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Data statement

The data associated with this manuscript has not been presented elsewhere.

ABSTRACT

Background: Cognitive decline in older adults has been attributed to reduced cerebral blood flow (CBF). Recently, the spatial coefficient of variation (sCoV) of ASL has been proposed as a proxy marker of cerebrovascular insufficiency. We investigated the association between baseline ASL parameters with cognitive decline, incident cerebrovascular disease and risk of vascular events and mortality.

Design, Setting and Participants: 368 memory-clinic patients underwent three-annual neuropsychological assessments and brain MRI scans at baseline and follow-up. MRIs were graded for white matter hyperintensities (WMH), lacunes, cerebral microbleeds (CMBs), cortical infarcts and intracranial stenosis. Baseline gray (GM) and white matter (WM) CBF and GM-sCoV were obtained with ExploreASL from 2D-EPI pseudo-continuous ASL images. Cognitive assessment was done using a validated neuropsychological battery. Data on incident vascular events (heart disease, stroke, transient ischemic attack) and mortality were obtained.

Results: Higher baseline GM-sCoV was associated with decline in the memory domain over three years of follow-up. Furthermore, higher GM-sCoV was associated with a decline in the memory domain only in participants without dementia. Higher baseline GM-sCoV was associated with progression of WMH and incident CMBs. During a mean follow-up of 3 years, 29 (7.8%) participants developed vascular events and 18 (4.8%) died. Participants with higher baseline mean GM-sCoV were at increased risk of vascular events.

Conclusions: Higher baseline GM-sCoV of ASL was associated with a decline in memory and risk of incident cerebrovascular disease and vascular events, suggesting that cerebrovascular insufficiency may contribute to accelerated cognitive decline and worse clinical outcomes in memory clinic participants.

KEYWORDS: cerebrovascular circulation, aged, brain, spin labels, perfusion, memory-clinic, cerebral blood flow, coefficient of variation

List of Non-standard Abbreviations and Acronyms

ASL	Arterial spin labeling
AD	Alzheimer's disease
ACA	Anterior cerebral artery
β	Standardized regression coefficient
CBF	Cerebral blood flow
CeVD	Cerebrovascular disease
CIND	Cognitive impairment no dementia
CMBs	Cerebral microbleeds
CI	confidence interval
DSM-IV	Diagnostic and statistical manual of mental disorders
FLAIR	Fluid-attenuated inversion recovery
HR	Hazard ratio
IQR	Inter quartile range
MARS	Microbleed Anatomical Rating Scale
MCA	Medial cerebral artery
MRI	Magnetic resonance imaging
MRA	Magnetic resonance angiography
NCI	No cognitive impairment
OR	Odds ratio
PCA	Posterior cerebral artery
SWI	susceptibility weighted image
SD	Standard deviation;
sCoV	Spatial coefficient of variation
TIA	Transient ischemic attack
VCIND	Vascular cognitive impairment no dementia
VaD	Vascular demeinta
WMH	White matter hyperintensities

INTRODUCTION

Reduced cerebral blood flow (CBF) is considered to be one of the potential underlying mechanisms for cognitive impairment and dementia.[1] There are very few longitudinal studies demonstrating the role of cerebral perfusion on cognition. One study reported an association of lower CBF at baseline with the decline in processing speed and memory in healthy elderly participants in 4-year follow-up,[2] whereas another study reported a decline in memory domain in participants with mild cognitive impairment (MCI) in 2.7-year follow-up.[1] Furthermore, there is an under-representation of participants with mixed neurodegenerative and cerebrovascular pathology in current literature.[3] Despite growing understanding of mixed-pathology,[4] current literature often categorizes participants into pure neurodegenerative or pure vascular groups.[3]

CBF can be measured quantitatively and non-invasively at the tissue level by arterial spin labeling (ASL) perfusion MRI.[5] However, in older adults with high burden of cerebrovascular disease (CeVD), the arterial transit time (ATT) of labeled blood to the tissue is often extended. Longer ATT makes it difficult to quantify gray matter (GM) and white matter (WM) CBF in ASL scans due to the presence of vascular artefacts and absence of tissue-perfusion signal.[6] Recently, the spatial coefficient of variation (sCoV) of ASL has been proposed to quantify the presence of vascular artefacts.[6] It has been shown that sCoV of ASL highly correlates with ATT, age,[7] sex,[6] and presence of CeVD,[8] making it a potential proxy marker of vessel insufficiency. Hence, it has been hypothesized that sCoV of ASL can better quantify perfusion-related changes in the brain than ASL-derived CBF, in populations with a high prevalence of vascular artifacts, and be associated with clinical outcomes.

Therefore, our objective is to investigate the association of baseline ASL parameters (total and regional) with global and domain-specific cognitive decline over 3-year follow-up in a memory-clinic sample with mixed pathology in overall population and stratified by with and

without dementia. Furthermore, we aim to analyze the association between baseline ASL parameters with incident CeVD on brain MRI and risk of vascular events [history of stroke, transient ischemic attack (TIA), and heart diseases] and mortality during the follow-up.

METHODS

Study Sample

Data for this study was drawn from an ongoing memory-clinic study, which recruits participants from National University Hospital, Singapore. Patients diagnosed with no cognitive impairment (NCI), cognitive impairment no dementia (CIND), vascular CIND (VCIND), and Dementia [(Alzheimer's disease (AD), mixed-dementia and vascular dementia (VaD)] at baseline were eligible for inclusion in this study **[Supplemental Digital Content-1].**

All individuals underwent clinical, physical and neuropsychological assessments along with a 3T-MRI examination at the National University of Singapore (NUS). These assessments were performed annually except for MRI, which was offered every 2 years. From August, 2010, to November, 2017, a total of 579 participants were recruited for this study, of whom 464 participants with baseline ASL sequence and those with a minimum of 2 cognitive scores including baseline and one of the follow-up scores were included in the further analysis. On quality analysis of 464 ASL images; 96 were labeled as unusable (incomplete scans, ASL labeling failed/severe motion artefacts), 52 as angiography (scans with dominant vascular artefacts, and no or minimal tissue perfusion contrast), 151 acceptable (scans with minor artefacts and reasonable tissue perfusion contrast) and 156 as good (artifact-free scans with tissue perfusion contrast). For analysis with sCoV and clinical outcomes (cognitive decline, incident CeVD, vascular events and mortality), a total of 368 participants were included (participants with ASL sequence labeled as angiography, acceptable, and good scans). For the

CBF and clinical outcomes analysis, 316 participants were included (participants with ASL sequence labeled as acceptable and good) [**Figure 1**].

Ethics approval was obtained from the National Healthcare Group Domain-Specific Review Board. Written informed consent was obtained from all participants before they participated in this study. This study was conducted in accordance with Helsinki Declaration.

Neuropsychological Assessment

All participants completed a detailed neuropsychological battery based on the recommendation of the National Institute of Neurological Disorders and Stroke and the Canadian Stroke Network (NINDS-CSN) at baseline (BL), year-1 (Y1), year-2 (Y2), and year-3 (Y3): Executive function, Attention, Language, Visuospatial function, Visuomotor speed, Memory [**Supplemental Digital Content**-**2**].

All individual test raw scores on the NINDS-CSN battery were transformed to standardized *Z*-scores using the means and standard deviations (SD) of the comparison group (NCI). The score for each domain was created by averaging the *Z*-scores of individual tests and standardized using the composite mean and SD of the comparison group. To obtain the global cognition score for each patient, the domain *Z*-scores were averaged and standardized using the mean and SD of the comparison group. Z-scores at follow-up were obtained similarly using the data of the comparison group at baseline.[9]

Vascular events

At baseline, history of stroke, TIA, and heart diseases were assessed during the study interviews and reviewing medical records. During the follow-up, medical reports were reviewed to verify any new occurrence of stroke, TIA, and heart diseases. The definition of stroke and TIA followed the World Health Organization criteria.[10] Heart disease was defined as the previous

history of atrial fibrillation, congestive heart failure, cardiac intervention, or myocardial infarction.

Follow-up started after the date of the baseline assessment. Participants were followed-up until the date of stroke, TIA, heart disease, death, date of the last contact in case of loss to follow-up, or end of this study (September 2018), whichever came first. For the analysis, the history of stroke, TIA, and heart disease at follow-up were combined as the incident vascular event.

Mortality

All-cause mortality was defined as participants who died from any cause during the follow-up period.

Covariates

A detailed questionnaire was administered to all participants to document age, sex, race, and education. Any previous history of hypertension, hyperlipidemia, and diabetes mellitus were noted and verified by medical records. Hypertension was defined as a previous diagnosis of hypertension, or the use of antihypertensive medications. Hyperlipidemia was defined as a previous diagnosis of hyperlipidemia, or the use of lipid-lowering medications. Diabetes mellitus was defined as a previous diagnosis of diabetes mellitus, or the use of glucose-lowering medications.

Neuroimaging

All participants underwent MRI at the Clinical Imaging Research Center of NUS, using a 3T Siemens Magnetom Trio Tim Scanner system, with a 32-channel head coil. Identical MRI protocol at the same 3-T scanner was used for both baseline and follow-up MRIs. MRI markers of CeVD such as cerebral microbleeds, lacunes, white matter hyperintensities, cortical infarcts, intracranial stenosis were graded at baseline and follow-up based on the Standards for Reporting Vascular Changes on Neuroimaging criteria.[11] **[Supplemental Digital Content-3].**

Baseline ASL Parameters

Pseudo-continuous ASL (PCASL) with a 2D gradient-echo echo-planar imaging (EPI) readout was used with the following parameters: voxel size=3x3x5mm^3, 24 slices, labeling duration=1656ms, initial post-labeling delay=1500ms, slice readout time=49.94ms, leading to a PLD range of 1500-2649ms across all slices with a mean PLD of 2074ms, TR/TE=4000/9ms, and generalized auto-calibrating partially parallel acquisitions (GRAPPA) factor=3. Two ASL volumes of 23 control-label pairs each were acquired with a 1-hour interval and were concatenated into one ASL time series to decrease physiological fluctuations. Details of ASL image processing have been described elsewhere.[6, 12] In brief, ASL image processing was performed with ExploreASL and included motion correction, quantification according to the ASL consensus paper,[13] rigid-body registration of the CBF map to a GM map from a segmented T1-weighted image, and spatial normalization to MNI space via the segmented T1-weighted image.[14] These T1 segmented images were then multiplied by 45 and 15mL/100g/min for GM and WM, respectively (assuming that CBF is homogeneously distributed across these tissue types)[15]. These GM and WM images were further smoothed to achieve the same resolution as the ASL acquisition resolution (3x3x7mm). Three ASL parameters were derived from the CBF maps: GM-CBF, WM-CBF and GM-sCoV. CBF reflects perfusion in mL blood/100g tissue/min and was calculated in total GM and WM regions of interest, whereas GM-sCoV was defined as the standard deviation of the CBF divided by mean CBF within GM.[6] Three parameters were obtained separately for the anterior and posterior flow territories, which were defined using a vascular-territory atlas as territories corresponding to the anterior (ACA) and middle (MCA) cerebral artery, and posterior (PCA) cerebral artery, respectively.[16] Quality assessments of the ASL scans were performed blinded to clinical diagnosis.

Statistical analysis

The baseline ASL parameters (GM-CBF, WM-CBF and GM-sCoV), and ASL parameters obtained from vascular territories (CBF and sCoV in ACA, MCA and PCA) were logarithmically transformed (log10) to ensure a normal distribution. We used a linear regression model with generalized estimating equations to assess associations between ASL parameters and cognitive decline as measured by detailed neuropsychological assessment. GEE was used because it allows us to account for within-subject variation as the correlation of repeated measurements and obtain population mean estimates for the effects of ASL parameters on cognition at each time point including baseline. We specified the correlation structure to be first-order autoregressive and robust variance estimators and identity link functions were used.

To investigate whether the effect of the baseline ASL parameter on cognitive scores differs between time points, regression models were constructed between cognition (global and domain-specific cognitive tests) with ASL parameters time and the interaction term 'ASL parameter x time' where the following markers were included in separate models: GM-CBF, WM-CBF, and GM-sCoV. These models adjusted for age, sex, and education, while subsequently models additionally adjusted for cardiovascular risk factors (hypertension, hyperlipidemia, and diabetes) and other MRI markers (total intracranial volume, baseline and incident CeVD markers including CMBs, lacunes, WMH, cortical infarcts, and intracranial stenosis). Similar analysis was done for ASL parameters obtained from vascular territories (CBF and sCoV in ACA, MCA and PCA) to analyze the association between region-specific ASL parameters with cognitive decline.

In our secondary analysis, between the participants with and without dementia at baseline, we divided our study participants into two groups, i.e., participants with dementia (AD, VaD and mixed-dementia) and with dementia (NCI, CIND and VCIND). The interaction term (ASL

parameters*diagnosis) was significant for analysis with global cognition (p=0.027), visuospatial function (p=0.007), visuomotor speed (p=0.023) and memory (p=0.006). Hence, we went on further to perform the stratified analysis for with and without dementia. All the analyses were performed for global and domain-specific cognitive tests as outcomes in all participants and separately for participants with and without dementia.

For each regression model, the standardized regression coefficient (β) and its 95% confidence interval (CI) were obtained. Because of the multiple testing performed within 6 cognitive domains, Bonferroni correction was applied (0.05/6~0.0083). This correction was applied to the overall p-value from the interaction factor and also the *p*-value from the effect of ASL parameters at each time point (BL, Y1, Y2 and Y3) respectively for each cognitive domain.

Logistic regression models with odds ratios (OR) and 95%CI were used to determine the association between ASL parameters with incident and progression of CeVD markers. All the models were adjusted for age, sex, cardiovascular risk factors, and scan interval. We additionally constructed, cox proportional hazard models to obtain estimated hazard ratios (HR) and 95%CI for the association between ASL parameters with incident vascular events and separately for mortality. All models were adjusted for age and sex, cardiovascular risk factors, and additionally for the history of stroke (in vascular event analysis).

RESULTS

Baseline characteristics of 368 participants are shown in **Table 1**. The median age of participants was 73±11years and 51.9% were female. There was a high prevalence of vascular risk factors and a high proportion of subjects at baseline had MRI markers of CeVD. Of 368 participants; 94(25.5%) were diagnosed as NCI, 100(27.2%) CIND, 65(17.7%) VCIND and 109(29.6%) dementia [AD: 45(12.2%), mixed-dementia: 44(12%), VaD: 20(5.4%)].

Higher GM-sCoV at baseline was associated with the decline in global cognition as well as the decline in memory domain at years 1, 2, and 3 after adjusting for age, sex, and education [**Supplementary Table-1].** Upon adjustment for cardiovascular risk factors, these associations remained significant **[Supplementary Table-2]**. However, on further adjusting for baseline and incident MRI markers of CeVD in model 3 only the association with GM-sCoV and memory remained significant (BL:β=-1.68, 95%CI=-2.76,-0.60, p=0.002, Y1:β=-2.66, 95%CI=-3.88,-1.44, p<0.001, Y2:β=-2.69, 95%CI= -3.95,-1.44, p<0.001, Y3:β= 3.21, 95%CI=-4.54,-1.89, p<0.001, p for interaction<0.001) [**Table 2]**. On applying Bonferroni correction, this association survived multiple testing. A negative association was observed between higher GM-sCoV and a decline in global cognition, executive function, and visuomotor speed but the interaction between GM-sCoV and time was not significant. Although there was a significant interaction between reduced GM-CBF and decline in memory domain (*p* for interaction=0.002), the majority of the decline observed across the different time points were not significant after adjusting for multiple testing. There was no association between WM-CBF and global or domain-specific cognitive decline. Region-specific analysis with ACA, MCA and PCA, we found that lower baseline CBF in PCA is associated with decline in memory **[Supplementary Table-3]**, and higher sCOV in ACA, MCA and PCA is associated with decline in memory **[Supplementary Table-4].**

The association between GM-sCoV and decline in global cognition and memory was observed in all years in participants without dementia. The interaction term between GM-sCoV and time was significant for memory (*p* =0.004), but for global cognition, it was significant only without Bonferroni correction (p=0.012) **[Supplementary Table-5].** There was no significant interaction of ASL parameters and time with global and domain-specific cognition in participants with dementia **[Supplementary Table-6].**

During the mean follow-up of 24.5 months, higher GM-sCoV was associated with progression of WMH (OR=6.11, 95%CI=1.01,37.01, p=0.049) and incident CMBs (OR=38.41, 95%CI= 5.67,260.16, p<0.001) independent of age, sex, cardiovascular risk factors, and scan interval. However, higher GM-sCoV was not associated with incident lacunes, cortical infarcts, or intracranial stenosis. Lower GM and WM CBF were not associated with incident CeVD markers **[Table 3].**

With respect to clinical events, 29 participants (7.8%) developed incident vascular events and 18 (4.8%) died over 3-year follow-up. Higher mean GM-sCoV was associated with increased risk of vascular events (HR=7.44, 95%CI=1.03,53.58, p=0.046) independent of age, sex, cardiovascular events and history of stroke, but not with increased risk of mortality. Lower GM and WM CBF were not associated with incident vascular events or mortality [**Table 5**].

DISCUSSION

In this study, we showed that baseline GM-sCoV, was associated with cognitive decline in the memory domain over 3-year follow-up. This cognitive decline was more obvious in participants without dementia. Region-specific analysis showed that lower baseline CBF in PCA and higher sCOV in ACA, MCA and PCA territories were associated with memory decline. Finally, higher GM-sCoV at baseline was associated with progression of WMH, incident CMBs and vascular events at follow-up.

Our finding that higher GM-sCoV, as well as sCoV in ACA, MCA and PCA flow territories, were associated with the decline in memory domain is partly in line with previous studies.[1] It has been shown that reduced cerebral perfusion has detrimental consequences on brain structure and function[17] such as, disruption of white matter structural integrity and damage in fronto-subcortical network which may affect complex cognitive functions such as memory.[18, 19] Furthermore, reduced cerebral perfusion in PCA and MCA may disrupt thalamo-cortical

tract affecting thalamic nuclei, which is important in storage and short-term memory.[20] Chronic hypoperfusion is related to cerebrovascular dysfunction, such as increased cerebrovascular stiffening, increased endothelial dysfunction, reduced autoregulation and impaired perivascular drainage, which affects brain function by promoting amyloid-beta and tau accumulation as well as CeVD.[21] These subsequently affect cognitive function.[21] Similarly, chronic cerebral hypoperfusion promotes neurodegeneration through several mechanisms; such as increased oxidative stress, increased synaptic dysfunction, as well as neuroinflammation.[22, 23] Such neurodegeneration and reduced perfusion especially in hippocampus affects memory function.[24] Hippocampus is essential in formation of new memories and recall.[25] Hippocampus is supplied by branches of PCA.[25] Hence, reduced perfusion in PCA territory may affect hippocampus blood supply and function. This is supported by our finding that lower CBF and higher sCoV in PCA flow territory are associated with memory decline.

Moreover, we found that higher GM-sCoV of ASL was associated with the decline in memory in participants without dementia. A previous study on the healthy individual over 4-year follow-up showed a strong association between reduced cerebral perfusion with memory impairment.[2] Memory impairment has been considered as an early sign of AD.[26] It has been shown that baseline ASL perfusion can predict conversion of MCI to dementia.[1] Hence, our findings are in accordance with previous studies and suggest that sCoV of ASL can be an early biomarker of cognitive decline in non-demented participants. However, we were not able to find any association between sCoV and cognitive decline in patients with dementia. This may be because the effect of hypoperfusion may be stronger in the early stage of the disease but at the later stage, the effect of vascular insufficiency may be predominated by non-vascular risk factors such as amyloid, tau, neuroinflammation and other comorbidities.[27]

In contrast to previous studies,[1, 2, 28] we were not able to find any significant association between lower GM and WM-CBF and cognitive decline. It has been shown that low CBF

values in WM, as well as higher ATT, especially in deep WM, makes WM-CBF difficult to assess by ASL[29] hence, absolute CBF values may not be fully reliable, which may have affected our findings. However, there was significant association between lower CBF and higher sCoV in posterior brain region with decline in memory which is partially in line with previous study which shows that GM-CBF, more specifically CBF in frontal region is associated with memory.[2] Higher sCoV means that a higher spatial heterogeneity was observed in the ASL signal most likely due to a higher presence of macrovascular artefact, which can be in most cases explained by increased ATT.[14] Moreover, higher sCoV means that there is less certainty about the accuracy of the CBF measurement. Although no direct conclusion can be drawn with respect to CBF and cognitive decline from the present study, further studies with a longer duration of follow-up are required to confirm these findings.

We also found that higher baseline GM-sCoV was associated with progression of WMH and incident CMBs, as well as increased risk of future vascular events. Increased sCoV and incident CeVD in this study may be due to amyloid deposition or arteriosclerosis in the blood vessel which contributes to the narrowing of vessels lumen and manifests as brain hemorrhagic and ischemic changes.[30] Reduced cerebral perfusion reflects the presence of vascular pathologies in both brain and heart, hence increased sCoV of ASL may be associated with increased risk of vascular events.[31] Moreover, cardiac events such as atrial fibrillation, congestive heart failure, and myocardial infarction are associated with reduced cardiac output which may result in reduced cerebral perfusion.[32] A growing body of evidence showed that patients with cardiovascular diseases are at risk of developing cerebral vascular disease and cognitive decline which is partly due to reduced cerebral perfusion.[9, 33, 34] Our study, thus, aligns with previous findings suggesting that participants with higher sCoV are at risk of incident vascular event.

Previous studies have reported that reduced cerebral perfusion not only is associated with neuronal injury and cell death but also increases the risk of mortality affecting immune and

stress response, energy balance and endocrine regulation.[31, 35] However, we were not able to prove the hypothesis that the lower CBF and higher sCoV at baseline are associated with increased risk of mortality. This may be because of small sample size (only 4.8% of participants died during this study) as well as the relatively short duration of follow-up. Hence, our hypothesis needs to be further tested in future studies investigating the long-term clinical outcome of cerebral hypoperfusion using ASL images.

This study has several limitations. Firstly, we only analyzed ASL parameters at baseline and longitudinal changes in ASL parameters were not studied. Second, even though we accounted for most of the confounders in the regression analysis, non-traditional vascular risk factors, such as hyperhomocysteinemia, inflammation, oxidative stress, amyloid and tau deposition have been linked to cognitive impairment.[36] Third, most of the participants in this study are elderly and have CeVD, which compromised the quality of the ASL images. We excluded those scans labeled as angiography and unusable. Those participants who were excluded from the analysis were significantly older (75 vs 73, p=0.004), less educated (6 vs 8, p<0.001), had a higher burden of cardiovascular risk factors and were more demented (55.9% vs 29.6%) **[Supplementary Table-7].** Finally, the effect estimates and confidence intervals of GM-sCoV with WMH progression, incident CMBs and mortality are wide. Hence it is difficult to interpret the clinical relevance of these findings and should be interpreted with caution. The strengths of this study are, first, to our best knowledge this is the only longitudinal study from a memory-clinic sample analyzing the association between baseline ASL parameters with cognitive decline over 3-year follow-up in patients with mixed-pathology. Second, an identical neuropsychological test battery was used for baseline and follow-up visits which made it possible to capture cognitive decline over time.

CONCLUSIONS

In this study, we found that baseline sCoV of ASL was associated with the decline in memory domain over 3-year follow-up, more specifically in participants without dementia. Higher baseline was associated with WMH progression, incident CMBs and increased risk of vascular events at follow-up. These findings encourage the potential of ASL sCoV as a surrogate endpoint marker for vascular brain injury.

AUTHOR CONTRIBUTION STATEMENT

BG is responsible for study concept and design, participated in data acquisition, performed the statistical analysis, drafting and revising the manuscript. HJMMM processed the ASL images and revised the manuscript. OKR participated in statistical analysis and revising the manuscript. JP was provided intellectual advice and revised the manuscript. CC was responsible for study concept and design, obtaining funding, and revising the manuscript. SH was responsible for study concept and design, data acquisition, obtaining funding, and revising the manuscript.

DISCLOSURE

The authors declare that they have no conflict of interest

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FIGURE LEGENDS
Figure 1

Title: Selection of participants

Legends: ASL, arterial spin labeling; CBF, cerebral blood flow; sCoV, spatial coefficient of variation of ASL

Table 1. Characteristics of study participants

Baseline characteristics	**Study participants (n=368)**
Age, years, median (IQR)	73 (11)
Female, n (%)	191 (51.9)
Education, years, median (IQR)	8 (8)
Hypertension, Yes, n (%)	253 (68.8)
Hyperlipidemia, Yes, n (%)	270 (73.4)
Diabetes, Yes, n (%)	124 (33.7)
Presence of CMBs, n (%)	159 (43.2)
Presence of lacunes, n (%)	94 (25.5)
Presence of WMH, modified Fazekas≥2, n (%)	200 (54.3)
Presence of cortical stroke, n (%)	38 (10.3)
Presence of intracranial stenosis, n (%)	49 (13.3)
Presence of incident CMBs, n (%)	65 (17.7)
Presence of incident lacunes, n (%)	17 (4.6)
Presence of WMH progression, n (%)	194 (52.7)
Presence of incident cortical stroke, n (%)	7 (1.9)
Presence of incident intracranial stenosis, n (%)	8 (2.2)
Gray matter CBF, median (IQR) (n=316)	45.6 (21.5)
White matter CBF, median (IQR) (n=316)	9.4 (7.7)
Gray matter sCoV, median (IQR)	0.6 (0.2)

IQR, interquartile range; CMBs, cerebral microbleeds; CBF, cerebral blood flow, ASL; arterial spin labeling, sCoV; spatial coefficient of variation of ASL, NCI; no cognitive impairment, CIND; cognitive impairment no dementia

Table 2: The effect of baseline ASL parameters on cognitive scores across time

		Global Z-score β (95%CI)	**Executive function β (95%CI)**	**Attention β (95%CI)**	**Language β (95%CI)**	**Visuospatial function β (95%CI)**	**Visuomotor speed β (95%CI)**	**Memory β (95%CI)**
CBF gray matter†	Baseline	-2.24 (-4.28, -0.20) p=0.031	-2.43 (-4.42, -0.44) p=0.017	-1.10 (-2.34, 0.14) p=0.082	-2.62 (-5.64, 0.39) p=0.088	-1.36 (-3.28, 0.55) p=0.163	-0.96 (-2.05, 0.13) p=0.084	-0.90 (-2.47, 0.67) p=0.264
	Year 1	-2.41 (-4.69, -0.13) p=0.038	-2.28 (-4.31-0.26) p=0.027	-0.55 (-1.74, 0.65) p=0.369	-3.11 (-6.85, 0.64) p=0.104	-1.39 (-3.31, 0.53) p=0.156	-1.20 (-2.30, -0.09) p=0.033	-1.59 (-3.26, 0.08) p=0.063
	Year 2	-3.34 (-5.80, -0.87) p=0.008#	-3.16 (-5.31, -1.02) p=0.004	-1.27 (-2.56, 0.02) p=0.055	-4.45 (-8.80, -0.09) p=0.045	-1.67 (-3.52, 0.18) p=0.076	-1.29 (-2.38, -0.20) p=0.021	-2.26 (-3.96, -0.56) p=0.009
	Year 3	-3.43 (-6.10, -0.75) p=0.012	-2.28 (-4.51, -0.05) p=0.045	-1.53 (-2.96, -0.09) p=0.037	-6.51 (-11.29, -1.73) p=0.008	-0.74 (-2.76, 1.27) p=0.469	-1.21 (-2.40, -0.02) p=0.047	-2.17 (-4.00, -0.34) p=0.020
	p*	0.090	0.109	0.179	0.142	0.591	0.469	0.002#
CBF white matter†	Baseline	-0.63 (1.63, -0.38) p=0.221	-0.72 (11.70, 0.26) p=0.148	-0.65 (-1.27, -0.02) p=0.042	0.03 (-1.56, 1.62) p=0.970	-0.46 (-1.36, 0.45) p=0.321	-0.46 (-0.94, 0.02) p=0.063	-0.36 (-1.12, 0.41) p=0.360
	Year 1	-0.64 (1.71, -0.44) p=0.245	-0.65 (-1.66, 0.35) p=0.201	-0.49 (-1.04, 0.06) p=0.082	0.17 (-1.68, 2.02) p=0.857	-0.57 (-1.45, 0.32) p=0.208	-0.47 (-0.96, 0.03) p=0.064	-0.64 (-1.42, 0.14) p=0.108
	Year 2	-0.79 (1.94, -0.37) p=0.183	-0.84 (-1.92, 0.24) p=0.127	-0.71 (-1.36, -0.07) p=0.030	-0.11 (-2.12, 1.91) p=0.918	-0.54 (-1.39, 0.32) p=0.218	-0.45 (-0.95, 0.05) p=0.080	-0.67 (-1.51, 0.16) p=0.115
	Year 3	-1.03 (2.34, -0.28) p=0.122	-0.30 (-1.45, 0.85) p=0.610	-1.07 (, -1.92, -0.22) p=0.013	-1.43 (-3.69, 0.84) p=0.217	-0.45 (-1.37, 0.46) p=0.330	-0.49 (-1.02, 0.04) p=0.072	-0.66 (-1.56, 0.25) p=0.154
	p*	0.593	0.319	0.384	0.132	0.957	0.990	0.440

sCoV gray matter‡	Baseline	-2.40 (-3.92, -0.88) p=0.002[#]	-2.56 (-3.96, -1.16) p<0.001[#]	-0.79 (-1.76, 0.17) p=0.108	-2.23 (-5.07, 0.62) p=0.125	-1.54 (-2.77, -0.31) p=0.014	-1.21 (-1.92, -0.50) p=0.001[#]	-1.68 (-2.76, -0.60) p=0.002[#]
	Year 1	-2.75 (-4.29, -1.21) p<0.0001[#]	-2.64 (-4.03, -1.25) p<0.001[#]	-0.47 (-1.45, 0.51) p=0.348	-2.87 (-5.75, 0.01) p=0.051	-1.52 (-2.75, -0.29) p=0.015	-1.31 (-2.05, -0.58) p<0.001[#]	-2.66 (-3.88, -1.44) p<0.001[#]
	Year 2	-3.14 (-4.80, -1.49) p<0.001[#]	-3.32 (-4.80, -1.84) p<0.001[#]	-0.82 (-1.92, 0.28) p=0.145	-3.19 (-6.31, -0.07) p=0.045	-1.77 (-2.93, -0.60) p=0.003	-1.40 (-2.13, -0.66) p<0.001[#]	-2.69 (-3.95, -1.44) p<0.001[#]
	Year 3	-3.53 (-5.55-, 1.52) p=0.001[#]	-2.73 (-4.30, -1.16) p=0.001[#]	-1.25 (-2.43, -0.06) p=0.039	-5.35 (-9.92, -0.78) p=0.022	-0.82 (-2.19,0.56) p=0.245	-1.44 (-2.20, -0.68) p<0.001[#]	-3.21 (-4.54, -1.89) p<0.001[#]
	p*	0.142	0.251	0.354	0.344	0.302	0.691	<0.001[#]

Linear regression model with generalized estimating equations for the associations between ASL parameters and cognitive decline at each time point [values represent as standardized regression coefficient (β) and 95% confidence interval (CI), degree of freedom=16]

† β represents mean difference in cognitive scores per decrease in 1og10 of CBF

‡ β represents mean difference in cognitive scores per increase in 1og10 of sCoV

All values adjusted for age, sex, education, cardiovascular risk factors, total brain volume, presence of baseline, and incident CeVD

p*=p value two-way interaction factor between ASL parameters and time (p*<0.05, significant decline in specific cognitive domain over time)

p<0.05 considered as statistically significant association [[#] significant after Bonferroni correction (0.05/6~0.0083)]

CBF, cerebral blood flow; ASL, arterial spin labeling; sCoV, spatial coefficient of variation of ASL; CeVD, cerebrovascular disease

Table 3: Association between ASL parameters with progression and incidence of CeVD

	WMH progression OR (95%CI) (n=166†, 194‡)	Incident lacunes OR (95%CI) (n=14†, 17‡)	Incident CMBs OR (95%CI) (n=47†, 65‡)	Incident cortical infarcts OR (95%CI) (n=5†, 7‡)	Incident intracranial stenosis OR (95%CI) (n=6†, 8‡)
CBF gray matter	6.12 (0.64, 57.84) p=0.114	8.38 (0.08, 927.28) p=0.376	5.29 (0.30, 93.06) p=0.254	0.30 (0.01, 147.56) p=0.782	0.01 (0.01, 0.01) p=0.145
CBF white matter	1.15 (0.36, 3.67) p=0.805	1.20 (0.08, 17.35) p=0.895	1.30 (0.31, 5.48) p=0.724	2.74 (0.03, 237.24) p=0.658	0.01 (0.01, 1.38) p=0.065
sCoV gray matter	6.11 (1.01, 37.01) p=0.049	6.89 (0.33, 143.06) p=0.212	38.41 (5.67, 260.16) p<0.001	0.19 (0.01, 52.36) p=0.559	0.01 (0.01, 8.69) p=0.188

Linear regression model for the association between ASL parameters with WMH progression, incident lacunes, incident CMBs, incident cortical infarcts and incident intracranial stenosis [values represent as odds ratio (OR) and 95% confidence interval (CI)]

All values adjusted for age, sex, cardiovascular risk factors and scan interval

† numbers of incident CeVD in CBF analysis

‡ numbers of incident CeVD in sCoV analysis

$p<0.05$ considered as statistically significant association

CBF, cerebral blood flow; ASL, arterial spin labeling; sCoV, spatial coefficient of variation of ASL; CeVD; cerebrovascular disease, WMH, white matter hyperintensities; CMBs, cerebral microbleeds

Table 4: Association between ASL parameters with incident vascular events and mortality

Cardiac biomarkers	Vascular events[#] HR (95%CI) (n=19[†], 29[‡])	Mortality* HR (95%CI) (n=12[†], 18[‡])
CBF gray matter	4.97 (0.10, 241.22) p=0.418	0.01 (0.01, 1.23) p=0.059
CBF white matter	2.71 (0.39, 18.67) p=0.310	0.43 (0.02, 7.38) p=0.559
sCoV gray matter	7.44 (1.03, 53.58) p=0.046	5.77 (0.39, 85.94) p=0.203

Cox proportional hazard regression model for the association between ASL parameters with incident vascular events and mortality [values represent hazard ratio (HR) and 95% confidence interval (CI)]

[#]adjusted for age, sex, cardiovascular risk factors, and history of stroke

*adjusted for age, sex, cardiovascular risk factors

[†] numbers of vascular events and mortality in CBF analysis

[‡] numbers of vascular events and mortality in sCoV analysis

$p<0.05$ considered as statistically significant association

HR: hazard ratio; OR: odds ratio; CI, confidence interval; CBF, cerebral blood flow; ASL, arterial spin labeling; sCoV, spatial coefficient of variation of ASL

LIST OF SUPPLEMENTAL DIGITAL CONTENT