ free Krebs solution, passive arteriolar diameter). Arterioles (50 mmHg intraluminal pressure) were pre-constricted with thromboxane A₂ analog, U46619 (3×10⁻⁸ mol/L) to induce ~30% of arteriolar tone. Cumulative concentrations of endothelium-dependent agonists, acetylcholine (ACh, 10⁻⁹–10⁻⁶ mol/L), histamine (10⁻⁹–10⁻⁶ mol/L) and the endothelium-independent nitric oxide (NO) donor, sodium nitroprusside (SNP, 10⁻⁹–10⁻⁶ mol/L), were administered and arteriolar diameter changes measured. Maximally-developed percent changes in vasodilatation were calculated $[(ID_{maximal} - ID_{initial}) / (ID_{passive} - ID_{initial}) \times 100]$. Arterioles responding with >50% dilation to the highest concentrations of either ACh or histamine were analyzed.

Inclusion criteria for vessel viability were >50% vasoconstriction to U46619 (10⁻⁷ mol/L) and >50% vasodilation either to the pharmacological agonists (to 10⁻⁶ mol/L) ACh, SNP or histamine. Of the 38 cases studied, 34 pial and 25 WM arterioles met these inclusion criteria. Of the identified 20 cases with low AD pathology, three additional cases with No

VBI and four cases with mVBI were excluded, because either the pial or the WM penetrating vessels were not viable in that particular specimen.

Validation of Functional Assessment of Postmortem Human Arterioles

To validate our rapid human autopsy-vascular protocol, we assessed with IACUC approval vasoreactivity and viability of postmortem adult male Wistar rat cerebral arteries handled similarly to human vessels. After death, rats were kept at room temperature for 3 h. Thereafter, brains were stored overnight in aCSF at 4°C after which vasoreactivity of middle cerebral artery branches (MCA) was assessed. MCA vasoreactivity was also analyzed immediately after death and at 3 h postmortem.

Immunohistochemistry

After antigen-retrieval,¹⁶ three tissue sections per case were incubated 48 h at 2–4°C in each of the following primary antibodies: mouse anti-human GFAP (1:500; Merck-Millipore), goat anti-human platelet-derived growth factor receptor (PDGFR α) (1:70; R&D-Systems), rabbit anti-human Nogo-A (1:500; Cell-Signaling), or rabbit anti-human Iba-1 (1:500; Wako). Tissue sections were incubated in the appropriate biotinylated secondary antibody: goat anti-mouse or goat anti-rabbit (1:200; Vector) or donkey anti-goat (1:200; Jackson-ImmunoResearch) and processed for immunoperoxidase staining.¹⁶

Stereological Quantification of N_v

Estimation of numerical cell densities (N_v) for GFAP, Nogo-A, PDGFR α , and Iba-1-labeled somata was accomplished with the optical fractionator using detailed protocols for: (1) delineation and systematic random sampling of regions-of-interest; (2) section thickness measurements; (3) counting brick construction; (4) counting procedures.¹⁶

Statistical Analysis

Vascular Studies: Data are expressed as mean $\pm$ SEM. Statistical analysis employed GraphPad Prism Software by one-way ANOVA followed by Tukey post-hoc test, or Student's t-test. If normal data distribution was met (Kolmogorov-Smirnov), Pearson's correlation and linear regression analyses were performed.

Oligodendroglial studies: Statistical analysis employed SAS software 9.2. Group analyses were tested for independent effects of post-conceptual age, sex, and post-mortem interval. If confounding effects were found, adjusted value was reported. To compare group means, unpaired two-tailed Student's t-tests were performed. The following cell density studies tested for an association across the entire cohort using a general linear model (GLM): Iba1/microglia vs. GFAP/astrocyte; PDGFR/OPC vs. astrocyte; PDGFR vs. Iba1; Nogo-A/mature OL vs. astrocyte; Nogo-A vs. Iba1.

Results

Selectively impaired WM-arteriole dilation in mVBI

We determined whether human WM-arterioles displayed vascular reactivity differences relative to pial arterioles in mVBI. To validate our approach, we analyzed adult rodent

vessels subjected to autopsy conditions similar to human vessels. Figure 1 demonstrates preserved vasodilation to acetylcholine (ACh) in rat MCA segments isolated immediately (Fig. 1A), at 3-h postmortem or under conditions similar to human vessels (Fig. 1B). Vasodilation of rat vessels to ACh and histamine (not shown) did not differ significantly from control human WM-arterioles (Fig. 1C).

We analyzed brains from individuals with or without mVBI, but no macroscopic infarcts or hemorrhages and no or low levels of commonly co-morbid neuropathologic changes seen in older individuals. From 38 prospective cases, we identified mVBI (n=8) and controls (No VBI, n=5) with our pathologic and vascular viability inclusion criteria (Methods and Table 1). Baseline diameters of pial and WM arterioles were similar in mVBI and controls in the presence of Ca^{2+} (active diameters at 50 mmHg; pial arterioles, mVBI vs. No mVBI: 86 ± 22 vs. 80 ± 6 μm and WM arterioles, mVBI vs. No mVBI: 79 ± 5 vs. 69 ± 7 μm) and the absence of Ca^{2+} (passive diameters at 50 mmHg; pial arterioles, mVBI vs. No mVBI: 127 ± 19 vs. 129 ± 11 μm and WM arterioles, mVBI vs. No mVBI: 108 ± 18 vs. 112 ± 24 μm). Pial and WM arterioles were similar in the diameters measured at intraluminal pressures that ranged from 20 to 120 mmHg, as well as vasoconstrictions to the thromboxane A2 analog U46619 (30 nM).

Vasodilation in response to ACh (Fig. 2A), SNP (Fig. 2B) and histamine (Fig. 2C) were similar in pial arterioles in no VBI and mVBI groups. However, dilation of WM-arterioles to increasing concentrations of ACh was significantly reduced in the mVBI group (Fig. 2A). Response to SNP was also reduced, but did not reach significance ($p=0.109$, Fig. 2B), whereas histamine-induced dilation was similar in both groups (Fig. 2C). There was no association between pial and WM vasodilation responses to ACh and SNP and a weak association in response to histamine, which suggested that selectively impaired vasodilation to ACh is an intrinsic abnormality of WM arterioles. Arteriole responses to ACh were not significantly influenced by patient age or post mortem interval.

We next determined if the mVBI cohort that displayed selective WM-arteriolar dysfunction also displayed increased WMI. To estimate WMI, we quantified GFAP-labeled astrocytes in prefrontal WM (Fig. 3A,B). We found a trend towards a significant increase in astrocyte density for mVBI vs. no VBI (Fig. 3C, $p=0.077$; two tailed Students t-test with Welch's correction).

WMI in mVBI Triggers Astrogliosis and Microgliosis

Since WM arteriolar dysfunction appeared to associate with increased WMI, we analyzed prefrontal WM in a separate retrospective cohort of 36 cases with mVBI (n=21) or no VBI (n=15) (Table 2). We hypothesized that WMI in mVBI is related to aberrant OPC maturation. We confirmed increased WMI in mVBI cases by quantifying GFAP-labeled astrocytes (Fig. 4A–C) or Iba-1-labeled microglia (Fig. 4D–F).¹⁶ Astrocyte density was significantly higher in mVBI cases (Fig. 4B; mVBI: 15405 ± 694 ; No mVBI: 11257 ± 768 , mean $\pm$ SEM; $p = 0.0004$). Total astrocyte process length was also significantly higher in mVBI (Fig. 4C). Similarly, microglia were significantly increased in mVBI cases (Fig. 4E; mVBI: 22540 ± 1163 ; No mVBI: 17567 ± 1287 ; $p = 0.0087$). However, there were no

significant associations between astrocyte vs. microglia density for either the mVBI ($r=-0.19$; $p=0.396$) or No VBI ($r=0.035$; $p=0.902$) groups (Fig. 4F).

Premyelinating OPCs Accumulate in VBI lesions

We previously reported that mVBI lesions displayed an increase in cells labeled with Olig2, a marker of all oligodendrocyte (OL) lineage stages. To determine if this increase is related to expansion of the OPC pool, we quantified the OPC marker, PDGFR α (Fig. 5A–C). The mVBI group displayed a significant increase in OPC density (Fig. 5D; No VBI: 48,403 $\pm$ 5293; mVBI: 87,341 $\pm$ 9257; $p=0.0024$).

To determine whether the increase in OPCs in the mVBI group is related to WMI, we analyzed for associations between the density of OPCs and that of astrocytes or microglia. The density of PDGFR α + OPCs displayed a trend toward a significant association with astrocyte density for the mVBI group (Fig. 5E; $r=0.395$; $p=0.077$; $n=21$), but there was no significant association with microglia density (Fig. 5F; $r=0.196$; $p=0.396$; $n=21$).

Reduced Mature Oligodendrocytes in mVBI Lesions

We determined the effect of WMI on OPC maturation to OLs. To quantify OL somata, we first visualized the OL marker, CC1,¹⁹ which not only detected numerous OL somata in controls, but labeled numerous astrocytes in mVBI lesions. We next evaluated the mature-OL marker, Nogo-A.²⁰ We confirmed the selectivity of Nogo-A for mature-OL somata (Fig. 6A)²⁰ and detected no labeling of astrocytes or microglia. When we quantified Nogo-A-labeled somata, the mVBI group exhibited a significant reduction in density (Fig. 6B; No VBI: 171,603 $\pm$ 9689; mVBI: 131,785 $\pm$ 9669; $p=0.0078$).

To determine whether the decrease in mature-OLs in the mVBI group was a response to WMI, we analyzed for an association between mature-OL and astrocyte or microglia density. Nogo-A-labeled mature-OLs were significantly negatively associated with astrocyte density for the mVBI group (Fig. 6C; $r=-0.505$; $p=0.0196$; $n=21$), but there was no significant association with microglia density (Fig. 6D; $r=0.1923$; $p=0.2612$; $n=36$).

OPC and Mature Oligodendrocyte Density are Negatively Associated

To determine whether the decrease in OLs was related to the increase in OPCs in the mVBI group, we determined how OPC density was associated with mature-OL density. The mVBI group, but not the no VBI, displayed a significant negative association between OPC and OL density across the entire cohort ($r=-0.4507$; $p=0.0058$; $n=36$), and for the mVBI group alone (Fig. 6E; $r=-0.447$; $p=0.042$).

Discussion

Our findings support a model (Fig. 7) in which selective vasodilator dysfunction of WM penetrating arterioles in mVBI results in WMI. We previously reported that mVBI is accompanied by significant oxidative stress and MRI diffusion abnormalities that involve axons and myelin.¹³ A consequence of WMI is reactive gliosis that involves astrocytes and microglia. In response to WMI, OPCs proliferate but fail to differentiate to myelinating OLs.

Diffuse WMI in preterm neonates²¹ and older adults²² similarly appears to involve OPC maturation arrest as well as aberrant remodeling of the extracellular matrix with accumulation of astrocyte-derived inhibitory forms of hyaluronic acid that block OPC maturation.^{23–25} Future studies are needed to further define these and other related molecular pathways that impede WM regeneration and repair in VCID.

We found that human mVBI involves an unexpected imbalance in vascular reactivity between pial and WM microvessels. Whereas previous human cerebrovascular reactivity studies focused on larger caliber vessels such as the middle cerebral^{8, 9} and pial arteries,¹² we analyzed WM-arteriolar dysfunction. Although pial arterioles displayed no defects in vascular reactivity, WM-arterioles displayed impaired reactivity to the endothelium-dependent agonist, acetylcholine. Since pial arterioles regulate downstream changes in local WM flow,¹ our results suggest that additional compromise of pial arteriolar blood flow in VCID may further disrupt flow to intrinsically less reactive WM arterioles. The predilection for WMI thus appears to involve dysregulation of blood flow at the level of penetrating WM-arterioles.

The mechanisms that contribute to selectively decreased vascular reactivity of WM-arterioles are unclear. Transgenic mice overexpressing amyloid precursor protein display pronounced disturbances in cerebral autoregulation.¹⁰ Despite evidence from experimental models that AD contributes to VCID pathogenesis, our studies support that human vasodilator dysfunction can occur with low AD and primary mVBI. Endothelial synthesis of vasodilator nitric oxide by the endothelial NO synthase is central to ACh-induced dilation of cerebral arteries²⁶ and plays key roles in cerebrovascular regulation related to cognitive impairment.²⁷ NAD(P)H oxidases are one important source of superoxide anion production, which interferes with nitric oxide-mediated vasodilation and thus contributes to the development of cerebrovascular dysfunction in transgenic mice that overexpress amyloid precursor protein.^{28, 29} Consistent with a role for vasodilator dysfunction in mVBI, we previously found a significant elevation in WM oxidative stress in VCID, independent of the burden of AD, but associated with aberrant accumulation of OL lineage cells in WM lesions.
13

Analysis of myelination in chronic WMI in VCID has been limited to descriptive studies with classical myelin stains (e.g., Bodian; Luxol Fast Blue)³⁰ that do not provide information regarding mechanisms of myelination failure and are relatively insensitive to milder forms of demyelination. We employed specific OPC and mature OL markers to determine whether chronic WMI involves disturbances in OL lineage progression. The accumulation of OL lineage cells in WM lesions¹³ was directly related to an expansion in the pool of OPCs. Studies from animal models of preterm WMI support that OPC proliferation is part of a generalized injury response of glial cells, including astrocytes and microglia, that appears to occur in response to acute degeneration of late OL progenitors (preOLs).³¹ Although OPCs do not appear to degenerate in mVBI lesions, the susceptibility of preOLs in mVBI remains unresolved. Although remyelination failure in WM strokes also may be initiated by acute ischemic degeneration of myelinating OLs,³² our findings support that *chronic* remyelination disturbances arise from arrested maturation of OPCs that fail to terminally differentiate to myelinating OLs in astrocyte-enriched lesions. Astrocyte-derived

factors have been linked to failure of OPC maturation.³¹ Consistent with this mechanism, the increase in OPCs in mVBI was significantly associated with the magnitude of WMI defined by reactive astrogliosis but not microgliosis. Moreover, WMI was negatively associated with the density of Nogo-A+ cells, which supported that WMI is accompanied by a decrease in myelinating OLs. Hence, myelination failure in chronic WM lesions in mVBI appears to involve a failure of OPC maturation to mature OLs.

Chronic human preterm WMI similarly involves OPC maturation arrest,²¹ which suggests a common response to WMI in both the preterm and aging brain. Hypoxia-ischemia triggers a spectrum of preterm WMI that ranges from focal necrosis to diffuse non-necrotic lesions.³³ Whereas myelination failure in necrotic lesions involves axonal and glial degeneration, non-necrotic WMI generates milder diffuse injury where OPC maturation arrest predominates.³¹ Similarly, micro or macro infarcts were uncommon in the frontal diffuse WM lesions in our VCID cases. Hence, in cases where mVBI predominates, WMI appears to involve diffuse, non-necrotic lesions accompanied by OPC maturation arrest.

Our study underscores the challenges and limitations to analyze the independent contribution of mVBI to VCID. Since there are no reliable tissue biomarkers for mVBI, the extent of mVBI in each case was defined by neuropathological criteria. Diagnosis of mVBI was based upon cerebral microinfarcts that were not necessarily in prefrontal WM and were likely to be variable across the brain in terms of injury burden and chronicity. Nevertheless, our findings support that diffuse mVBI commonly occurs in prefrontal WM, but may not be identified without application of quantitative stereological approaches.

The sensitivity of MRI to detect diffuse WMI arising mostly from mVBI is unclear. We previously analyzed frontal WM lesions by diffusion-based ex vivo high field MRI and found that mVBI lesions with low AD displayed no abnormalities, whereas a significant loss of fractional anisotropy was restricted to cases with mVBI and high AD pathology.¹³ Although MRI-defined WM hyperintensities are commonly detected in periventricular as well as frontal WM, the pathological features of these lesions remain poorly defined.^{30, 34} Future studies are needed to define the spectrum of pathology associated with MRI-defined hyperintensities and the extent to which they involve myelination failure related to OPC maturation arrest.

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Figure 1. Validation of protocols for functional assessment of human cerebral arterioles in postmortem samples

Original traces (A, B) and summary data (C, n=3/group) of vasodilation in response to ACh (10⁻⁷ M). (A) rat middle cerebral arteries isolated either freshly, at 3-hours postmortem or 3-hours postmortem and stored 12-hours at 4°C behaved similarly to human cerebral microvessels (B, C) obtained by rapid autopsy.

Figure 2. mVBI displays a selectively impaired dilation of WM penetrating arterioles
 Summary data of vasodilation in response to increasing concentrations of ACh (A), SNP (B) and Histamine (C) are shown in pial surface (left panels) versus WM penetrating arterioles (right panels) in the No mVBI (n=5) and mVBI (n=8) groups.

Figure 3. WM arteriole dysfunction is accompanied by astrogliosis in mVBI subjects
 Representative photomicrographs of GFAP-labeled astrocytes in No mVBI (A) vs. mVBI cases (B). (C) Astrocytes displayed a near-significant increase in density in the mVBI (n=8) vs. No mVBI groups (n=5). Scale bar: 100 μ m.

Figure 4. mVBI displays WMI defined by significant astrogliosis and microgliosis

(A) GFAP-labeled cells in a no VBI vs. mVBI case. Insets in A show the typical increase in astrocyte processes in the mVBI white matter relative to no VBI. In response to mVBI, astrocytes displayed increased density (B) and process length (C). (D) Iba-1-labeled cells appeared more activated in mVBI vs. no VBI. In response to mVBI, microglia displayed increased density (E) but there were no significant associations between astrocyte vs. microglia density (F). Scale bar: A,D:100 μ m.

Figure 5. Premyelinating OPCs are increased in mVBI

(A–C) PDGFR α -labeled OPCs were typically lower in density in no mVBI (A) vs. mVBI cases (B). A high-power image (C) shows representative cell membrane labeling of OPCs. (D) OPCs density was significantly greater in response to mVBI. (E) The density of OPCs and astrocytes displayed a near-significant association in response to VBI, but no associations were found for PDGFR α -labeled OPCs vs. Iba-1-labeled microglia (F). Scale bars: A, 100 μ m; C, 10 μ m.

Figure 6. Mature OLs are decreased, consistent with a failure of OPC maturation in mVBI
 (A) Nogo-A-labeled oligodendrocytes (OLs) were typically decreased in density in mVBI vs. no VBI cases. A high-power image shows representative Nogo-A membrane labeling of OLs. (B) OLs were significantly decreased in response to mVBI (C). The density of Nogo-A-labeled OLs and displayed a significant negative association in response to mVBI for astrocytes but not for microglia (D). (E) Nogo-A-labeled OLs displayed a significant negative association with the density of PDGFR α -labeled OPCs, consistent with arrested OPC maturation in mVBI. Scale bars in A: 100 μ m (left); 10 μ m (right).

We propose that vasomotor dysfunction of WM penetrating arterioles results in hypoxia, ischemia, inflammation and oxidative/nitrosative stress that contribute to axon and myelin damage. Additional axon degeneration also may arise from focal or diffuse retrograde degeneration from stroke. WMI triggers reactive microgliosis (Iba1⁺) and astrogliosis (GFAP⁺) and recruitment of proliferating PDGFRα⁺ OPCs. Astrocytes are a primary source of megadalton (HMW) hyaluronic acid (HA) that is digested to HA fragments by CNS hyaluronidases. Multiple mechanisms may contribute to block OPC maturation to myelinating oligodendrocytes (NogoA⁺).³³ Prior studies support a role for HA as an inhibitor of OPC maturation.³³

Table 1
Patient demographics for cases with or without vascular brain injury studied for cerebral arteriole vasoreactivity

Data presented as number (N) percent (%), or mean $\pm$ standard deviation. No VBI is defined as no infarcts (territorial, lacunar, or CMIs). VBI is defined as any CMIs. No territorial or lacunar infarcts were observed in the VBI cohort. The presence of cystic infarcts and subacute and acute infarcts are part of the determining criteria for mVBI.

	No VBI	VBI	p value [†]
Number (N) of Cases	5	8	
Age (years)	88.4 $\pm$ 6.1	89.3 $\pm$ 4.2	0.771
Female:Male	4:1	4:4	0.565
Postmortem Interval (hours)	4.39 $\pm$ 0.9	4.60 $\pm$ 0.6	0.612
Brain Weight (grams)	1167 $\pm$ 101	1179 $\pm$ 80	0.807
Ventricular Diameter (cm)	2.20 $\pm$ 0.82	2.16 $\pm$ 1.14	0.950
Cystic infarcts, N, (%)	3 (60)	6 (75)	1.000
Acute or subacute infarcts, N, (%)	2 (40)	2 (25)	0.560
NIA-AA Low, N, (%)	5 (100)	8 (100)	1.000
CAA None, N, (%)	4 (80)	5 (63)	1.000
Mild, N, (%)	1 (20)	3 (38)	1.000

Abbreviations: CMI, cerebral microinfarct; VBI, vascular brain injury; NIA-AA: National Institute on Aging and Alzheimer's Association classification; CAA, cerebral amyloid angiopathy. Age: average age at death; Ventricular Diameter: left ventricular diameter at temporal tips.

[†] p value is based on Fisher's Exact test for categorical variables and on 2-sample t-test for continuous variables.

Table 2
Summary for Cases Studied for Oligodendrocyte Lineage Maturation

Data presented as number (N) percent (%), or mean $\pm$ standard deviation. No VBI is defined as no infarcts (territorial, lacunar, or CMIs). VBI is defined as any CMIs. No territorial or lacunar infarcts were observed in the VBI cohort. See Table 1 for details.

	No mVBI	mVBI	p value [†]
Number (N) of Cases	15	21	
Age (years)	87.7 $\pm$ 8	89.4 $\pm$ 7	0.47
Female:Male	7:8	10:11	0.96
Postmortem Interval (hours)	4.7 $\pm$ 1.6	4.6 $\pm$ 1.6	0.80
Brain Weight (grams)	1249 $\pm$ 111	1221 $\pm$ 120	0.49
Ventricular Diameter (cm)	1.12 $\pm$ 0.93	1.37 $\pm$ 1.03	0.46
Cystic infarcts, N, (%)	2 (13)	11(52)	0.033
Acute, subacute infarcts, N, (%)	0 (0)	7 (33)	0.027
Braak I–III, N, (%)	15 (100)	21 (100)	1.00
IV–VI, N, (%)	0 (0)	0 (0)	1.00
CAA None, N, (%)	11 (73)	19 (90)	0.21
Mild, N, (%)	2 (13)	2 (10)	1.00
Moderate, N, (%)	1 (7)	0 (0)	0.42
Severe, N, (%)	1 (7)	0 (0)	0.42