MINocyclinE to Reduce inflammation and blood brain barrier leakage in small Vessel diseAse (MINERVA) trial study protocol

Robin B Brown1, Daniel J Tozer1, Laurence Loubière1, Young T Hong1,2, Tim D Fryer1,2, Guy B Williams1,2, Martin J Graves3, Franklin I Aigbirhio1,2, John T O’Brien4 and Hugh S Markus1

Abstract

Background: Cerebral small vessel disease (SVD) is a common cause of stroke and cognitive impairment. Recent data has implicated neuroinflammation and increased blood-brain barrier (BBB) permeability in its pathogenesis, but whether such processes are causal and can be therapeutically modified is uncertain. In a rodent model of SVD, minocycline was associated with reduced white matter lesions, inflammation and BBB permeability.

Aims: To determine whether blood-brain barrier permeability (measured using dynamic contrast-enhanced MRI) and microglial activation (measured by positron emission tomography using the radioligand 11C-PK11195) can be modified in SVD.

Design: Phase II randomised double blind, placebo-controlled trial of minocycline 100 mg twice daily for 3 months in 44 participants with moderate to severe SVD defined as a clinical lacunar stroke and confluent white matter hyperintensities.

Outcomes: Primary outcome measures are volume and intensity of focal increases of blood-brain barrier permeability and microglial activation determined using PET-MRI imaging. Secondary outcome measures include inflammatory biomarkers in serum, and change in conventional MRI markers and cognitive performance over 1 year follow up.

Discussion: The MINERVA trial aims to test whether minocycline can influence novel pathological processes thought to be involved in SVD progression, and will provide insights into whether central nervous system inflammation in SVD can be therapeutically modulated.

Keywords
Stroke, small vessel disease, arteriosclerosis, neuroinflammation, bloodbrain barrier, clinical trial, experimental medicine

Date received: 4 April 2022; accepted: 24 April 2022

Introduction
Cerebral small vessel disease (SVD) causes a quarter of strokes and is the commonest cause of vascular cognitive impairment. SVD refers to vasculopathy affecting small arteries and venules which show pathological changes including both focal microatheroma and more diffuse arterial deposits sometimes described as lipohyalinosis. Age, hypertension and diabetes are important risk factors for sporadic SVD. Characteristic MRI findings include small subcortical infarcts (lacunar infarcts), white matter hyper-intensities (WMHs), cerebral microbleeds (CMBs) and enlarged perivascular spaces.

A major obstacle to developing treatments for SVD is a gap in understanding of the pathogenesis and the lack of suitable surrogate disease markers. The conventional hypothesis is that the arteriopathy leads to a reduction in cerebral blood flow, and an impairment in cerebral
autoregulation, which results in hypoperfusion. This hypothesis is supported by the finding that cerebral blood flow is reduced in the white matter of patients with SVD\(^3\) and that this reduction is seen not only in WMHs, but also in apparently normal appearing white matter.\(^4\)

However, treatment of typical cardiovascular risk factors has proved largely unsuccessful in clinical trials,\(^5\) with the exception of intensive blood pressure treatment which reduced the progression of WMHs in hypertensive stroke-free individuals in the SPRINT-MIND study.\(^6\) Accordingly, other pathophysiological mechanisms have been investigated in an attempt to provide possible options for disease modifying treatment.

Two processes that have been implicated in the pathogenesis of SVD are inflammation, both systemic and central nervous system (CNS), and increased blood brain barrier (BBB) permeability. The role of systemic inflammation in cardiovascular disease is becoming better understood, and is thought to involve both vascular endothelium activation and cell-mediated components.\(^7\) Endothelial dysfunction and activation has been implicated in SVD and could mediate both the reduced flow and autoregulation.\(^8\) The endothelium in cerebral small arteries is abnormal on post mortem studies,\(^9\) and circulating endothelial markers are elevated in patients with SVD\(^10\); it has been further suggested that at least in some patients there may be a systemic endotheliopathy with additional abnormalities in systemic vessels.\(^11\) Systemic inflammatory markers were also found to predict SVD progression in longitudinal studies.\(^12\)

In addition to evidence of peripheral inflammation in SVD, several studies have demonstrated inflammation in the CNS. Positron emission tomography (PET) imaging using the radioligand \(^{11}\)C-PK11195, which binds to activated microglia, has shown an association between whole brain inflammatory response and markers of SVD, including WMHs.\(^13\) Focal areas of increased binding (‘hotspots’) have also been demonstrated in patients with moderate to severe symptomatic SVD.\(^14\)

Recently it has also been suggested that alterations in BBB permeability may be associated with the abnormalities in endothelial function and activation described above. Neuropathological studies show fibrinogen leakage around vessels consistent with BBB leakage,\(^15\) and a number of studies have reported evidence of low grade BBB permeability using dynamic contrast-enhanced MRI (DCE-MRI) in lacunar stroke\(^16,17\) and vascular cognitive impairment.\(^18\) More recent development of the technique allows maps of BBB permeability to be produced,\(^18\) and foci of increased BBB permeability have been shown in the white matter of patients with symptomatic SVD.\(^14\)

A pathway linking hypoperfusion and hypoxia to BBB disruption and inflammation has been proposed based on studies in a rodent model of white matter ischaemia.\(^19\) This hypothesises that:

1. Chronic hypertension causes vessel lumen narrowing and loss of cerebral autoregulation, leading to hypoperfusion and hypoxia in the vulnerable deep white matter
2. Hypoxia then leads to production of HIF-1\(\alpha\), inducing an inflammatory response
3. Matrix metalloproteinases (MMPs) are produced as part of this response, which disrupts the tight junctions and extracellular matrix of the vascular endothelium leading to opening of the BBB.

In the same rodent model minocycline administration was associated with a significant reduction in white matter damage and improved behavioural and survival outcomes.\(^20\) Minocycline is known to have anti-inflammatory properties within the brain, reducing the activation of microglia,\(^21\) and may be effective in stabilising the BBB.\(^22\)

In the MINocyclinE to Reduce inflammation and blood brain barrier leakage in small Vessel disease (MINERV A) study, we are using a randomised controlled double-blind methodology to determine whether minocycline reduces neuroinflammation and BBB permeability, assessed using \(^{11}\)C-PK11195 PET and DCE-MRI respectively.

### Methods

#### Aims and objectives

To determine whether minocycline reduces BBB permeability and microglial activation in patients with symptomatic SVD.

#### Trial design

Phase II randomised, double-blind placebo-controlled trial of minocycline 100 mg twice daily for 3 months duration with surrogate outcome based on neuroimaging.

#### Outcome measures

1. Primary co-endpoints:
   - Volume of ‘hotspots’ (see definition in Image Analysis below) of white matter BBB permeability measured on MRI
   - Volume of ‘hotspots’ of \(^{11}\)C-PK11195 binding in the white matter measured on PET

2. Secondary endpoints:
   - Mean BBB permeability and microglial activation of white matter measured using transfer constant and \(^{11}\)C-PK11195 binding potential respectively
   - Blood endothelial and inflammatory markers using the Olink proteomics platform (cardiovascular-III biomarker panel, www.olink.com/products-services/target/cardiovascular-iii-panel/)

---

\(^1\) Chronic hypertension causes vessel lumen narrowing and loss of cerebral autoregulation, leading to hypoperfusion and hypoxia in the vulnerable deep white matter
\(^2\) Hypoxia then leads to production of HIF-1\(\alpha\), inducing an inflammatory response
\(^3\) Matrix metalloproteinases (MMPs) are produced as part of this response, which disrupts the tight junctions and extracellular matrix of the vascular endothelium leading to opening of the BBB.
Inclusion criteria

- Clinical evidence of cerebral small vessel disease as evidenced by one or more of:
  - a lacunar stroke syndrome (e.g. pure motor stroke, pure sensory stroke, sensorimotor stroke or ataxic hemiparesis, or clumsy hand dystaxia syndrome) with a corresponding acute lacunar infarct on diffusion weighted imaging (DWI) for cases imaged (clinically) within 3 weeks of stroke or an anatomically compatible lacunar infarct on FLAIR/T1 MRI for cases imaged later after stroke (⩽1.5cm diameter).
  - Symptoms of cognitive impairment
  - Gait apraxia

AND

- Confluent white matter hyper-intensities on T2 weighted MRI (Fazekas scale score ≥ 2)

- If a past history of stroke, baseline MRI will be scheduled at least 3 months after last stroke to exclude BBB changes secondary to acute infarction.

Exclusion criteria

- Unable/unwilling to consent
- Recorded diagnosis of dementia for consent issues and to ensure cognitive testing is possible (or lack of capacity to consent and complete testing via clinician assessment)
- Age <18
- Lacunar infarcts >1.5 cm – as many of these are striatocapsular infarcts caused by embolism
- Evidence of cortical stroke
- Any stroke cause other than SVD including:
  - Cardiogenic source
  - Carotid or vertebral stenosis >50% measured on NASCET criteria
- Probable cerebral amyloid angiopathy defined by the modified Boston criteria
- Known or suspected monogenic form of small vessel disease
- Estimated glomerular filtration rate (eGFR) \(\leq 60 \text{ml/min/1.73m}^2\) within past 3 months.
  - Estimated GFR will be calculated using the Modification of Diet in Renal Disease (MDRD) equation:
    \[186 \times (\text{Creatinine} / 88.4)^{1.154} \times (\text{Age}^{-0.203}) \times (0.742 \text{ if female}) \times (1.210 \text{ if black})\]
- Contraindications to taking part in MRI study, e.g., pacemaker
- Women of child bearing potential, pregnant or breastfeeding
- Meeting exclusions related to minocycline consumption, in particular:
  - Allergic to minocycline hydrochloride, other similar antibiotics
  - Suffer from myasthenia gravis, have impaired liver or kidney function,
  - Systemic lupus erythematosus (SLE)
  - Are sensitive to sunlight or artificial light (e.g. sunbeds)
- Taking medication contraindicated with minocycline

Figure 1. MINERVA inclusion and exclusion criteria.

(c) Brain volume and WMM lesion volume change, and, change in markers of white matter microstructural integrity measured using diffusion tensor imaging (DTI) assessed on MRI at 12 months

(d) Cognitive and behavioural metrics using a panel of neurospsychometric tests that we have previously optimised for use in this population based on assessments at baseline and 12 months (see Supplemental Table 1 for a list of tests used and domains assessed).

Patient selection

Participants will be recruited from inpatient and outpatient stroke services at Cambridge University Hospitals NHS Foundation Trust and will have symptomatic small vessel disease as defined by a clinical syndrome compatible with SVD (lacunar stroke, cognitive impairment or gait apraxia) and at least moderately severe WMHs (Fazekas scale ≥ 2) Figure 1 shows the inclusion and exclusion criteria for the study.

We are not recruiting patients with cerebral amyloid angiopathy, which might have different pathological mechanisms and will exclude any participants with probable cerebral amyloid angiopathy defined using the modified Boston criteria. We are also excluding patients with known or suspected monogenic forms of SVD such as cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).

Trial procedures and interventions

Patients are randomised to intervention or placebo in the ratio 1:1 with a random permuted block randomisation design (block size of 2/4). Randomisation is performed via a web-based system managed by Sealed Envelope Ltd (www.sealedenvelope.com). Participants in the intervention arm will take minocycline 100 mg orally twice daily;
participants in the placebo arm will take a matching cellulose capsule. Participants and investigators are blind to treatment allocation.

Participants undergo visits at baseline (for phlebotomy, neuropsychometry and PET-MRI imaging), 6 weeks (for clinical check-up), 3 months (for post-treatment data collection, phlebotomy and PET-MRI imaging) and again at 1 year for non-contrast MRI and repeat neuropsychometry. The trial design is summarised in Figure 2.

**Imaging acquisition**

The trial neuroimaging protocol includes PET and MRI which is co-acquired on a 3T GE SIGNA PET-MRI scanner (GE Healthcare, Waukesha, WI) at the Wolfson Brain Imaging Centre in Cambridge, UK using sequences that we have previously optimised in a similar cohort. Baseline and 3 month (post-treatment) imaging includes:

1. PET data acquisition for 75 min following the injection of $^{11}$C-PK11195 (target injection activity 500MBq) produced at the Wolfson Brain Imaging Centre Radiopharmaceutical Unit
2. Simultaneous whole brain non-contrast MRI using a 32-channel head coil (Nova Medical) including T1- and T2-weighted images, FLAIR, DTI and susceptibility weighted images. Sequence details are given in Supplemental Table 2.
3. Dynamic T1 maps acquired using DCE-MRI in a sub-volume of the brain chosen to reflect characteristic damage due to SVD. A Gadolinium-based contrast agent in the form of gadoterate meglumine (Dotarem®) is injected at a sub-clinical dose of 0.025 mmol/kg. The dynamic T1 relaxation time is mapped prior to injection and is followed by eight cycles of post injection T1 mapping using an in-house developed pulse sequence that repeatedly acquires six 3D radiofrequency spoiled gradient echo images with different flip angles to calculate each T1 map.

Follow-up MRI only imaging is performed at 1 year and includes T1- and T2-weighted images, FLAIR, DTI and susceptibility-weighted images acquired on the same scanner.

**Image analysis**

WMH lesions will be marked using Jim version 8.0 (http://xinapse.com/j-im-software/), a semi-automated program in which a region of interest is selected by the rater and voxels within this contour are selected. Pre- and post-treatment images will be marked slice by slice on a parallel split screen and displayed randomly in terms of order of acquisition to reduce the risk of bias with the rater blinded to image timepoint. T1 images will be processed to produce tissue probability maps for each tissue class after removal of the WMH mask. WMH and normal appearing white matter masks will then be eroded by 3 mm to eliminate contamination from CSF or grey matter.

The T1 maps from the DCE-MRI will be calculated using the standard radiofrequency spoiled-gradient echo signal equation and used to estimate the gadolinium concentration in tissue using a Patlak graphical analysis to determine influx rate ($K_i$) as a metric of permeability. As there is no artery in the field of view, we will use the superior sagittal sinus as an arterial input function, corrected by the factor (1–haematocrit), which is assumed to be representative of the arterial concentration of contrast agent.
Voxels of increased BBB permeability (‘hotspots’) will be defined as those with a $K_i$ greater than the 95th percentile of permeability derived from an existing cohort of stroke-free control participants.

List-mode PET data will be histogrammed into time bins and reconstructed using time-of-flight ordered subsets expectation-maximisation.\(^27\) Attenuation correction will include the use of a multi-subject atlas method\(^28\) and improvements to the MRI brain coil component. Image reconstruction will also correct for random coincidences, dead time, normalisation, scattered coincidences, radioactive decay, and sensitivity. SPM12 will be used to realign each dynamic image series which will be co-registered with the T1 MRI sequence using a mean realigned PET image.

The specific binding of $^{11}$C-PK11195 will be estimated by determining the binding potential relative to a non-displaceable reference tissue (BP\(_{ND}\)) using a basis function implementation of the simplified reference tissue model that incorporates correction for vascular binding.\(^29\) The white matter reference tissue input will be estimated with supervised cluster analysis\(^30\) using library data determined from healthy control participant $^{11}$C-PK11195 scans using the same PET-MRI scanner. Binding hotspots will be defined as those above the 95th percentile of control participants as for the BBB permeability measurements above.

Figure 3 shows an example of the maps of $^{11}$C-PK11195 binding and BBB permeability hotspots that can be produced using this technique.

DTI images will be analysed using FSL software (‘FDT’; FMRIB’s Diffusion Toolbox, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT) to correct for eddy current effects and to create a binary brain mask in DTI space. Fractional anisotropy (FA) and mean diffusivity (MD) maps will be created from this data using the DTIFIT tool. Spurious cerebrospinal fluid voxels based on thresholds of MD values above $2.6 \times 10^{-4} \text{mm}^2 \text{s}^{-1}$ and FA $> 1$ will be removed. For each participant, the FMRIB Linear Image Registration Tool will be used to register the FLAIR and B0 images. Tissue segments and WMH masks will then be transformed into DTI space and used to create tissue specific FA and MD histograms.

**Statistical analysis and power calculations**

Participant recruitment will be reported in a Consolidated Standards of Reporting Trials (CONSORT) diagram (template shown in Figure 4). Differences between the treatment and placebo groups will be tested using $\chi^2$ tests (categorical data) and one way ANOVA or Mann-Whitney $U$ tests (for normal or non-normal continuous data) as appropriate.

Primary outcome analysis will be performed on an intention to treat basis, including all randomised participants. As our primary outcomes consider the treatment with minocycline as an experimental probe rather than a clinical endpoint, intention to treat analysis might bias the results towards the null hypothesis and so we will also perform a per-protocol analysis including only participants who complete the treatment course. Outcomes will be tested using standard regression models both unadjusted and adjusted for age, sex and demographic or clinical variables that are significantly different between groups.

Using the data from our observational study\(^14\) we calculated that in order to show a 20% reduction in $^{11}$C-PK11195 binding metrics with power of 80% and $\alpha = 0.05$, we require 17 participants in each arm. To demonstrate a 20% reduction in BBB permeability with these constraints, we require 21 participants per arm. Our target sample size of 22 per arm encompasses these requirements.

**Safety and adverse event reporting**

The radiation dose during PET imaging is approximately 2.6 mSv (the equivalent of 1 year of background environmental radiation). Participant information states that this confers a small additional risk of developing cancer, and patients consent explicitly to radiation exposure. 3T MRI does not have any adverse clinical effects, and as gadolinium contrast usage can lead to nephrogenic systemic fibrosis in participants with renal disease, only patients with an estimated glomerular filtration rate of $60 \text{ml/min}/1.73 \text{m}^2$ will be recruited.

Minocycline is a safe and well-tolerated medication but potential side effects include gastrointestinal disturbance, dizziness and skin rashes/discolouration. Patients are provided with an alert card and emergency contact details for the study clinician in case of any possible side effects. Data will be collected on adverse reactions in keeping with the Summary of Product Characteristics for minocycline.

**Figure 3.** Sample FLAIR MRI image from a patient with sporadic SVD, with hotspots of microglial activation (yellow) and BBB permeability (blue) overlaid.
during the treatment period. Additional safety outcomes include recurrent stroke or other cardiovascular events during the treatment period and at 1 year, and change in neuropsychometric test performance.

Data capture/data access

Data will be recorded electronically using an online research data management tool (REDCap) hosted at the University of Cambridge. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing validated data capture, audit trails for tracking data manipulation and export procedures, and procedures for importing data from external sources. After study completion, cleaning and database finalisation and our pre-specified analyses, anonymised data will be available for secondary uses on reasonable request.

Ethical and regulatory approval

Approval for the MINERVA trial was granted by the East of England – Cambridge Central Research Ethics Committee (reference 18/EE/0237) and it has been classified as a non-CTIMP (clinical trial of investigational medical product) by the Medicines and Healthcare products Regulatory Authority. The use of $^{11}$C-PK11195 was approved by the UK Administration of Radioactive Substances Advisory Committee (ARSAC, Research ID 176; 19/09/2018). The study was registered prospectively on the International Clinical Trials Registry Portal (reference ISRCTN15483452).

Discussion

The MINERVA study is testing the hypothesis that minocycline reduces measures of neuroinflammation and BBB permeability in a cohort of patients with symptomatic and moderate to severe SVD. It aims to test whether similar results can be demonstrated in patients with SVD to those reported in an rodent model of ischaemic white matter injury. Enrolment commenced in September 2019, with 34 participants enrolled by 01/03/2022, and is projected to finish midway through 2022. Our primary outcomes will provide evidence as to whether this intervention can influence the inflammatory response in SVD based on $^{11}$C-PK11195 binding, and whether it can affect BBB permeability measured by DCE-MRI. If positive, it would imply that both processes can be altered in parallel, whereas if only one of the $^{11}$C-PK11195 PET or DCE-MRI outcomes are positive it would provide
further evidence that these changes occur at different points in the disease process and should be targeted by different disease modifying strategies.

In addition to primary endpoints of microglial activation and BBB permeability, our study will also provide assessments of the effect of this intervention on radiological measurements of SVD severity and the activation of the systemic immune response using conventional MRI markers of SVD and immunophenotyping of peripheral blood respectively. Follow up after 1 year will allow us to assess any mid-term effects on brain structure/pathology and cognitive performance, though deteriorating cognition becomes evident over a longer timescale in SVD\(^2\) and longer term follow up would be required to accurately model the risk of incident stroke or cognitive impairment/dementia.

Potential limitations of our study include that the time-course of the inflammatory response in SVD has not been fully characterised and in vivo evidence that microglial activation drives the progression of white matter damage is unclear. In addition, the PET radioligand we are using is known to have off-target binding\(^3\) and its receptor is expressed in multiple cell lines in addition to microglia\(^4\), our choice of reference tissue model aims to mitigate this by accounting for endothelial binding and thereby increasing the specificity for binding in brain parenchyma.

A final consideration is that the dose of minocycline that was effective in the rodent model\(^2\) may not translate to a human study. Minocycline has previously been tested unsuccessfully in neurodegenerative conditions such as Parkinson’s disease,\(^5\) and Alzheimer’s disease,\(^6\) but has not yet been assessed in SVD which has a different pathological mechanism to neurodegenerative causes of dementia.

Although both CNS inflammation and increased BBB permeability have been implicated in SVD, whether they play a causal role and whether they can be therapeutically modulated in man is uncertain. The MINERVA study will provide novel information in this area, and if positive will inform phase 3 trials of immunomodulatory therapy in SVD.

Acknowledgements

The authors would like to thank staff at the Clinical Trials Pharmacy, Addenbrooke’s Hospital and at the Wolfson Brain Imaging Centre for their assistance in carrying out study interventions.

Declaration of conflicting interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: There are no personal, professional or financial relationships that would constitute a potential conflict of interest.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study is funded by an Association of British Neurologists Clinical Research Training Fellowship funded by the Guarantors of Brain. Hugh Markus is supported by a National Institute for Health Research (NIHR) Senior Investigator Award. Recruitment to the study is supported by the NIHR Clinical Research Network (study no 39159). This research is supported by infrastructural support from the NIHR Cambridge Biomedical Research Centre (BRC-1215-20014), and the Cambridge BHF Centre of Research Excellence (RE/18/1/34212). The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Ethical approval

Approval for the MINERVA trial was granted by the East of England – Cambridge Central Research Ethics Committee (reference 18/EE/0237).

Informed consent

All participants provide informed written consent prior to enrolment.

Guarantor

HSM.

Contributorship

RBB, DJT, MJG, FIA, JTO’B and HSM conceived and designed the study. RBB is the clinician responsible for recruitment and drafted the manuscript. LL manages the trial. DJT, YTH, TDF, GBW and MJG designed the neuroimaging sequences. DJT, TDF, GBW, FIA, JTO’B and HSM obtained funding. All authors critically reviewed the manuscript and will be involved in trial analysis.

Trial registration

The study was registered prospectively on the International Clinical Trials Registry Portal (reference ISRCTN15483452).

ORCID iDs

Robin B Brown https://orcid.org/0000-0003-0431-7841
Guy B Williams https://orcid.org/0000-0001-5223-6654

Supplemental material

Supplemental material for this article is available online.

References

1. Wardlaw JM, Smith C and Dichgans M. Small vessel disease: mechanisms and clinical implications. Lancet Neurol 2019; 18: 684–696.
2. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol 2013; 12: 822–838.
3. O’Sullivan M, Lythgoe DJ, Pereira AC, et al. Patterns of cerebral blood flow reduction in patients with ischemic leukoaraisos. Neurology 2002; 59: 321–326.
4. Marstrand JR, Garde E, Rostrup E, et al. Cerebral perfusion and cerebrovascular reactivity are reduced in white matter hyperintensities. *Stroke* 2002; 33: 972–976.

5. Smith EE and Markus HS. New treatment approaches to modify the course of cerebral small vessel diseases. *Stroke* 2020; 51: 38–46.

6. Nasrallah IM, Pajewski NM, Auchus AP, et al. Association of intensive vs standard blood pressure control with cerebral white matter lesions. *JAMA* 2019; 322: 524.

7. Libby P, Loscalzo J, Ridker PM, et al. Inflammation, immunity, and infection in atherothrombosis: JACC review topic of the week. *J Am Coll Cardiol* 2018; 72: 2071–2081.

8. Markus HS. Genes, endothelial function and cerebral small vessel disease in man. *Exp Physiol* 2008; 93: 121–127.

9. Giwa MO, Williams J, Elderfield K, et al. Neuropathologic evidence of endothelial changes in cerebral small vessel disease. *Neurology* 2012; 78: 167–174.

10. Poggesi A, Pasi M, Pesce F, et al. Circulating biologic markers of endothelial dysfunction in cerebral small vessel disease: a review. *J Cereb Blood Flow Metab* 2016; 36: 72–94.

11. Thompson CS and Hakim AM. Living beyond our physiological means: small vessel disease of the brain is an expression of a systemic failure in arteriolar function: a unifying hypothesis. *Stroke* 2009; 40: e322–e330.

12. Low A, Mak E, Rowe JB, et al. Inflammation and cerebral small vessel disease: a systematic review. *Ageing Res Rev* 2019; 53: 100916.

13. Low A, Mak E, Malpetti M, et al. In vivo neuroinflammation and cerebral small vessel disease in mild cognitive impairment and Alzheimer’s disease. *J Neurol Neurosurg Psychiatry* 2020; 92: 45–52.

14. Walsh J, Tozer DJ, Sari H, et al. Microglial activation and blood–brain barrier permeability in cerebral small vessel disease. *Brain* 2021; 144: 1361–1371.

15. Simpson JE, Wharton SB, Cooper J, et al. Alterations of the blood–brain barrier in cerebral white matter lesions in the ageing brain. *Neurosci Lett* 2010; 486: 246–251.

16. Wardlaw JM, Doublé F, Armitage P, et al. Lacunar stroke is associated with diffuse blood-brain barrier dysfunction. *Ann Neurol* 2009; 65: 194–202.

17. Topkian R, Barrick TR, Howe FA, et al. Blood-brain barrier permeability is increased in normal-appearing white matter in patients with lacunar stroke and leucoaraisis. *J Neurol Neurosurg Psychiatry* 2010; 81: 192–197.

18. Taheri S, Gasparovic C, Shah NJ, et al. Quantitative measurement of blood-brain barrier permeability in human using dynamic contrast-enhanced MRI with fast T1 mapping. *Magn Reson Imaging* 2011; 65: 1036–1042.

19. Rosenberg GA, Bjerké M and Wallin A. Multimodal markers of inflammation in the subcortical ischemic vascular disease type of vascular cognitive impairment. *Stroke* 2014; 45: 1531–1538.

20. Jalal FY, Yang Y, Thompson JF, et al. Hypoxia-induced neuroinflammatory white-matter injury reduced by minocycline in SHR/SP. *J Cereb Blood Flow Metab* 2015; 35: 1145–1153.

21. Nikodemova M, Duncan ID and Watters JJ. Minocycline exerts inhibitory effects on multiple mitogen-activated protein kinases and IκBα degradation in a stimulus-specific manner in microglia. *J Neurochem* 2006; 96: 314–323.

22. Yang F, Zhou L, Wang D, et al. Minocycline ameliorates hypoxia-induced blood-brain barrier damage by inhibition of HIF-1α through SIRT-3/PHD-2 degradation pathway. *Neuroscience* 2015; 304: 250–259.

23. Lawrence AJ, Patel B, Morris RG, et al. Mechanisms of cognitive impairment in cerebral small vessel disease: multimodal MRI results from the St George’s cognition and neuroimaging in stroke (SCANS) study. *PLoS One* 2013; 8: e61014.

24. Fazekas F, Chawluk J, Alavi A, et al. MR signal abnormalities at 1.5 T in Alzheimer’s dementia and normal aging. *Am J Roentgenol* 1987; 149: 351–356.

25. Linn J, Halpin A, Demaerel P, et al. Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy. *Neurology* 2010; 74: 1346–1350.

26. Patlak CS and Blasberg RG. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations. *J Cereb Blood Flow Metab* 1985; 5: 584–590.

27. Hudson HM and Larkin RS. Accelerated image reconstruction using ordered subsets of projection data. *IEEE Trans Med Imaging* 1994; 13: 601–609.

28. Burgos N, Cardoso MJ, Thiemelans K, et al. Attenuation correction synthesis for hybrid PET-MR scanners: application to brain studies. *IEEE Trans Med Imaging* 2014; 33: 2322–2341.

29. Tomasi G, Edison P, Bertoldo A, et al. Novel reference region model reveals increased microglial and reduced vascular binding of 11C-(R)-PK11195 in patients with Alzheimer’s disease. *J Nucl Med* 2008; 49: 1249–1256.

30. Yaqub M, van Bercelk BN, Schuitemaker A, et al. Optimization of supervised cluster analysis for extracting reference tissue input curves in (R)-[11C]PK11195 Brain PET Studies. *J Cereb Blood Flow Metab* 2012; 32: 1600–1608.

31. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009; 42: 377–381.

32. Zeestraeten EA, Lawrence AJ, Lambert C, et al. Change in multimodal MRI markers predicts dementia risk in cerebral small vessel disease. *Neurology* 2017; 89: 1869–1876.

33. Bader S, Wolf L, Milenkovic VM, et al. Differential effects of TSPO ligands on mitochondrial function in mouse microglia cells. *Psychoneuroendoocrinology* 2019; 106: 65–76.

34. Nutma E, Ceyzériat K, Amor S, et al. Cellular sources of TSPO expression in healthy and diseased brain. *Eur J Nucl Med Mol Imaging* 2021; 49: 146–163.

35. NINDS NET-PD Investigators. Fagan SC, Hart RG, et al. A randomized, double-blind, futility clinical trial of creatine and minocycline in early Parkinson disease. *Neurology* 2006; 66: 664–671.

36. Howard R, Zubko O, Bradley R, et al. Minocycline at 2 different dosages vs placebo for patients with mild Alzheimer disease: a randomized clinical trial. *JAMA Neurol* 2020; 77: 164–174.