Associations of Blood Cardiovascular Biomarkers With Brain Free Water and Its Relationship to Cognitive Decline: A Diffusion-MRI Study

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ABSTRACT

Background and Objectives: There is an increasing awareness of the “Heart-Brain Connection”, whereby cardiovascular function is connected with cognition. Diffusion MRI studies reported higher brain free-water (FW) was associated with cerebrovascular disease (CeVD) and cognitive impairment. In current work, we investigated whether higher brain FW was related to blood cardiovascular biomarkers and whether FW mediated the associations between blood biomarkers and cognition.

Methods: Participants recruited from two Singapore memory clinics between 2010 and 2015 underwent collection of blood samples and neuroimaging at baseline and longitudinal neuropsychological assessments up to 5 years. We examined the associations of blood cardiovascular biomarkers (high-sensitivity cardiac troponin-T (hs-cTnT), N-terminal pro-hormone B-type natriuretic peptide (NT-proBNP), growth/differentiation factor 15 (GDF-15)) with brain white matter (WM) and cortical grey matter (GM) FW derived from diffusion-MRI using whole brain voxel-wise general linear regression. We then assessed the relationships among baseline blood biomarkers, brain-FW, and cognitive decline using path models.

Results: 308 older adults (76 no cognitive impairment, 134 cognitive impairment no dementia, and 98 Alzheimer’s disease dementia and vascular dementia; mean [SD] age: 72.1 [8.3]) were included. We found that blood cardiovascular biomarkers were associated with higher FW in widespread WM regions and in specific GM networks including the default mode, executive control, and somatomotor networks at baseline ($p < 0.01$, family-wise error corrected). Baseline FW in widespread WM and network-specific GM fully mediated the
associations of blood biomarkers with longitudinal cognitive decline over 5 years. Specifically, in GM, higher FW in the default mode network mediated the relationship with memory decline (hs-cTnT: $\beta=-0.115$, SE=0.034, $p=0.001$; NT-proBNP: $\beta=-0.154$, SE=0.046, $p=0.001$; GDF-15: $\beta=-0.073$, SE=0.027, $p=0.006$); in contrast, higher FW in the executive control network was responsible for executive function decline (hs-cTnT: $\beta=-0.126$, SE=0.039, $p=0.001$; NT-proBNP: $\beta=-0.110$, SE=0.038, $p=0.004$; GDF-15: $\beta=-0.117$, SE=0.035, $p=0.001$). Similar full mediation effects of brain FW were also identified for baseline cognition.

**Discussion:** Results suggested a role of brain-FW in linking cardiovascular dysfunction to cognitive decline. These findings provide new evidence for brain-heart interactions, paving the way for prediction and monitoring of domain-specific cognitive trajectory.

**Keywords**

Circulating cardiovascular biomarker; free-water; cognitive decline, dementia, cerebrovascular disease

**Glossary**

AD = Alzheimer’s disease; CeVD = cerebrovascular disease; CIND = cognitive impairment no dementia; DTI = diffusion tensor imaging; DMN = default mode network; ECN = executive control network; FW = free-water; GDF-15 = growth/differentiation factor 15; GM = grey matter; hs-cTnT = high-sensitivity cardiac troponin-T; NCI = no cognitive impairment; NT-proBNP = N-terminal pro hormone B-type natriuretic peptide; VaD = Vascular dementia; WM = white matter
Introduction

Cerebrovascular disease (CeVD) is related to increased risk of developing cognitive impairment and dementia\(^1\). There is increasing awareness of a “Heart-Brain Connection”\(^2\), where cardiac disease and vascular function may potentially contribute to CeVD, dementia due to Alzheimer’s disease (AD), and vascular cognitive impairment\(^3\)-\(^5\). Studies have demonstrated that peripheral cardiovascular dysfunction may lead to blood vessel damage and neurovascular alterations via both vascular and AD pathophysiological pathways in dementia, which eventually cause neuronal injury and cognitive dysfunction\(^6\). For example, the established circulating markers of cardiac diseases such as high-sensitivity cardiac troponin-T (hs-cTnT) and N-terminal pro hormone B-type natriuretic peptide (NT-proBNP) exhibit up-regulation in the early phases of cardiac dysfunction and myocardial injury\(^7\). These cardiovascular blood biomarkers have been associated with concomitant CeVD MRI markers like cortical microinfarcts\(^8\),\(^9\) and cognitive dysfunction\(^10\). BNP, in particular, was found to predict vascular cognitive impairment, independent of cardiovascular risk factors\(^7\),\(^10\).

Growth/differentiation factor 15 (GDF-15), a cardiovascular biomarker with protective and trophic bioactivity in cardiomyocytes\(^11\), was related to small vessel CeVD in dementia\(^10\). However, the relationships between circulating cardiovascular markers and cerebrovascular function underlying cognitive decline are not yet fully understood.

Diffusion MRI (dMRI) has emerged as an important method for studying CeVD and dementia\(^12\). Free-water (FW) volume derived from dMRI\(^13\) using a bi-tensor model reflects the relative contribution of freely diffusing extracellular water molecules which are unrestricted by their local microenvironment\(^13\). Higher FW in the white matter (WM) was found in patients with CeVD or AD dementia compared to controls and associated with dementia severity and cognitive decline\(^14\)-\(^17\). Interestingly, a recent study demonstrated that FW alternations in the grey matter (GM) may indicate neuronal microstructure perturbations...
in the AD continuum\textsuperscript{18}, and was associated with cognition\textsuperscript{19}. However, there is a lack of understanding of whether cardiovascular dysfunction is related to these CeVD-related FW abnormalities and eventually leads to general and domain-specific cognitive impairment. Furthermore, it is unclear if such relationships are specific to certain brain networks or regions.

Accumulating evidence suggests that the executive control network (ECN) and somatomotor network changes were related to cerebrovascular dysfunction\textsuperscript{20-22}. In contrast, AD pathology (i.e. amyloid plaques and neurofibrillary tangles) leads to targeted large-scale brain network disorganization specifically in the default mode network (DMN)\textsuperscript{23,24}. Reduced network connectivity (via resting-state fMRI) and metabolism (via FDG-PET) in the ECN and DMN were associated with deficits in executive function and memory\textsuperscript{25-27}. Nevertheless, it remains unknown whether brain cortical FW changes relate to cardiovascular dysfunction and domain-specific cognitive decline in a network-specific manner.

To investigate these research questions, we examined the associations of brain FW in WM and GM with cardiovascular blood biomarker levels and cognitive measures in an Asian memory clinic population with a high cerebrovascular disease burden. We hypothesized that 1) higher cardiovascular biomarkers levels would be associated with higher FW mainly in the frontal-parietal regions related to executive and somatomotor function; 2) both baseline GM and WM FW would mediate the associations of cardiovascular biomarkers with baseline and longitudinal changes of global cognition; and 3) cortical GM FW would influence cognitive function in a brain network-specific manner.
Methods

Participants

This study was part of an ongoing prospective memory clinic study. Participants with no cognitive impairment (NCI), cognitive impairment with no dementia (CIND), Alzheimer's disease (AD) dementia and vascular dementia (VaD) were recruited from the National University Hospital of Singapore and Saint Luke’s Hospital. AD dementia was diagnosed in accordance with Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV for dementia and internationally established criteria for the clinical diagnosis of AD dementia. Vascular dementia (VaD) was diagnosed using the DSM-IV criteria for dementia and internationally established criteria for the clinical diagnosis of VaD. CIND was determined based on objective impairment in at least one domain of the neuropsychological assessment, but did not meet the DSM-IV criteria for dementia. Participants were classified as NCI if they had no objective impairment in the neuropsychological assessment. Participants of the cohort study were aged 50 years and above, and had no major vascular risk factor related encephalopathy or significant neurologic comorbid conditions, or loss of functional independence (Detailed diagnoses, significant CeVD and inclusion/exclusion criteria are provided in eMethods in the Supplement).

Out of 471 participants enrolled between August 2010 and August 2015, we studied 308 participants (76 NCI, 134 CIND, 98 with AD and vascular dementia) according to the following criteria (see flowchart in eFigure 1 in the Supplement): (i) passed the MRI data quality control (details in imaging data processing), and (ii) had blood and cognitive test information (Table 1). 271 participants (68 NCI, 121 CIND, 82 with AD and vascular dementia) with baseline cognitive scores and at least one follow-up were included in the longitudinal analysis (eTable 1). Characteristics of the included and excluded participants were similar (eTable 2).
Neuropsychological assessments

Neuropsychological assessments were performed using a locally validated comprehensive neuropsychological battery\textsuperscript{32} at baseline, year 2, 4, and 5, which assesses memory, executive function, language, attention, visuomotor speed and visuoconstruction (individual subtests in each domain are summarised in eTable 3 in the Supplement). Standardized domain scores were calculated following previous study\textsuperscript{33} (see eMethods).

Vascular risk factor assessment and medications

Data on various risk factors associated with vascular health were collected through a combination of clinical interview, examination of medical records, and physical examination\textsuperscript{9}. Hypertension was defined as systolic blood pressure≥140 mm Hg and/or diastolic blood pressure≥90 mm Hg or use of antihypertensive medication. Hyperlipidaemia was defined as total cholesterol levels≥4.14 mmol/L or use of lipid-lowering medication. Diabetes mellitus was defined as glycated haemoglobin≥6.5% or use of diabetic medication. Heart disease was defined as presence of coronary artery disease, ischemic heart disease, or atrial fibrillation. History of stroke was defined as having a clinical history of rapid-onset focal or global neurologic deficits for>24 hours and confirmed on medical records. Antiplatelet therapy was defined as use of antiplatelet medication. Additionally, smoking history and body mass index (BMI) were also recorded. BMI was calculated by participant’s weight divided by the square of height.

Blood cardiovascular biomarkers

Non-fasting blood was drawn from study participants. NT-proBNP and hs-cTnT were measured using electrochemiluminescence immunoassays on an automated Cobas-e411
analyser, while GDF-15 were measured using quantitative sandwich immunoassays (see eMethods in the Supplement).

Image acquisition and processing

Each participant underwent MRI scanning at the Centre for Translational MR Research, National University of Singapore (3-T MAGNETOM Trio™, Siemens, Germany). High-resolution T1-weighted structural MRI was performed using a magnetization-prepared rapid gradient echo (MPRAGE). Diffusion-MRI (dMRI) scans were acquired using a single-shot fast echo-planar imaging sequence (b-value=1150 s/mm², 61 diffusion directions, and 7 b0). Fluid attenuated inversion recovery (FLAIR) was also acquired.

The dMRI pre-processing was following previous work including correction for head movements, eddy current distortions, and geometric distortions. We employed the free-water imaging method on the pre-processed dMRI data to estimate the fractional volume of freely diffusing extracellular water molecules (FW) and the tissue compartment fractional anisotropy (FAt). Tract-based spatial statistics (TBSS) was applied to carry out the WM FW maps, while surface-based approach was employed to derive cortical GM FW maps of each participant. Please see eMethods in the Supplement for details of image acquisition and processing.

Statistical analyses

Associations between cardiovascular biomarker levels and brain FW

To identify region-specific associations between brain FW and the three logarithmically transformed blood cardiovascular biomarkers within each clinical group (CIND+dementia and NCI), we built general linear models (GLMs) for each blood biomarker separately (Figure 1, Step 1). The FW in the vertex-wise surfaced GM or voxel-wise skeletonized WM
images were entered as the dependent variables. Each blood biomarker level was the independent variable of interest. We included age, sex, education, ethnicity, total intracranial volume (TIV), cognitive status, and CeVD status as additional covariates for both GLMs. We tested the interaction effects of cognitive status (i.e., CIND/dementia) and CeVD status (i.e., with and without CeVD) for the GLM of CIND+dementia. We also tested the interaction effects of CeVD status for the GLM of NCI. For WM FW measures, skeleton regions were examined for statistical significance using threshold-free cluster enhancement and permutation-based non-parametric testing (FSL, Randomise). For GM FW measures, cortical regions were tested for significance using a Monte-Carlo simulation with 10,000 repeats (Freesurfer, Gmlfit). GLM results for both WM and GM were reported at \( p < 0.01 \), family-wise error corrected.

To mitigate possible confounds due to regional atrophy, we included GM cortical thickness, or WM volume as additional covariates in vertex/voxel-based statistical models. To control for the influence of vascular-related factors, we included the eight vascular-related covariates (hypertension, hyperlipidaemia and diabetes mellitus, history of heart disease, history of stroke, antiplatelet therapy, smoking history, BMI) as nuisance variables. Lastly, to minimize potential confounds due to WMH, we derived FW in normal-appearing WM after excluding regions with WMH and repeated the association analyses.

We also compared the participant characteristics across the three cognitive groups (see eMethods in the Supplement and Table 1).

**Associations between cardiovascular biomarker levels and cognition**

Based on the previous evidence that memory and executive dysfunctions are most prevalent in dementia with concomitant AD and CeVD\(^{1,4,28,34}\), we conducted correlation analyses between logarithmically transformed cardiovascular biomarker levels and cognition decline
over time in all patients (CIND+dementia), with a priori interest in global cognition, memory, and executive function. Linear regression was calculated between the baseline cognitive scores/longitudinal rate of changes and cardiovascular biomarker levels across all patients. The main model adjusted for age, sex, years of education, ethnicity, TIV, cognitive stage, and CeVD status. We also validated the results in crude model (no covariates adjusted) and a model with further adjusted vascular-related covariates. The threshold was set at $p<0.05$ (two-tailed).

To calculate the annual rate of change in cognitive outcomes over time (mean=3.81, SD=1.52 years), linear mixed models were conducted (see eMethods in the Supplement).

**Path analyses**
To evaluate whether and how FW in the grey and white matter mediates the effects of higher blood marker levels on baseline global cognitive deficits and longitudinal decline, we first performed path analyses by including each blood biomarker (NT-proBNP, hs-cTnT, or GDF-15) as a predictor, both white and grey matter FW as mediators, and baseline global cognitive scores or longitudinal global cognitive rate of changes as outcomes (Figure 1, Step 2). We used structural equation modelling method (R (v3.3.1) packages Lavaan (v0.5–20)) controlling for age, sex, years of education, ethnicity, TIV, cognitive stage and CeVD status following our previous work. We built one model for each of the three blood biomarkers and each of the two outcomes (baseline or longitudinal cognition) variables (i.e., in total six models). For each model, to represent grey and white matter FW, we created brain masks containing only the regions that were significantly correlated with each blood cardiovascular biomarker. Path analyses were used to simultaneously consider the direct effect (blood biomarker on cognition) and the indirect effect (each blood marker on cognition through mediators).
Second, we further evaluated how FW influenced the association of higher blood marker levels on individual cognitive domains (executive function and memory) using path models. Based on the work from our group and others, we expected that the role of FW in WM would be widespread while the contribution from GM would be specific to cognitive networks. Therefore, we parcellated blood biomarker-related GM regions (from the previous step) into regions of interests (ROIs) according to the existing cortical functional parcellation. This parcellation employed clustering approach to identify and replicate 7 networks of functionally coupled regions across the cerebral cortex, which have been shown corresponding to individual cognitive performance. In these models, blood biomarker was the predictor; mean GM FW of each network and mean WM FW values derived from previous significant regions were the mediators, and baseline memory and executive function scores or longitudinal rate of changes were the outcomes. Age, sex, years of education, TIV, cognitive stages, and CeVD status were also included in the models. To mitigate possible confounds due to regional atrophy, we also repeated the same path analyses using the ratio of mean WM FW divided by WM volume and network-specific GM FW divided by network-specific cortical thickness as mediators. We built the path model, evaluated the model fits, and reported direct and indirect effects following the criteria in the previous work (see eMethods in the Supplement).

**Standard protocol approvals, registrations, and patient consents**

Ethics approval was obtained from National Healthcare Group Domain-Specific Review Board (2015/00406-AMD0012). Participants gave informed consent according to the Declaration of Helsinki.
Data availability

Data are available upon reasonable request. The data sets generated for this study are available on request to the senior author, for non-commercial academic studies, but may be subject to some restrictions according to consent and confidentiality.

RESULTS

Group differences in blood cardiovascular markers and brain FW

Blood cardiovascular markers levels were higher in CIND and patients with dementia compared with NCI. Patients with dementia had greater blood cardiovascular marker levels than the CIND group (Table 1). Within the same cognitive stage, participants with CeVD had higher cardiovascular markers levels than the non-CeVD participants (see eMethods and eTable 4 in the Supplement). FW averaged across all the WM regions was greater in CIND participants with and without CeVD compared with their NCI counterparts (eFigure 2A). Furthermore, patients with dementia had greater WM FW than CIND. CeVD participants had greater WM FW compared with non-CeVD participants among NCI, CIND and dementia groups. Similarly, FW averaged across all the GM regions was increased along the dementia continuum. However, CeVD participants did not show higher GM FW than their non-CeVD counterparts (eFigure 2B). These results remained in an age, sex, and education matched sub-cohort (see eResults and eFigure 3).

Associations of white matter free-water with circulating cardiovascular biomarker levels

The voxel-wise analysis on the WM FW metrics showed that greater cardiovascular biomarker levels (NT-proBNP, hs-cTnT, GDF-15) were associated with higher FW in multiple WM regions (including projection, association, commissural, limbic and brainstem...
fibres) in all the patients with CIND and dementia at baseline (Figure 2, eTable 5 in the Supplement). There was no interaction effect of cognitive stage or CeVD status on such association. There was no region showing association of WM FW with blood biomarkers in NCI regardless of the CeVD status.

The results remained when 1) controlling for regional WM volume (eFigure 4A in the Supplement), 2) controlling for vascular-related covariates (eFigure 4B), and 3) using FW in normal-appearing WM after excluding regions with WMH (eResults).

In addition, there was no association of blood cardiovascular biomarker levels with WM tissue compartment fraction anisotropy (fAt). GDF-15, but not NT-proBNP and hs-cTNT, was associated with total WMH volume ($r=0.19$, 95% confidence interval=[0.07,0.31], $p<0.05$).

**Associations of grey matter free-water with circulating cardiovascular biomarker levels**

All three cardiovascular biomarkers were related to higher FW in GM at baseline, primarily in the executive control network (ECN), default mode network (DMN) and somatomotor network. Specifically, higher hs-cTnT was associated with greater FW in bilateral middle frontal and temporal regions, mainly within the DMN, ECN, somatomotor and parts of dorsal/ventral attention and limbic networks (Figure 3A, eTable 6 in the Supplement). Similarly, greater NT-proBNP was associated with higher FW in bilateral frontal-parietal and left temporal regions, within the ECN, DMN, somatomotor and attention networks (Figure 3B, eTable 6). Higher GDF-15 levels were associated with higher FW in the bilateral superior frontal and anterior cingulate regions and right temporal-occipital regions, which contain bilateral DMN, ECN, attention networks, right limbic and visual networks (Figure 3C, eTable 6). There was no interaction effect of cognitive stage or CeVD status on this
relationship. For the NCI with and without CeVD, no association was found between GM FW and blood biomarkers.

The results remained when controlling for 1) the regional cortical thickness (eFigure 5A in the Supplement), and 2) vascular-related covariates (eFigure 5B). Besides, we did not find any associations between cardiovascular biomarker levels and cortical thickness.

**Brain free-water mediated the association of cardiovascular biomarkers with cognition**

We found that baseline cardiovascular biomarker levels were associated with baseline global cognition, executive functioning, and memory impairment as well as longitudinal cognitive decline (Table 2). The results remained after further adjusting for vascular-related covariates and in the crude model (no covariates adjusted) (eTable 7 in the Supplement).

The baseline mean FW levels in both GM and WM mediated the association of hs-cTNT with baseline global cognitive impairment (Figure 4A, eTable 8 in the Supplement) and the rate of global cognitive decline over time (Figure 4B, eTable 9). Given the significant indirect effects (see eTables 5 and 6) of hs-cTNT on global cognition via GM FW and WM, and non-significant direct paths from blood markers levels to global cognition, we observed a complete mediation effect of both GM and WM FW. Similar mediation effects of brain FW on the association of NT-proBNP and GDF-15 levels with both baseline global cognition and rate of change in global cognition over time were observed (eResults, eFigure 6, eTables 8 and 9).

For the cognitive domain-specific (executive function and memory) path analysis, FW in the ECN mediated the effect of hs-cTnT levels on baseline (eFigure 7, eTable 10 in the Supplement) and the rate of executive function decline (Figure 5, eTable 11). ECN FW had no effect on memory. In contrast, FW in the DMN mediated the effect of hs-cTnT levels on
baseline and longitudinal memory decline but did not influence executive function. Lastly, the mean WM FW was the mediator of both pathways (executive function and memory).

Notably, the direct paths between hs-cTnT and cognition were not significant during the model pruning stage. All mediators exerted a full mediation effect because the indirect effects of hs-TNT on cognition via all FW measures were significant. Similar findings were observed in the path analysis of NT-proBNP and CDF-15 (eResults, eTables 10 and 11 in the Supplement). All results remained after controlling for WM volume and network-specific cortical thickness (eTables 12 and 13).

DISCUSSION
The present study demonstrates that baseline circulating cardiovascular biomarker levels were associated with higher baseline FW in multiple WM regions as well as in the DMN, ECN, and somatomotor GM networks. Moreover, we found that the associations of circulating cardiovascular marker levels with baseline and longitudinal cognitive decline were fully mediated by both higher WM and GM FW. Specifically, in GM, the association of cardiovascular markers with executive function was mediated by higher FW in the ECN, while the association of blood markers with memory was mediated by greater FW in the DMN, both at baseline and longitudinally. Widespread higher WM FW mediated the same association for both domains. Our findings provided new evidence supporting increased brain FW as a proxy of cerebrovascular integrity largely accounting for the linkage between cardiovascular dysfunction and cognitive impairment.

FW as a brain imaging marker associated with circulating cardiovascular function
Our study demonstrates that FW measures in the brain are associated with circulating cardiovascular biomarkers. Recently, FW alterations have gained increasing attention
because of their capability of detecting early brain abnormalities in neurodegenerative disease and vascular cognitive impairments\textsuperscript{14,38}. The findings suggest a substantial increase of FW which is most likely originating from extracellular water characteristics\textsuperscript{13}, but independent of regional atrophy. However, the precise factors leading to the observed increase of extracellular water and hence increased FW signal in vascular-related cognitive impairment are not yet clear. One possible explanation is cerebrovascular-related damage\textsuperscript{16,17,38}. Our study thus provided important support for this hypothesis by uncovering the associations between brain FW and circulating cardiovascular biomarkers. In dementia, concomitant cardiovascular dysfunction may lead to ischaemia (reduced blood supply to the brain) and cardioembolic stroke (thrombus from the heart dislodging went into the cerebral vasculature)\textsuperscript{4}. These processes in turn lead to vascular inflammation, endothelial and blood brain barrier (BBB) dysfunction\textsuperscript{5,39,40}, which may cause FW increases. Moreover, these cerebrovascular dysfunctions may further lead to circulating inflammatory cytokines and other blood-borne mediators of neurotoxicity infiltrating remote brain areas, causing global inflammation, widespread microvascular burden, and brain tissue damage\textsuperscript{6,41,42}, and thus further increase brain FW\textsuperscript{16}. However, future work is needed to determine the temporal causality between these processes.

Interestingly, we observed that higher cardiovascular biomarker levels were related to higher FW in a region-specific pattern in GM, which was in contrast to the widespread association in WM. These findings are consistent with the previous clinical observation that GM is less vulnerable to CeVD, possibly because GM receives more collateral circulation and has more extensive blood supply than WM\textsuperscript{43}. Secondly, GM regions closely linked with cardiovascular markers included the anterior cingulate and somatosensory cortex, which are known to be heart function controlling brain regions\textsuperscript{44}. We can thus speculate that a vicious circle may occur in CeVD progression, where cardiovascular-derived embolism or ischaemia
leads to damage in the brain regions of heart controlling centre, thus leading to further derangement of cardiovascular function as well as cognitive function. Future studies using refined cardiac markers (i.e., cardiac imaging markers) could be performed to test this hypothesis and provide further insights into the mechanism of brain-heart interaction. Lastly and importantly, these GM regions overlapped with the canonical cognitive brain networks including DMN and ECN as well as somatomotor networks. This is aligned with the previous work demonstrating that neurodegenerative disease and cerebrovascular disease could lead to network-specific dysfunction, for example, the DMN in Alzheimer’s disease while ECN and somatomotor networks in cerebrovascular disease. Our findings suggested that FW alterations might be one of the early basis of brain network degeneration, which is worth further investigation in combination with other disease pathology.

Brain network-specific free-water mediate the associations of circulating cardiovascular biomarkers with cognition

Previous studies have demonstrated associations of circulating cerebrovascular biomarkers with cerebrovascular burden and cognition. Higher FW were related to cognitive deficits and longitudinal decline in dementia. Our study put the pieces together by demonstrating that the effects of circulating cardiovascular biomarkers on longitudinal cognitive decline were mediated through higher FW in both WM and GM. Critically, we found brain FW entirely mediated the association of blood cardiovascular biomarkers with cognitive decline, suggesting that FW could be a key brain proxy linking the periphery cardiovascular dysfunction to cognitive decline. According to the two-hit vascular hypothesis of dementia, concomitant cardiac and peripheral endothelial dysfunction would lead to damage to small arteries, arterioles and brain capillaries (e.g. hypoperfusion and BBB breakdown) as well as neurovascular alterations via both vascular and AD pathophysiology pathways (hits).
pathways interact and converge on these cerebrovascular dysfunction processes, and can independently or synergistically lead to neuronal damage, synaptic loss and neurodegeneration, resulting in dementia and cognitive decline\(^ {47}\). FW increases in diffusion MRI capture these processes of cerebrovascular dysfunction\(^ {17,38,48}\), likely leading to the full mediation effect observed here. Our results underscore the importance of cerebrovascular function in the connection between heart and cognition. Further longitudinal studies should take into account other dementia-related pathologies such as Aβ, tau, or TDP43 to fully understand the intricate interactions among these processes.

Furthermore, we demonstrated ECN FW mediated the influence of cardiovascular biomarkers on executive functioning decline, while DMN FW played a role in memory decline. These findings were supported by prior studies that the DMN is important for episodic memory and DMN dysfunction is widely implicated in AD dementia\(^ {21,23}\). In parallel, the ECN connectivity alteration was associated with executive function deficits in patients with CeVD\(^ {25}\). Such dissociable correspondence between cardiovascular-related network-specific GM FW abnormality at baseline and longitudinal decline in cognition suggests that cardiovascular-related cerebrovascular dysfunction may target specific brain networks for specific cognitive domains. Early cardiovascular changes might induce cerebral hypoperfusion, endothelial dysfunction and ischemic damage in specific brain networks, eventually causing BBB leakage and neuroinflammation which may manifest as higher GM FW\(^ {38}\). The reduced blood supply and entry of potentially harmful compounds may cause further injury to axons and neurons resulting in longitudinal cognitive dysfunction in domains supported by the targeted network\(^ {34}\). Moreover, with reference to the two-hit vascular hypothesis of dementia, the detected GM pattern might also suggest AD pathophysiology could potentially be more active in the DMN, underlying memory impairment, while the vascular hit might be the leading pathway in the ECN, underlying executive dysfunction.
Future investigation could combine blood cardiovascular biomarkers and a neuroimaging scan to identify vascular abnormalities as well as brain network-specific alterations in order to predict disease progression and domain-specific cognitive decline.

**Limitations and future directions**

There are several limitations of this study. First, our results were based on diffusion imaging data obtained with a single shell\(^{13}\). More advanced acquisitions including multi b-values and FW modelling method could further increase the sensitivity and specificity of the derived measures and potentially tease apart various microstructural and vascular changes\(^{49}\). Second, although we performed visual quality control to minimize the possible misalignment between the T1 and dMRI data, partial volume with surrounding CSF cannot be completely ruled out, in other words, brain atrophy may also affect the FW values in the GM. To mitigate these concerns, we performed partial volume correction and controlled for TIV and regional atrophy. Besides, we did not find any association of blood biomarkers with cortical thickness. Moreover, though we accounted for age, gender, and years of education in our analyses, the potential influence of these demographics on brain measures cannot be ruled out. Further investigation in a larger cohort with matching demographics across different groups are necessary. Lastly, participants were recruited from memory clinics, which might be confounded by selective survival bias. Although we have controlled for a number of vascular risk factors, no physical activity and alcohol consumption information was available for this cohort. More works in other clinical and community cohorts with comprehensive lifestyle evaluation are needed.

There are three future directions. First, more advanced diffusion imaging and FW models can be used to potentially tease apart different microstructural and vascular changes. Second, given the two-hit vascular hypothesis, future work in animal models and human
studies is needed to determine the temporal patterns (or even causality) of vascular damage, neurovascular dysfunction, AD pathologies like amyloid, and neuronal dysfunction\textsuperscript{47}. It is also important to combine FW with other biomarkers to determine the relative relevance of vascular pathology to the overall multi-aetiology picture. 3) Further developed, the new evidence on brain-heart interactions provided here may pave the way for effective strategy of early detection and prediction of domain-specific cognitive trajectories. Given that brain FW is a sensitive measure for early and mild vascular-related alteration\textsuperscript{50}, combining cardiovascular biomarkers with brain FW measurement may help monitor response to pharmacological and non-pharmacological therapies in vascular-related treatment of dementia.

**Conclusion**

In conclusion, we found higher circulating cardiovascular marker levels were associated with higher WM FW in a widespread pattern and higher GM FW in the DMN, ECN and somatomotor networks at baseline. Importantly, baseline FW in both GM and WM fully mediated the association of cardiovascular biomarker levels with cognitive decline over time. The association of blood markers with executive function decline was mediated by higher FW in the ECN, while the same association with memory decline was mediated by the DMN. In other words, the effects of cardiovascular dysfunction proxied by the blood biomarkers on the cognition may be accounted by cerebrovascular pathophysiology detected by higher FW in specific brain networks. Our results suggest that higher FW could underlie the heart-brain interactions. Developed further, assessment of FW in specific brain networks together with circulating cardiovascular assays would be helpful for prediction and monitoring of cardio/cerebrovascular disease progression and domain-specific cognitive decline.

WNL-2023-000247_sup -- http://links.lww.com/WNL/C813
Reference:

Table 1. Demographic, cognition, and circulating cardiovascular biomarker levels of participants.

<table>
<thead>
<tr>
<th></th>
<th>NCI (n = 76)</th>
<th>CIND (n = 134)</th>
<th>Dementia (n = 98)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), years</td>
<td>68.5 (6.1)</td>
<td>71.0 (8.2)</td>
<td>76.3 (8.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, Female/Male</td>
<td>44/32</td>
<td>62/72</td>
<td>62/36</td>
<td>0.03</td>
</tr>
<tr>
<td>Ethnicity, Chinese/Non</td>
<td>67/9</td>
<td>103/31</td>
<td>73/25</td>
<td>0.07</td>
</tr>
<tr>
<td>Education, mean (SD), years</td>
<td>10.3 (4.7)</td>
<td>7.6 (4.9)</td>
<td>4.8 (4.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Handedness, Right/Left</td>
<td>73/3</td>
<td>131/3</td>
<td>98/0</td>
<td>0.12</td>
</tr>
<tr>
<td>MMSE (Max=30), median (IQR)</td>
<td>28.0 (3.0)</td>
<td>25.0 (4.0)</td>
<td>16.0 (7.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Global CDR, mean (SD)</td>
<td>0.1 (0.2)</td>
<td>0.3 (0.2)</td>
<td>1.2 (0.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, Yes/No</td>
<td>43/33</td>
<td>91/43</td>
<td>83/15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyperlipidaemia, Yes/No</td>
<td>51/25</td>
<td>105/29</td>
<td>71/27</td>
<td>0.19</td>
</tr>
<tr>
<td>Diabetes mellitus, Yes/No</td>
<td>17/59</td>
<td>51/83</td>
<td>42/56</td>
<td>0.02</td>
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<tr>
<td>History of heart disease, Yes/No</td>
<td>5/71</td>
<td>15/119</td>
<td>8/90</td>
<td>0.50</td>
</tr>
<tr>
<td>History of stroke, Yes/No</td>
<td>12/64</td>
<td>51/83</td>
<td>27/71</td>
<td>0.003</td>
</tr>
<tr>
<td>Antiplatelet therapy, Yes/No</td>
<td>18/58</td>
<td>48/86</td>
<td>34/64</td>
<td>0.17</td>
</tr>
<tr>
<td>Smoking, Yes/No</td>
<td>20/56</td>
<td>40/94</td>
<td>25/73</td>
<td>0.74</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>24.6 (4.1)</td>
<td>24.1 (3.6)</td>
<td>23.8 (3.9)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>n = 76</th>
<th>n = 134</th>
<th>n = 98</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDF15, median (IQR), pg/mL</td>
<td>827.1 (350.9)</td>
<td>1132.2 (1057.0)</td>
<td>1555.4 (1549.7)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NT-proBNP, median (IQR), pg/mL</td>
<td>n = 45</td>
<td>n = 93</td>
<td>n = 80</td>
<td></td>
</tr>
<tr>
<td>hs-cTnT, median (IQR), pg/mL</td>
<td>6.2 (4.1)</td>
<td>9.6 (7.9)</td>
<td>13.9 (10.4)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Note: Superscript letters indicate whether group mean was significantly different compared with \(^a\)NCI; \(^b\)CIND; following one-way ANOVA or nonparametric Kruskal–Wallis ANOVA (for MMSE and blood biomarkers). Chi-square tests were carried out on sex and ethnicity, binarized vascular related covariates, while Fisher’s exact test was carried out for handedness.

**Abbreviations**: MoCA, Montreal Cognitive Assessment; CDR: Clinical Dementia Rating; MMSE, Mini-Mental State Examination; BMI, Body Mass Index; GDF-15, growth/differentiation factor 15; NT-proBNP, N-terminal pro hormone B-type natriuretic peptide; hs-cTnT, high-sensitivity cardiac troponin-T; ANOVA, analyses of variance; IQR: interquartile range; SD: standard deviation.
Table 2 Associations of cardiovascular biomarker levels with baseline and longitudinal cognitive scores.

<table>
<thead>
<tr>
<th></th>
<th>hs-cTnT (r, 95%CI)</th>
<th>NT-proBNP (r, 95%CI)</th>
<th>GDF 15 (r, 95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample size</td>
<td>173</td>
<td>173</td>
<td>232</td>
</tr>
<tr>
<td>Global cognition</td>
<td>-0.24 (-0.41 -0.07) **</td>
<td>-0.26 (-0.45 -0.07) **</td>
<td>-0.22 (-0.36 -0.09) **</td>
</tr>
<tr>
<td>Executive function</td>
<td>-0.25 (-0.40 -0.09) **</td>
<td>-0.28 (-0.45 -0.11) ***</td>
<td>-0.21 (-0.35 -0.06) **</td>
</tr>
<tr>
<td>Memory</td>
<td>-0.35 (-0.52 -0.19) ***</td>
<td>-0.25 (-0.40 -0.11) **</td>
<td>-0.28 (-0.45 -0.11) ***</td>
</tr>
<tr>
<td><strong>Longitudinal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample size</td>
<td>155</td>
<td>155</td>
<td>203</td>
</tr>
<tr>
<td>Global cognition</td>
<td>-0.22 (-0.37 -0.07) **</td>
<td>-0.23 (-0.38 -0.08) **</td>
<td>-0.24 (-0.39 -0.11) ***</td>
</tr>
<tr>
<td>Executive function</td>
<td>-0.21 (-0.35 -0.06) **</td>
<td>-0.22 (-0.37 -0.07) **</td>
<td>-0.20 (-0.34 -0.06) **</td>
</tr>
<tr>
<td>Memory</td>
<td>-0.30 (-0.45 -0.16) ***</td>
<td>-0.29 (-0.43 -0.15) ***</td>
<td>-0.22 (-0.35 -0.08) **</td>
</tr>
</tbody>
</table>

** indicated p<0.01, *** indicated p<0.001. **Abbreviations**: hs-cTnT, high-sensitivity cardiac troponin-T; NT-proBNP, N-terminal pro hormone B-type natriuretic peptide; GDF-15, growth/differentiation factor 15; CI, confidence interval.

Figure legends

**Figure 1 Study design schematic.** 308 participants with either no cognitive impairment (NCI), cognitive impairment with no dementia (CIND) or dementia were studied. General linear models (GLMs) were performed to identify region-specific associations between brain FW and the three blood cardiovascular biomarkers within the CIND+dementia group or NCI at baseline **(Step 1)**. Path analyses were used to evaluate whether and how FW in the grey and white matter mediated the effects of higher blood marker levels on baseline global cognitive deficits and longitudinal decline **(Step 2)**. Furthermore, the influences of network-specific GM FW on the association of higher blood marker levels with individual cognitive domains were also evaluated. **Abbreviations**: FW, free-water; GM, grey matter; WM, white matter.
Figure 2 Higher white matter free-water (FW) correlated with circulating cardiovascular marker levels. The whole-brain voxel-wise linear regression indicated that higher FW values in widespread brain white matter regions were associated with increased levels of (A) hs-cTNT, (B) NTpro-BNP, and (C) GDF-15. Results are TFCE enhanced, reported at $p<0.01$, FWE corrected. Abbreviations: hs-cTnT, high-sensitivity cardiac troponin-T; NT-proBNP, N-terminal pro hormone B-type natriuretic peptide; GDF-15, growth/differentiation factor 15.
Figure 3 Higher grey matter free-water (FW) correlated with circulating cardiovascular marker levels. The whole-brain vortex-wise linear regression indicated that higher FW values in middle frontal, temporal lobes and cingulate regions were associated with increased levels of (A) hs-cTNT, (B) NTpro-BNP, and (C) GDF-15. (D) Yeo’s functional intrinsic networks parcellation. Results are reported at $p<0.01$, FWE corrected. **Abbreviations:** ECN, executive control network; DMN, default mode network; DAN dorsal attention network; SN/VA, ventral attention network; SMN, somatomotor network; hs-cTnT, high-sensitivity cardiac troponin-T; NT-proBNP, N-terminal pro hormone B-type natriuretic peptide; GDF-15, growth/differentiation factor 15.
Figure 4 Effects of circulating cardiovascular biomarker levels on global cognition through mediators. Schematic diagram of the path analyses for hs-cTNT. hs-cTNT was entered as a predictor in each model. Mean WM FW and mean GM FW were added as mediators. (A) global cognition baseline impairment and (B) rate of decline over time (5 years) were treated as the outcome. Numbers on the paths indicate standardized coefficients that were statistically significant. Abbreviations: GM, grey matter; WM, white matter; FW, free-water; hs-cTnT, high-sensitivity cardiac troponin-T.

Figure 5 Effects of circulating cardiovascular biomarker levels on longitudinal executive function and memory decline through mediators. Schematic diagram of the path analyses. hs-cTNT was inputted as a predictor. Mean WM FW, and mean GM FW derived from 7 Yeo’s intrinsic networks were added as mediators (see parcellations with coloured boundary in the right bottom). Rates of changes in executive and memory domains over time (5 years) were treated as outcomes. Numbers on the paths indicate standardized coefficients that were statistically significant. Abbreviations: GM, grey matter; WM, white matter; FW, free-water; ECN, executive control network; DMN, default mode network; DAN dorsal attention network; SN/VA, ventral attention network; SMN, somatomotor network; hs-cTnT, high-sensitivity cardiac troponin-T.