Published Ahead of Print on March 7, 2023 as 10.1212/WNL.0000000000207113

OPEN

Neurology®

The most widely read and highly cited peer-reviewed neurology journal
The Official Journal of the American Academy of Neurology

AMERICAN ACADEMY OF NEUROLOGY®

Neurology Publish Ahead of Print
DOI:10.1212/WNL.0000000000207113

Associations Between CSF Markers of Inflammation, White Matter Lesions, and Cognitive Decline in Individuals Without Dementia

Author(s):
Eske Christiane Gertje, MD[1,2]; Shorena Janelidze, PhD[1]; Danielle van Westen, MD, PhD[3,4]; Nicholas Cullen, BS[1,5]; Erik Stomrud, MD, PhD[1,6]; Sebastian Palmqvist, MD, PhD[1,6]; Oskar Hansson, MD, PhD[1,6]; Niklas Mattsson-Carlgren, MD, PhD[1,5,7]

Corresponding Author:
Eske Christiane Gertje, eske.gertje@med.lu.se

Affiliation Information for All Authors: 1. Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University, Sweden; 2. Department of Internal Medicine, Skåne University Hospital, Lund, Sweden; 3. Diagnostic Radiology, Department of Clinical Sciences Lund, Lund University; 4. Imaging and Function, Skåne University Hospital, Lund, Sweden; 5. Memory Clinic, Skåne University Hospital, Malmö, Sweden; 6. Department of Clinical Sciences Lund, Neurology, Lund University, Skåne University Hospital, Lund, Sweden; 7. Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden.

Equal Author Contribution:
Oskar Hansson MD, PhD, and Niklas Mattsson-Carlgren MD, PhD, contributed equally to this work as senior authors

Contributions:
Eske Christiane Gertje: Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data
Shorena Janelidze: Study concept or design; Analysis or interpretation of data
Danielle van Westen: Major role in the acquisition of data
Nicholas Cullen: Analysis or interpretation of data
Erik Stomrud: Major role in the acquisition of data
Sebastian Palmqvist: Analysis or interpretation of data
Oskar Hansson: Study concept or design; Analysis or interpretation of data
Niklas Mattsson-Carlgren: Study concept or design; Analysis or interpretation of data

Figure Count:
3

Table Count:
3

Search Terms:
[2] All Cerebrovascular disease/Stroke, [26] Alzheimer's disease, [120] MRI, [319] Cerebrospinal Fluid

Acknowledgment:
The authors thank Anika Wüstefeld and Nicola Spotorno for technical assistance.

Study Funding:
This study was supported by the Swedish Research Council (2016-00906), the Knut and Alice Wallenberg foundation (2017-0383), the Marianne and Marcus Wallenberg foundation (2015.0125), the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson's disease) at Lund University, the Swedish Alzheimer Foundation (AF-939932), the Swedish Brain Foundation (FO2019-0326), The Parkinson foundation of Sweden (1280/20), the Skåne University Hospital Foundation (2020-O000028), Regionalt Forskningsstöd (2020-0314), and the Swedish federal government under the ALF agreement (2018-Projekt 0279).

Disclosures:
O. Hansson has acquired research support (for the institution) from AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, GE Healthcare, Pfizer, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Alzpath, Biogen, Cerveau and Roche. S. Palmqvist has served on scientific advisory boards and/or given lectures in symposia sponsored by F. Hoffmann-La Roche, Biogen, Eli Lilly, and Geras Solutions. The other authors report no disclosures relevant to the manuscript.

Preprint DOI:

Received Date:
2022-04-12

Accepted Date:
2023-01-11

Handling Editor Statement:
Submitted and externally peer reviewed. The handling editor was Associate Editor Linda Hershey, MD, PhD, FAAN.

Abstract:

Background and Objectives

Small vessel disease (SVD) and neuroinflammation both occur in Alzheimer's disease (AD), and other neurodegenerative diseases. It is unclear if these processes are related or independent mechanisms in AD, especially in the early stages of disease. We therefore investigated the association between white matter lesions (WML; the most common manifestation of SVD), and CSF biomarkers of neuroinflammation and their effects on cognition in a population without dementia.

Methods

Individuals without dementia from the Swedish BioFINDER study were included. CSF was analyzed for proinflammatory markers (interleukin [IL]–6, IL-8), cytokines (IL-7, IL-15, IL-16), chemokines (interferon-γ–induced protein 10 [IP-10], monocyte chemoattractant protein 1, markers of vascular injury (soluble intercellular adhesion molecule 1, soluble vascular adhesion molecule 1), and markers of angiogenesis (placental growth factor [PlGF], soluble fms-related tyrosine kinase 1 [sFlt-1], vascular endothelial growth factors [VEGF-A, and VEFG-D]), and Aβ42, Aβ40 and P-tau 217. WML volumes were determined at baseline and

longitudinally over six years. Cognition was measured at baseline and follow-up over eight years. Linear regression models were used to test associations.

Results

495 cognitively unimpaired (CU) elderly and 247 patients with mild cognitive impairment (MCI) were included. There was significant worsening in cognition over time, measured by MMSE, CDR and mPACC in CU and MCI, with more rapid worsening in MCI for all cognitive tests. At baseline, higher levels of PlGF (β=0.156, p<0.001), lower levels of sFlt-1 (β=-0.086, p=0.003), and higher levels of IL-8 (β=0.07, p=0.030) were associated with more WML in CU. In MCI, higher levels of PlGF (β=0.172, p=0.001), IL-16 (β=0.125, p=0.001), IL-8 (β=0.096, p=0.013), IL-6 (β=0.088, p=0.023), VEGF-A (β=0.068, p=0.028), and VEGF-D (β=0.082, p=0.028) were associated with more WML. PlGF was the only biomarker that was associated with WML independent of Aβ status and cognitive impairment. Longitudinal analyses of cognition showed independent effects of CSF inflammatory markers and WML on longitudinal cognition, especially in people without cognitive impairment at baseline.

Discussion

Most neuroinflammatory CSF biomarkers were associated with WML in individuals without dementia. Our findings especially highlight a role for PlGF, which was associated with WML independent of Aβ status and cognitive impairment.

Introduction

White matter lesions (WML) are part of the small vessel disease (SVD) spectrum, and associated with cognitive decline and dementia.[1,2] They may be caused by reduced cerebral blood flow and loss of autoregulation resulting in chronic and diffuse subclinical ischemia, causing demyelination and axonal loss.[3] The etiology of SVD is not fully understood. Previous studies suggest a role of endothelial dysfunction in SVD, especially in WML.[4,5]

The pathophysiological mechanisms of neurodegenerative diseases involve changes in neuronal- and non-neuronal cells in the brain, e.g. in microglia and astrocytes, which modulate immunological responses and neuroinflammation.[6-8] Neuroinflammation is linked to impairment of the blood-brain barrier (BBB), which is part of the neurovascular unit.[9] Disruption of the BBB can be observed in neurodegenerative diseases, including Alzheimer's disease (AD), and in cerebrovascular disease. It can be induced by hypoxia or ischemia.[10-12]

Cerebrospinal fluid (CSF) biomarkers of neuroinflammation and cerebrovascular dysfunction are associated with cognitive decline.[9,13,14] According to previous studies, markers of endothelial dysfunction are more strongly associated with SVD than those of systemic inflammation.[15] Associations between vascular endothelial growth factor (VEGF-A) and placental growth factor (PlGF) with WML have been reported in patients with Parkinson's disease.[16] In addition, plasma VEGF-D correlated with greater cerebral SVD burden in individuals without dementia or stroke.[15,17] However, it is unclear to what degree neuroinflammatory CSF biomarkers are correlated with vascular pathology in people with or without AD pathology.

We investigate the relationship between SVD and neuroinflammation and their impact on cognition by studying the association between WML, CSF biomarkers of inflammation, and cognitive tests. Our primary aim was to estimate the effect of inflammation

on WML, both cross-sectionally and longitudinally. We hypothesized that markers involved in endothelial dysfunction such as soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular adhesion molecule 1 (sVCAM-1)[5] as well as markers of the VEGF family are associated with WML.[9,13,18] Reduced blood flow and hypoxia due to SVD such as WML, might trigger angiogenesis (which is associated with upregulation of markers of the VEGF family).[17,19] Findings from previous studies have been inconsistent on associations between pro-inflammatory markers and WML.[15,16] Here we tested specific hypothesis that pro-inflammatory markers such as interleukin-6 and 8 (IL-6, IL-8) are associated with WML. Lastly, we hypothesized that a combination of neuroinflammation and cerebrovascular injury increases the risk for more aggressive neurodegenerative disease, such as AD. Our secondary aim was therefore to test whether interactions between inflammatory markers and WML lesions predict longitudinal cognitive decline and if there are differences depending on Aβ status (indicating presence of AD brain changes). In symptomatic patients, we also stratified by tau status.

Methods

Study population

In this cohort study, cognitively unimpaired (CU) elderly, patients with subjective cognitive decline (SCD), and patients with mild cognitive impairment (MCI) were included. CU were recruited from the population-based Malmö Diet Cancer Study, and SCD and MCI patients were enrolled at three memory outpatient clinics between 2010 and 2014 as part of the Swedish BioFINDER study (http://www.biofinder.se).[13] Inclusion criteria for cognitively healthy controls were (1) age ≥60 years, (2) Mini-Mental State Examination (MMSE) 28–30 points at the screening visit, (3) absence of cognitive symptoms as evaluated by a physician, (4) fluent in Swedish, and (5) not fulfilling the criteria of MCI or any dementia. Exclusion

criteria were (1) significant neurologic or psychiatric disease (e,g. stroke, Parkinson's disease, multiple sclerosis, major depression), (2) significant systemic illness making it difficult to participate, (3) significant alcohol abuse, or (4) refusing lumbar puncture. SCD and MCI patients were thoroughly examined by physicians specialized in dementia disorders. Inclusion criteria for SCD and MCI were (1) cognitive symptoms, (2) not fulfilling the criteria for dementia, (3) MMSE 24–30 points, (4) age 60–80 years, and (5) fluent in Swedish. Patients with (1) cognitive impairment that without doubt could be explained by another condition (other than prodromal dementia), (2) severe somatic disease, and (3) refusing lumbar puncture or neuropsychological investigation were excluded. Patients with SCD vs MCI were assessed with a neuropsychological battery assessing the cognitive domains of verbal ability, visuospatial construction, episodic memory, and executive functions and the clinical assessment of a senior neuropsychologist.[20] In agreement with guidelines, cognitively normal individuals and study participants with SCD were included in the CU group.[13,21]

CSF collection and analysis

CSF samples were collected and handled according to a standardized protocol.[22] Samples were taken from non-fasting individuals by lumbar puncture at three different centers. They were centrifuged (2,000 g, +4°C, 10 minutes), 1 mL was aliquoted into polypropylene tubes (Sarstedt AG & Co, Nümbrecht, Germany), and aliquots were stored at −80°C. Samples went through one freeze–thaw cycle before the analysis when 200 μL were further aliquoted into LoBind tubes (Eppendorf Nordic A/S, Hørsholm, Denmark).

An ultrasensitive Mesoscale Discovery immunoassay and a customized V-PLEX kit was used to analyze CSF concentrations of proinflammatory markers (IL–6, IL-8), cytokines (IL-7, IL-15, IL-16), chemokines (interferon-γ–induced protein 10 [IP-10], MCP-1), markers of vascular injury (sICAM-1, sVCAM-1), and markers of angiogenesis (PlGF,

sFlt-1, VEGF-A, and VEGF-D) as previously described[13] (**Table 1**). Assays were selected from the preconfigured V-PLEX Neuroinflammation Panel 1 Human Kit (combining proinflammatory, cytokine, chemokine, and angiogenesis panels), restricted to analytes with intra-assay and inter-assay coefficients of variation below 20% and to assays sensitive enough for CSF analysis in test runs. Samples were analyzed with the customized kit according to the manufacturer's recommendations with one modification: for chemokine and proinflammatory panels, samples and calibrators were incubated overnight at +4°C. CSF concentrations of Aβ42 and Aβ40 were measured using ELISA kits according to the manufacturer's recommendations (Aβ42, Aβ40, EUROIMMUN AG, Lübeck, Germany). All analyses were performed using one batch of reagents. Samples were randomized across plates/runs to minimize effects of run-to-run variation.[13] CSF P-tau 217 was measured using a Mesoscale Discovery immunoassay developed by Lilly Research Laboratories. Samples were analyzed as previously described for plasma samples.[23] For CSF analysis, we used a different calibrator range and 1:4 sample dilution.

MRI

MR imaging was performed with a 3T Siemens (Erlangen, Germany) Trio system equipped with a standard 12-channel head coil. The protocol comprised an axial T2 fluid-attenuated inversion recovery (FLAIR) imaging and sagittal MPRAGE sequence. Automated segmentation of WML was performed using the Lesion Segmentation Tool (LST) implemented in SPM8; this generated a total lesion volume (named WML volume), for each individual. The hippocampal volume (HCV) [total HCV: (right HCV + left HCV)/2] and the intracranial volume (ICV) were determined by FreeSurfer (version 5.3) using the MPRAGE images. Longitudinal follow up was done with MR imaging after two, four, and six years from baseline.

Cognitive testing

Cognitive assessment included the Mini-Mental State Examination (MMSE)[24], global Clinical Dementia Rating (CDR)[25], the 10-word delayed recall test from the Alzheimer's disease Assessments scale – cognitive subscale (ADAS-cog)[26], the Trail Making Test B (TMTB)[27], and the animal and letter S fluency tests.[28] A modified preclinical Alzheimer's composite score (mPACC) was calculated based on the average of z-scores from different tests (mPACC=(MMSE (z) + 2*ADAS-cog (z) + animal fluency (z) + TMTB (z))/5). The ADAS-cog was counted twice to preserve the weight of memory from the original PACC.[29,30] Tests were repeated after two, four, six, and eight years for CU, and after one, two, three, four, six, and eight years for MCI and SCD.

Statistical analyses

The subgroups CU and MCI were analyzed separately, since these groups may differ in terms of brain- and cognitive changes. All participants had WML data, and data on at least one CSF biomarker (a list of missing data is available in **eTable 1** in the Supplement). CU and MCI baseline characteristics were compared using Student's t-test (continuous variables) or Chi-square test (categorical variables). Variables that were not normally distributed (IP-10, IL-6, IL-8, and WML volume), were log10 transformed. CSF biomarker and WML volume data were standardized to z-scores based on the entire population when used as predictors. In accordance with definitions on the Alzheimer's continuum, following definitions from the National Institute of Aging-Alzheimer's Association (NIA-AA) in Jack et al. 2018,[21] study participants were categorized into groups with normal (Aβ−) or pathologic (Aβ+) CSF signature using the CSF Aβ42/Aβ40 ratio (cutoff ≤0.1). Aβ+ were sufficient to indicate that individuals were on the Alzheimer's continuum.[31] In a sensitivity analysis, we validated

results in a further specified MCI group, including those that were positive for Aβ and a tau biomarker (A+T+). For T+ we used CSF P-tau 217, which has been shown to be highly correlated to tau pathology in the brain.[32] We derived a cutoff value from P-tau 217 at the mean + 2 SD in Aβ- CU individuals of a larger population from the BioFINDER study (n=403). Using this cutoff, our MCI population was divided into A-T- MCI (N=93), A-T+ MCI (N=8), A+T- MCI (N=17), A+T+ MCI (N=113).

The statistical analyses were done in three steps. First, associations between baseline CSF neuroinflammatory biomarkers (used as predictors) and baseline WML volume (used as outcome) were tested in linear regression models for each neuroinflammatory biomarker separately, univariately and multivariately (including covariates age, gender, ICV, and Aβ status). In a sensitivity analyses, we also added CSF Aβ40 as an additional covariate to the models to correct for individual differences in CSF production.[33] We then evaluated vascular risk factors as possible confounding factors. All vascular risk factors that were univariately associated with WML in the subgroups of CU and MCI were included as covariates for the association between CSF biomarkers and WML. Regression analyses showed no evidence of multicollinearity. Next, a multivariable regression analysis was performed including all univariately significant neuroinflammatory markers and covariates in the same model. Then, associations between neuroinflammatory markers and baseline WML were analyzed by Aβ status.

Associations between baseline CSF biomarkers, covariates, and longitudinal WML volumes were analyzed using linear mixed effect models (LME), with age, gender, ICV, and Aβ status as covariates, and including random intercepts and slopes. Third, the effect of baseline neuroinflammatory markers and baseline WML volumes on longitudinal changes in cognition (MMSE, global CDR and mPACC) was tested. LME were used to test longitudinal changes in cognitive scores, and differences between diagnostic groups, and then

to test for effects of WML and neuroinflammatory markers on cognitive changes, in separate models, and in models that included both (and their interaction with time) simultaneously. All models were adjusted for age, gender, education, ICV, HCV, and Aβ status, including random intercepts and slopes. Adjustment for multiple comparisons was performed using the false discovery rate (FDR) according to Benjamini-Hochberg; $p_{FDR}<0.05$, indicating statistical significance for all regression models as well as LMEs. All subjects with available data were included in linear regression models and LME models, except one outlier on PlGF, which was excluded from analyses including PlGF concentration.

Statistical analyses were performed using SPSS version 26 (IBM SPSS Statistics for 240 Windows, Version 24.0, Armonk, NY: IBM Corp), and R (version 4.0.0).

Standard Protocol Approvals, Registrations, and Patient Consents

The study has been approved by the Regional Ethic Committee at Lund University, Sweden (Dnr 2010/156, and Dnr 695/2008). All participants gave written informed consent to participate in the study. Methods were carried out in accordance with the approved guidelines.

Data Availability

By request from a qualified academic investigator anonymized data will be shared for the sole purpose of replicating procedures, and results presented in the article. The data transfer needs to be in agreement with EU legislation on the general data protection regulation, and decisions by the Ethical Review Board of Sweden and Region Skåne, which should be regulated in a material transfer agreement.

Results

Demographics

The study population included 495 CU, and 247 MCI patients. Baseline characteristics are summarized in **Table 2.** The CU group was older (t=2.66, p=0.008), had more females ($\chi(1)=27.85$, p<0.001), longer education (t=4.10, p<0.001), more cases with hyperlipidemia ($\chi(1)= 45.23$, p<0.001), higher MMSE scores (t=15.76, p<0.001), less ICV (t=-5.55, p<0.001), and larger HCV (t=6.24, p<0.001) than the MCI group. The MCI group had more Aβ positive individuals ($\chi(1)=54.47$, p<0.001), more patients with a stroke ($\chi(1)=24.99$, p<0.001) and ischemic heart disease (IHD) ($\chi(1)=12.39$, p<0.001), and more WML (t=-7.30, p<0.001) than CU. The A+T+ MCI group did not differ from the A+ MCI group on any covariate.

Associations between CSF neuroinflammatory markers and WML at baseline

In univariate analyses, age, gender and ICV were significantly associated with WML, but there were no associations for CSF Aβ status. In multivariable analyses, associations for age and ICV with WML remained stable, while associations for gender and WML were attenuated (**eTable 2** in the Supplement). In CU, higher levels of IL-8 (β=0.07, p=0.03) were associated with greater WML volume in adjusted models (every standard deviation higher level of IL-8 was associated with 0.07 mm^3 increase in log 10 of WML volume). Higher levels of PlGF (β=0.17, p=0.001), and lower levels of sFlt-1 (β=-0.09, p=0.02) were also associated with greater WML volume. In the MCI group, higher levels of IL-6 (β=0.09, p=0.023), IL-8 (β=0.10, p=0.013), IL-16 (β=0.13, p=0.001), PlGF (β=0.17, p=0.001), VEGF-A (β=0.07, p=0.028), and VEGF-D (β=0.08, p=0.028) were associated with greater WML volume **(Figure 1**).

In a sensitivity analysis, CSF Aβ40 was added to the model as an additional covariate. The majority of the associations were not affected by this additional adjustment. But, in CU, the association for IL-16 (β=0.16, p=0.001), MCP-1 (β=0.05, p=0.048), VEGF (β=0.07, p=0.021) and VEGF-D (β=0.11, p=0.003) became significant, while the association for sFlt-1(β=-0.01, p=0.906) was lost. In MCI, the associations for IL-6 (β=0.03, p=0.431), IL-16 (β=0.02, p=0.818), VEGF-A (β=0.04, p=0.431), and VEGF-D (β=0.00, p=0.972) were lost, while PlGF and IL-8 remained significant (**eTable 3** in the Supplement).

In a further sensitivity analysis, we tested the associations between CSF biomarkers and WML when adjusting for potential confounding vascular risk factors. Atrial fibrillation, hypertension, and stroke were associated with WML in CU, whereas diabetes and stroke were associated with WML in MCI (Pearson correlation tests, P<0.05, r-coefficients 0.1 to 0.3). Therefore, these vascular risk factors were added as additional covariates to the main models. The associations with WML remained significant with this adjustment in CU for IL-8, PlGF and sFlt-1. In MCI, associations remained significant for IL-6, IL-8, PlGF, and VEGF-A (all p<0.05), but the association between VEGF-D and WML was attenuated (p=0.074). In those models, stroke (but not the other vascular risk factors) remained significantly associated with WML.

Independent effects of CSF neuroinflammatory markers on baseline WML

Significant biomarkers from univariate analyses were used simultaneously as predictors together with the covariates. In CU, PlGF (β=0.17, p<0.001), IL-8 (β=0.08, p=0.009), and sICAM-1 (β=-0.10, p=0.022) remained significant (R^2 = 0.36), meaning that 36% of WML volume can be explained by the predicting biomarker variables and the covariates with PlGF here being the strongest predictive biomarker. In MCI, PlGF (β=0.15, p<0.001), IL-16

(β=0.15, p=0.002), and sICAM-1 (β=-0.11, p=0.030), remaining significant (R^2 = 0.36). The R^2 for each marker separately is shown in **eTable 4** in the Supplement.

CSF neuroinflammatory markers and baseline WML by Aβ status

Finally, associations between neuroinflammatory markers and baseline WML were analyzed by Aβ status (**eTable 5** in the Supplement**)**. Higher PlGF was associated with more WML across all subgroups independent of Aβ status and cognitive state (**Figure 2**): Aβ+ CU PlGF (β=0.19, p=0.013), Aβ- CU PlGF (β=0.16, p=0.001), Aβ+ MCI PlGF (β=0.18, p=0.001), Aβ- MCI PlGF (β=0.16, p=0.007). In Aβ + MCI, this association remained significant, when restricting to the A+T+ MCI group (β=0.17, p=0.026) in a sensitivity analysis. Furthermore, in Aβ- CU lower sFlt-1(β=-0.12, p=0.039) was associated with more WML and in Aβ- MCI, higher IL-8 (β=0.14, p=0.039), higher IL-16 (β=0.18, p=0.009), and higher VEGF-A (β=0.18, p=0.007) were associated with more WML (**eFigure 1** in the Supplement).

CSF neuroinflammatory markers and longitudinal WML volume

Our data showed an increase of WML over time, both in subjects with low and high WML burden at baseline (**eFigure 2** in the Supplement). When using longitudinal WML as outcome, lower PlGF (β=-0.01, p=0.021) was associated with increasing WML over time in CU. In MCI, lower IL-6 (β=-0.01, p=0.028), IL-8 (β=-0.01, p=0.033), IL-16 (β=-0.01, p=0.028), MCP-1 (β=-0.01, p=0.052), PlGF (β=-0.01, p=0.004), and VEGF-A (β=-0.01, p=0.028) were associated with increasing WML over time. We also tested these analyses when stratifying by Aβ-status. Associations between lower PlGF and increase in WML volume were seen in Aβ+ CU (β=-0.02, p=0.032), and Aβ+ MCI (β=-0.03, p=0.031), but not in Aβ- subgroups. Greater age and ICV were associated with more longitudinal WML (p<0.05) in subgroups, both when including biomarkers and when used alone without

biomarkers. Female sex and positive Aβ status were associated with more longitudinal WML in CU ($p<0.05$) in both models, but not in MCI. The effect of positive Aβ status on WML in CU was stable in models without biomarker (β =0.012, p=0.014), and when used together with PlGF (β =0.011, p=0.025). In CU, PlGF had no effect in the unadjusted model due to the lack of ICV as a covariate (**eTable 6** in the Supplement).

CSF neuroinflammatory markers and baseline WML predicting longitudinal cognitive decline separately

Cognition generally declined over time in both groups (**eFigure 3** in the Supplement). A decline in MMSE was observed in CU (β=-0.26, $p<0.001$, meaning a decline in 0.26 units per year), with a steeper decline in MCI (group*time interaction term: β=-1.39, $p<0.001$, meaning that MMSE declined 1.39 units more per year in MCI than in CU, on average). For CDR, an increase (indicating worse cognition) over time in the CU group (β=0.03, $p<0.001$), with a steeper increase in the MCI group (group*time: β=0.16, $p<0.001$) was observed. Cognitive decline was also seen in mPACC scores in CU (β=-0.08, $p<0.001$) with a steeper decline in MCI (group*time in: β=-0.27, $p<0.001$).

We then tested associations between neuroinflammatory markers, WML volume, and longitudinal MMSE score. When testing for the univariate effect in subgroups, no associations were found between neuroinflammatory markers and longitudinal MMSE. However, greater baseline WML volume was associated with longitudinal decline in MMSE in CU (β=-0.11, p=0.001), and MCI (β=-0.19, p=0.028) (**Table 3**).

Next, we tested global CDR as outcome. In the CU group, associations were seen between higher IL-8 (β=0.01, p=0.001), MCP-1 (β=0.01, p=0.001), sICAM-1 (β=0.01, p=0.005), sVCAM-1 (β=0.01, p=0.001), and VEGF-A (β=0.01, p=0.024), and increased longitudinal global CDR. No associations were seen in the MCI group. Associations between

more WML and higher longitudinal CDR were seen in CU (β=0.01, p=0.004), and MCI (β=0.03, p=0.012) (**Table 3**).

No associations were seen between neuroinflammatory markers and mPACC composite score in any groups Main effects between more WML and declining mPACC score in CU (β=-0.03, p<0.001), but not in MCI were found (**eTable 7** in the Supplement).

Combining CSF neuroinflammatory markers and baseline WML to predict longitudinal cognitive decline

We then tested to what degree associations in CU for WML and neuroinflammatory markers with CDR were independent. For all neuroinflammatory markers that were significant univariately, we tested models that also included WML (and its interaction with time) as an additional predictor of longitudinal decline. Associations for both, biomarkers and WML, with longitudinal CDR in CU remained significant, when adjusting for the other modality (**Table 3**). These analyses were not relevant for MMSE or mPACC, since there were no univariate associations for neuroinflammatory biomarkers and longitudinal MMSE or mPACC in the groups.

Discussion

The current study investigated associations between CSF biomarkers of neuroinflammation and cerebrovascular dysfunction, SVD, and longitudinal cognitive decline, in both CU and patients with MCI.

The proinflammatory markers IL-8, and biomarkers of cerebrovascular dysfunction such as PlGF and sFlt-1 were associated with greater WML volume in CU. In MCI, not only IL-8 and PlGF, but also IL-6, IL-16, VEGF-A, and VEGF-D were significantly

associated with greater WML volume. We note that the general levels of WML were greater in MCI than in CU, which may increase the power to detect associations with neuroinflammatory biomarkers. Associations between higher levels of serum IL-8 and WML in cognitively impaired no dementia, and AD have been described before,[34] as well as involvement of IL-8, sFlt-1 and VEGF-A in BBB impairment.[9,13,18] We therefore suggest that upregulation of those cytokines could be involved in BBB impairment and contribute to the progress of WML (**Figure 3**). IL-16 is a cytokine, which may be involved in cell recruitment and activation at sites of inflammation. It seems to increase in areas of neurodegeneration and inflammation in the brain.[35] In a recent study, a higher trend of CSF IL-16 concentration was observed in BBB impairment (defined as CSF albumin index ≥9).[9]

According to other studies, the proinflammatory markers IL-8 and IL-6 are produced by microglia, astrocytes, and endothelial cells, and could be involved in stimulating growth factor production.[16,36] In our study, elevated concentrations of different members of the VEGF family were associated with greater WML volume. PlGF and VEGF-A are thought to mediate angiogenesis and regulate vascularization, as well as induce vascular permeability.[37,38] Our findings suggest that markers of the VEGF family, especially PlGF, may be involved in the pathophysiology around WML, possibly by its contribution to vascular permeability and neuroinflammation.[37] When examining groups based on presence of the Alzheimer´s continuum (Aβ positive versus Aβ negative) separately, the above described inflammatory makers were only associated with more WML in Aβ negative subgroups, except for PlGF. Most strikingly, higher PlGF levels were associated with greater WML volumes across all groups, independently of Aβ status and cognitive impairment, supporting converging pathways between neuroinflammation and cerebrovascular pathology. Therefore, PlGF seems to be a general marker of WML. Interestingly, Winder et al. found that plasma PlGF and VEGF were associated with cerebral amyloid angiopathy (CAA), another

marker of SVD which was obtained by post mortem neuropathological examination.[39] This supports our findings that there is an association between processes of angiogenesis and SVD. PlGF upregulation can be induced by different stimuli including hypoxia, other inflammatory cytokines, growth factors, or oncogenes.[40] Compared to VEGF-A (which binds to VEGFR-1 receptor and VEGFR-2 receptor), PlGF only binds to VEGFR-1 receptor, which is considered the main signaling receptor in angiogenesis.[40] The soluble receptor isoform of VEGFR-1, sFlt-1, which can be spliced from VEGFR-1 can bind and inhibit the action of PlGF and VEGF-A, followed by reduced blood vessel growth. Our results showed associations between lower concentrations of sFlt-1 (which is thought to counterbalance PlGF) and WML, suggesting that it is possible that there is increased angiogenesis in individuals with more WML (**Figure 3**).

Growth factors of the VEGF family play an important role in protection and recovery after ischemia, regulating angiogenesis, and neurogenesis.[41,42] Our longitudinal results unexpectedly showed that lower levels of PlGF were associated with more rapid longitudinal WML volume increase in both groups, with a slightly stronger association in MCI. Also, lower IL-6, IL-8, IL-16, MCP-1, and VEGF-A were associated with longitudinal increase in WML in MCI. This was a surprising finding, since we expected that higher biomarker levels would be associated with more WML over time. Dobrynina et al. described lower VEGF-A levels at baseline being associated with more WML in a group of patients with increased prevalence of periventricular WML as well as more atrophy in general.[43] One possibility is that there are associations between widespread vascular wall damage destroying endothelial cells, and lower growth factor production.[43,44] This could then be followed by a decline in cerebrovascular angiogenesis resulting in hypoxia-induced capillary loss and more WML.[45] In a postmortem analyses decreased concentrations of several cytokines including IL-16, IL-8, and IL-6 in the frontal white matter across different dementia groups compared to

controls without dementia were described.[46] Venous collagenosis – which is associated with WML – is thought to cause venous insufficiency resulting in vessel leakage and vasogenic edema, which may contribute to dynamic changes in WML volumes over time.[47] Taken together, the literature may be in agreement with our observation of associations between lower biomarker levels and increased WML over time. The magnitudes of the associations between biomarkers and longitudinal WML in our study were small. Since there were associations at baseline between higher biomarker levels and more WML, the longitudinal results may also partly be caused by a regression to the mean phenomenon. We also analyzed the effect of covariates in univariate analyses. We conclude that positive Aβ status predicts more WML over time in CU (but not in MCI), in a manner that seems to be independent of the biomarkers tested here. Considering all of the above, the associations between biomarkers and longitudinal WML need to be validated in an independent cohort.

Finally, we analyzed independent contributions of inflammatory markers and WML to predict longitudinal cognitive decline. IL-8, IL-15, MCP-1, sICAM-1, sVCAM-1, sFlt-1, and VEGF-A have been described previously to be associated with cognitive decline and may be involved in BBB impairment.[9,13,18] We found that the effects of WML on cognitive decline were independent of biomarker levels. Some biomarkers (IL-8, MCP-1, sICAM-1, sVCAM-1, and VEGF-A) had independent effects on cognition in the CU group even when adjusting for WML, suggesting that this represents a partly distinct pathway leading to cognitive decline.

The strengths of this study include a large sample size of CU and MCI subjects as well as the multimodal design with a broad panel of neuroinflammatory markers. Studies with these neuroinflammatory markers in CSF in relation to vascular pathology are rare. Previous studies mainly focused on plasma measurements, which may be less accurate for changes in the central nervous system.[17,39] A weakness is the lack of patients with a diagnosis of vascular dementia. Since the cohort was recruited from a Swedish memory clinic (mainly

of European ancestry), results may not be completely generalizable to the general population or people with other ethnical backgrounds. In addition, it is possible that different pathological processes in the brain may affect CSF levels of neuroinflammatory markers. We tried to overcome this by adjusting for different relevant factors (including biomarkers representing both Aβ pathology, and in MCI also tau pathology). We adjusted associations between biomarkers and WML for possible confounding vascular risk factors. Most associations (all but WML versus CSF VEGF-D in MCI) remained significant. Finally, we only had cross-sectional data on CSF biomarkers, and could not study how these CSF biomarkers change dynamically in relation to cognition and WML.

There is a lack of validated fluid biomarkers for different aspects of vascular pathology in neurodegenerative diseases. Our findings strongly support CSF PlGF as a biomarker of vascular brain changes independent of AD co-pathology, which may have clinical importance. However, current results are not conclusive on how to use the studied biomarkers in clinical practice. Future studies may specifically test the additional value of CSF PlGF in clinical management of patients with brain diseases and its use together with fluid biomarkers of other brain pathologies, e.g. CSF Aβ42 and P-tau for AD. Truly longitudinal biomarker studies are needed to determine the role of CSF neuroinflammatory markers (especially PlGF), in relation to WML and other markers of SVD.

In conclusion, this study strengthens the role of CSF PlGF as a potential biomarker for WML, independent of AD pathology. In addition, we provide a comprehensive analysis on the relation between inflammatory markers and WML, showing that several proinflammatory markers and markers of angiogenesis are associated with WML. Surprisingly, there were inverse associations between some of the baseline measures of biomarkers and longitudinal WML changes, which need replication. Longitudinal analyses of cognition showed independent effects of CSF inflammatory markers and WML on longitudinal cognition, especially in people without cognitive impairment at baseline.

Appendix 1 Authors

Name	Location	Contribution
Eske Christiane Gertje, MD	University of Lund, Sweden	Co-designed the study, analyzed and interpreted the data; wrote and drafted the manuscript for intellectual content
Shorena Janelidze, PhD	University of Lund, Sweden	Co-designed the study, performed biochemical analyses, gave advice on data analysis, and reviewed the manuscript for intellectual content
Danielle van Westen, MD, PhD	University of Lund, Sweden	Visually rated WML in T2 MRIs, gave advice on data analysis, reviewed the manuscript for intellectual content
Nicholas Cullen, BS	University of Lund, Sweden	Wrote the R script for mixed effect model analyses
Erik Stomrud, MD, PhD	University of Lund, Sweden	Collected clinical data, oversaw study logistics, and reviewed the manuscript for intellectual content
Sebastian Palmqvist,	University of Lund,	Acquired clinical data, gave advise on data analysis, and reviewed the manuscript for

MD, PhD	Sweden	intellectual content
Niklas Mattsson-Carlgren, MD, PhD	University of Lund, Sweden	Co-designed the study, gave advise on data analysis; reviewed the manuscript for intellectual content
Oskar Hansson, MD, PhD	University of Lund, Sweden	Co-designed the study, gave advice on data analysis; reviewed the manuscript for intellectual content

WNL-2023-000028_sup --- http://links.lww.com/WNL/C673

References

1. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. *Lancet Neurol*. May 2013;12(5):483-97. doi:10.1016/s1474-4422(13)70060-7

2. Gunning-Dixon FM, Raz N. The cognitive correlates of white matter abnormalities in normal aging: a quantitative review. *Neuropsychology*. Apr 2000;14(2):224-32. doi:10.1037//0894-4105.14.2.224

3. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol*. Jul 2010;9(7):689-701. doi:10.1016/s1474-4422(10)70104-6

4. Arba F, Giannini A, Piccardi B, et al. Small vessel disease and biomarkers of endothelial dysfunction after ischaemic stroke. *Eur Stroke J*. Jun 2019;4(2):119-126. doi:10.1177/2396987318805905

5. Markus HS, Hunt B, Palmer K, Enzinger C, Schmidt H, Schmidt R. Markers of endothelial and hemostatic activation and progression of cerebral white matter hyperintensities: longitudinal results of the Austrian Stroke Prevention Study. *Stroke*. Jul 2005;36(7):1410-4. doi:10.1161/01.STR.0000169924.60783.d4

6. Heneka MT, Carson MJ, El Khoury J, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol*. Apr 2015;14(4):388-405. doi:10.1016/s1474-4422(15)70016-5

7. Hampel H, Caraci F, Cuello AC, et al. A Path Toward Precision Medicine for Neuroinflammatory Mechanisms in Alzheimer's Disease. *Front Immunol*. 2020;11:456. doi:10.3389/fimmu.2020.00456

8. Singh D. Astrocytic and microglial cells as the modulators of neuroinflammation in Alzheimer's disease. *J Neuroinflammation*. Aug 17 2022;19(1):206. doi:10.1186/s12974-022-02565-0

9. Bowman GL, Dayon L, Kirkland R, et al. Blood-brain barrier breakdown, neuroinflammation, and cognitive decline in older adults. *Alzheimers Dement*. Dec 2018;14(12):1640-1650. doi:10.1016/j.jalz.2018.06.2857

10. Al-Bachari S, Naish JH, Parker GJM, Emsley HCA, Parkes LM. Blood-Brain Barrier Leakage Is Increased in Parkinson's Disease. *Front Physiol*. 2020;11:593026. doi:10.3389/fphys.2020.593026

11. Iadecola C. The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. *Acta Neuropathol*. Sep 2010;120(3):287-96. doi:10.1007/s00401-010-0718-6

12. Knowland D, Arac A, Sekiguchi KJ, et al. Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. *Neuron*. May 7 2014;82(3):603-17. doi:10.1016/j.neuron.2014.03.003

13. Janelidze S, Mattsson N, Stomrud E, et al. CSF biomarkers of neuroinflammation and cerebrovascular dysfunction in early Alzheimer disease. *Neurology*. Aug 28 2018;91(9):e867-e877. doi:10.1212/wnl.0000000000006082

14. Popp J, Oikonomidi A, Tautvydaitė D, et al. Markers of neuroinflammation associated with Alzheimer's disease pathology in older adults. *Brain Behav Immun*. May 2017;62:203-211. doi:10.1016/j.bbi.2017.01.020

15. Low A, Mak E, Rowe JB, Markus HS, O'Brien JT. Inflammation and cerebral small vessel disease: A systematic review. *Ageing Res Rev*. Aug 2019;53:100916. doi:10.1016/j.arr.2019.100916

16. Janelidze S, Lindqvist D, Francardo V, et al. Increased CSF biomarkers of angiogenesis in Parkinson disease. *Neurology*. Nov 24 2015;85(21):1834-42. doi:10.1212/wnl.0000000000002151

17. Kapoor A, Gaubert A, Marshall A, et al. Increased Levels of Circulating Angiogenic Cells and Signaling Proteins in Older Adults With Cerebral Small Vessel Disease. *Front Aging Neurosci*. 2021;13:711784. doi:10.3389/fnagi.2021.711784

18. Taipa R, das Neves SP, Sousa AL, et al. Proinflammatory and anti-inflammatory cytokines in the CSF of patients with Alzheimer's disease and their correlation with cognitive decline. *Neurobiol Aging*. Apr 2019;76:125-132. doi:10.1016/j.neurobiolaging.2018.12.019

19. Quick S, Moss J, Rajani RM, Williams A. A Vessel for Change: Endothelial Dysfunction in Cerebral Small Vessel Disease. *Trends Neurosci*. Apr 2021;44(4):289-305. doi:10.1016/j.tins.2020.11.003

20. Gertje EC, van Westen D, Panizo C, Mattsson-Carlgren N, Hansson O. Association of Enlarged Perivascular Spaces and Measures of Small Vessel and Alzheimer Disease. *Neurology*. Jan 12 2021;96(2):e193-e202. doi:10.1212/wnl.0000000000011046

21. Jack CR, Jr., Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. Apr 2018;14(4):535-562. doi:10.1016/j.jalz.2018.02.018

22. Palmqvist S, Zetterberg H, Blennow K, et al. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid β-amyloid 42: a cross-validation study against amyloid positron emission tomography. *JAMA Neurol*. Oct 2014;71(10):1282-9. doi:10.1001/jamaneurol.2014.1358

23. Binette AP, Janelidze S, Cullen N, et al. Confounding factors of Alzheimer's disease plasma biomarkers and their impact on clinical performance. *medRxiv*. 2022:2022.05.30.22275718. doi:10.1101/2022.05.30.22275718

24. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. Nov 1975;12(3):189-98. doi:10.1016/0022-3956(75)90026-6

25. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology*. Nov 1993;43(11):2412-4. doi:10.1212/wnl.43.11.2412-a

26. Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. *Am J Psychiatry*. Nov 1984;141(11):1356-64. doi:10.1176/ajp.141.11.1356

27. Reitan R, Wolfson D. *The Halstead–Reitan Neuropsycholgical Test Battery: Therapy and clinical interpretation*. 1985.

28. Strauss E, Sherman E, Spreen O. *A compendium of neuropsychological tests: Administration, norms and commentary (3rd ed).* 2006.

29. Mattsson-Carlgren N, Janelidze S, Palmqvist S, et al. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain*. Dec 5 2020;143(11):3234-3241. doi:10.1093/brain/awaa286

30. Papp KV, Rentz DM, Orlovsky I, Sperling RA, Mormino EC. Optimizing the preclinical Alzheimer's cognitive composite with semantic processing: The PACC5. *Alzheimers Dement (N Y)*. Nov 2017;3(4):668-677. doi:10.1016/j.trci.2017.10.004

31. Janelidze S, Zetterberg H, Mattsson N, et al. CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol*. Mar 2016;3(3):154-65. doi:10.1002/acn3.274

32. Leuzy A, Janelidze S, Mattsson-Carlgren N, et al. Comparing the Clinical Utility and Diagnostic Performance of CSF P-Tau181, P-Tau217, and P-Tau231 Assays. *Neurology*. Oct 26 2021;97(17):e1681-e1694. doi:10.1212/wnl.0000000000012727

33. Guo T, Korman D, La Joie R, et al. Normalization of CSF pTau measurement by Aβ(40) improves its performance as a biomarker of Alzheimer's disease. *Alzheimers Res Ther*. Aug 15 2020;12(1):97. doi:10.1186/s13195-020-00665-8

34. Zhu Y, Chai YL, Hilal S, et al. Serum IL-8 is a marker of white-matter hyperintensities in patients with Alzheimer's disease. *Alzheimers Dement (Amst)*. 2017;7:41-47. doi:10.1016/j.dadm.2017.01.001

35. Guo LH, Mittelbronn M, Brabeck C, Mueller CA, Schluesener HJ. Expression of interleukin-16 by microglial cells in inflammatory, autoimmune, and degenerative lesions of the rat brain. *J Neuroimmunol*. Jan 2004;146(1-2):39-45. doi:10.1016/j.jneuroim.2003.09.017

36. Loeffler S, Fayard B, Weis J, Weissenberger J. Interleukin-6 induces transcriptional activation of vascular endothelial growth factor (VEGF) in astrocytes in vivo

and regulates VEGF promoter activity in glioblastoma cells via direct interaction between STAT3 and Sp1. *Int J Cancer*. Jun 10 2005;115(2):202-13. doi:10.1002/ijc.20871

37. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. *Proc Natl Acad Sci U S A*. Nov 15 1993;90(22):10705-9. doi:10.1073/pnas.90.22.10705

38. Keck PJ, Hauser SD, Krivi G, et al. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science*. Dec 8 1989;246(4935):1309-12. doi:10.1126/science.2479987

39. Winder Z, Sudduth TL, Anderson S, et al. Examining the association between blood-based biomarkers and human post mortem neuropathology in the University of Kentucky Alzheimer's Disease Research Center autopsy cohort. *Alzheimers Dement*. Mar 10 2022;doi:10.1002/alz.12639

40. Albonici L, Giganti MG, Modesti A, Manzari V, Bei R. Multifaceted Role of the Placental Growth Factor (PlGF) in the Antitumor Immune Response and Cancer Progression. *Int J Mol Sci*. Jun 18 2019;20(12)doi:10.3390/ijms20122970

41. Geiseler SJ, Morland C. The Janus Face of VEGF in Stroke. *Int J Mol Sci*. May 4 2018;19(5)doi:10.3390/ijms19051362

42. Ma Y, Zechariah A, Qu Y, Hermann DM. Effects of vascular endothelial growth factor in ischemic stroke. *J Neurosci Res*. Oct 2012;90(10):1873-82. doi:10.1002/jnr.23088

43. Dobrynina LA, Zabitova MR, Shabalina AA, et al. MRI Types of Cerebral Small Vessel Disease and Circulating Markers of Vascular Wall Damage. *Diagnostics (Basel)*. May 29 2020;10(6)doi:10.3390/diagnostics10060354

44. Lee S, Chen TT, Barber CL, et al. Autocrine VEGF signaling is required for vascular homeostasis. *Cell*. Aug 24 2007;130(4):691-703. doi:10.1016/j.cell.2007.06.054

45. Brown WR, Thore CR. Review: cerebral microvascular pathology in ageing and neurodegeneration. *Neuropathol Appl Neurobiol*. Feb 2011;37(1):56-74. doi:10.1111/j.1365-2990.2010.01139.x

46. Chen A, Oakley AE, Monteiro M, et al. Multiplex analyte assays to characterize different dementias: brain inflammatory cytokines in poststroke and other dementias. *Neurobiol Aging*. Feb 2016;38:56-67. doi:10.1016/j.neurobiolaging.2015.10.021

47. Black S, Gao F, Bilbao J. Understanding white matter disease: imaging-pathological correlations in vascular cognitive impairment. *Stroke*. Mar 2009;40(3 Suppl):S48-52. doi:10.1161/strokeaha.108.537704

48. Carmeliet P, Moons L, Luttun A, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med*. May 2001;7(5):575-83. doi:10.1038/87904

Table 1 Neuroinflammatory and cerebrovascular biomarkers

Group	Biomarker	Functions	Cell origin in the brain
Proinflammatory markers	**IL-6**	Regulate immune reactivity, the acute-phase response, inflammation, oncogenesis and hematopoiesis	Secreted by microglia and astrocytes, or endothelial cells and fibroblasts
	IL-8	Initiate acute inflammation, induces chemotaxis in target cells, migrating neutrophils, basophils, and T cells to the site of infection	Produced by macrophages and endothelial cells
Cytokines	**IL-15**	Induce natural killer cells	Expressed by microglia
	IL-16	Contributes to the regulatory process of CD4+ cell recruitment and activation at sites of inflammation	Produced by microglia
	IL-7	Involved in the development of an effective immune system, and in the generation and maintenance of strong and effective cellular immune responses; increases numbers of astroglia and microglia	Secreted by immune and non-immune cells
Chemokines	**IP-10**	Trafficking of immune cells to inflammatory sites	Produced by monocytes, lymphocytes, endothelial cells
	MCP-1	Guide cells towards inflammation, induce chemotaxis in nearby responsive cells (induces microglia activation)	Secreted by microglia and astrocytes
Markers of vascular injury	**sICAM-1**	Endothelial activation, leukocyte adhesion to endothelial cells	Secreted by microglia and astrocytes
	sVCAM-1	Endothelial activation, leukocytes adhesion to endothelial cells	Secreted by microglia and astrocytes
Markers of angiogenesis	**PlGF**	Mediate angiogenesis associated with ischemia, hematologic diseases and cancer;	Produced by endothelial cells
	sFlt-1	Counterbalance VEGF family members, and is involved in negative regulation of angiogenesis, as well as in glia cell development and neurogenesis	Receptor of the VEGF family with co-expression on endothelial cells. Expressed in microglia cells
	VEGF-A	Mediate angiogenesis and induces vascular permeability	Produced by endothelial cells and astrocytes
	VEGF-D	Promote angiogenesis and the remodeling of lymphatics	Produced by endothelial cells

Abbreviations: IL, interleukin; IP-10, interferon-gamma induced protein-10; MCP-1, monocyte chemoattractant protein 1; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular adhesion molecule 1; PlGF, placental growth factor; sFlt-1, soluble fms-related tyrosine kinase 1; VEGF, vascular endothelial growth factor (VEGF-A, and VEGF-D). A version of this table, with references included to literature, is available as **eTable 8** in the Supplement.

Table 2 Demographics

	CU (n=495)	MCI (n=247)
Age (years)	72.02 (5.52)	70.89 (5.45)**
Gender (F/M (%))	294/201 (59/41)	96/151 (39/61)***
Education (years)	12.29 (3.60)	11.14 (3.59)***
MMSE (score)	28.86 (1.17)	27.09 (1.89)***
Aβ pathologic CSF ratio (yes/no (%))	153/342 (31/69)	146/101 (59/41)***
***APOE* ε4 (pos/neg (%))**	160/333 (32/68)	129/118 (52/48)***
CSF/plasma albumin ratio	5.87 (2.30)	6.28 (3.95)
HCV (mm^3)	3410.55 (427.92)	3188.82 (469.85)***
ICV (mm^3)	1487242.43 (145273.62)	1551066.34 (152606.68)***
Anti-inflammatory drugs (yes/no (%))	67/428 (13/87)	23/224 (9/91)
Stroke (yes/no (%))	22/473 (4/96)	37/210 (15/85)***
Hypertension (yes/no (%))	180/315 (36/64)	79/168 (32/68)
Diabetes (yes/no (%))	45/450 (9/91)	24/223 (10/90)
Ischemic heart disease (yes/no (%))	37/458 (7/93)	39/208 (16/84)***
Atrial Fibrillation (yes/no (%))	13/481 (3/97)	12/234 (5/95)
Congestive heart failure (yes/no (%))	6/488 (1/99)	4/242 (2/98)
Hyperlipidemia (yes/no (%))	149/345 (30/70)	20/226 (8/92)***
Smoking (yes/no (%))	40/454 (8/92)	26/220 (11/89)
WML volume (mm^3)	11.53 (14.00)	25.67 (28.78)***
IL-6 (pg/ml)	0.71 (1.11)	0.82 (1.17)
IL-8 (pg/ml)	35.09 (19.10)	37.87 (15.10)*
IL-15 (pg/ml)	3.16 (0.90)	3.18 (1.04)
IL-16 (pg/ml)	7.26 (2.12)	7.57 (2.60)
IL-7 (pg/ml)	1.34 (0.60)	1.36 (0.53)
IP-10 (µg /ml)	0.31 (0.20)	0.350 (0.59)
MCP-1 (µg /ml)	0.37 (0.11)	0.393 (0.12)**
sICAM-1 (µg/ml)	3.10 (0.95)	3.24 (1.35)
sVCAM-1 (µg/ml)	9.43 (2.69)	9.76 (3.56)
PlGF$^+$ (µg /ml)	0.15 (0.05)	0.17 (0.06)***
sFlt-1 (pg/ml)	32.43 (10.76)	31.93 (12.46)
VEGF-A (pg/ml)	4.81 (1.51)	5.10 (2.42)
VEGF-D (pg/ml)	37.38 (13.15)	38.49 (14.42)

Abbreviations: CU, cognitively unimpaired; MCI, mild cognitive impairment; MMSE, Mini Mental State Examination; HCV, hippocampal volume; ICV, intracranial volume; WML, white matter lesions; IL, interleukin; IP-10, interferon-gamma induced protein-10; MCP-1, monocyte chemoattractant protein 1; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular adhesion molecule 1; PlGF, placental growth factor; sFlt-1, soluble fms-related tyrosine kinase 1; VEGF, vascular endothelial growth factor (VEGF-A, and VEFG-D). *** $p<0.001$ significant difference between groups, ** $p<0.01$ significant difference between groups, * $p<0.05$ significant difference between groups; $^+$ One subject was an outlier in PlGF concentrations and was excluded from PlGF analyses.

Table 3 Associations between neuroinflammatory markers, WMLs and MMSE and global CDR.

	MMSE				CDR			
	CU		MCI		CU		MCI	
Univariate	**Effect Size (95% CI)**	***p* Value**	**Effect size (95% CI)**	***p* Value**	**Effect Size (95% CI)**	***p* Value**	**Effect size (95% CI)**	***p* Value**
IL-6*	0.02 (-0.04- 0.08)	0.787	-0.10 (-0.28- 0.08)	0.562	2.27E-03 (-5.04E-03, 9.58E-03)	0.642	6.77E-03 (-2.07E-02, 3.43E-02)	0.683
IL-8*	-0.05 (-0.11- 0.01)	0.299	-0.15 (-0.33- 0.03)	0.562	1.41E-02 (6.99E-03, 2.12E-02)	0.001	2.41E-02 (-3.06E-03, 5.13E-02)	0.615
IL-15	0.01 (-0.05- 0.07)	0.870	-0.03 (-0.21- 0.14)	0.924	1.31E-03 (-6.61E-03, 9.24E-03)	0.746	1.14E-02 (-1.51E-02, 3.78E-02)	0.581
IL-16	-0.05 (-0.12- 0.01)	0.299	-0.16 (-0.34- 0.02)	0.562	9.48E-03 (5.30E-04, 1.84E-02)	0.076	3.35E-02 (5.44E-03, 6.16E-02)	0.203
IL-7	-0.05 (-0.11- 0.01)	0.299	-0.12 (-0.30- 0.06)	0.562	6.10E-03 (-4.60E-04, 1.27E-02)	0.090	3.18E-02 (3.02E-03, 6.06E-02)	0.203
IP-10*	-0.01 (-0.07- 0.06)	0.870	-0.11 (-0.29- 0.08)	0.562	7.63E-03 (1.60E-04, 1.51E-02)	0.076	2.67E-02 (-5.70E-04, 5.40E-02)	0.243
MCP-1	-0.08 (-0.15- -0.02)	0.138	-0.11 (-0.29- 0.07)	0.562	1.39E-02 (6.38E-03, 2.13E-02)	0.001	1.21E-02 (-1.42E-02, 3.84E-02)	0.581
sICAM-1	-0.05 (-0.11- 0.02)	0.355	-0.05 (-0.22- 0.13)	0.882	1.34E-02 (5.19E-03, 2.17E-02)	0.005	1.24E-02 (-1.02E-02, 3.51E-02)	0.581
sVCAM-1	-0.06 (-0.12- 0.01)	0.299	0.06 (-0.12- 0.24)	0.882	1.47E-02 (6.82E-03, 2.26E-02)	0.001	8.09E-03 (-1.65E-02, 3.27E-02)	0.615
PlGF	-0.01 (-0.08- 0.05)	0.855	0.01 (-0.17- 0.19)	0.942	7.25E-03 (-3.60E-04, 1.49E-02)	0.090	9.15E-03 (-1.67E-02, 3.50E-02)	0.615
sFlt-1	0.03 (-0.03- 0.10)	0.552	-0.06 (-0.25- 0.12)	0.882	-1.61E-03 (-9.89E-03, 6.67E-03)	0.746	1.20E-02 (-1.50E-02, 3.91E-02)	0.581
VEGF-A	-0.02 (-0.08- 0.05)	0.855	0.02 (-0.16- 0.19)	0.924	1.15E-02 (2.88E-03, 2.01E-02)	0.024	1.45E-02 (-5.55E-03, 3.46E-02)	0.411
VEGF-D	-0.01 (-0.07- 0.06)	0.870	0.01 (-0.17- 0.19)	0.924	7.35E-03 (1.20E-04, 1.46E-02)	0.076	1.99E-03 (-2.39E-02, 2.79E-02)	0.881
WML	-0.11 (-0.17, -0.05)	0.001	-0.19 (-0.37 -0.02)	0.028	1.00E-02 (3.00E-03, 1.70E-02)	0.004	3.40E-02 (8.00E-03, 6.10E-02)	0.012
Multivariate								
IL-6*	NA	NA	NA	NA	NA	NA	NA	NA
IL-8*	NA	NA	NA	NA	1.20E-02 (5.00E-03, 1.90E-02) [a]	0.002	NA	NA
IL-15	NA	NA	NA	NA	NA	NA	NA	NA
IL-16	NA	NA	NA	NA	NA	NA	NA	NA
IL-7	NA	NA	NA	NA	NA	NA	NA	NA
IP-10*	NA	NA	NA	NA	NA	NA	NA	NA
MCP-1	NA	NA	NA	NA	1.20E-02 (5.00E-03, 1.90E-02) [a]	0.002	NA	NA
sICAM-1	NA	NA	NA	NA	1.10E-02 (4.00E-03, 1.80E-02) [a]	0.003	NA	NA
sVCAM-1	NA	NA	NA	NA	1.20E-02 (5.00E-03, 1.90E-02) [a]	0.002	NA	NA
PlGF	NA [a]	NA	NA	NA	NA	NA	NA	NA
sFlt-1	NA	NA	NA	NA	NA	NA	NA	NA
VEGF-A	NA	NA	NA	NA	8.00E-03 (1.00E-03, 1.50E-02) [a]	0.022	NA	NA
VEGF-D	NA	NA	NA	NA	NA	NA	NA	NA

The top part of the table shows univariate analyses for individual biomarkers and WML to predict MMSE and CDR. For significant neuroinflammatory biomarkers, we also tested combined models where they were used together with WML as predictors, shown in the lower part of the table. Abbreviations: MMSE, Mini-Mental State Examination; global CDR, global Clinical Dementia Rating; CU, cognitively unimpaired; MCI, mild cognitive impairment; interleukin (IL); IP-10, interferon-gamma induced protein-10; MCP-1, monocyte chemoattractant protein 1; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular adhesion molecule 1; PlGF, placental growth factor; sFlt-1, soluble fms-related tyrosine kinase 1;VEGF, vascular endothelial growth factor (VEGF-A, and VEFG-D). *log10 transformed.[a] In these multivariate analyses, effects of WML on longitudinal cognitive measurement remained significant (with $p<0.05$). All *p* values reported are FDR corrected. Linear regression models were adjusted for age, gender, intracranial volume, and CSF Aβ status. and all p-values corrected for multiple comparison.

Figure 1 Associations between different neuroinflammatory markers and WML summarized in forest plots for CU and MCI.

Abbreviations: WML, white matter lesions; CU, cognitively unimpaired; MCI, mild cognitive impairment; IL, interleukin; IP-10, interferongamma induced protein-10; MCP-1, monocyte chemoattractant protein 1; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular adhesion molecule 1; PlGF, placental growth factor; sFlt-1, soluble fms-related tyrosine kinase 1; VEGF, vascular endothelial growth factor (VEGF-A, and VEFG-D).*log10 transformed. RED= significant after correction for multiple comparison with $p<0.05$. Linear regression models were adjusted for age, gender, intracranial volume, and CSF Aβ status, and all p-values corrected for multiple comparison.

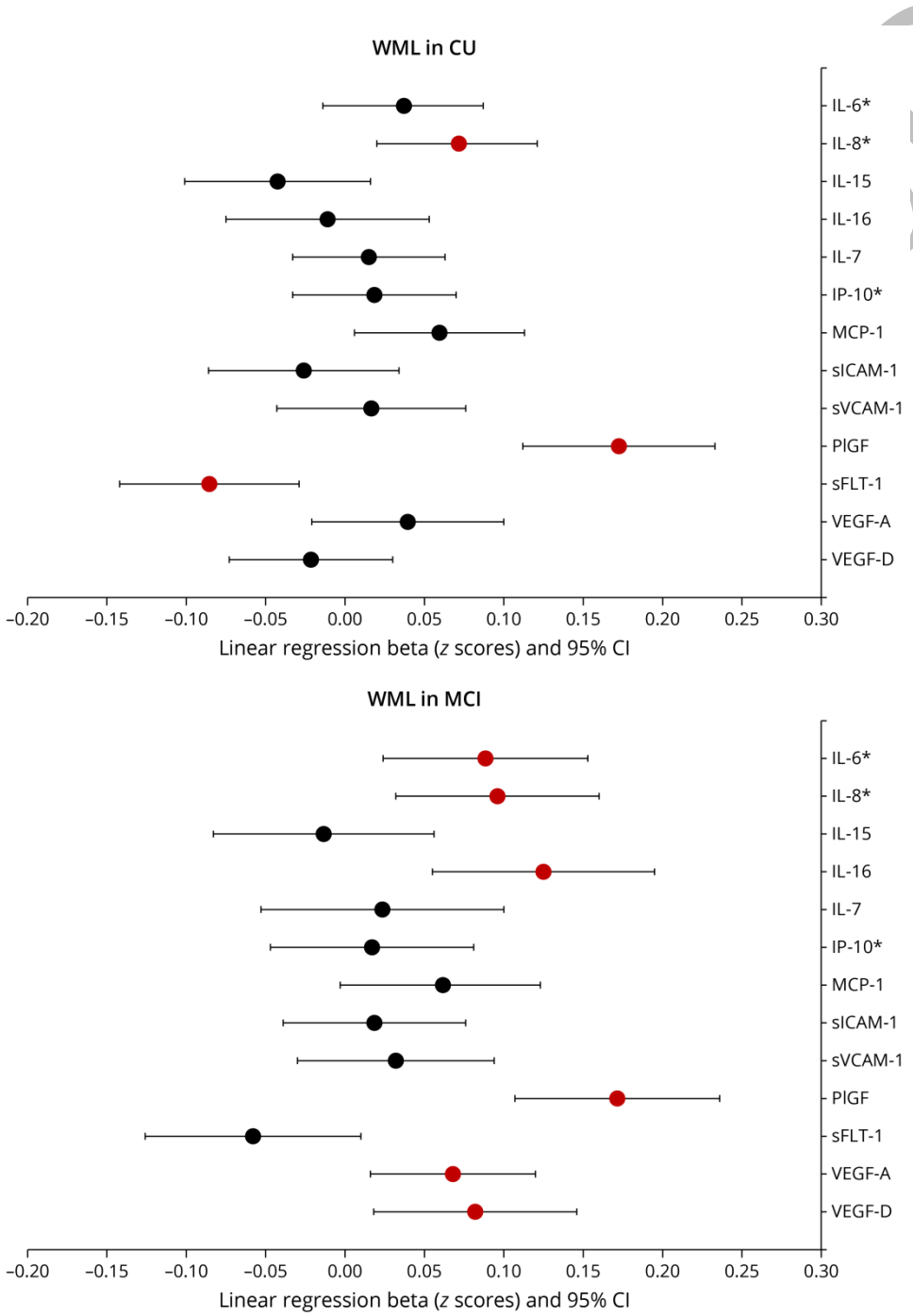


Figure 2 PlGF is associated with WML in CU and MCI independent of cognitive impairment and Aβ status

Abbreviations: WML, white matter lesions; CU, cognitively unimpaired; MCI, mild cognitive impairment; PlGF, placental growth factor. Linear regression models were adjusted for age, gender, and intracranial volume, and all p-values corrected for multiple comparison.

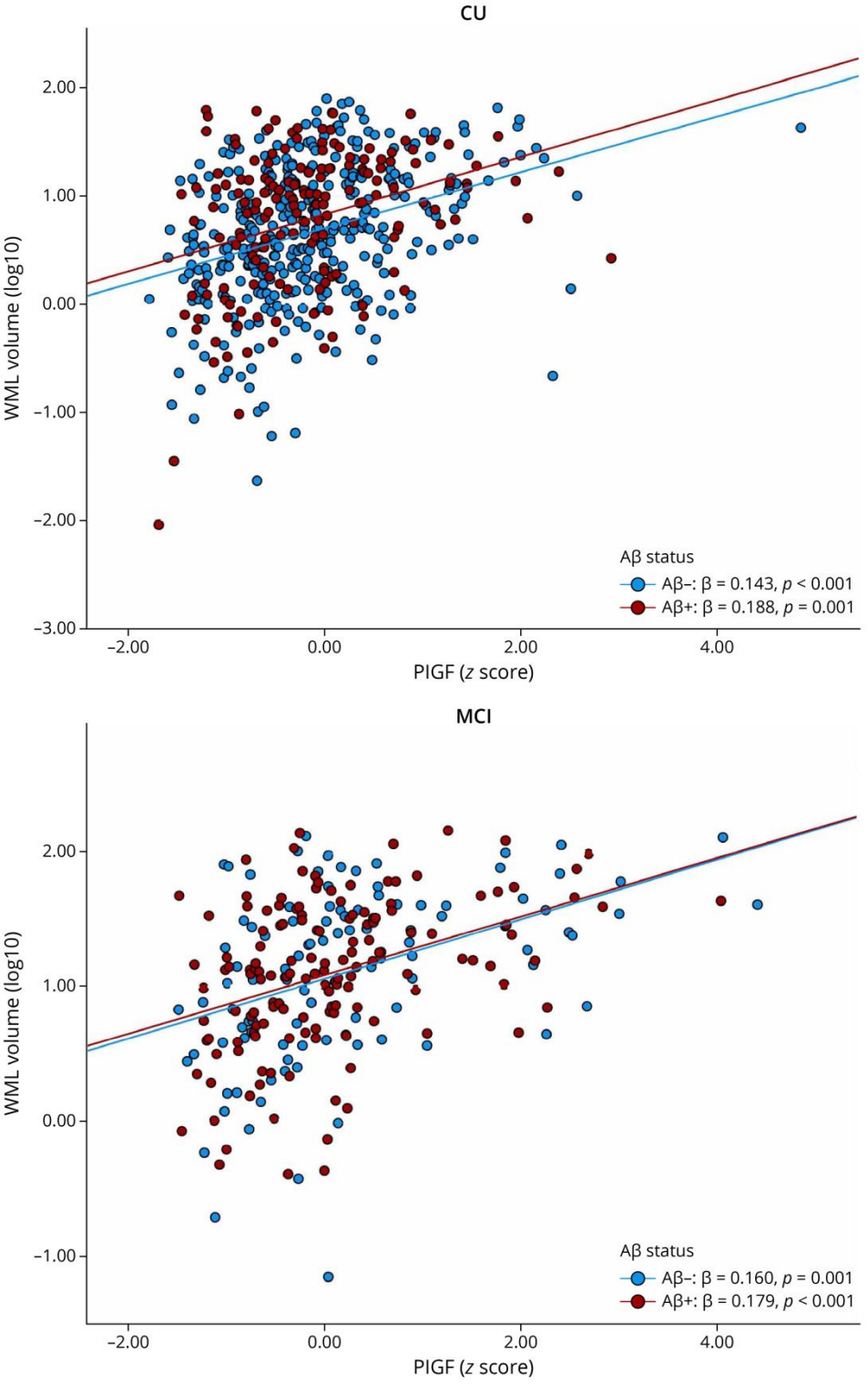
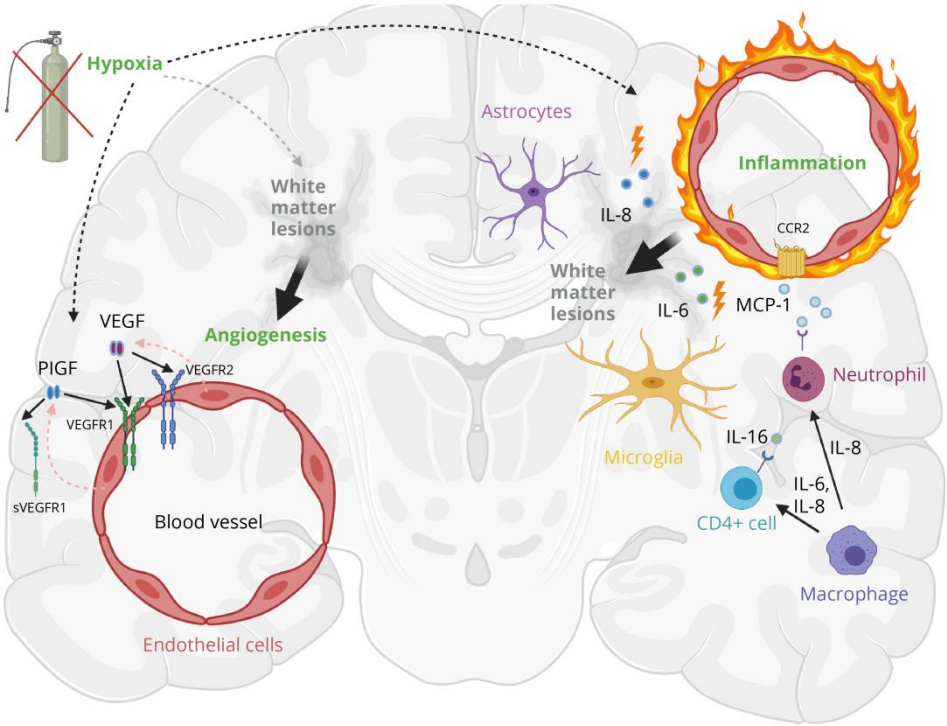


Figure 3 Theoretical model of the interaction between WML and markers of neuroinflammation and angiogenesis based on current results and review of the literature

Hypoxia induces neuroinflammation and endothelial cells, microglia and astrocytes release proinflammatory markers IL-8 and IL-6 as well as IL-16 and MCP-1. Those cytokines could be involved in WML pathology by BBB disruption and disruption of the neurovascular unit resulting in gliosis and thus WML.[19] Hypoxia and neuroinflammation could stimulate upregulation of PlGF and VEGF inducing pathological angiogenesis in damaged white matter in the acute phase.[11,19,48] This original figure was designed for this manuscript and created with BioRender.com. Abbreviations: WML, white matter lesions; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; PlGF, placental growth factor; VEGF, vascular endothelial growth factor (VEGF-A, and VEFG-D); sVEGFR1, soluble VEGF receptor 1 (same as sFlt-1); VEGFR1/ VEGFR2, VEGF receptor 1/ 2.



Associations Between CSF Markers of Inflammation, White Matter Lesions, and Cognitive Decline in Individuals Without Dementia

Eske Christiane Gertje, Shorena Janelidze, Danielle van Westen, et al.

Neurology published online March 7, 2023
DOI 10.1212/WNL.0000000000207113