1 Specific Aims: Cerebral small vessel disease (CSVD) is the most prevalent etiology underlying vascular contributions to cognitive impairment and dementia (VCID), the 2nd leading cause of dementia, and a major pathologic contributor to Alzheimer disease (AD). While vascular risk factors such as **age**, hypertension, and diabetes are closely linked to CSVD, the precise mechanisms underlying its development and progression are poorly understood. It is postulated that risk factor-induced endothelial injury may instigate a pathophysiologic cascade further injuring cerebral arterioles, impairing cerebrovascular autoregulation and provoking ischemia. In recent work utilizing MR oxygen extraction fraction (OEF), I have identified a signature of hypoxia-ischemia in the region of nadir white matter perfusion (physiologic watershed) in CSVD. Watershed OEF was strongly associated with two major neuroimaging manifestations of CSVD: (1) white matter hyperintensity (WMH) burden and (2) loss of microstructural integrity on diffusion tensor imaging (DTI), further supporting the role of active hypoxia-ischemia in CSVD.

Beyond small vessel ischemia, however, multiple lines of evidence support an independent role of neuroinflammation in CSVD. Neuroimaging and tissue studies have shown evidence of blood brain barrier breakdown (BBB) and leakage of plasma proteins into surrounding tissue, promoting inflammation. In humans, elevated systemic inflammatory markers such as C-reactive protein, matrix metalloproteinases, and pro-inflammatory cytokines indirectly implicate inflammation. Elevation of CNS-specific immune mediators involved in microglial and astrocytic activation, respectively, soluble form of triggering receptor expressed on myeloid cells 2 (sTREM2) and YKL-40, are well-studied in AD but less so in CSVD. Autopsy studies of brain tissue from CSVD patients have shown the presence of activated microglia in proportion to CSVD severity. Although these data indirectly implicate neuroinflammation in CSVD, the lack of noninvasive imaging markers of neuroinflammation has been a major barrier to measuring its presence *in vivo*.

Building upon PET methods utilizing radiotracers that measure binding of the 18-kDA translocator protein as a measure of microglial activation, my preliminary data demonstrate elevated uptake of [^{11}C] PK11195 in association with WMH burden. PET, however, is limited by the effects of radiation, requirement of a cyclotron, low voxel resolution, and high inter-subject variability in tracer binding. To overcome these limitations, novel MR method, diffusion basis spectrum imaging (DBSI) can be utilized which employs a multi-tensor diffusion approach to measure extra-axonal surrogates of inflammation independent from fiber pathologies such as demyelination or axon loss. This method has been validated in animal models and autopsy studies in multiple sclerosis, though it has not been correlated with cerebrospinal fluid (CSF) and serum markers of inflammation, nor utilized to study neuroinflammation in CSVD. In this proposal, I will test DBSI as a non-invasive marker of neuroinflammation in CSVD alongside CSF and serum biomarkers of inflammation, ultimately using DBSI to stratify patients at high risk of VCID progression. In a cohort of older adults across a range of CSVD burden, I will test my hypotheses in the following aims:

Aim 1: Determine if biomarkers of neuroinflammation are associated with MRI metrics of neuroinflammation. In a cohort of older adults across a range of CSVD severity, I hypothesize that CSF levels of sTREM2 and YKL-40 will be associated with white matter DBSI measures of neuroinflammation thereby linking specific CNS immunologic responses to DBSI metrics of neuroinflammation in CSVD. Using an exploratory, data-driven approach, I will also study the relationship between serum and CSF biomarkers of inflammation with DBSI measures of inflammation using a commercially-available multiplexed immunoassay of inflammatory analytes.

Aim 2: Determine if MRI metrics of neuroinflammation independently predict severity of white matter injury and cognitive impairment in older adults with VCID. I hypothesize that DBSI measures of neuroinflammation will be associated with severity of CSVD imaging endpoints, including WMH burden and loss of microstructural integrity on DTI, as well as cognitive impairment, measured by global and domain-specific cognitive dysfunction. This will elucidate whether neuroinflammation is independent from or adds to hypoxia ischemia in VCID progression. I will determine if DBSI imaging metrics of neuroinflammation interact with CSF sTREM2 and YKL-40 levels to predict CSVD severity. Using an exploratory, data-driven approach, I will also study these relationships using serum and CSF biomarkers of inflammation.

Scope of work and relevance of project to the LLF mission: It is increasingly clear that the VCID is a frequent cause of dementia in the elderly. Furthermore, the contribution of vascular pathology to what was previously thought as Alzheimer disease (AD) specific dementia has become more apparent. As our population continues to age, the societal ramifications of dementia will be costly and unsustainable. Unfortunately, we still do not fully understand the pathogenic underpinnings of VCID. Completion of this work will aid in validating a noninvasive, imaging biomarker of neuroinflammation in VCID. Furthermore, it will help elucidate the role of neuroinflammation in the pathogenesis of VCID, independent of hypoxic-ischemic physiology. Ultimately, these deliverables will guide **development of mechanism-based, novel therapeutic targets** for treatment of VCID when none

currently exists. This is **directly in line with the goals of the LLF** as my work will work on new discoveries of disease mechanisms, ultimately leading to prevention and treatment strategies and improving quality and length of life in the elderly.

2 Background and Significance

1.1 The crisis of VCID. The WHO estimates 50 million people are affected by dementia worldwide, and project dramatic increases in coming decades.[1] As the 2nd leading cause of dementia after AD, VCID is broadly defined as cognitive decline resulting from progressive cerebrovascular disease.[2] VCID progresses steadily and insidiously due to accumulating CSVD. While early signs may be subtle, dementia inevitably results in disability due to a loss of independence, ending with premature death.[3-7]

1.2. Imaging endpoints of VCID progression. VCID encompasses multiple pathologies represented by several neuroimaging endpoints, including: WMH, loss of white matter microstructural integrity on DTI, lacunar stroke, microbleeds, atrophy, and enlarged perivascular spaces. Of these, WMH are an attractive endpoint for evaluating biomarkers of CSVD pathogenesis: (1) WMH are strongly associated with clinical outcomes including incident stroke, dementia, and all-cause mortality.[8-15] (2) Progression of WMH is superior to other endpoints such as microbleeds, lacunar infarcts, and atrophy in predicting progression to dementia.[16,17] (3) WMH are prevalent, occurring in 90% of adults older than 65, in contrast to lacunes and microbleeds which occur in < 20%.[18-20] 75% of older individuals have WMH progression at 3 year follow-up, [18] with annual increases in volume of up to 25% depending on age and baseline lesion load. [21] In contrast, other imaging endpoints and cognitive decline progress more slowly with greater variability requiring 50-200X greater sample sizes to detect disease progression. [22] Disruption in microstructural integrity of normal appearing white matter (NAWM) on DTI has been shown to predate progression of WMH.[23-26] Increased mean diffusivity (MD) and reduced fractional anisotropy are closely linked to age-related cognitive decline and may be more sensitive to the early effects of CSVD.[27,28] Similar to WMH, white matter MD is superior to other endpoints for predicting progression to dementia.[16]

1.3 The pathophysiology underlying WMH is poorly understood and unlikely due to ischemia alone. The severity and progression of WMH are associated with vascular risk factors, notably, age, hypertension, and diabetes; [29-36] however, the direct link between risk factors and clinical endpoints of CSVD are unclear, as they account for 1.4 - 2% of the variance in WMH. [37,38] It is postulated that vascular risk factors injure the end arterioles leading to loss of vasoreactivity and disrupted cerebral autoregulation, resulting in ischemia when cerebral perfusion pressure transiently falls below the autoregulatory curve.[18,20,39] In support, pathologic work show thickening of the penetrating arterioles to the subcortical white matter (mean increase 5.5 μm) in individuals with WMH compared to those without.[40] My recent work has shown OEF elevation in the physiologic watershed, a region we defined by the lowest 10% of white matter perfusion, is associated with severity of imaging endpoints in CSVD. This elevation in regional OEF suggests active hypoxia-ischemia is a central pathomechanism in CSVD.[19,20,37] While endpoint pathology, including decreased vascular density, microinfarcts, and arteriolosclerosis also implicate ischemia as a central pathomechanism in CSVD, there are compelling studies to support an additional role of inflammation.[41-44] Whether inflammation occurs independently or is part of a pathologic cascade, however, is unclear. It is hypothesized that microvascular changes result in chronic hypoxia-ischemia, leading to endothelial dysfunction.[45] The resultant vascular inflammation may lead to increased monocyte infiltration, increase of pro-inflammatory cytokines, BBB leakage of plasma contents into the interstitium and worsening inflammation and tissue destruction. [46,47]

1.4 Neuroinflammation as a pathomechanism underlying CSVD. Normally, central nervous system (CNS) inflammation defends against injury and infection. In response to insult, microglia and astrocytes are activated and participate in a cascade of pro-inflammatory factors, with eventual resolution to a resting state.[48] However, a dysfunctional immune response may lead to excessive activation of inflammatory cascades and chronic injury.[49] Rat studies have shown inflammatory infiltration in CSVD. [50,51] Postmortem studies show inflammatory changes such as microgliosis and astrogliosis in VCID; however, these are identified in the late stages demonstrating severe chronic ischemia (*e.g.,* arteriolosclerosis, focal infarcts, spongiform changes). [43,44,52,53]

1.5. Fluid biomarkers of neuroinflammation in CSVD. Larger human studies have shown an association of inflammatory biomarkers in blood and cerebrospinal fluid (CSF) in adults with CSVD/VCID such as interleukin-6 (IL-6), C-reactive protein (CRP), albumin, and markers of endothelial dysfunction such as P-selectin and matrix metalloproteinases (MMPs), but these are nonspecific markers, often associated with systemic inflammation. [47,54-56] Elevation of putative neuroinflammatory mediators involved in microglial and astrocytic activation, the soluble form of triggering receptor expressed on myeloid cells 2 (sTREM2) and chitinase-3-like protein-1 (YKL-40), respectively, have been associated with CSVD severity and vascular dementia but have not been studied in relation to neuroimaging metrics of neuroinflammation. [57,58] In contrast, candidate markers of inflammation, such as CSF sTREM2, YKL-40, and plasma glial fibrillary acidic protein (GFAP), have been well studied in

preclinical AD, progression of AD and alongside well-established AD biomarkers such as Aβ 42/40 isoforms and total/phosphorylated tau. [57,59-67] In contrast to the field of AD, fluid biomarkers remain understudied in CSVD/VCID, yet hold promise to inform distinct pathomechanisms.

Taken together, I postulate that CNS inflammation plays a central role in the pathogenesis and progression of CSVD. Given the limitations of previous studies, however, which have been restricted to autopsy specimens and testing of peripheral blood-only biomarkers, strong evidence for this hypothesis is lacking due to inadequate tools for measuring patient- and tissue-specific inflammation in the CNS.

1.6. Non-invasive imaging biomarkers of neuroinflammation in VCID. Imaging BBB permeability using modeling of slow leakage of gadolinium-based contrast agents on MRI indirectly indicates vulnerability to neuroinflammation in CSVD but does not provide a direct measure of regional inflammation.[68] Currently, the most robust imaging method for studying neuroinflammation in-vivo is with positron emission tomography (PET). Several PET radiotracers have been developed as putative markers of CNS inflammation including against the 18 kDA translocator protein (TSPO). [69,70] The most common of these ligands, ^{11}C-PK11195 has been used to image neuroinflammation in humans for decades.[55] ^{11}C-PK11195 PET imaging has implicated the role of neuroinflammation both in AD as well as VCID (see preliminary data). [49,71,72] Several limitations, however, prevent their widespread use: (1) genetic polymorphisms that affect binding affinity, (2) low signal to noise ratio, (3) low voxel resolution, (4) requirement of an on-site cyclotron and (5) adverse effects of radiation. [69,70,73,74] Recent advances in MR imaging have allowed for non-invasive imaging of neuroinflammation while overcoming these obstacles. To overcome these challenges in imaging surrogates of neuroinflammation, my collaborator, Dr. Song developed Diffusion Basis Spectrum Imaging (DBSI) which employs a multi-tensor diffusion approach capable of measuring extra-axonal surrogates of cellularity and edema independent from fiber pathologies such as demyelination or axon loss. Conventional DTI reflects a more global measure of diffusion restriction but cannot resolve signals measuring neuroinflammation (*i.e.,* inflammatory cells and extracellular vasogenic edema) from crossing fibers and coexisting axonal injury and demyelination. This method has been validated in animal models and autopsy studies in multiple sclerosis, [75-81] and studied in cervical spondylotic myelopathy,[82] HIV,[83] and obesity.[84] However, DBSI parameters have not been correlated with CSF markers of neuroinflammation and they have not been employed to examine the presence of neuroinflammation in patients with CSVD. If DBSI is shown to correlate with CSF biomarkers of microglial activation and astrogliosis, it would be an ideal noninvasive neuroimaging method to measure, quantify and track CNS inflammation. This would be the first step to improving participant selection for clinical trials, to track subclinical disease activity (especially early on), and to inform development of targeted therapeutics for prevention and treatment of CSVD.

2 Approach

2.1 Study Design and Protocol. This is a prospective neuroimaging/neurocognitive study to elucidate the role of neuroinflammation in CSVD/VCID progression.

2.2 Participant Recruitment. *The Cerebrovascular Disease population at Barnes-Jewish Hospital (BJH).* BJH became the first comprehensive stroke center in Missouri and is a high volume center, seeing 1700 stroke patients per year. Our inpatient stroke service is housed in an 80-bed neuroscience unit, a 24-bed step-down unit, and a 32-bed neurointensive care unit. All study participants (N=132) will be recruited from our outpatient clinics (**Table 1**) using the Stroke Patient Access Core, an infrastructure for screening and recruitment into all clinical stroke studies. I will work closely with our dedicated study coordinator, to recruit participants from a range from *low to high risk* of having CSVD. Enrolled participants will be stratified across three categories with three strata each (using a block permutation design)[85] to capture a range from low to high CSVD risk defined by: (1) age from 50-90, (2) volume of WMH, and (3) vascular risk factors (lacunar stroke, hypertension, diabetes, hyperlipidemia, cardiovascular disease requiring medication, and tobacco use). Vascular risk factors were selected based on Framingham Stroke Risk Score (FSRS) which has been validated to predict ischemic stroke, WMH progression, and cognitive impairment in older (>55 years)[29,30,86] and young adults (≤55 years).[30] Low risk individuals will be recruited from partners or friends of patients and from the same outpatient clinic to avoid recruitment bias. Siblings and other first degree relatives will be avoided. Enrolled participants will complete a baseline visit (MRI, clinical assessment, labs, lumbar puncture, and cognitive testing) but for an expanded **longitudinal analysis that will be derived from the data collected in this grant,** they will return for 15-18 month (cognitive battery only) and 30-36 month follow-ups (MRI and cognitive battery). This follow-up duration

Table 1. Participant Enrollment Criteria

Inclusion Criteria:
1. Adult participants age 50-90 stratified by age, WMH volume (range 0-30cc), and vascular risk factors (range 0-4)
2. Provide written informed consent

Exclusion Criteria:
1. Contraindication to MRI
2. Acute stroke within 6 months
3. History of non-lacunar ischemic stroke
4. Conditions that increase the risk of non-lacunar stroke (i.e. atrial fibrillation, cardiomyopathy, hypercoagulable state, etc.)
5. History of other neurological illness (i.e. Alzheimer's disease, Parkinson's disease, seizures, post-stroke dementia) [Note: subjective cognitive decline and mild cognitive impairment can be included]
6. Intra/extracranial vascular stenosis > 50% of internal carotid or proximal circle of Willis arteries
7. Current or past alcohol or substance abuse
8. Concurrent sedating medications (e.g., benzodiazepine) that may affect CBF or OEF

was chosen based on work estimating detectable MRI changes as a function of sample size.[22,87]

2.3 Clinical assessments. Participants will have a detailed medical history and assessment. Demographic, medical history, medication (*e.g.,* antithrombotics, statins, anithypertensives), EKG, and vital signs will be obtained at the baseline and follow-up visits. **Laboratory Data:** I will obtain hemoglobin (OEF calculation), fasting glucose, hemoglobin A1c, and lipid panel at each scan visit. Blood will also be banked for batch processing for inflammatory analytes. **CSF:** Dr. Kang will perform lumbar puncture (LP) during the baseline study visit or within 3 months. He is an expert critical care proceduralist, including with difficult LPs. Based on prior studies conducted at our institution, I estimate ~50% of participants will consent to CSF collection.[88] CSF will be analyzed by ELISA for sTREM2 (INNOTEST, Fujirebio, Malvern, PA) and YKL-40 (MicroVue, Quidel, San Diego, CA).[57,62,89] Serum and cerebrospinal fluid will be batch processed (Rules Based Medicine, Austin, MN) using the InflammationMAP® Multiplex Immunoassay panel which tests for 54 antigen measurements. Unused samples will be banked for future work. Biospecimens will be stored in -80°C freezers with alert features and backup storage available. **Neurocognitive testing:** I will utilize a cognitive battery designed specifically for VCID which includes the *Montreal Cognitive Assessment (MoCA), Free and Cued Selective Reminding Test (FCSRT), Digit Symbol Substitution Test, Category Fluency, Trailmaking A and B, and the Slosson Oral Reading Test. The Geriatric Depression Scale, the State-Trait Anxiety Inventory, household income and years of education* will be collected to aid in adjusting analyses for independent effects of socioeconomic status on cognitive outcomes.

2.4 MRI Protocol. All MRIs will be acquired on a single Siemens Prisma 3T MRI scanner with 64 channel head coil. ***DTI and DBSI.*** A diffusion MRI scan with 99 variable b values and non-collinear diffusion directions will be used.[79] The maximal b value is 1500 s/mm². This diffusion MRI protocol is designed for optimal accuracy of DBSI. Restricted fraction (***RF***, cellularity) and hindered fraction (***HF,*** vasogenic edema) maps will be generated. This scan will also be used to compute conventional DTI parameters such as MD to evaluate microstructural integrity using the diffusion toolbox (FDT) by FSL (Oxford University, UK). ***MR-CBF*** will be measured using 3D background suppression pseudocontinuous arterial spin labeling (pCASL) with post-labeling delay of 2 seconds as previously described. pCASL images will be motion-corrected prior to calculating MR-CBF maps.[90-92] ***MR-OEF*** will be obtained with an asymmetric spin eco (ASE) sequence, developed and validated by mentor, Dr. An, as previously described [93-100]. Partial volume correction will be performed for both CBF and OEF using a local regression method.[101] *Magnetic resonance angiography (MRA)* will be obtained to screen for large vessel cervical and intracranial vasculopathy (if not already assessed by clinically). In accordance with the STRIVE Standards, diffusion weighted imaging, T1, T2, FLAIR, and *susceptibility weighted imaging* will be utilized to characterize recent small subcortical infarcts, lacunes, enlarged perivascular spaces, cerebral microbleeds as well as cortical superficial siderosis.[102] Participants who meet criteria for probable amyloid angiopathy will be excluded.[103] Anonymized images will be sent to the WUSM Central Neuroimaging Data Archive, where images will be downloaded and processed. **2.5 Image Processing.** MPRAGE, 3D FLAIR, DTI/DBSI, OEF, and CBF images will be aligned using linear image registration. CBF and OEF will be computed using the pCASL and ASE scans as detailed previously.[104] **2.6 Tissue Segmentation**. Gray and white matter will be segmented from whole brain using T1 MPRAGE. **3.7 WMH delineation and removal of infarcted tissue:** I will manually segment WMH independently using FLAIR maps to create lesion masks using medical imaging processing, analysis and visualization (https://mipav.cit.nih.gov/) software according to the STRIVE standards.[102]. WMH masks will be manually outlined and excluded from regional analyses when appropriate, allowing creation of both a WMH mask and normal appearing white matter (NAWM) region of interest (ROI). **3.8 Two Neuroimaging Endpoints of CSVD progression Used in Aim 2.** (1) To account for variations in brain volume across patients and over time, I will use a ratio of WMH volume to total brain volume. (2) Normalized peak height of MD (NPH-MD) histogram was selected as a stable, sensitive metric of change in white matter microstructure and has high sensitivity in predicting progression to dementia compared to other surrogate endpoints of CSVD. [16]

3 Preliminary Data

3.1 Microglial activation, measured by PET ^{11}C-PK11195 imaging, implicates neuroinflammation in VCID.

In a preliminary study (manuscript in preparation), 24 elderly participants with a range of vascular risk factors and varying degrees of WMH burden underwent multimodal PET and MRI scans with longitudinal cognitive evaluations. We found that WMH lesion volume was associated with whole brain ^{11}C-PK11195 (PK) standardized uptake value ratio (SUVR, β = 0.320, p = 0.039) after controlling for age and vascular risk

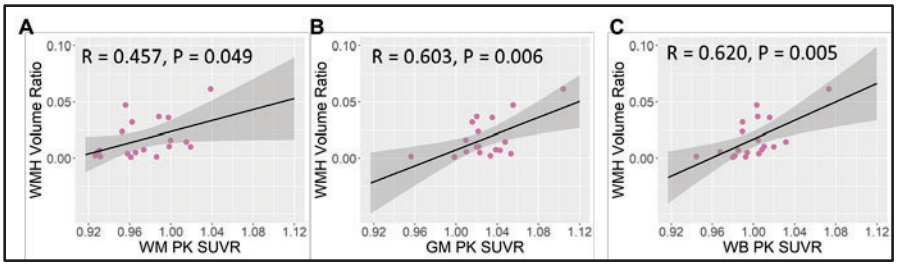

Figure 1. *PK SUVR in cerebral white matter, gray matter and whole brain is significantly associated with WMH volume ratio (WMH volume in mL divided by total intracranial volume)*

factors. **Figure 1** demonstrates the relationship of WM, GM and whole brain PK SUVR and WMH volume. Elevated ^{11}C-PK11195 uptake and older age predicted global cognitive (p=0.019) and processing speed (p=0.0027) decline. These findings suggest that neuroinflammation is associated with both WMH burden and cognitive decline in CSVD. However, ^{11}C-PK11195 PET has several limitations, making MRI diffusion-based methods an attractive alternative (**section 1.6**). The innovation of DBSI allows us to overcome these limitations.

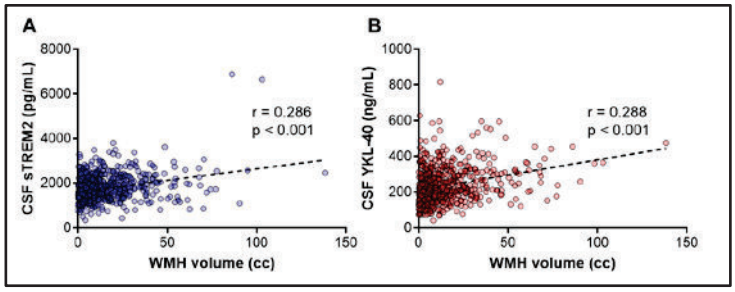

Figure 2. *WMH volume is associated with levels of CSF sTREM2 (A, n = 653) and YKL-40 (B, n = 672) in a cohort from the Knight ADRC*

3.2 CSF biomarkers of microglial and astrocytic activation are elevated in proportion to WMH burden in older adults with MCI or AD. In addition, I evaluated a cohort of ~650 participants from the Knight Alzheimer Disease Research Center (ADRC) with MRI and CSF analytes. I found that both sTREM2 and YKL-40, as markers of microgliosis and astrocytosis, respectively, were associated with WMH burden (**Figure 2**). These participants differ from the proposed study because they have fewer vascular risk factors and many are diagnosed with mild cognitive impairment (MCI) or AD, however, these data suggest that sTREM2 and YKL-40 levels may be important in our study population. **3.3. DBSI-based measures of neuroinflammation are elevated in CSVD.** DBSI is capable of quantifying inflammatory cell infiltration, inflammatory vasogenic edema, axonal injury, and demyelination with the summation of each of these fractions corresponding to the pathologic processes in a voxel. My preliminary data show that increased WM isotropic diffusion fraction (HF, reflecting vasogenic edema) is notably elevated in older adults with CSVD, and shows a milder elevation due to age alone **(Figure 3)**. In patients with CSVD, WMH volume was associated with increasing HF **(Figure 4)**, suggesting that neuroinflammation may be proportional with CSVD disease severity. Building upon my previous work in understanding ischemic vulnerability in patients with CSVD, I now propose to investigate an additional role of neuroinflammation using novel MRI methods in relation to fluid biomarkers of neuroinflammation in CSVD and VCID progression.

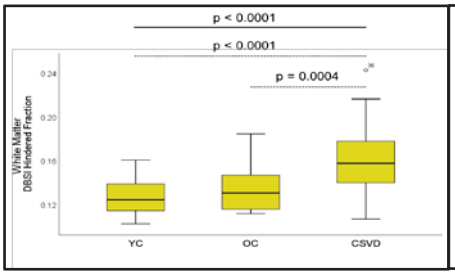

Figure 3. *DBSI HF significantly differs between young healthy controls (YC, age < 50), older healthy controls (OC, age 50-80), and patients with CSVD*

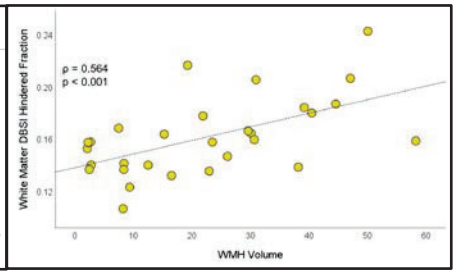

Figure 4. *White matter DBSI hindered fraction is significantly associated with WMH volume*

4 Aim 1: Determine if biomarkers of neuroinflammation are associated with MRI metrics of neuroinflammation. sTREM2 and YKL-40, which reflect microgliosis and astrocytosis, respectively, have been associated with disease severity in patients with AD. [57,59-66] Neuroinflammation in AD is likely secondary to an aberrant innate immune response to promote Aβ clearance. While several inflammatory biomarkers have been studied, sTREM2 and YKL-40 levels have been associated with worsening cognition as well as greater burden of Aβ and tau. [61,105,106] In contrast, fluid biomarkers of CNS inflammation associated with CSVD and VCID are less well established and include nonspecific markers of inflammation such as IL-6, CRP, endothelial dysfunction markers, and matrix metalloproteinases. [54] sTREM2 and YKL-40 were chosen due to a strong body of literature linking them with neuroinflammation in AD and because they may differentiate between distinct components of the innate immune system, microglial activation (sTREM2) and astrocytosis (YKL-40).

4.1. Determine if Serum and CSF biomarkers are associated with white matter injury. I will investigate the correlations between CSF sTREM2 and both WMH volume as well as WM microstructural integrity (DTI NPH-MD) on MRI using a Pearson's correlation coefficient. I will perform the same analysis with CSF YKL-40. Next, I will perform linear regression to determine if CSF sTREM2 and YKL-40 independently predict WMH volume and NPH-MD adjusting for **age** due to the strong effect of age in these outcomes. I will assess these relationships with 54 inflammatory antigens using serum/CSF InflammationMAP® Multiplex Immunoassay panels.

4.2 Determine if Serum and CSF biomarkers are associated with MRI metrics of neuroinflammation. On the baseline scans, I will correlate CSF sTREM2 and DBSI metrics of neuroinflammation (**HF**, inflammatory vasogenic edema and **RF**, inflammatory cell infiltration) within three ROIs: WM, WMH, and the physiologic watershed, a region of ischemic vulnerability in CSVD patients (see attached paper). I will do the same with CSF YKL-40. This will be accomplished first with Pearson's correlation coefficients. I will then perform separate linear regression models to determine if CSF sTREM2 and YKL-40 independently predict DBSI HF and RF in the three

ROIs after adjusting for **age**. As an exploratory analysis, I will also assess univariate relationships between the 54 serum and CSF analytes on the multiplex panel with regional DBSI metrics.

4.4. Pitfalls and Alternative Approaches. *Sample size considerations for CSF analysis:* The rate of consent for lumbar puncture from participants is approximated to be 50% based on prior experiences at our institution.[88] We will recruit 132 participants, resultant in an estimated 66 participants for CSF analysis. This will limit my ability to detect a smaller effect size. Even with a limited sample size, this work is novel and promises to aid in validating the MR metric of neuroinflammation and elucidate disease mechanisms.

5 Aim 2: Determine if MRI metrics of neuroinflammation independently predict severity of WM injury and cognitive impairment in older adults with VCID. My work supports a central role of active hypoxia-ischemia in CSVD. Further, my data and work from others utilizing fluid biomarkers, histopathology as well as neuroimaging, implicate an additional role of neuroinflammation in CSVD. In order to further elucidate these mechanisms of disease, I will prospectively obtain DBSI in this cohort of patients to determine whether they are associated with WMH severity and disruption of WM microstructural integrity. Additionally, the cognitive consequences of CSVD are most closely linked to the loss of independence and long-term disability associated with VCID. My preliminary data with the PET ^{11}C-PK11195 cohort (section 4.1) suggest that neuroinflammation may play a central role in cognitive decline in VCID. I will also determine if DBSI measures are associated with cognitive dysfunction, focusing on cognitive domains known to be affected in VCID.[87,107]

5.1. Determine if DBSI HF (vasogenic edema) and RF (cellular infiltration) are associated with WMH burden and WM microstructural injury: I will investigate associations of DBSI metrics of inflammation and CSVD endpoints of WM injury on neuroimaging. WMH may represent a later stage of WM injury in CSVD. Prior work has shown that diffusion MRI changes occur prior to WMH, representing latent microstructural injury not seen on FLAIR MRI. [23,24,26,108] Therefore, in addition to WMH, I will also be looking at NPH-MD as an earlier, more sensitive marker of injury. First, I will analyze our baseline visit scans to investigate the association between white matter DBSI HF and RF with WMH burden as well as NPH-MD cross-sectionally using a Pearson's correlation coefficient. Next, I will determine if these DBSI measures are associated with WMH volume in two separate models (white matter DBSI HF and RF) using multivariate linear regression, adjusting for **age**. This will determine the role of neuroinflammation independent from hypoxia-ischemia. I will also test these same models with DTI NPH-MD as the outcome variable.

5.2. Determine if DBSI metrics of inflammation area associated with cognitive dysfunction. I will investigate associations of DBSI metrics of inflammation and cognitive dysfunction using global composite cognitive performance (primary) as well as in executive function, processing speed, and working memory (secondary outcomes), each calculated as z-score standardized to baseline mean (SD). I will mirror the analysis in 5.1 but will use the cognitive measures as the outcomes. I will also test supplemental models controlling for Geriatric Depression Score, State-trait Anxiety Inventory, socio-economic status and highest level of education.

5.3 Exploratory biomarker subanalysis: I will examine the contribution of CSF sTREM2 and YKL-40 levels as well as the 54 serum and CSF analytes on the multiplex panel independent from DBSI by investigating the statistical interaction. This will help determine if novel fluid and imaging biomarkers synergistically predict WMH volume and cognitive impairment in patients with CSV.

6.5. Pitfalls and Alternative Approaches. *Measuring active (primary) or reactive (secondary) neuroinflammation:* It is unknown whether neuroinflammation is a primary active disease mechanism or if it is reactive to other processes such as ischemia. To help elucidate this, I will be adjusting for ischemia in my models. However, this may not be sufficient to parse the separate mechanisms apart. Whether inflammation is an active/primary mechanism or reactive/secondary, it will still result in tissue injury and will be an impediment to normal microvascular repair mechanisms, regardless.

7 Sample Size, Power, and Statistical Considerations. Participants undergoing multi-modal MRI, cognitive testing, and blood biobanking (N=132) will be recruited. This sample size yields 80% power to detect an effect size R^2=0.06 after covariate adjustment, to detect a relationship between DBSI with WMH burden and NPH-MD (loss of microstructural integrity. Based on expected recruitment projections for participants consenting to LP (n=66), I will have 80% power to detect a moderate association (R^2=0.15) between CSF biomarkers (sTREM2 and YKL-40) and imaging endpoints of CSVD (WMH volume and DTI NPH-MD) and a moderate to large effect size (R^2=0.20) of association between CSF biomarkers and DBSI, after adjusting for all covariates.

Estimates of achievable effect size and statistical power were calculated using G*Power and R Statistical Software. Multivariate linear regression models will be assessed for collinearity using the variance inflation factor (VIF threshold>5) and for normality of residuals. Threshold for statistical significance set to 0.05 after statistical adjustment for multiple comparisons.

Plan for obtaining additional extramural funding arising from the proposed research

As an early career clinical investigator, I am fully cognizant of the important academic metrics and milestones needed for success in this career path I have chosen. One important part of this is dissemination of data in the form of manuscripts and scientific talks. However, continued funding is equally as important and ultimately, the science cannot be done without financial backing from extramural sources. Ultimately this means that without successful funding, I cannot make the scientific advances I hope to make in order to improve to lives of the patients that I am seeing at the end stages of the CSVD/VCID spectrum when they are admitted to the ICU with severe, life-threatening illness. Therefore, I have carefully considered the pathways towards research independence. Firstly, I have taken what I have learned from successfully competing for grants in the past and applied it into a K23 resubmission that is currently under review with NINDS (the initial submission was reviewed but the score was not fundable). As I have addressed all the reviewer criticisms in concrete ways, I am optimistic I will be funded in this application cycle. I will be able to publish 1-2 strong papers from the data generated from this grant in the short term (1-2 years). This preliminary data will also serve as the foundation for longitudinal analyses that have the potential for publication in high impact journals. I plan to apply for an R21 in 2023-2024 and eventually an R01 in 2025-2026 to do this important longitudinal work.

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