# Blood–brain barrier link to human cognitive impairment and Alzheimer's disease

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**Abstract**

Vascular dysfunction is frequently seen in disorders associated with cognitive impairment, dementia and Alzheimer's disease (AD). Recent advances in neuroimaging and fluid biomarkers suggest that vascular dysfunction is not an innocent bystander only accompanying neuronal dysfunction. Loss of cerebrovascular integrity, often referred to as breakdown in the blood-brain barrier (BBB), has recently shown to be an early biomarker of human cognitive dysfunction and possibly underlying mechanism of age-related cognitive decline. Damage to the BBB may initiate or further invoke a range of tissue injuries causing synaptic and neuronal dysfunction and cognitive impairment that may contribute to AD. Therefore, better understanding of how vascular dysfunction caused by BBB breakdown interacts with amyloid-β and tau AD biomarkers to confer cognitive impairment may lead to new ways of thinking about pathogenesis, and possibly treatment and prevention of early cognitive impairment, dementia and AD, for which we still do not have effective therapies.

## Introduction

Alzheimer's disease (AD) is associated with vascular dysfunction[1,2]. Leaks in a protective filter called the blood-brain barrier (BBB) are found in AD and other dementias[3]. Whether or not they contribute to disease pathogenesis is a matter of debate.

Here, we examine recent human neuroimaging and fluid biomarker studies suggesting that loss of cerebrovascular integrity initiating breakdown in the BBB may lead to early cognitive dysfunction, mild cognitive impairment (MCI) and AD. Cerebral blood flow (CBF) changes are also frequently observed in AD[2], but how they influence disease process is beyond the scope of the present perspective.

## Blood-brain barrier

The BBB was discovered more than 100 years ago. Initial studies with vital dyes injected into the blood stream were shown to permeate all peripheral organs while the brain remained uncoloured. This led to definition of the BBB as a biological membrane between blood and brain, which in contrast to relatively "leaky" capillaries in peripheral organs, does not allow free solute exchanges across the capillary endothelium[2]. Today, the barrier function remains one of the many critical functions that BBB plays for the brain. Physiological, cellular and molecular studies, and recent studies in the living human brain have revealed that the BBB plays a key role in brain metabolism and function, has an important role in disease process, and is yet poorly explored as a therapeutic target, as recently reviewed[1].

### *Physiology*

The BBB is formed by a continuous endothelial monolayer at the level of brain capillaries[1] (**Fig. 1**), which provides 85% of endothelial surface area of the brain or 12 $m^2$ in the human brain[1]. The endothelial monolayer extends along the arterioles, small arterial vessels, and venules. As recently reviewed in detail elsewhere[1,4,5], the pioneering electron microscopy studies in rodents revealed the presence of tight junctions (TJs) between the neighbouring endothelial cells of the BBB that helped establish the anatomical basis of the BBB as a tightly sealed endothelial monolayer. The follow-up molecular and genetic studies identified several TJ proteins in endothelium including zonula occludens-1 (ZO1), a critical node in the organization of many protein complexes associated with TJs such as occludins, which regulate paracellular endothelial permeability, and claudins, including low molecular weight size-selective claudin-5. Other contacts between endothelial cells include adherens junctions (AJs), typified by proteins such as VE-cadherin. Importantly, a recent single-nucleus RNA-sequencing study of human brain vasculature has shown for the first time that human BBB endothelium expresses all key TJ and AJ proteins that previously had only been shown in the brains of other mammalian species[6]. These include adhesion molecules such as different cadherins and proto-cadherins, contactins and catenins that contribute to BBB integrity[6]. For further details on TJs and AJs proteins and original articles describing their function and how they were discovered over the last few decades, we refer to recent reviews[1,4,5].

In contrast to the relatively permeable systemic capillaries, healthy and normal brain capillaries exhibit a low rate of transendothelial bulk flow by transcytosis. This together with expression of TJs and AJs restricts the entry of most blood-derived molecules into the brain, unless they have specialized carriers and/or receptors in the brain endothelium that facilitate their transport across the BBB. In this sense, the BBB can be viewed as a selective semipermeable barrier

allowing specific molecules to pass in and out of the brain. Recent studies have identified over 10,000 transcripts in the murine BBB endothelium with preferential expression of transporters in the capillary endothelium[7,8]. Similar data sets on BBB transporters have recently been reported in human brain endothelium[6].

The selective substrate-specific transport systems at the BBB include carrier-mediated transport (CMT) of carbohydrates (e.g., glucose), amino acids, monocarboxylic acids (e.g., lactate, ketone bodies), hormones, fatty acids, nucleotides, inorganic anions, amines, choline and vitamins. These CMT systems enable transport of their respective substrates to cross BBB bi-directionally according to their concentration gradients. Some larger molecules including certain proteins and peptides can use receptor-mediated transport (RMT) to cross the BBB from blood-or brain, as for example insulin, insulin-like growth factors, transferrin, leptin and some others. RMT systems including lipoprotein receptors mediate clearance from brain of proteinaceous neurotoxic molecules that are produced in the brain such Alzheimer's amyloid-$\beta$ (A$\beta$) or Parkinson's $\alpha$-synuclein. Endothelial ATP-binding cassette transporters prevent brain accumulation of drugs, xenobiotics, drug conjugates, and nucleosides in the brain by active efflux from endothelium to blood. And endothelial ion transporters, such as sodium pumps, control ion concentrations in the brain. Thus, in addition to protecting the brain parenchyma from blood-derived toxic molecules, cells and microorganisms, the endothelial monolayer of the BBB regulates transport of nutrients and essential molecules across brain endothelium into the brain, and clearance into the blood of metabolic end products and endogenous neurotoxins produced by the brain. For more details, on BBB transport systems in healthy brain, and how they are affected by the disease process see recent reviews[1,4,5].

Pericytes, mural cells that lie along brain capillaries, share a common basement membrane with endothelial cells. As reviewed recently[1,4], N-cadherin forms peg-and-socket contacts between endothelial cells and pericytes, whereas the gap junction connexin (CX) 43 hemichannels mediate intercellular communications between pericytes and endothelial cells. Astrocytes also express gap junction proteins, some of which are important for maintaining BBB integrity, such as CX30 and CX43. These adhesion molecules originally found in murine pericytes and astrocytes were recently confirmed in human pericytes and astrocytes[6].

Finally, in contrast to peripheral organs such as liver, brain does not have a storage capability for larger energy-saving molecules[1,2]. Its energy metabolism depends on delivery of metabolites such as glucose by CBF and transport across the BBB. Vascular smooth muscle cells and pericytes regulate CBF by constricting and dilating arterioles and capillaries, respectively[2,9–11] (**Fig. 1**). Importantly, pericytes maintain BBB integrity, and their loss leads to BBB disruption[12–16].

*BBB dysfunction and neurological disorders in humans*

That intact BBB is required for normal brain function is best illustrated by examples of rare monogenic human neurological disorders where the genetic mutations or defects are found to originate exclusively within brain endothelial cells, and/or BBB-associated pericytes and vascular smooth muscle cells. For example, inactivating mutations in the Solute Carrier Family 2 Member 1 (*SLC2A1*) gene encoding GLUT1 glucose transporter in brain endothelial cells, lead to GLUT1-deficiency syndrome, a paediatric neurological disease with early onset of seizures and microcephaly, BBB breakdown, and neuron loss[17]. Inactivating mutations in the Major Facilitator Superfamily Domain Containing 2A (*MSFD2A*) gene encoding transporter for essential omega-3 fatty acids that is enriched in brain endothelial cells, lead to BBB breakdown[18,19] and microcephaly syndrome[20,21]. Mutations in genes encoding the BBB TJ

proteins, cerebral cavernous malformation proteins or collagens lead to uncontrolled leakage of proteins and other content from blood into the brain causing neuroinflammatory response, increased microvessel fragility, cerebral haemorrhages and small vessels disease (SVD), resulting in focal neurological deficits, seizures and headaches, and/or lacunar ischemic strokes[1]. Mutations in *NOTCH3* gene that is expressed in vascular smooth muscle cells and pericytes lead to cerebral autosomal dominant arteriopathy with subcortical infracts and leukoencephalopathy (CADASIL), a major cause of genetically inherited stroke in humans associated with loss of blood vessels integrity[22].

Altogether, about 20 rare neurological monogenic disorders identified offer insights into causal pathogenic links between BBB dysfunction and neurological disease in humans, supporting the idea that BBB dysfunction can have neurological consequences.

**Blood-brain barrier breakdown and cognitive dysfunction**

Several neuropathological studies have shown BBB breakdown in AD[1]. However, the point at which individuals suffering from MCI and AD develop BBB breakdown has not been clear until recently. Using dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) with gadolinium-based contrast agents (GBCA), recent studies indicated that BBB breakdown occurs early in individuals with MCI and AD-type dementia, and is an early biomarker of cognitive dysfunction[23,24]. The presence of gadolinium in brain reflects subtle BBB "leaks" of plasma components, and is typically caused by loss of TJ or AJ proteins, and/or increased trans-endothelial fluid transcytosis of plasma components across the BBB. According to experimental studies BBB leaks could be related to loss of pericyte coverage[12,14].

Loss of BBB integrity has also been shown in MCI and AD dementia by susceptibility weighted imaging (SWI) MRI detecting early cerebral microhaemorrhages[25,26]. This greater and more focal degree of BBB breakdown leads to extravasation of red blood cells into the brain. Lobar microhaemorrhages seen by SWI are often due to cerebral amyloid angiopathy (CAA) that is present in many cases of MCI and AD along with amyloid deposition in brain[27]. However, microhaemorrhages are also seen in deep infratentorial regions related to hypertensive SVD and vascular dementia[28].

The BBB dysfunction in MCI and AD is not limited only to "mechanical" types of BBB breakdown described above, and may also include dysfunction in the BBB transporters and/or receptors, such as, to name a few, loss of GLUT1 glucose transporter and P-glycoprotein 1 (P-gp) key efflux transporter of toxins, as discussed below. For details of how BBB transport systems are affected by different neurological disorders see recent review[5].

The link between BBB and AD is further supported by a recent nuclear RNA sequencing study of major human brain vascular and perivascular cell types from hippocampus and cortex, which revealed that 30 of the top 45 AD genes identified by genome wide association studies (GWAS) are expressed in brain vasculature[6]. Vascular GWAS genes mapped to endothelial protein transport, adaptive immune and extracellular matrix pathways.

Below, we discuss recent neuroimaging studies demonstrating BBB breakdown in individuals with early cognitive impairment, MCI and AD-type dementia, and in relation to AD biomarkers amyloid-$\beta$ (A$\beta$), tau and neurodegeneration[29]. But before we proceed with this discussion, we would like also to mention that earlier neuroimaging studies using computed tomography (CT)[30,31], positron emission tomography (PET) with [$^{68}$Ga]EDTA[32], and DCE-MRI semi-quantitative analysis[33] failed to detect higher BBB permeability in AD. In contrast to these earlier

studies from late eighties and nineties, more recent neuroimaging studies from several groups over the last five to six years have shown age-related BBB breakdown[23,34–40], BBB breakdown in MCI[23,24,35,36,41,42], AD[43–46], cerebral SVD[47–53] and in other neurodegenerative disorders[54,55]. The discrepancy between earlier and recent studies could likely be attributed to use of more advanced techniques and analysis in recent studies. This includes use of MRI sequences with higher spatial and temporal resolution[23,24,35,43,56], direct measurements of individual vascular input functions from the arterial inflow[23,24,35,36] or the venous outflow[34,37–42,44–47,49,50,52], and use of quantification methods, such as the Patlak model[57], which has not been used in previous studies[30–33]. Some earlier studies[31,33] measured only signal changes after contrast injection without applying the pharmacokinetic analysis that takes into account the tracer's concentration in blood. One CT study[30] and one PET study[32] adopted pharmacokinetic models, but did not detect BBB leaks likely due to a lower ability of CT and PET to resolve cerebral anatomical structures compared to recent MRI sequences, and generally much lower spatial resolution of PET.

## Mild cognitive impairment

*BBB breakdown by DCE-MRI and analysis of cerebrospinal fluid*

DCE-MRI studies revealed that individuals with MCI develop BBB breakdown in the hippocampus, a centre for learning and memory[23] (**Table 1**), which correlated with increased levels of biochemical biomarkers of BBB breakdown in the cerebrospinal fluid (CSF) such as CSF/serum albumin ratio, $Q_{alb}$, fibrinogen and plasminogen[23,24] (**Table 2**). Increased BBB leaks (i.e., $K_{trans}$ values) correlated with increased CSF levels of soluble platelet-derived growth factor β (sPDGFRβ), a biomarker of pericyte injury[23,24,58]. DCE-MRI approach also revealed a more widespread BBB breakdown in MCI in the grey and normally-appearing white matter[44]. BBB breakdown in the hippocampus was also found during physiological aging, but to a lesser degree than in MCI[23], and in grey and white matter regions vulnerable to age-related deteriorations, suggesting it is likely an underlying mechanism of age-related cognitive decline[35,37,38], particularly associated with loss of memory retrieval[39].

Since $A\beta^2$ and tau[59,60] are both vasculotoxic, several studies have investigated the relationship between BBB permeability and Aβ and tau CSF biomarkers[24,35,61]. These studies revealed that neither increase in the BBB permeability in the hippocampus and parahippocampal gyrus by DCE-MRI, nor increased levels of pericyte injury biomarker sPDGFRβ in the CSF, depended on Aβ and tau CSF status[24], and were found both in individuals with and without positive AD biomarkers in CSF and/or brain by PET[24,35] (**Table 1**, **Table 2**). These data suggest a link between early BBB dysfunction and cognitive impairment in individuals that are in early stages in the AD continuum, but also in those that have not yet developed alterations in Aβ and tau biomarkers. Whether this latter group will develop vascular dementia, AD or mixed dementia at a later stage remains presently unknown. This should be investigated by future longitudinal studies.

A few MCI studies reported that the BBB breakdown was not influenced by vascular risk factor (VRF) burden[24,35]. Since the studied cohorts excluded participants with substantial cerebrovascular pathology, it is possible that interactions between traditional VRFs and BBB dysfunction in cohorts with more severe vascular lesions and vascular cognitive impairment will lead to synergistic effects. Again, this remains to be determined by future studies. Some studies have shown that BBB breakdown in MCI individuals precedes hippocampal degeneration[7,18],

suggesting that early BBB dysfunction may occur prior to brain atrophy. These cross-sectional findings remain to be confirmed, however, by longitudinal studies.

A recent DCE-MRI study indicated that BBB breakdown in the hippocampus and parahippocampal gyrus begins in cognitively unimpaired (CU) *APOE4* carriers (ε3/ε4 and ε4/ε4), which further increases with cognitive impairment, irrespective of Aβ and tau biomarker changes in the CSF or brain by PET[35]. Since hippocampal volumes were not different between CU *APOE4* and *APOE3* carriers, these findings additionally suggest that BBB breakdown in CU *APOE4* carriers preceded hippocampal atrophy that was observed only in *APOE4* carriers at MCI stage[35]. Again, future longitudinal studies should confirm and extend these cross-sectional findings.

Interestingly, high baseline CSF levels of sPDGFRβ, a BBB pericyte injury biomarker, predicted future cognitive decline in *APOE4* carriers, but not *APOE3* homozygotes, and remained a significant predictor of cognitive decline after correcting for Aβ and tau status[35]. Elevated levels of sPDGFRβ correlated with activation of the BBB-degrading cyclophilin A (CypA)-matrix metalloproteinase 9 (MMP9) pathway in the CSF[35], similar as shown before in *APOE4* knock-in mice[62]. Since pharmacologic inhibitors of CypA have been used for non-neurological applications in humans[63], it is possible that CypA inhibitors may also suppress CypA in cerebral blood vessels of *APOE4* carriers, which in turn could improve vascular integrity and the associated neuronal and synaptic deficits, potentially slowing cognitive impairment.

*Microbleeds by SWI-MRI*

MCI patients develop microbleeds that can be detected by SWI-MRI sequences and T2* as small, round hypointense foci representing perivascular deposits of blood-derived hemosiderin phagocytosed by macrophages. **Table 1** lists MCI studies showing early microhaemorrhages reflecting breakdown in the BBB mainly in the cortex and deep gray matter regions[25,27,64–66]. Several studies in AD linked lobar microbleeds to CAA[25,27], whereas deep infratentorial microbleeds have been linked to hypertensive arteriopathy[28]. On 7T MRI, >75% of MCI individuals were found to develop microhaemorrhages likely of capillary and/or pre-capillary origin[67,68]. These are typically missed when studied by lower resolution 3T MR scanners, detecting only 21-45% microbleeds in MCI[25,27,64–66], or on 1.5T detecting microbleeds in 10-15% of MCI patients.

Consistent with DCE-MRI findings[35], the prevalence of microbleeds was higher in *APOE4* carriers[27,65,66], and was associated with increased CSF/serum albumin ($Q_{alb}$) ratio[66], suggesting a link between microbleeds and BBB dysfunction. Recent studies indicated that the appearance of microhaemorrhages was associated with cognitive decline and/or higher risk for dementia[69–71].

Interestingly, the occurrence of microbleeds was not influenced by tau[66], and in some studies preceded medial temporal lobe atrophy[25,64,66]. In a few studies, the incidence of lobar microbleeds was higher in participants with higher Aβ brain load on PET[27], but was not associated with lower CSF Aβ42 levels[66]. Since most studies on microbleeds in MCI did not evaluate simultaneously Aβ pathology by PET or in the CSF, the association between regional BBB permeability changes on DCE-MRI, microbleeds, and AD biomarkers needs to be investigated by future studies.

**Alzheimer's Disease**

BBB breakdown in the cortex, white matter, and some deep grey matter regions has been shown by DCE-MRI during early stages of AD[44]. Compared to MCI, early AD patients present with a higher prevalence of cerebral microbleeds on 3T MRI[64], often localized in the occipital and parietal lobes, sites of CAA (**Table 1**). Cerebral microbleeds are commonly found with more advanced AD with the prevalence as high as 45% at 3T[25,27,65] and up to 78% at 7T[68]. Although, the majority of microbleeds was typically lobar and CAA-related, the CAA-unrelated microhaemorrhages in the subcortical gray matter and infratentorial regions were also found.

Recent studies found that patients with epilepsy and AD, as well as aging mice, develop BBB leaks associated with slower cortical activity[43]. Moreover, these BBB leaks were related to activation of transforming growth factor-β (TGFβ) in astrocytes, as shown in humans and mice[56].

P-gp, an active efflux transporter at the luminal side of the BBB endothelium removes drugs, xenobiotics and Aβ from brain[72]. Studies using $^{11}$C-verapamil, a PET ligand for P-gp, indicated diminished P-gp activity in early AD in multiple region including hippocampus and cortex[73], suggesting impaired BBB clearance.

In addition to increased CSF sPDGFRβ and $Q_{alb}$ in large cohort studies in AD[74,75], increased CSF sPDGFRβ correlated with increased sPDGFRβ in the serum and increased CSF/serum $Q_{alb}$ ratio suggestive of BBB breakdown[76] was also found (**Table 2**). Increased CSF levels of biomarkers of angiogenesis and endothelial dysfunction, including vascular endothelial growth factor (VEGF) and VEGF/soluble VEGF receptor 1 (sVEGFR-1) ratio, were also found in AD[75]. These biomarkers were not associated with Aβ load[75], suggesting that BBB endothelial dysfunction is likely independent of amyloid pathology (**Table 2**).

Reduced FDG-PET is often interpreted as brain hypometabolism. However, several investigators support the view that reduced transport across the BBB also contributes to reduced FDG-PET as recently reviewed[77]. In brief, glucose enters the brain via transport across the BBB mediated by GLUT1 glucose transporter, and if GLUT1 is deficient, deleted from the BBB, blocked genetically, inhibited pharmacologically or suppressed by disease, glucose cannot reach the brain[1]. Several earlier FDG dynamic PET studies have shown diminished BBB transport of glucose in AD, as reviewed elsewhere[77].

## Blood-brain barrier and perivascular spaces

BBB breakdown during early cognitive decline in people at risk for AD[24] could lead to increased perivascular spaces (PVS) as in CADASIL[22,78]. The suggestion of direct leakage across the perforating vessel wall into the PVS[79] is supported by work in pericyte-deficient mice which develop BBB leakage associated with increase in the size and number of PVS[80]. Subtle diffuse BBB leaks on DCE-MRI correlated with increasing numbers of PVS[79]. When enlarged, PVS in the white and deep grey matter become visible by MRI[81]. PVS increases at older age, with cerebral SVD[82], and BBB breakdown[80], indicating that they are likely markers of BBB-related vascular dysfunction. Systematic reviews of population, vascular, and neurodegenerative diseases indicate that higher number of PVS is associated in cross-sectional studies with cognitive decline, AD-type dementia, and executive dysfunction[83–86].

## Conclusions and future directions

The NIA-AA Research Framework classifies individuals in the AD continuum by the AT(N) biomarkers for Aβ (A), tau (T) and neurodegeneration (N)[29,87]. Based on recent developments in fluid and neuroimaging biomarkers, the AT(N) biomarker matrix is now expanding towards ATX(N) system, where X could represent novel candidate biomarkers for additional pathophysiological mechanisms such as neuroimmune dysregulation, synaptic dysfunction and/or BBB alterations[88].

Here, we suggest the ATv(N) matrix to monitor early stages of cognitive dysfunction by adding neuroimaging and fluid biomarkers caused by an early vascular BBB breakdown (v) (**Fig. 2**). This model is supported by recent findings examined in this review. How biomarkers of early BBB breakdown interact with the AT biomarkers to predict cognitive decline during early preclinical stage[89–92], and clinical progression from CU to MCI, and MCI to dementia, remains unclear. These interactions should be evaluated by future longitudinal studies. The ATv(N) matrix allows a possibility for each of the studied pathways, i.e., the A, T and v, to contribute to early cognitive dysfunction and neurodegeneration independently of each other or acting synergistically. In this regard, we expect that the biomarkers of BBB breakdown (v) should be helpful in predicting early cognitive dysfunction in individuals within the AD pathway, as well as in those with negative AT biomarkers that may develop a different type of dementia, such as vascular and mixed dementia, and/or convert to AD at a later stage. This should be addressed by future and ongoing longitudinal studies. The current model also does not take into account the effects of other comorbid AD vascular pathologies such as white matter changes, lacunes, microinfarcts, ischemic changes and others not discussed here. Future models incorporating other vascular changes are also warranted in the context of addressing a full picture of vascular comorbidity and interaction with A and T.

We acknowledge limits of DCE-MRI for clinical use in AD[26,93], such as lack of standardized multivendor protocol and evidence of repeatability and reproducibility. However, the DCE-MRI technique has been in clinic for more than 35 years. GBCA are injected approximately 30 million times annually for evaluation of patients with multiple sclerosis, brain tumours, and other neurological disorders[94]. Advanced versions of this technique have been used recently by multiple centres in research studies in individuals with cognitive problems during physiological aging, MCI, AD, SVD and other neurodegenerative disorders. It is noteworthy, the BBB leakage detected by DCE-MRI in MCI and AD is almost an order of magnitude lower than leakage seen in grey matter after acute ischemic brain injury and large arterial infracts in stroke, and/or during relapsing acute episodes in the white matter in multiple sclerosis. Nevertheless, subtle chronic leakages in the BBB that may persist over longer periods of time for decades during preclinical decline and clinical progression to dementia and AD may importantly contribute to cognitive impairment.

Developing neuroimaging biomarkers for brain endothelial dysfunction, pericytes, and vascular smooth muscle cells, and new PET ligands that selectively track BBB transport of glucose, such as 3-O-$^{11}$C-methyl glucose[95], will advance our understanding of the multiple BBB dysfunctions in MCI and AD. Using ultrahigh-field 7T MR scanners will substantially improve detectability of BBB capillary microbleeds in CU and MCI individuals. More effort should be directed at developing biomarkers of BBB injury in the blood, similarly as it has been recently done with phosphorylated tau to distinguish individuals developing neuronal injury with AD pathology from those with non-AD pathology, and/or with other neurodegenerative disorders[96–99]. Indeed, there are national efforts to create biomarkers for vascular dysfunction, i.e. MarkVCID, a consortium of US academic medical centres whose mission is to identify and validate biomarkers for SVD that produce vascular contributions to cognitive impairment and dementia[100]. We expect the

proposed ATv(N) matrix will stimulate more researchers and clinicians to analyse the BBB in studies and trials for early stages of cognitive impairment, MCI, AD and other dementias.

## Contributors

G.B., A.M., K.K. and B.V.Z. prepared the figures and wrote the manuscript. All authors performed literature search, edited the text, provided critical reading of the manuscript, and approved the final version for submission. B.V.Z. provided final edits of the manuscript.

## Declaration of interests

Drs. Barisano, Montagne, Kisler, and Zlokovic declare no competing interests related to this work. Dr. Schneider reports personal fees from National Hockey League, from National Football League, outside the submitted work; Dr. Wardlaw reports grants from UK Dementia Research Institute (MRC, Alzheimers Society, ARUK), grants from Fondation Leducq, grants from EU Horizon 2020, grants from Row Fogo Charitable Trust, grants from Selfridges Group Foundation, during the conduct of the study; grants from British Heart Foundation, grants from Stroke Association, grants from Wellcome Trust, outside the submitted work.

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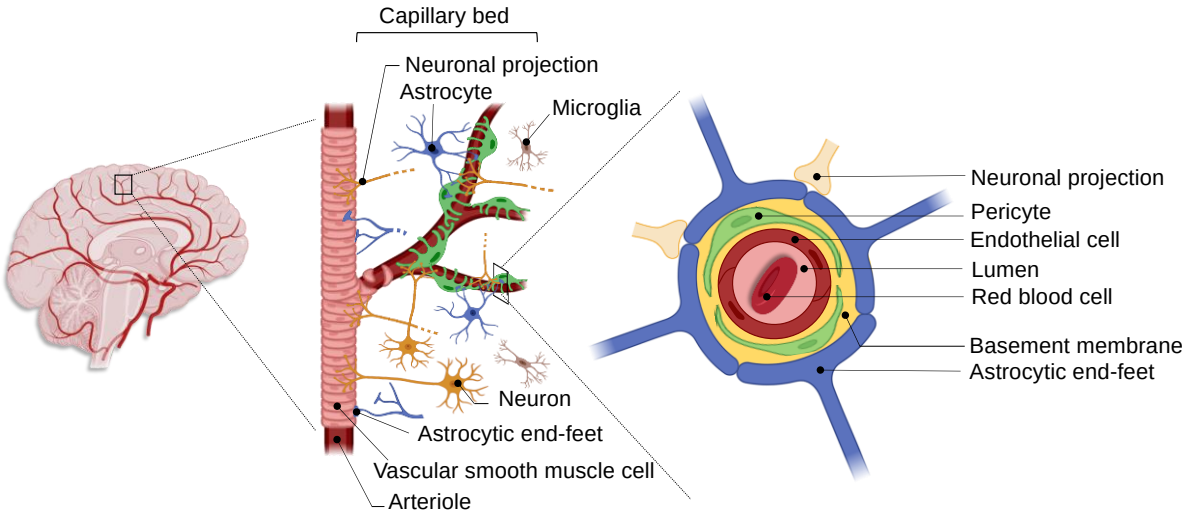
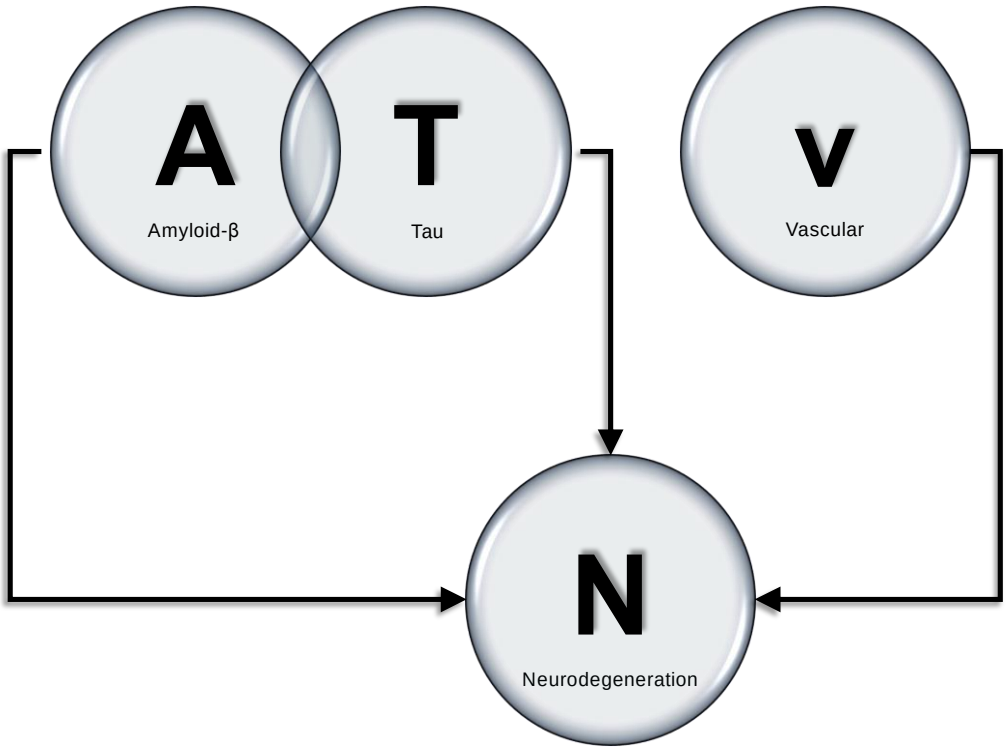



**Figure 1. Blood-brain barrier and the associated cell types.** The brain is amongst the highest vascularized organs in the body. Oxygenated blood, nutrients and regulatory molecules are delivered to the brain via arterial and arteriolar blood vessels that branch out into brain capillaries. Carbon dioxide and metabolic end products are removed from the brain by venous drainage system. Tightly-sealed brain capillary endothelium is the key site of the blood–brain barrier (BBB). The endothelial BBB monolayer extends along the arterioles, small arterial vessels and venules. Middle inset: An arteriole branching out into small capillaries. Vascular smooth muscle cells (pink) and pericytes (green) wrap around the arterioles and capillaries, respectively. Pericytes are embedded into the basement membrane encircling endothelial cells of the capillary vessel wall. Astrocyte endfeet (blue) wrap around the capillary wall, and in places not covered by pericytes are separated from endothelial cells by the basement membrane. Together with perivascular microglia (light brown) and macrophages, and neurons (orange), these different cell types form the neurovascular unit (NVU). Right inset: Capillary cross-section illustrates the cellular composition of the NVU at the level of brain capillary.

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724 **Figure 2. ATv(N) matrix.** The AT(N) system classifies individuals in the Alzheimer's disease
725 (AD) continuum by monitoring biomarkers for amyloid-β (A), tau (T), and neurodegeneration (N).
726 Recent neuroimaging and fluid biomarker studies suggest that novel candidate biomarkers (X)
727 for additional pathophysiological mechanisms should be incorporated within the AT(N) system.
728 Adding vascular dysfunction caused by an early breakdown in the blood-brain barrier (v) should
729 be helpful when evaluating preclinical decline, and clinical progression from cognitively
730 unimpaired to mild cognitive impairment (MCI), and MCI to dementia both in individuals along
731 the AD continuum and in those with negative AT biomarkers that may develop at a different
732 type of dementia, such as vascular dementia and mixed dementia, and/or convert to AD a later
733 stage. The 'v' could be chosen from neuroimaging and/or molecular biomarkers of blood-brain
734 barrier breakdown. The ATv(N) matrix allows for A, T, and v pathways to contribute to early
735 cognitive dysfunction and neurodegeneration independently of each other or acting
736 synergistically. The 'v' does not take into account the effects of other comorbid AD vascular
737 pathologies such white matter changes, lacunes, microinfarcts, and/or ischemic changes, and
738 may not apply to late stage progressive AD dementia, as discussed in this Perspective.

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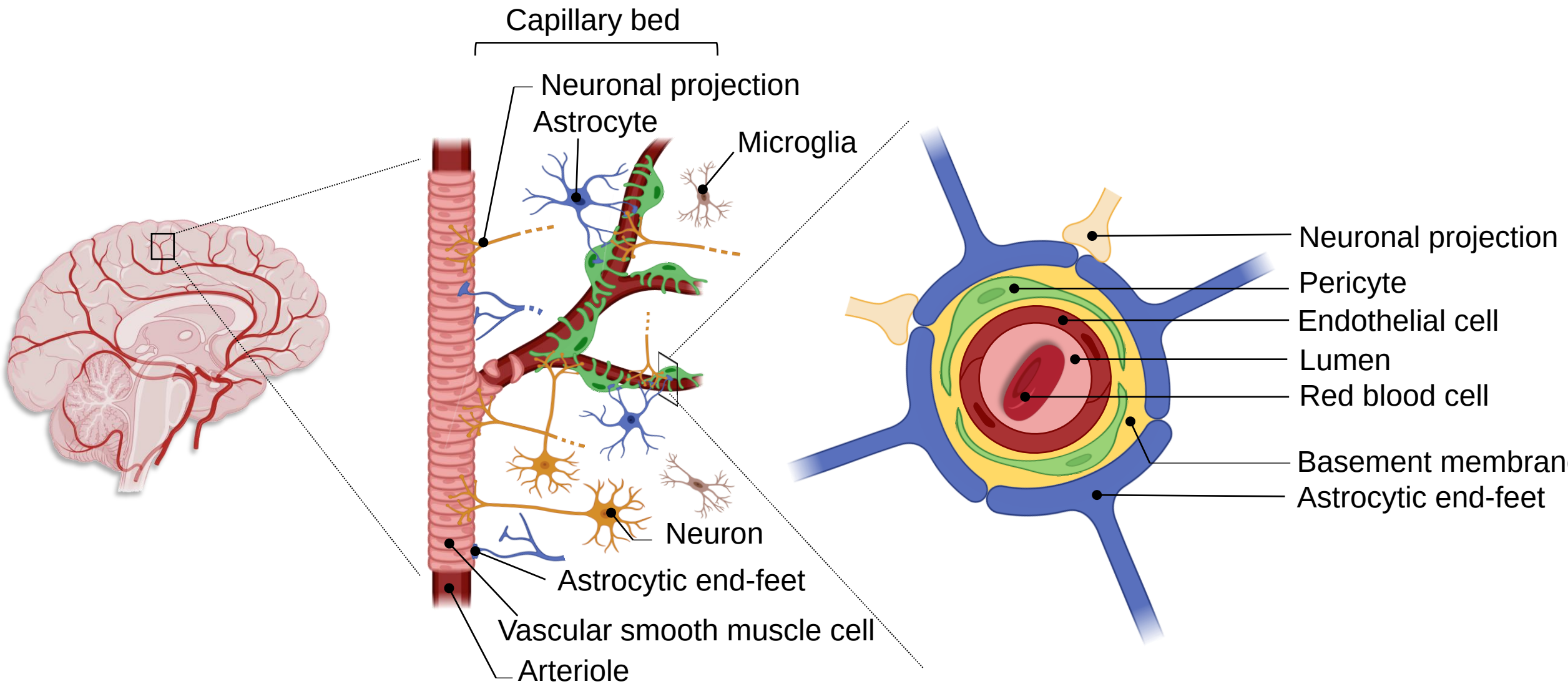
Capillary bed
Neuronal projection
Astrocyte
Microglia
Neuron
Astrocytic end-feet
Vascular smooth muscle cell
Arteriole
Neuronal projection
Pericyte
Endothelial cell
Lumen
Red blood cell
Basement membrane
Astrocytic end-feet

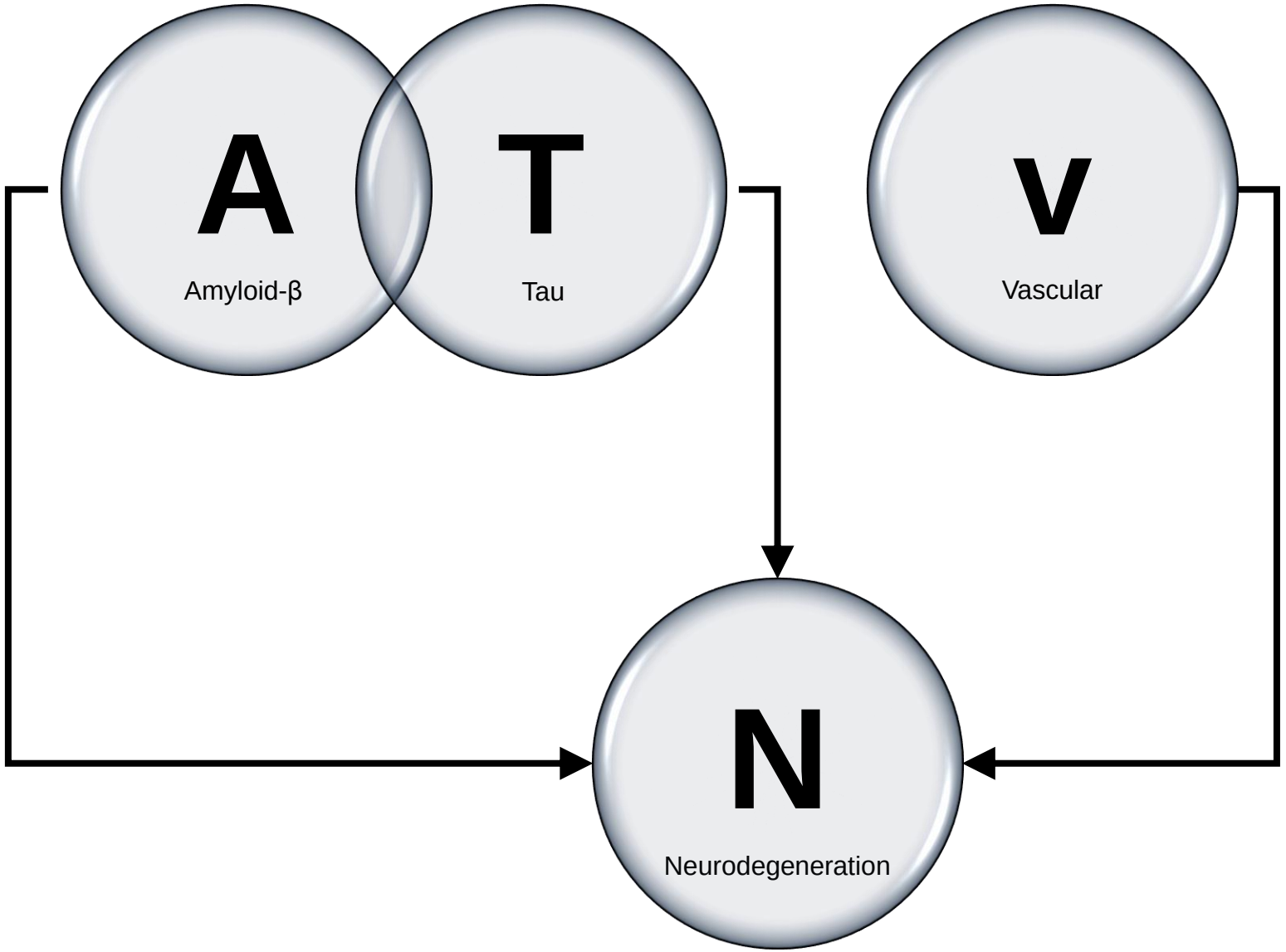
A
Amyloid-β
T
Tau
V
Vascular
N
Neurodegeneration

***Table 1:*** **Blood-brain barrier breakdown in MCI and Alzheimer disease dementia detected by neuroimaging**

| | Ref. | BBB Breakdown | | Sample size | Key risk factors | | | AT(N) biomarkers | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | DCE-MRI | SWI-MRI | | Age | VRFs | *APOE4* | A | T | N |
| **MCI** | [44], C | GM, NAWM | | 33 | ✓ | ✓ | Not studied | Not studied | | Yes* |
| | [24], C | Hippocampus<br>Parahippocampus<br>Caudate nucleus | | 73 | ✓ | ✓ | Not studied | Yes*<br>CSF, PET | Yes*<br>CSF, PET | Yes* |
| | [35], C | Hippocampus<br>Parahippocampus<br>Caudate nucleus | | 245 | ✓ | ✓ | ✓ | Yes*<br>CSF, PET | Yes*<br>CSF, PET | Yes* |
| | [41], C | GM, NAWM,<br>Hippocampus | | 80 | ✓ | ✓ | ✓ | Not studied | | Yes* |
| | [64], C | | Cortex<br>GM (deep)<br>Infratentorial | 67 | ✓ | Not studied | | Not studied | | Yes* |
| | [65], C | | Cortex (siderosis) | 809 | ✓ | Not studied | ✓ | Not studied | | Yes |
| | [27], L | | Lobes | 174 | ✓ | ✓ | ✓ | Yes<br>PET | Not studied | |
| | [25], C | | Lobes<br>GM (deep)<br>Infratentorial | 1504 | ✓ | ✓ | Not studied | Not studied | | |
| | [66], C | | Lobes<br>GM (deep)<br>Infratentorial | 136 | ✓ | Not studied | ✓ | Yes*<br>CSF | Yes*<br>CSF | Yes* |
| **Early AD** | [44], C | GM, NAWM | | 33 | ✓ | ✓ | Not studied | Not studied | | Yes |
| | [64], C | | Lobes<br>GM (deep)<br>Infratentorial | 67 | ✓ | Not studied | | Not studied | | Yes* |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **AD** | [41], C | GM, NAWM, Hippocampus | | 80 | ✓ | ✓ | ✓ | Not studied | Yes |
| | [65], C | | Lobes Cortex (siderosis) | 809 | ✓ | Not studied | ✓ | Not studied | Yes |
| | [27], L | | Lobes | 174 | ✓ | ✓ | ✓ | Yes PET | Not studied |
| | [25], C | | Lobes GM (deep) Infratentorial | 1504 | ✓ | ✓ | Not studied | Not studied | Not studied |

The AT(N) system monitors changes in amyloid-β (A), tau (T) and neurodegeneration (N) biomarkers. “Yes” indicates that BBB breakdown was found in individuals positive for the A and T biomarkers in the cerebrospinal fluid (CSF) and/or brain by positron emission tomography (PET), and/or N by MRI. ”Yes*” with asterisks indicates that BBB breakdown was found in individuals positive for the A, T and/or N biomarkers as well as in those that have not developed AT(N) biomarkers abnormalities. *C*, and *L*, indicate cross-sectional and longitudinal study, respectively. ✓, factor has been studied.

Mild cognitive impairment (MCI) was defined by clinical dementia rating scale of 0.5 and impairment in neuropsychological test scores in one or more cognitive domains selected from memory, attention/executive function, language tests, and global cognition; AD dementia was defined by the clinical criteria of the National Institute of Neurological and Communicative Disorders, Stroke–Alzheimer’s Disease and Related Disorders Association and/or the National Institute on Aging–Alzheimer’s Association guidelines.
**Abbreviations:** AD, Alzheimer disease; *APOE4,* variant of apolipoprotein E; BBB, blood-brain barrier; DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; SWI, susceptibility-weighted imaging sequence; GM, the entire grey matter; NAWM, the entire normal appearing white matter; VRFs, vascular risk factors.

***Table 2:*** **Blood-brain barrier breakdown in MCI and Alzheimer disease dementia detected by CSF biomarkers**

| | Ref. | CSF | Sample size | Key risk factors | | | AT(N) biomarkers | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Age | VRFs | *APOE4* | Amyloid | Tau | Neurodegeneration |
| **MCI** | [23], C | ↑ sPDGFRβ<br>↑ $Q_{alb}$ | 64 | ✓ | Not studied | | Not studied | | Yes* |
| | [24], C | ↑ sPDGFRβ | 141 | ✓ | ✓ | Not studied | Yes*<br>CSF, PET | Yes*<br>CSF, PET | Yes* |
| | [35], C | ↑ sPDGFRβ, ↑CypA, ↑MMP9<br>*APOE4* vs. *APOE3* | 350 | ✓ | ✓ | ✓ | Yes*<br>CSF, PET | Yes*<br>CSF, PET | Yes |
| **AD** | [74], M | ↑ $Q_{alb}$ | 1295 | Not studied | | | Not studied | | |
| | [75], L | ↑ sPDGFRβ, ↑ VEGF,<br>↑ VEGF/sVEGFR-1 ratio,<br>↑ $Q_{alb}$ | 1015 | ✓ | Not studied | ✓ | Yes*<br>CSF, PET | Not studied | |
| | [76], C | ↑ sPDGFRβ (CSF and serum)<br>↑ albumin | 78 | ✓ | Not studied | | Yes*<br>CSF | Yes<br>CSF | Not studied |

**Abbreviations:** CypA, cyclophilin A; MMP9, matrix metalloproteinase-9; $Q_{alb}$, CSF/plasma and/or CSF/serum albumin quotient; sPDGFRβ, soluble platelet-derived growth factor receptor-β; sVEGFR, soluble vascular endothelial growth factor receptor; VEGF, vascular endothelial growth factor; For definitions of MCI, AD, AT(N) biomarkers system, CSF, PET, *APOE*, VRFs, "Yes" and "Yes*" see footnote to Table 1. ✓: factor has been studied. *C*, *L*, and *M* indicate cross-sectional, longitudinal, or meta-analysis study, respectively.