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Developing blood-brain barrier arterial spin labelling as a non-invasive early biomarker of Alzheimer's disease (DEBBIE-AD): a prospective observational multicohort study protocol

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ABSTRACT

Introduction Loss of blood-brain barrier (BBB) integrity is hypothesised to be one of the earliest microvascular signs of Alzheimer's disease (AD). Existing BBB integrity imaging methods involve contrast agents or ionising radiation, and pose limitations in terms of cost and logistics. Arterial spin labelling (ASL) perfusion MRI has been recently adapted to map the BBB permeability non-invasively. The DEveloping BBB-ASL as a non-Invasive Early biomarker (DEBBIE) consortium aims to develop this modified ASL-MRI technique for patient-specific and robust BBB permeability assessments. This article outlines the study design of the DEBBIE cohorts focused on investigating the potential of BBB-ASL as an early biomarker for AD (DEBBIE-AD).

Methods and analysis DEBBIE-AD consists of a multicohort study enrolling participants with subjective cognitive decline, mild cognitive impairment and AD, as well as age-matched healthy controls, from 13 cohorts. The precision and accuracy of BBB-ASL will be evaluated in healthy participants. The clinical value of BBB-ASL will be evaluated by comparing results with both established and novel AD biomarkers. The DEBBIE-AD study aims to provide evidence of the ability of BBB-ASL to measure BBB permeability and demonstrate its utility in AD and AD-related pathologies.

Ethics and dissemination Ethics approval was obtained for 10 cohorts, and is pending for 3 cohorts. The results of the main trial and each of the secondary endpoints will be submitted for publication in a peer-reviewed journal.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ DEveloping BBB-ASL as a non-Invasive Early biomarker-Alzheimer's disease (DEBBIE-AD) is a large prospective observational study that uniquely focuses on testing a single promising imaging biomarker in multiple cohorts.
⇒ The outcomes of DEBBIE-AD may gain insights into the underlying mechanisms of cognitive impairment and may even present a novel imaging biomarker for disease prediction.
⇒ Although most research questions can be addressed within individual cohorts, the need for harmonisation may arise to ensure consistency and comparability.
⇒ Factors including patient motion and varying levels of atrophy may affect the acquisition and image analysis, but it is unsure yet to what extent the image quality will be degraded.

INTRODUCTION

Ageing-related cognitive impairment has emerged as one of the major public health challenges of our time, with Alzheimer's disease (AD) being one of the primary causes.[1] While the diagnosis of AD can be made based on biomarkers for amyloid-β (Aβ) plaques and τ tangles alone,[2 3] other biomarkers are still needed to help unravel the complex pathological cascade of AD.[4–7]

Novel biomarkers may help to improve prognosis and AD subtype stratification and eventually monitor the effects of potential disease-modifying treatments.[8]

One of the earliest microvascular observations in the pathogenesis of AD and related dementias is the loss of integrity of the blood-brain barrier (BBB).[9 10] The BBB is a cellular structure that protects the brain by regulating the transport of molecules between the blood and the interstitial fluid in the brain.[11] While BBB dysfunction in AD has been recognised for some time, its importance in neurodegenerative diseases has recently been redefined as a potential biomarker implicated in vascular, inflammation and glymphatic pathways of AD pathogenesis.[12 13]

Encouraged by promising findings of altered albumin cerebrospinal fluid (CSF)/serum ratio in AD,[14] novel BBB imaging biomarkers may uncover spatial patterns of BBB vulnerability in the brain. Compared with invasive methods to probe BBB integrity, such as positron emission tomography (PET) with radioactive isotopes and dynamic contrast-enhanced (DCE) MRI with gadolinium chelated agents, arterial spin labelling (ASL) perfusion MRI is fully non-invasive as well as cost-effective and easy to use. ASL uses magnetically labelled blood water as an endogenous tracer and can be extended to quantify BBB water exchange dynamics by separating the ASL signal into intravascular and extravascular compartments based on differences in the MRI signal characteristics of the two compartments. The BBB-ASL technique probes to quantify BBB water permeability and potentially employ it as a new biomarker of BBB dynamics.

Therefore, the DEveloping BBB-ASL as a non-Invasive Early biomarker (DEBBIE) consortium was initiated in 2020 through the Joint Programming Neurodegenerative Disease funded project 'Novel imaging and brain stimulation methods and technologies related to neurodegenerative diseases'. Here, we describe the DEBBIE-AD study design to investigate the clinical value of BBB-ASL as an early biomarker of AD.

Objectives

Our study design is based on specific methodological and clinical research questions (RQ1A-C and RQ2A-C, respectively, as defined below).

Reproducibility (RQ1A)

Is the BBB water permeability measured with BBB-ASL reproducible in healthy subjects? The within-subject coefficient of variation of ASL cerebral blood flow (CBF) has been established to be around 10–20%, and similar reproducibility was found for the gold standard CBF acquisition technique oxygen-15-labelled water (^{15}O-H_2O) PET.[15 16] Nevertheless, the reproducibility of BBB-ASL measurements still needs to be established. One pilot study[17] has shown encouraging BBB-ASL reproducibility in a cohort of 10 healthy volunteers. RQ1A will investigate the reproducibility of BBB-ASL in a larger cohort of healthy volunteers (n=50). Additionally, we aim to compare the two most-used BBB-ASL acquisition techniques: multi-echo (ME) and diffusion-weighted (DW) ASL.[17 18]

Accuracy (RQ1B)

What is the accuracy of BBB-ASL compared with PET in measuring BBB water permeability? Currently, the measurement of blood flow with ^{15}O-H_2O-PET and ^{11}C-butanol-PET is considered the reference standard for in vivo BBB water permeability measurements. ^{11}C-butanol is freely diffusible through the BBB, and, in contrast, water transport is mediated by aquaporin-4 (AQP-4) channels.[19] By comparing water permeability values derived by BBB-ASL with PET acquired with a simultaneous PET-MRI device, we will investigate the accuracy of our biomarker.

Normal variability (RQ1C)

What is the normal range of BBB-ASL derived values across age and sex in a cognitively healthy cohort? Haemodynamic parameters such as CBF and arterial transit time (ATT) are known to have high physiological variability across healthy volunteers.[20] CBF is not only known to have short-term variability related to physiological changes such as caffeine and exercise[21] but also changes significantly with age and sex.[22 23] This variability may impact the BBB-ASL values in older adults. Thus, to identify abnormal patterns of BBB water permeability in pathological status, the normal variability of BBB water permeability measurements needs to be established in age and by sex.

Patients versus controls (RQ2A)

Can BBB-ASL differentiate patients with AD from healthy controls? Increased and decreased CBF patterns have already been recognised for both AD and prodromal AD stages.[24] Additionally, ATT estimates have been shown to help differentiate patients with AD and controls, even when PET was already present in the model.[25] For RQ2A, we will investigate if BBB water permeability differs between patients with AD and healthy controls in both regions of interest and pattern-based analysis.

BBB-ASL versus established AD biomarkers (RQ2B)

Does BBB-ASL correlate with current AD biomarkers? Recent studies have found associations between the BBB-breakdown marker CSF/blood albumin ratio and Aβ deposition,[26] suggesting that BBB breakdown may play a role in amyloid-related AD pathophysiology. The accepted clinical biomarkers of AD are defined mainly by Aβ and τ pathology (neurofibrillary tangles).[3] These biomarkers can also be used to stage patients across the AD pathophysiology.[27 28] Furthermore, apolipoprotein E-4 (APOE-4) is established as the most prominent genetic risk factor of AD. Therefore, we will investigate associations of BBB water permeability with more established AD biomarkers: Aβ, τ, cortical atrophy and robust risk factors such as APOE-4, aiming to localise BBB-ASL alterations in the AD continuum.[29]

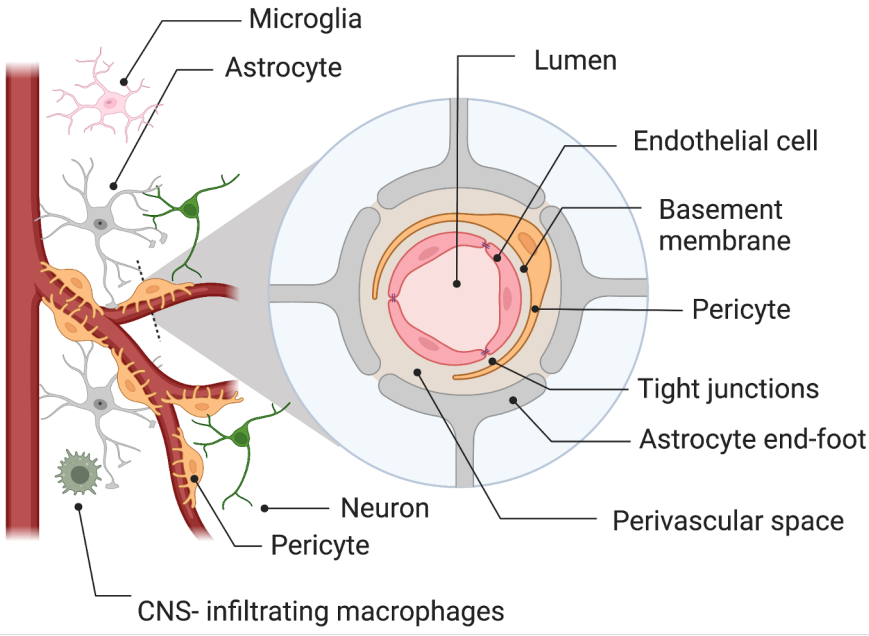

Figure 1 Schematic representation of the blood-brain barrier. Adapted with permission from Moyaert *et al*.[68] CNS, central nervous system.

BBB-ASL in novel AD pathways (RQ2C)

Is BBB-ASL associated with vascular, inflammation and glymphatic AD markers? Cardiovascular risk factors and neuroinflammatory markers have been shown to accelerate BBB dysfunction as well as AD pathophysiology,[7 30 31] and investigating small vessel disease (SVD) in relation to AD has been gaining attention.[32 33] Furthermore, activated microglia as a neuroinflammatory response has been found in patients with AD,[34] and BBB breakdown has been correlated with activated microglia and cognitive decline in older adults[35] as well as in patients with SVD.[36] Additionally, compromised functioning of the glymphatic system has been correlated with AD,[37] impairing Aβ and toxin clearance. Furthermore, disturbed sleep and increased wakefulness have been found to acutely elevate Aβ production and impede Aβ clearance,[38 39] concluding that unstable sleep is associated with an increased risk of AD. We will investigate the associations of cardiovascular and cerebrovascular, neuroinflammatory and sleep disturbance factors with BBB water permeability.

BACKGROUND

The blood-brain barrier

The BBB is a cellular structure that tightly regulates the transport of molecules between the blood and the central nervous system.[11] At the cellular level, the BBB comprises continuous endothelial cells surrounded by pericytes, smooth muscle cells, astrocytes and microglia (figure 1), constituting the neurovascular unit. In a healthy and intact BBB, the endothelial cells are held together by tight junctions, eliminating paracellular transport across the BBB. While small lipophilic molecules, such as O_2, can passively diffuse through the cell membrane, water and small ions are transported through dedicated channels regulating their exchange. AQP-4 channels are responsible for water transport and play an essential role in the glymphatic system of the brain.[40]

Measuring the permeability of the BBB to water with ASL-MRI

BBB imaging with ASL may be a viable alternative to address the aforementioned shortcomings of PET due to its non-invasiveness by using magnetically labelled blood water as an endogenous contrast agent. A detailed explanation of ASL can be found elsewhere.[41] Briefly, the water of the blood in the vertebral and internal carotids is magnetically labelled. When the labelled water reaches the microvasculature, at any given time—post-labelling delay (PLD)—a fraction of the label remains in the intravascular compartment, and a fraction passes the BBB and becomes part of the interstitial fluid, that is, the extravascular compartment.[42 43] Similarly to DCE, the measured MR signal can originate from the intravascular and extravascular compartments. Various characteristics of these two compartments, such as the diffusion coefficient[44] and T2 time,[43] can be measured by either DW- or ME-ASL, respectively, to estimate the contribution of each compartment to the total signal and the exchange time between these compartments that characterise the BBB permeability to water. If the labelled water molecules remain within the blood compartment without crossing the BBB, they will retain the diffusion and T2 relaxation properties of blood. Conversely, if these labelled water molecules cross the BBB, they will assume the properties of the grey matter. By employing various diffusion gradients (DW-ASL) or acquiring data from multiple echo times (ME-ASL), it becomes possible to characterise these two compartments and estimate the

amount of water that has traversed from the blood to the brain parenchyma. Previous DW-ASL and ME-ASL studies have shown promising results for measuring this intra/extravascular compartment ratio in patients who had a stroke and animals.[45–47] The water exchange time (Tex) corresponds to the average time it takes for the labelled water at the capillary bed to transit from the intravascular to extravascular space by crossing the BBB. Thus relatively high/low Tex represents a relatively low/high BBB permeability to water, respectively.

Relevance in AD

The focus on AD research is mostly based on the disease-specific molecular biomarkers amyloid (A) and τ (T) within the AT(N) framework.[3] The new NIA-AA criteria for AD diagnosis consider biomarkers of cerebrovascular health (V), by incorporating MRI findings of cerebrovascular disease.[7 48 49] BBB dysfunction is increasingly acknowledged as a potential early risk factor for AD,[7 50 51] including increased leakage through disrupted tight junctions, decreased AQP-4 expression and reduced clearance of Aβ.[52 53] BBB disruption has been correlated with Aβ burden in mice,[54] and albumin levels have been found to correlate with Aβ levels.[26] However, the association between BBB disruption and τ has yet to be elucidated.

Additionally, multiple novel AD pathology pathways/hypotheses (vascular, inflammatory and glymphatics mechanisms) all likely contribute to reduced BBB integrity,[55 56] making this a nexus for the major pathologies involved in age-related cognitive impairment and dementia.[50 55] Focal BBB breakdown, as seen in early cerebral microhaemorrhages[57 58] detected with susceptibility-weighted imaging (SWI) MRI, leads to the extravasation of red blood cells to the central nervous system (CNS). Lobar microhaemorrhages are often due to cerebral amyloid angiopathy (CAA), present in many cases of mild cognitive impairment (MCI) and AD, along with Aβ deposition in the brain parenchyma.[59] The BBB is involved in neuro-inflammatory processes in AD, which include microglia activation[34 60] and astrocytes' response[13 61] through reactive gliosis, consequently upregulating glial fibrillar acidic protein (GFAP) and other inflammatory mediators.

Finally, studies have highlighted the role of the brain's glymphatic system in AD by contributing to Aβ clearance.[37 62] AQP-4 water channels support the function of the glymphatic system,[40] and their dysfunction can negatively affect Aβ clearance.[63] Recent findings also described that genetic variations of AQP-4 can negatively impact sleep quality and clearance of Aβ,[50] contributing to the development of AD.[64] Therefore, investigating the permeability to water of the BBB and its relation to AD biomarkers could be of significant importance as an early-stage vascular biomarker of AD and related dementias.

Current BBB biomarkers

A widely used fluid BBB biomarker is the CSF/plasma albumin ratio, as albumin is a protein naturally present in the blood but not the CSF. Thus, the presence of albumin in the CSF is used to measure BBB breakdown.[65] This technique's limitations include the absence of spatial information and its invasiveness—as it requires a lumbar puncture. Plasma BBB biomarkers include the platelet-derived growth factor β (PDGFRβ; related to neuroinflammation) and GFAP (related to astrocytosis). Injured pericytes in the neurovascular unit release PDGFRβ into the CSF and CSF PDGFRβ has been recently correlated with worse BBB integrity measured by the CSF/plasma albumin ratio,[66] concluding that it may be involved in age-related BBB disruption together with neuroinflammation. GFAP is a marker of reactive astrocytes—considered critical glial cells in the support of vital CNS functions—and has been found to be significantly increased in all Aβ-positive groups compared with participants without Aβ pathology.[67]

Several imaging markers exist to study BBB function; a more detailed overview can be found elsewhere.[68] One of the most commonly used methods is gadolinium-based contrast-enhanced MRI. Gadolinium chelates cannot cross the intact BBB due to their relatively large molecular size[69] and will only leak paracellularly through disrupted tight junctions. Gadolinium alters local magnetic properties, which can be measured over time with DCE. However, gadolinium's relatively large molecular size makes DCE-MRI less suited for measuring subtle BBB breakdown. Moreover, concerns about patient safety, comfort and environmental hazards[70 71] make DCE unsuitable for repeated measurements.

An alternative approach is to measure the BBB permeability to water. One way to measure BBB permeability to water is with PET imaging combining oxygen-15-labelled water (^{15}O-H_2O) and ^{11}C-butanol isotopes.[72–74] As alcohol, ^{11}C-butanol is a freely diffusible tracer, whereas ^{15}O-H_2O's transport is typically limited to AQP-4 channels. Therefore, the ratio of ^{15}O-H_2O to ^{11}C-butanol transport-based PET measurements yields an index of BBB function. However, this approach is impractical for widespread use due to its costs, invasiveness, radiation burden and logistical demands.

METHODS AND ANALYSIS

DEBBIE consortium

The collaborators of the DEBBIE consortium are shown in figure 2. DEBBIE builds on existing successful collaborations between consortium members and external collaborators on several international projects involving (1) dementia imaging using ASL (ASL-European Cooperation in Science and Technology (ASL-COST) – action BM113: ASL in dementia, in which ASL sequence standards and multisite reproducibility of ASL were established,[75] (2) automatic processing and interpretation of ASL-CBF (through the ExploreASL[76] initiative, and Eurostars 'ASPIRE' project, http://aspire-mri.eu/) and (3) efforts to develop best practices for perfusion MRI image processing to accelerate ASL clinical integration[77] (the

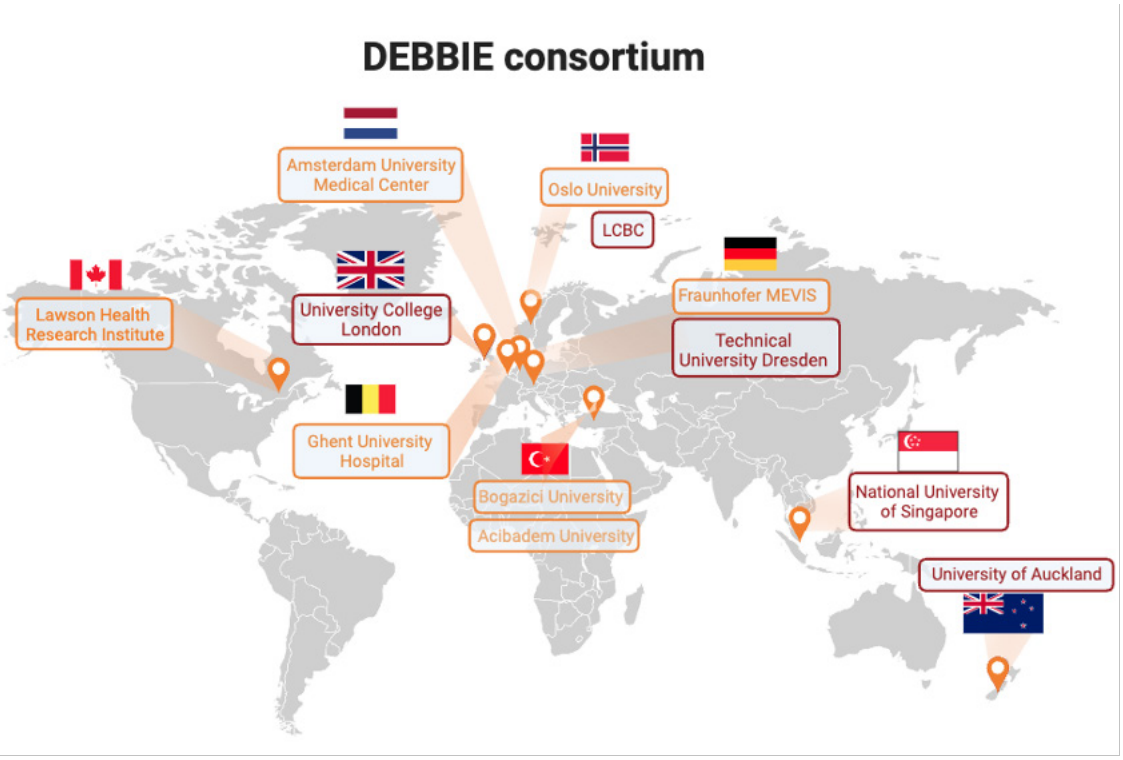

Figure 2 A geographical overview of the DEBBIE (DEveloping BBB-ASL as non-Invasive Early biomarker of Alzheimer's Disease) consortium partners (orange box) and external collaborators (violet box). Franhofer MEVIS, Fraunhofer-Institut für Digitale Medizin (Fraunhofer MEVIS);LCBC, Centre for Lifespan Changes in Brain and Cognition.

International Society for Magnetic Resonance in Medicine (ISMRM) – Open Science Initiative for Perfusion Imaging (OSIPI), http://osipi.ismrm.org/).

BBB-ASL acquisition

ME-ASL probes the T2 relaxation time of labelled water at different inflow times (TI). As the T2 differs between blood and the extravascular compartment at 3T, it is possible to perform BBB water permeability analysis.[78 79] The ME acquisition allows the assessment of the transversal relaxation time T2, which is longer for blood than for tissue. Since the ASL images with and without labelling are subtracted, all intravascular and extravascular signal is removed except for the signal coming from the labelled water molecules. If these molecules still reside in the intravascular compartment at the moment of acquisition, the measured signal will have a long T2. The more these molecules have passed the BBB into the extravascular compartment, the larger part of the measured signal will have a shorter T2.[17]

DEBBIE sequence

Our proposed ME-ASL uses time-encoded pseudo-continuous ASL acquisitions[42 80 81] with Walsh-ordered Hadamard (HAD)-encoding and an ME segmented 3D-GRASE readout.[82] Two protocols with different sub-bolus durations (SBD) are used,[17] where TI=SBD+PLD (online supplemental table 1). First, the single-echo HAD8 includes seven PLDs optimised for ATT and CBF quantification, and second, the ME HAD4, includes three PLDs optimised for BBB Tex quantification. The sequence was also implemented in a vendor-independent MRI sequence development framework gammaSTAR.[83 84] An illustration of perfusion-weighted images from single-TE HAD8 and ME HAD4 acquisitions is shown in online supplemental figure 1.

ME-ASL image processing

To harmonise the image processing, DEBBIE-AD uses ExploreASL.[41 76] CBF, ATT and Tex quantification are performed with FSL-FABBER,[76 85] implemented as a plug-in in ExploreASL. An example of the mean and SD Tex maps of two DEBBIE cohorts is shown in figure 3 to illustrate the similarities of the Tex patterns from two cohorts of similar-aged healthy adults from different sites.

Study participants

DEBBIE-AD includes cohorts with healthy as well as participants with cognitive impairment (table 1). The inclusion criteria for cognitively normal subjects are a global Clinical Dementia Rating (CDR) score of 0 or a score ≥27 points on the Mini-Mental State Examination (MMSE)[86] or equivalent on other similar tests. For defining subjects with MCI, a global CDR score ≥0.5 point (s) or an MMSE score of 23–26 points (inclusive) or equivalent will be used. As indicated in online supplemental table 2, these criteria vary slightly between cohorts. For AD, a global CDR score of ≥2 points or an MMSE score of <23 points will be used, as well as clinical consensus. Some cohorts (online supplemental table 2) also use Montreal

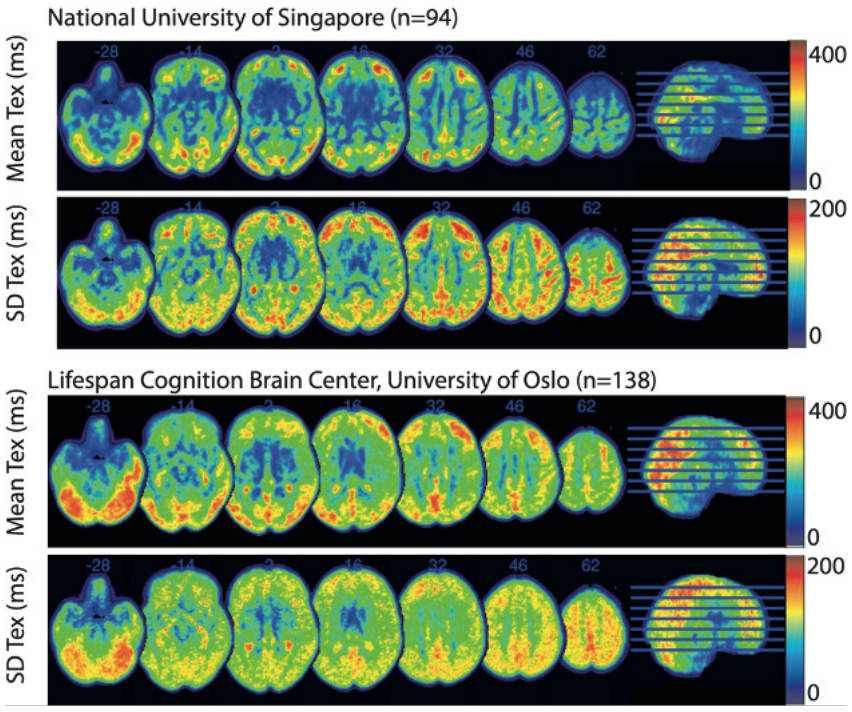

Figure 3 Scanner-average time of exchange (Tex) maps for the two populations—National University of Singapore mean age of 56.7±6.1 with 62% women and Centre for Lifespan Changes in Brain and Cognition mean age of 52.9±15.3 with 64% women—with mean Tex and the voxel-wise between-subject SD in Montreal Neurological Institute (MNI) space.

Cognitive Assessment (MoCA) scores for diagnosis (at least 26 for healthy controls, 18–26 for MCI and 12–26 for AD). Exclusion criteria differ between cohorts but generally include major brain lesions or psychiatric disorders, the inability to undergo all study procedures, visual or hearing impairment that would impair neuropsychological testing, severe depression (eg, Geriatric Depression Scale score ≥11 points),[87] other comorbidities or medication that could impair cognition at the discretion of the cohort investigator (eg, stroke, epilepsy or use of lithium carbonate) and contraindications to MRI scanning (eg, pacemaker/defibrillator, ferromagnetic metal implants).

Patient and public involvement

The patients were not involved in the design of the study.

Biomarkers

The biomarkers from the DEBBIE-AD cohorts include MRI and PET (table 2) as well as blood and CSF biomarkers (table 2) and neuropsychological assessments (online supplemental table 3). In addition to BBB-ASL, the core MRI scan types (T1w, T2w, fluid-attenuated inversion recovery and diffusion-weighted imaging) are conducted in all DEBBIE-AD participants. Other advanced MRI acquisitions differ between cohorts. This may include one or more of the following types of acquisitions: three-dimensional (3D) SWI or 3D-T2*-weighting, diffusion tensor imaging, resting-state and task-based functional MRI and quantitative susceptibility mapping. Five cohorts will also acquire amyloid-PET and τ-PET scans (RQ2A-C), and one cohort will acquire neuroinflammation PET scans (RQ2C). Eight cohorts include blood sample measurements (table 2) to allow APOE genotyping, inflammatory markers (RQ2C) such as C reactive protein, astrocyte neuroinflammation marker GFAP, microglial marker triggering receptor expressed on myeloid cells 2 (TREM2) and neurodegenerative marker neurofilament light chain. Plasma biomarkers (RQ2A-B) include Aβ peptides 1–40 and 1–42, as well as total-τ and phosphorylated-τ species. Additional routine blood analysis values (eg, lipids and glucose), vitamin status (B12 and folic acid) and albumin will also be obtained in two cohorts (table 2) to investigate their potential effect on the normal BBB permeability (RQ1B). The neuropsychological assessment for each cohort includes tests on several cognitive domains (table 2).

Analysis plan

The DEBBIE cohorts are designated a specific technical or clinical task to answer the research questions (table 1). The DEBBIE data collection has started in 2023 and is planned to finish by May 2025.

The *reproducibility* of BBB-ASL (**RQ1A**) will be investigated with healthy participants from the Ghent cohort. Good reproducibility of ME-ASL data was already shown in a pilot study of 10 participants.[17] We will extend this number to provide a more robust estimation of reproducibility by including 50 healthy controls for test–retest analysis. Additionally, the Dementia Prevention Research Clinic (DPRC) cohort aims to compare DW-ASL[18] with ME-ASL measurements in 40 healthy controls to investigate if the two ASL techniques used to measure BBB water permeability provide similar information.

Table 1 Cohort demographics of DEBBIE-AD

DEBBIE-AD cohort	Site	Anticipated sample size (n)		Age range (years)	RQ
		Healthy controls	Patients		
LCBC	Oslo University	150	–	20–90	1C/2B
DDI		50	50 (Aβ+)	40–80	2C
COGA	Amsterdam UMC	204 (SCD/MCI/AD)		60+	2A
VARIATION		50 (SCD/MCI/AD)		65+	2C
VVI		–	55 MCI	50+	2A/2C
SYNAPSE			20 Aβ+	50+	2B/2C
InflammAD		20	20 AD	50+	2A /2B/ 2C
GUH memory clinic	Ghent University Hospital (GUH)	–	30 MCI/20 AD	55–75	1A
Ghent healthy controls		50	–	18+	1A
Cognitive Neurology and Alzheimer Research Centre	Lawson Health Research Institute	12	12 Stroke 12 MCI 12 AD	50–90	1B
DPRC	University of Auckland	40	40 MCI 25 AD	55+	1A/2A /2B
NEURO-BMC	National University Hospital of Singapore	–	200 SCD or MCI	45–85	2C

AD, Alzheimer's disease; Aβ, amyloid-β; COGA, Centre Of Geriatrics Amsterdam; DDI, Dementia Disease Initiation; DEBBIE, DEveloping BBB-ASL as a non-Invasive Early biomarker; DPRC, Dementia Prevention Research Clinic; InflammAD, Imaging inflammation in Alzheimer's disease; LCBC, Centre for Lifespan Changes in Brain and Cognition; MCI, mild cognitive impairment; NEURO-BMC, NEUROlogical biomarkers of Blood, MRI and Cognition; RQ, research questions according to the Analysis Plan section; SCD, subjective cognitive decline; SYNAPSE, Synaptic density and τ pathology in Alzheimer's disease; UMC, University Medical Centre; VARIATION, Vascular Phenotypes in a Geriatric Population; VVI, Verloren Verbindingen Vinden ('finding lost connections'); WMH, white matter hyperintensities.

The *accuracy* of BBB-ASL values (**RQ1B**) will be evaluated by comparing ME-ASL with ^{15}O-H_2O-PET values in a subset of 10 pigs and 12 AD, 12 MCI patients and 12 age-matched controls from the Lawson Health Research Institute (LHRI) cohort, scanned with the same protocol and same scanner. Previous studies were able to establish the accuracy of ASL in comparison with ^{15}O-H_2O-PET with 14 subjects,[88] and ^{15}O-H_2O-PET has been used before to study the relation between water dynamics and early accumulation of Aβ.[89]

The *normal variability* of the BBB-ASL values (**RQ1C**) over age and sex will be investigated in 150 healthy participants from the Centre for Lifespan Changes in Brain and Cognition (LCBC) cohort to create an atlas of a healthy BBB across age groups and to investigate the ability of Tex to predict age above and beyond CBF and ATT.[90] Previous studies examining the age and sex-related variability of CBF in healthy adults achieved significant and reliable results when including between 50 and 100 participants.[91 92]

To investigate if *BBB-ASL can differentiate patients with AD from healthy controls* (**RQ2A**), BBB-ASL differences between 25 patients with AD, 40 MCI patients and 40 healthy controls will be investigated with data from the DPRC cohort. We will compare global and regional Tex values between the three groups and perform analysis to determine if spatial patterns of Tex distinguish the groups. We will test if the between-group results from the DPRC cohort are consistent with other cohorts in the consortium for the same groups, namely 55 MCI and 20 patients with AD from the Amsterdam cohorts (Verloren Verbindingen Vinden (VVI) and Imaging inflammation in Alzheimer's disease (InflammAD)), as well as 20 MCI and 20 patients with AD from the Technical University Dresden (UKD) cohort. Additionally, MRI and blood biomarkers data will be included from Centre Of Geriatrics Amsterdam (COGA) participants, which include from subjective cognitive decline (SCD) to patients with AD.

To investigate how BBB water permeability is related to the *established AD biomarkers* (**RQ2B**), we will compare the BBB-ASL values with established AD biomarkers, such as amyloid and τ, using CSF/blood, MRI and PET. The LCBC and DPRC cohorts will investigate Tex patterns with amyloid PET in subjects older than 50, for LCBC and in both 40 MCI and 20 patients with AD, for DPRC; the 'Imaging inflammation in AD' (InflammAD) cohort, will use blood and CSF biomarkers of amyloid and τ from 20 patients with AD; and the 'Synaptic density and tau pathology in AD' (SYNAPSE) cohort will include amyloid- and τ from PET, CSF and blood, from 20 Aβ-positive individuals.

Table 2 Neuroimaging and fluid biomarkers

Cohort	Participants	Neuroimaging			Fluid biomarkers	
		Common MRI (T1w, T2w, FLAIR, DWI)	Other MRI (fMRI, DTI, SWI)	Amyloid- PET	Blood	CSF
LCBC	Healthy	✓	task-fMRI	✓ (for age >50 y)	✓ (CRP, vitamin D and cholesterol)	–
DDI	SCD	✓	DTI, SWI	✓ (in MCI/AD ub-groups)	✓ (Aβ, p-τ, Nfl, GFAP, TREM2, albumin)	✓ (Aβ–38, Aβ–40/42, p-τ,t-τ, Nfl, albumin)
COGA / VARIATION	Healthy, MCI	✓	DTI, SWI	–	✓ (Hb, vit D, vit B12, folic acid, cholesterol, albumin, creatinine)	–
VVI	MCI	✓	DTI, fMRI	–	–	✓ (whole CSF proteome including amyloid and τ)
SYNAPSE	AD spectrum	✓	DTI, fMRI	✓	Aβ, t-τ, p-τ, Nfl, TREM2,	
InflammAD	Healthy, AD spectrum	✓	DTI, fMRI	–	✓ (to be determined)	
GUH	MCI, AD	✓	s-fMRI, DTI, SWI	–	–	–
Ghent healthy controls	Healthy	✓	rs-fMRI, DTI, SWI	–	–	–
LHRI	Healthy, MCI, AD	✓	DTI, SWI	–	–	–
UKD	MCI, AD	✓	DTI, SWI	–	✓ (Aβ, p-τ, Nfl)	✓ (Aβ–40, Aβ–42, Aβ–40/42Ratio, p-τ, t-τ, Nfl, albumin)
DPRC	Healthy, MCI, AD	✓	rs-fMRI, DTI, SWI, QSM	✓	✓ (Aβ1–42, Aβ1–40, Aβ–40/42 ratio, t-τ, p-τ, Nfl, GFAP, S100β, sPDGFRβ)	–
NEURO-BMC	SCD, MCI, AD	✓	rs-fMRI, DTI, SWI	–	–	–

AD, Alzheimer's disease; Aβ, amyloid β; COGA, Centre Of Geriatrics Amsterdam; CRP, C reactive protein; CSF, cerebrospinal fluid; DDI, Dementia Disease Initiation; DPRC, Dementia Prevention Research Clinic; DTI, diffusion tensor imaging; DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; GFAP, glial fibrillar acidic protein; GUH, Ghent University Hospital; InflammAD, Imaging inflammation in Alzheimer's disease; LCBC, Centre for Lifespan Changes in Brain and Cognition; LHRI, Lawson Health Research Institute; MCI, mild cognitive impairment; NEURO-BMC, NEUROlogical biomarkers of Blood, MRI and Cognition; Nfl, neurofilament light protein; p-τ, phosphorylated τ; rs-fMRI, resting state functional MRI; SCD, subjective cognitive decline; sPDGFRβ, soluble platelet derived growth factor receptor-β; SWI, susceptibility weighted imaging; SYNAPSE, Synaptic density and τ pathology in Alzheimer's disease; S100β, S100 calcium binding protein B; t-τ, total τ; UKD, Technical University Dresden; VARIATION, Vascular Phenotypes in a Geriatric Population; VVI, Verloren Verbindingen Vinden .

Finally, to study how the BBB is implicated in (1) vascular, (2) inflammation and (3) glymphatic AD pathways (**RQ2C),** we will include: (a) 200 SCD/MCI participants will be selected with quite extensive white matter hyperintensities, from the NEUROlogical biomarkers of Blood, MRI and Cognition (NEURO-BMC) cohort, as well as 50 participants from the Vascular Phenotypes in a Geriatric Population (VARIATION) study which will be used to look at other vascular biomarkers including, for example, cerebral small vessel disease, microvascular function, arterial stiffness and intima-media thickness. Additionally, the Dementia Disease Initiation (DDI) cohort will focus on the differences in BBB-ASL values between Aβ+ and Aβ– groups, to identify CAA in early stages; (b) 20 MCI and 20 patients with AD from UKD will be used to focus on investigating macrostructural and microstructural patterns of sleep in relation to BBB-ASL values in

participants with and without sleep disorders; and (c) 55 MCI subjects from the VVI ('Finding Lost Connections') cohort, 20 Aβ+ subjects from the SYNAPSE group, as well as 20 healthy controls and 20 patient with AD from the InflammAD cohorts (table 1) will be used to examine novel inflammation biomarkers (GFAP, TREM2) and a new PET tracer for activated microglia (^{11}C-SMW139 and UCBJ).

Regional analysis

We will investigate if there is a regional variability in Tex values related to tissue type (gray matter vs white matter) or vascular territory (Anterior, middle and posterior cerebral arteries, proximal vs distal). We hypothesise that BBB disruption patterns may follow amyloid and τ accumulation patterns seen in various stages of AD pathology.[93 94] Therefore, for the clinical RQs (RQ2A-C), we will focus on the regional variability of BBB-ASL values in AD—including the precuneus, cingulate cortex and orbital frontal gyrus[94]—as well as τ signature regions—transentorhinal, temporal and limbic regions such as the hippocampus.[93]

Data harmonisation

Although most of our research questions can be answered using single cohort data, we may need to combine cohorts for more statistical power for post hoc analyses ('Analysis plan' section). In the case of data pooling, we will attempt several harmonisation steps.

While the BBB-ASL acquisition protocols are consistent across cohorts, the other imaging techniques may vary. To harmonise the data structure, we will convert all DEBBIE-AD data to Brain Imaging Data Structure (BIDS) format[95] and investigate the metadata variability between data sets (eg, due to scanner hardware or software differences and updates). The structural and ASL image data will be analysed with one pipeline (ExploreASL)[76] in a standardised setting, including quantification with BASIL and FSL-FABBER.[85] We will perform harmonisation on MRI images by scaling the scanner-average mean and between-subject SD maps to be the same. Our amyloid-PET data will be harmonised using the centiloid method.[96]

Fluid biomarkers may also vary, and harmonisation strategies should be taken into account. CSF amyloid is often harmonised using, for example, the Aβ1–42/Aβ1–40 ratio compared with Aβ1–42 alone for improved concordance, since the division by Aβ1–40 is hypothesised to correct for interindividual biological variation in amyloid production and/or clearance. Recent literature has introduced standardised cut-offs for fluid biomarkers, highlighting all these aspects.[97 98]

While several cohorts have specific neuropsychological tests depending on their distinct aims, most/all studies have included the same MMSE and MoCA test.

Finally, the tabular derivatives can be harmonised in the statistics using neuroCombat.[99]

Expected impact

The DEBBIE-AD study aims to explore the potential use of BBB disruption as a novel early biomarker for AD. This project holds promise for various applications: (1) Research advancements: by gaining a deeper understanding of the role of BBB dysfunction in AD, this study can contribute to significant advances in AD research. Even if a plasma biomarker would be an easier/more cost-effective or otherwise desirable biomarker than BBB-ASL, this would provide knowledge about such biomarkers; (2) clinical trials require more and more biomarkers for the increasing heterogeneity in AD cases and their potential response to disease-modifying treatment. The BBB being implicated in many AD pathways makes it a potentially helpful biomarker for selecting patients in clinical trials or as a secondary endpoint; (3) personalised diagnosis and treatment: in the future, BBB permeability assessment could assist in prognosis and tailoring AD medication.

Our study includes six distinct research questions. We anticipate obtaining over 1000 BBB-ASL scans from participants from various groups. Specifically, we expect to include more than 500 healthy controls, over 250 individuals with MCI, approximately 100 patients with AD and the remaining participants representing Aβ+ and SCD cohorts. The research questions described here will be addressed without pooling data. Additionally, the strength of the DEBBIE consortium is that we have the opportunity to pool data, creating an extensive data set that enables comprehensive investigations into the value of BBB-ASL.

The focus of DEBBIE-AD is to investigate the impact of this potential biomarker across different cohorts, answering specific research questions. This aspect sets DEBBIE-AD apart as all cohorts within the consortium will measure the same biomarker to explore various aspects of AD. Furthermore, we ensure consistency by using the same sequence on the same scanner vendor, using gammaSTAR technology to ensure optimised uniformity across the consortium.

Although ME ASL has been proposed previously,[43] the significance of this work lies in its development of a time-efficient MRI sequence suitable for acquiring CBF, ATT and Tex in the clinical setting. To our knowledge, DEBBIE-AD is the first study protocol for evaluating a single promising MRI biomarker of AD across multiple cohorts.

This study also has some limitations. The main potential limitation of BBB-ASL as an early dementia biomarker is its methodological and physiological reliability. BBB-ASL has only been tested in healthy volunteers and not yet in patients. The inherently low signal-to-noise ratio (SNR) of the method may make the detection of clinically meaningful changes challenging. We will regularly perform quality control to assess the image quality, test preliminary associations and adapt the BBB-ASL sequence if required (eg, increasing the number of averages to boost SNR). Furthermore, BBB-ASL uses water as an endogenous tracer that has been shown to detect subtler—and

potentially earlier—BBB damage than tracers with larger molecular sizes. The downside could be that BBB-ASL has a higher physiological variability, requiring larger cohorts to achieve adequate statistical power. Additionally, patient motion, atrophy and haematocrit levels may affect the acquisition and image analysis. These factors can be critical as they vary between patients and controls—for example, patients typically move more—while haematocrit and atrophy can be both methodological and physiological confounders. We will attempt several options to tackle these challenges. To optimise the quality of quantification, we intend to incorporate a T2 map for each participant based on the control images, aiming to minimise errors associated with the assumption of standard T2 relaxation values. Also, we will investigate if the image quality of the CBF, ATT and Tex maps is related to (patho-)physiological parameters such as age, sex and AD staging. Our objective is to determine whether the quality of these maps varies with age and then compare CBF quantification across a reduced number of PLDs within the same subject group, investigating potential challenges posed by ATT for BBB Tex quantification in elderly subjects. Additionally, our analysis will include testing the reliability of motion correction, specifically for the joint analysis of HAD4 and HAD8 sequences.

In summary, the mission of DEBBIE is to establish a non-invasive biomarker related to BBB health in AD. This requires demonstrating the significance of the biomarker within the disease context by comprehensive investigations of repeatability, reproducibility, variability and associations with other disease markers. The successful validation of this biomarker could have far-reaching implications, including a better understanding of the underlying mechanisms of AD and lead to earlier detection and a more accurate diagnosis.

Ethics and dissemination

The Amsterdam Medical Ethics Review Committee approved the COGA, VARIATION, VVI, SYNAPSE and InflammAD studies, in accordance with the ethical conduct and juridical laws of the Declaration of Helsinki 64th WMA General Assembly, Fortaleza, Brazil, October 2013, (www.wma.net), and in accordance with the Medical Research Involving Human Subjects Act (WMO). For the Oslo cohorts (DDI and LCBC), the regional medical research ethics committee approved the study. All further study conduct was in line with the guidelines provided by the Helsinki declaration of 1964 (revised 2013) and the Norwegian Health and Research Act. For both the Ghent and UKD cohorts, study protocol amendments are pending and being prepared, respectively, for implementing the BBB-ASL sequence in the MRI protocols. For the LHRI cohort, the animal study will be conducted according to the regulations of the Canadian Council on Animal Care and was approved by the Animal Care Committee at Western University, and the human study will be conducted in accordance with the Declaration of Helsinki ethical standards and was approved by the University Research Ethics Board. DPRC study procedures were approved by appropriate ethical review boards (University of Auckland Human Participants Ethics Committee ref 020737 and The Health and Disability Ethics Committee ref 15/NTB/202). All participants provided informed written consent before taking part in accordance with the New Zealand National Ethical Standards. The National University of Singapore Institutional Review Board approved the study in Singapore for NEURO-BMC (NUS-IRB Reference Code: NUS-IRB-2021–531). All in accordance with the Human Biomedical Research Act and the applicable laws and regulations of Singapore. All study participants provided written informed consent. On each of the research questions, separate manuscripts will be written and submitted for publication in peer-reviewed journals.

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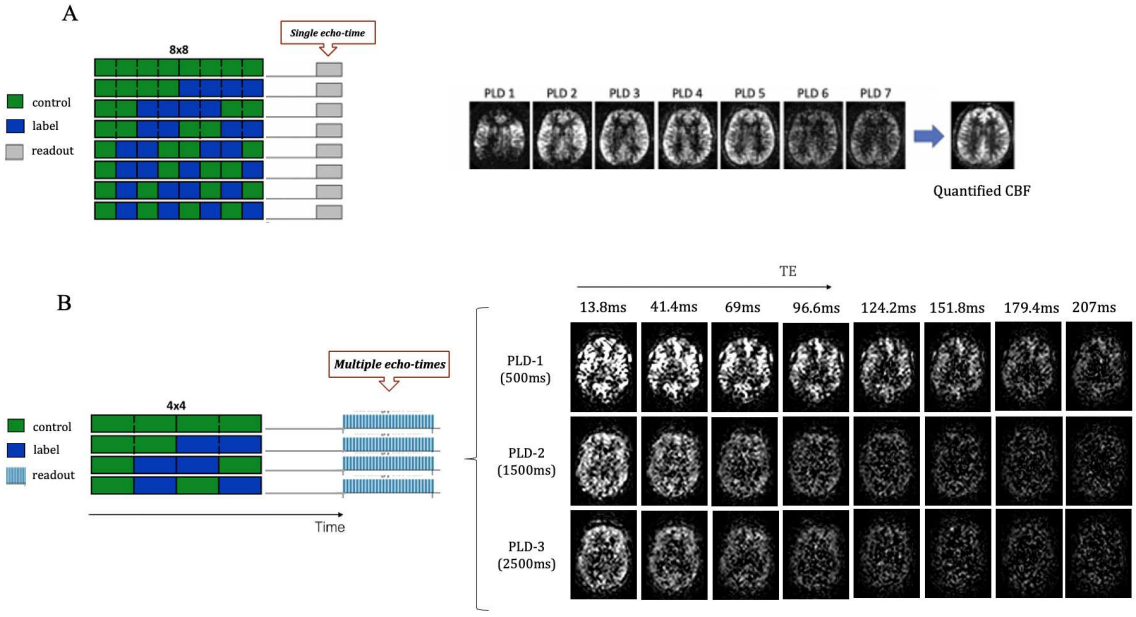

Supplementary figure 1: *Schematic representation of HAD8 (A) and HAD4 (B) labeling scheme[81] with a single- and multi-echo acquisition, respectively. Multi-post-labeling delay (PLD) used in HAD8 aims to accurately estimate the arterial arrival time, and multi-echo-time acquisition used in HAD4 is used to correctly reconstruct the T2 relaxation curves of the labeled water in the intra/extravascular spaces. All of the acquired information about the arrival time and relaxation of the label is used in a two-compartment model to estimate the water exchange time across BBB.*

Supplementary table 1: *Information about the single-echo Hadamard8 and multi-echo Hadamard4 acquisition parameters.*

MRI acquisition	SBD (ms)	Inflow times ([ms])	TEs (n [ms])	TR (ms)
Single-echo HAD8	400	2 repetitions [1000:400:3400] and [1200:400:3600]	1 TE = [13.2]	4000
Multi-echo HAD4	1000	[1500, 2500, 3500]	8 TEs = [13.8:27.6:207]	4500

PLD = post-labeling delay; SBD = sub-bolus duration; TE = echo-time; All times are provided in milliseconds except for the scan time; TR = Repetition time. Inflow times correspond to SBD+PLD.

Supplementary table 2: *Inclusion and exclusion criteria*

Cohort	Inclusion criteria	Exclusion criteria
LCBC	Healthy participants that have already been scanned and neuropsychologically tested at LCBC.	History of injury or disease known to affect central nervous system function, including neurological or psychiatric illness or serious head trauma, dementia, previous stroke with sequela, Parkinson's disease, and other neurodegenerative diseases likely to affect cognition, being under psychiatric treatment, use of psychoactive drugs known to affect central nervous system functioning, and MRI contraindications.
DDI	New onset symptoms of cognitive impairment or Parkinson's disease, Age: 40-80, local mother tongue.	Brain injury, including clinical stroke (TIA or silent infarction does not cause for exclusion); known dementia; serious psychiatric disorder (e.g. schizophrenia, bipolar disorder) that may influence cognitive capacity; serious somatic condition (e.g. liver or kidney failure, radiotherapy, chemotherapy); known neurodevelopmental disorder.
COGA	All patients visiting the Center Of Geriatric Amsterdam, an outpatient memory clinic at Amsterdam UMC for cognitive evaluation who consent for their data being used for scientific research.	MRI contraindications.
VARIATION	Substudy of COGA patients aged 65 and older, who additionally consent for vascular measurements.	Insufficient proficiency in the Dutch language or severe cognitive impairment (MMSE <16).
VVI	Clinical diagnosis of MCI due to AD; Abnormal CSF biomarker for aggregated amyloid; Signed informed consent for Amsterdam Dementia Cohort (ADC) and Amsterdam Dementia Biobank (P2016.061 and P2017.315); Age ≥50 years	No CSF collection or MRI-imaging performed at day screening memory clinic; Clinical diagnosis of dementia or subjective cognitive decline; Other neurological diagnosis, such as Parkinson's disease, symptomatic stroke, mental retardation, brain tumor or infection, likely to be cause of cognitive impairment; Major psychiatric disorder, such as psychosis, schizophrenia, depression with vital signs, severe personality disorder, abuse of alcohol or other substances, likely to be cause of cognitive impairment; Use of (oral) anticoagulants or other contraindications for lumbar puncture; Contraindications for MRI scan (e.g., metal implants, pacemaker)
SYNAPSE	At least 50 years of age; Biomarker evidence (CSF or PET) for the presence of Aβ pathology. Subjects must, in the opinion of the attending neurologist, be able to tolerate study procedures and be competent to make a well-informed decision to participate in this study; Signed informed consent for Amsterdam Dementia Cohort.	Contraindications for MRI scanning. Evidence of structural abnormalities such as major stroke or mass on MRI that is likely to interfere with the clinical presentation and/or interpretation of PET scan. Women of childbearing potential who are not surgically sterile, not refraining from sexual activity or not using reliable methods for contraception. Relevant history of severe drug allergy or hypersensitivity. Has ever participated in an experimental study with a tau, amyloid, or synapse targeting agent, unless it can be documented that the subject received only a placebo during the course of the trial. History of any clinically significant cardiovascular, endocrinology, hematologic, hepatobiliary, immunologic, metabolic, urologic, pulmonary, neurologic (with the exception of AD), psychiatric, renal or other major disease. Has been injected with a

		previously administered radiopharmaceutical within 6 terminal half-lives or when total yearly radiation exposure exceeds 11.3 mSv for females and 15.3 mSv for males. The following medications during the study and 4 weeks prior to [11C]UCB-J PET: - Use of anticonvulsant medications; Other medications that, in the opinion of the Investigator, may interfere with the study
InflamAD	For the Control group: 1. Have biomarker evidence (CSF or PET) for the Absence of Aβ pathology; 2. Have no objective cognitive impairment as determined by a neuropsychologist or neuropsychological testing; 3. At least 50 years of age; 4. Subjects must, in the opinion of the principal investigator/attending neurologist, be able to tolerate study procedures and be competent to make a well-informed decision to participate in this study; 5.Signed informed consent for Amsterdam Dementia Cohort (2016.061); For the AD continuum group: 1. Be along the AD continuum, defined as having positive biomarker evidence (CSF or PET) for the presence of Aβ pathology; 2. At least 50 years of age; 3. Subjects must, in the opinion of the principal investigator/attending neurologist, be able to tolerate study procedures and be competent to make a well-informed decision to participate in this study; 4. Signed informed consent for Amsterdam Dementia Cohort(2016.061);	Contraindications for MRI scanning. Evidence of gross structural abnormalities, such as a major stroke or a mass on MRI, could interfere with the interpretation of the PET scan. Unable to undergo PET-CT with the administration of the radioligand [11C]SMW139. Woman of childbearing potential who is not surgically sterile, not refraining from sexual activity, or not using reliable contraception methods. Additionally, women of childbearing potential must not be pregnant or breastfeeding during screening and imaging. Relevant history of severe drug allergy or hypersensitivity, including but not limited to hay fever, allergies to cats and dogs, and dust mite allergy. If previously participated in an experimental study involving a tau, amyloid, or neuroinflammation targeting agent, unless documented proof is available that they received only a placebo during the trial. History of any clinically significant cardiovascular, endocrinology, hematologic, hepatobiliary, immunologic, inflammatory, metabolic, urologic, pulmonary (including asthma), neurologic (excluding AD), psychiatric, renal, or other major diseases, as determined by the principal investigator. In male subjects, their hemoglobin (Hb) levels are below 8.0 g/dL, and in female subjects, their Hb levels are below 7.0 g/dL. If have been injected with a previously administered radiopharmaceutical within 6 terminal half-lives, or their total yearly radiation exposure exceeds 10 mSv; History of severe traumatic brain injury (TBI); Taking certain medications during the study or within 4 weeks prior to [11C]SMW139 PET, including anticonvulsant medications, anti-inflammatory medications (such as chronic NSAID use), or any other medications that, in the investigator's opinion, may interfere with the study.
GUH	Patients of the Ghent University Hospital Memory Clinic who consent with their data being released for scientific research.	Any pre-existing cognitive impairment, brain injury or serious psychiatric disorder.
Ghent healthy controls	Healthy volunteers, recruited from the general population	Any known cognitive impairment, brain injury, or serious psychiatric disorder.
LHRI	(MCI/AD/Stroke) patients of the Cognitive Neurology and Alzheimer Research Centre who consent to their data being released for scientific research.	Any neurological condition that may be contributing to cognitive impairment above and beyond that caused by the participant's Alzheimer's disease. Evidence of other clinically significant lesions on brain MRI at screening that could indicate a dementia diagnosis other than Alzheimer's disease. Severe visual or hearing impairment that would prevent the participant from performing psychometric tests accurately. Any psychiatric diagnosis or symptoms (e.g., hallucinations, major depression, or delusions) that could interfere with study procedures in the participant. Contraindications to MRI scanning, including cardiac pacemaker/defibrillator, and ferromagnetic metal implants.

UKD	MCI and AD patients from the memory clinic who consent to their data being released for scientific research.	Any neurological condition or evidence of other clinically significant lesions on brain MRI that could indicate a dementia diagnosis other than Alzheimer's disease. Any MRI contraindications.
DPRC	≥ 55 years, have memory or other cognitive difficulties noticed by themselves or others, and not living in a long-term care facility.	A significant history of psychiatric disorders, significant past or current alcohol problems, moderate-severe traumatic brain injury, a pacemaker or neurological conditions other than mild probable AD including moderate dementia.
NEURO-BMC (NUS)	1) suspected dementia (CDR>=0.5 or symptoms in cognition) or 2) cognitive assessment score below education-adjusted threshold in any of the assessments.	Any physical disability, prevalent dementia, significant neurological diseases, or any contraindications for MRI. Lack of mental capacity to function cognitively.

Supplementary table 3: *Neuropsychological measures*

Cohort	Neuropsychological measures
LCBC	MMSE, CVLT (verbal memory), Rey Complex Learning Test (visual memory), Mirror tracing task (implicit memory test), WASI (general cognitive ability), WAIS-III (working memory test), D-KEFS Stroop (executive function), ANT (attention/executive function), Visual n-back (working memory/executive function)
DDI	MMSE, CERAD 10 words test (Immediate recall, Delayed recall after 10 min), Clock Drawing test, TMT A and B, COWAD
COGA/ VARIATION	MMSE, MoCA, Rey Complex Learning Test, VAT, TMT and B, Clock Drawing test, Stroop, 15-word test,
VVI	MMSE, 15-word test, Trail Making Test A and B, Animal fluency, letter fluency, VAT, Forward and backward digit span, Stroop, GDS..
SINAPSE/ InflamAD	MMSE, 15-word test, Trail Making Test A and B, Animal fluency, letter fluency, VAT, Forward and backward digit span, Stroop, GDS..
GUH	MMSE, MoCA, FAQ
Ghent Healthy Controls	MoCA, COTESS Digits Forwards, COTESS Digits back, Auditory Verbal Learning Test, Rey-Osterrieth Complex Figures, Trail Making Test, D2, Stroop, Controlled Word Association Test, Hooper, Graded Naming
LHRI	MMSE, MoCA
UKD	MMSE, MoCA, CERAD-Plus
DPRC	**Global cognition:** ACE-III, **Attention**: WAIS-IV Digits Forwards, WAIS-IV Digits Back,CVLT-II Trial 1, **Processing speed:** WAIS-IV Digit Symbol Coding, DKEFS Stroop color naming & word reading, TMT A, **Memory - Verbal: Learning:** CVLT-II Total Trials 1-5, **Immediate Recall:** WMS-III Logical Memory I, CVLT—II Short Term Delay, WMS-III Logical Memory II, CVLT-II Long Term Delay, **Memory - Visual Learning:** BVMT-R Total, **Immediate Recall:** Rey Complex Figure Immediate, **Delayed Recall:** BVMT-R Delay, Rey Complex Figure delayedoutl, **Visuospatial**: Rey Complex Figure copy, Line Orientation Test, WAIS-IV Block Design. **Language/semantic: Naming**: Boston Naming Test, SydBat Naming, **Comprehension:** SydBat Comprehension, **Semantic:** SydBat Semantic Associates. **Executive function:** DKEFS Verbal Fluency letter, DKEFS Category Fluency, DKEFS Switching Accuracy, DKEFS Stroop Inhibition, Hayling Sentence Completion, TMT B. **Additional Measures**: WAIS-IV Matrix Reasoning, WAIS-IV Similarities
NEURO-BMC	MoCA, MMSE, CDR, GDS

ACE-III: Addenbrooke's Cognitive Examination–III; ANT: Attention Network Test; BVMT-R: Brief Visuospatial Memory Test Revised; COWAD = Control Oral Word Association Test; CVLT-II: California Verbal Learning Test – second edition; DKEFS: Delis-Kaplan Executive Function System; FAQ: Functional Activities Questionnaire; RCFT: Rey Complex Figure Test; SYDBAT: Sydney Language Battery; TMT: Trail Making Test; WAIS-IV: Wechsler Adult Intelligence Scale Fourth Edition; WASI: Wechsler Abbreviated Scale of Intelligence; WMS-III: Wechsler Memory Scale Third Edition.