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

1 **Blood-brain barrier link to human cognitive impairment and Alzheimer's Disease**

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25 **Abstract**
26 Vascular dysfunction is frequently seen in disorders associated with cognitive impairment,
27 dementia and Alzheimer's disease (AD). Recent advances in neuroimaging and fluid biomarkers
28 suggest that vascular dysfunction is not an innocent bystander only accompanying neuronal
29 dysfunction. Loss of cerebrovascular integrity, often referred to as breakdown in the blood-brain
30 barrier (BBB), has recently shown to be an early biomarker of human cognitive dysfunction and
31 possibly underlying mechanism of age-related cognitive decline. Damage to the BBB may
32 initiate or further invoke a range of tissue injuries causing synaptic and neuronal dysfunction
33 and cognitive impairment that may contribute to AD. Therefore, better understanding of how
34 vascular dysfunction caused by BBB breakdown interacts with amyloid-β and tau AD
35 biomarkers to confer cognitive impairment may lead to new ways of thinking about
36 pathogenesis, and possibly treatment and prevention of early cognitive impairment, dementia
37 and AD, for which we still do not have effective therapies.
38

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

90   allowing specific molecules to pass in and out of the brain. Recent studies have identified over
91   10,000 transcripts in the murine BBB endothelium with preferential expression of transporters in
92   the capillary endothelium[7,8]. Similar data sets on BBB transporters have recently been reported
93   in human brain endothelium[6].
94
95   The selective substrate-specific transport systems at the BBB include carrier-mediated transport
96   (CMT) of carbohydrates (e.g., glucose), amino acids, monocarboxylic acids (e.g., lactate, ketone
97   bodies), hormones, fatty acids, nucleotides, inorganic anions, amines, choline and vitamins.
98   These CMT systems enable transport of their respective substrates to cross BBB bi-directionally
99   according to their concentration gradients. Some larger molecules including certain proteins and
100   peptides can use receptor-mediated transport (RMT) to cross the BBB from blood-or brain, as
101   for example insulin, insulin-like growth factors, transferrin, leptin and some others. RMT systems
102   including lipoprotein receptors mediate clearance from brain of proteinaceous neurotoxic
103   molecules that are produced in the brain such Alzheimer's amyloid-β (Aβ) or Parkinson's α-
104   synuclein. Endothelial ATP-binding cassette transporters prevent brain accumulation of drugs,
105   xenobiotics, drug conjugates, and nucleosides in the brain by active efflux from endothelium to
106   blood. And endothelial ion transporters, such as sodium pumps, control ion concentrations in
107   the brain. Thus, in addition to protecting the brain parenchyma from blood-derived toxic
108   molecules, cells and microorganisms, the endothelial monolayer of the BBB regulates transport
109   of nutrients and essential molecules across brain endothelium into the brain, and clearance into
110   the blood of metabolic end products and endogenous neurotoxins produced by the brain. For
111   more details, on BBB transport systems in healthy brain, and how they are affected by the
112   disease process see recent reviews[1,4,5].
113
114   Pericytes, mural cells that lie along brain capillaries, share a common basement membrane with
115   endothelial cells. As reviewed recently[1,4], N-cadherin forms peg-and-socket contacts between
116   endothelial cells and pericytes, whereas the gap junction connexin (CX) 43 hemichannels
117   mediate intercellular communications between pericytes and endothelial cells. Astrocytes also
118   express gap junction proteins, some of which are important for maintaining BBB integrity, such
119   as CX30 and CX43. These adhesion molecules originally found in murine pericytes and
120   astrocytes were recently confirmed in human pericytes and astrocytes[6].
121
122   Finally, in contrast to peripheral organs such as liver, brain does not have a storage capability
123   for larger energy-saving molecules[1,2]. Its energy metabolism depends on delivery of metabolites
124   such as glucose by CBF and transport across the BBB. Vascular smooth muscle cells and
125   pericytes regulate CBF by constricting and dilating arterioles and capillaries, respectively[2,9–11]
126   (**Fig. 1**). Importantly, pericytes maintain BBB integrity, and their loss leads to BBB disruption[12–
127   16].
128
129   *BBB dysfunction and neurological disorders in humans*
130
131   That intact BBB is required for normal brain function is best illustrated by examples of rare
132   monogenic human neurological disorders where the genetic mutations or defects are found to
133   originate exclusively within brain endothelial cells, and/or BBB-associated pericytes and
134   vascular smooth muscle cells. For example, inactivating mutations in the Solute Carrier Family 2
135   Member 1 (*SLC2A1*) gene encoding GLUT1 glucose transporter in brain endothelial cells, lead
136   to GLUT1-deficiency syndrome, a paediatric neurological disease with early onset of seizures
137   and microcephaly, BBB breakdown, and neuron loss[17]. Inactivating mutations in the Major
138   Facilitator Superfamily Domain Containing 2A (*MSFD2A*) gene encoding transporter for
139   essential omega-3 fatty acids that is enriched in brain endothelial cells, lead to BBB
140   breakdown[18,19] and microcephaly syndrome[20,21]. Mutations in genes encoding the BBB TJ

141   proteins, cerebral cavernous malformation proteins or collagens lead to uncontrolled leakage of
142   proteins and other content from blood into the brain causing neuroinflammatory response,
143   increased microvessel fragility, cerebral haemorrhages and small vessels disease (SVD),
144   resulting in focal neurological deficits, seizures and headaches, and/or lacunar ischemic
145   strokes[1]. Mutations in *NOTCH3* gene that is expressed in vascular smooth muscle cells and
146   pericytes lead to cerebral autosomal dominant arteriopathy with subcortical infracts and
147   leukoencephalopathy (CADASIL), a major cause of genetically inherited stroke in humans
148   associated with loss of blood vessels integrity[22].
149
150   Altogether, about 20 rare neurological monogenic disorders identified offer insights into causal
151   pathogenic links between BBB dysfunction and neurological disease in humans, supporting the
152   idea that BBB dysfunction can have neurological consequences.
153
154   **Blood-brain barrier breakdown and cognitive dysfunction**
155
156   Several neuropathological studies have shown BBB breakdown in AD[1]. However, the point at
157   which individuals suffering from MCI and AD develop BBB breakdown has not been clear until
158   recently. Using dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) with
159   gadolinium-based contrast agents (GBCA), recent studies indicated that BBB breakdown occurs
160   early in individuals with MCI and AD-type dementia, and is an early biomarker of cognitive
161   dysfunction[23,24]. The presence of gadolinium in brain reflects subtle BBB "leaks" of plasma
162   components, and is typically caused by loss of TJ or AJ proteins, and/or increased trans-
163   endothelial fluid transcytosis of plasma components across the BBB. According to experimental
164   studies BBB leaks could be related to loss of pericyte coverage[12,14].
165
166   Loss of BBB integrity has also been shown in MCI and AD dementia by susceptibility weighted
167   imaging (SWI) MRI detecting early cerebral microhaemorrhages[25,26]. This greater and more
168   focal degree of BBB breakdown leads to extravasation of red blood cells into the brain. Lobar
169   microhaemorrhages seen by SWI are often due to cerebral amyloid angiopathy (CAA) that is
170   present in many cases of MCI and AD along with amyloid deposition in brain[27]. However,
171   microhaemorrhages are also seen in deep infratentorial regions related to hypertensive SVD
172   and vascular dementia[28].
173
174   The BBB dysfunction in MCI and AD is not limited only to "mechanical" types of BBB breakdown
175   described above, and may also include dysfunction in the BBB transporters and/or receptors,
176   such as, to name a few, loss of GLUT1 glucose transporter and P-glycoprotein 1 (P-gp) key
177   efflux transporter of toxins, as discussed below. For details of how BBB transport systems are
178   affected by different neurological disorders see recent review[5].
179
180   The link between BBB and AD is further supported by a recent nuclear RNA sequencing study
181   of major human brain vascular and perivascular cell types from hippocampus and cortex, which
182   revealed that 30 of the top 45 AD genes identified by genome wide association studies (GWAS)
183   are expressed in brain vasculature[6]. Vascular GWAS genes mapped to endothelial protein
184   transport, adaptive immune and extracellular matrix pathways.
185
186   Below, we discuss recent neuroimaging studies demonstrating BBB breakdown in individuals
187   with early cognitive impairment, MCI and AD-type dementia, and in relation to AD biomarkers
188   amyloid-β (Aβ), tau and neurodegeneration[29]. But before we proceed with this discussion, we
189   would like also to mention that earlier neuroimaging studies using computed tomography
190   (CT)[30,31], positron emission tomography (PET) with [^{68}Ga]EDTA[32], and DCE-MRI semi-
191   quantitative analysis[33] failed to detect higher BBB permeability in AD. In contrast to these earlier

192 studies from late eighties and nineties, more recent neuroimaging studies from several groups
193 over the last five to six years have shown age-related BBB breakdown[23,34–40], BBB breakdown in
194 MCI[23,24,35,36,41,42], AD[43–46], cerebral SVD[47–53] and in other neurodegenerative disorders[54,55]. The
195 discrepancy between earlier and recent studies could likely be attributed to use of more
196 advanced techniques and analysis in recent studies. This includes use of MRI sequences with
197 higher spatial and temporal resolution[23,24,35,43,56], direct measurements of individual vascular
198 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
199 quantification methods, such as the Patlak model[57], which has not been used in previous
200 studies[30–33]. Some earlier studies[31,33] measured only signal changes after contrast injection
201 without applying the pharmacokinetic analysis that takes into account the tracer's concentration
202 in blood. One CT study[30] and one PET study[32] adopted pharmacokinetic models, but did not
203 detect BBB leaks likely due to a lower ability of CT and PET to resolve cerebral anatomical
204 structures compared to recent MRI sequences, and generally much lower spatial resolution of
205 PET.

Mild cognitive impairment

BBB breakdown by DCE-MRI and analysis of cerebrospinal fluid

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

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

235 A few MCI studies reported that the BBB breakdown was not influenced by vascular risk factor
236 (VRF) burden[24,35]. Since the studied cohorts excluded participants with substantial
237 cerebrovascular pathology, it is possible that interactions between traditional VRFs and BBB
238 dysfunction in cohorts with more severe vascular lesions and vascular cognitive impairment will
239 lead to synergistic effects. Again, this remains to be determined by future studies. Some studies
240 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

291
292 BBB breakdown in the cortex, white matter, and some deep grey matter regions has been
293 shown by DCE-MRI during early stages of AD[44]. Compared to MCI, early AD patients present
294 with a higher prevalence of cerebral microbleeds on 3T MRI[64], often localized in the occipital
295 and parietal lobes, sites of CAA (**Table 1**). Cerebral microbleeds are commonly found with more
296 advanced AD with the prevalence as high as 45% at 3T[25,27,65] and up to 78% at 7T[68]. Although,
297 the majority of microbleeds was typically lobar and CAA-related, the CAA-unrelated
298 microhaemorrhages in the subcortical gray matter and infratentorial regions were also found.
299
300 Recent studies found that patients with epilepsy and AD, as well as aging mice, develop BBB
301 leaks associated with slower cortical activity[43]. Moreover, these BBB leaks were related to
302 activation of transforming growth factor-β (TGFβ) in astrocytes, as shown in humans and mice[56].
303
304 P-gp, an active efflux transporter at the luminal side of the BBB endothelium removes drugs,
305 xenobiotics and Aβ from brain[72]. Studies using ^{11}C-verapamil, a PET ligand for P-gp, indicated
306 diminished P-gp activity in early AD in multiple region including hippocampus and cortex[73],
307 suggesting impaired BBB clearance.
308
309 In addition to increased CSF sPDGFRβ and Q_{alb} in large cohort studies in AD[74,75], increased
310 CSF sPDGFRβ correlated with increased sPDGFRβ in the serum and increased CSF/serum
311 Q_{alb} ratio suggestive of BBB breakdown[76] was also found (**Table 2**). Increased CSF levels of
312 biomarkers of angiogenesis and endothelial dysfunction, including vascular endothelial growth
313 factor (VEGF) and VEGF/soluble VEGF receptor 1 (sVEGFR-1) ratio, were also found in AD[75].
314 These biomarkers were not associated with Aβ load[75], suggesting that BBB endothelial
315 dysfunction is likely independent of amyloid pathology (**Table 2**).
316
317 Reduced FDG-PET is often interpreted as brain hypometabolism. However, several
318 investigators support the view that reduced transport across the BBB also contributes to
319 reduced FDG-PET as recently reviewed[77]. In brief, glucose enters the brain via transport across
320 the BBB mediated by GLUT1 glucose transporter, and if GLUT1 is deficient, deleted from the
321 BBB, blocked genetically, inhibited pharmacologically or suppressed by disease, glucose cannot
322 reach the brain[1]. Several earlier FDG dynamic PET studies have shown diminished BBB
323 transport of glucose in AD, as reviewed elsewhere[77].
324
325 **Blood-brain barrier and perivascular spaces**
326
327 BBB breakdown during early cognitive decline in people at risk for AD[24] could lead to increased
328 perivascular spaces (PVS) as in CADASIL[22,78]. The suggestion of direct leakage across the
329 perforating vessel wall into the PVS[79] is supported by work in pericyte-deficient mice which
330 develop BBB leakage associated with increase in the size and number of PVS[80]. Subtle diffuse
331 BBB leaks on DCE-MRI correlated with increasing numbers of PVS[79]. When enlarged, PVS in
332 the white and deep grey matter become visible by MRI[81]. PVS increases at older age, with
333 cerebral SVD[82], and BBB breakdown[80], indicating that they are likely markers of BBB-related
334 vascular dysfunction. Systematic reviews of population, vascular, and neurodegenerative
335 diseases indicate that higher number of PVS is associated in cross-sectional studies with
336 cognitive decline, AD-type dementia, and executive dysfunction[83–86].
337
338 **Conclusions and future directions**
339

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

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

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

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

387 proposed ATv(N) matrix will stimulate more researchers and clinicians to analyse the BBB in
388 studies and trials for early stages of cognitive impairment, MCI, AD and other dementias.
389

390 **Contributors**
391 G.B., A.M., K.K. and B.V.Z. prepared the figures and wrote the manuscript. All authors
392 performed literature search, edited the text, provided critical reading of the manuscript, and
393 approved the final version for submission. B.V.Z. provided final edits of the manuscript.
394

395 **Declaration of interests**
396 Drs. Barisano, Montagne, Kisler, and Zlokovic declare no competing interests related to this
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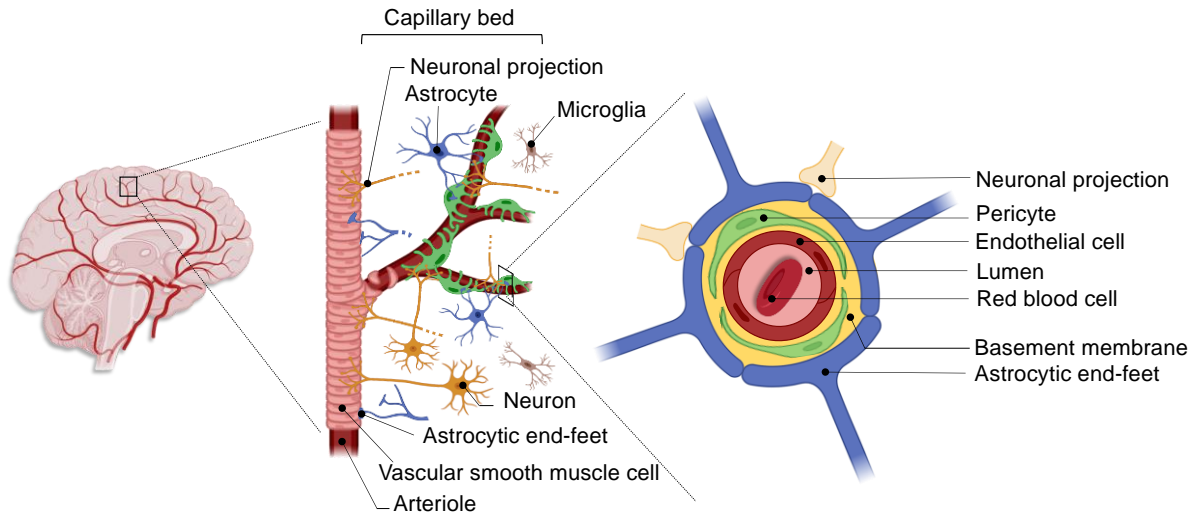700   doi:10.1038/s41380-020-0838-x.
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Capillary bed
Neuronal projection
Astrocyte
Microglia
Neuron
Astrocytic end-feet
Vascular smooth muscle cell
Arteriole

Neuronal projection
Pericyte
Endothelial cell
Lumen
Red blood cell
Basement membrane
Astrocytic end-feet

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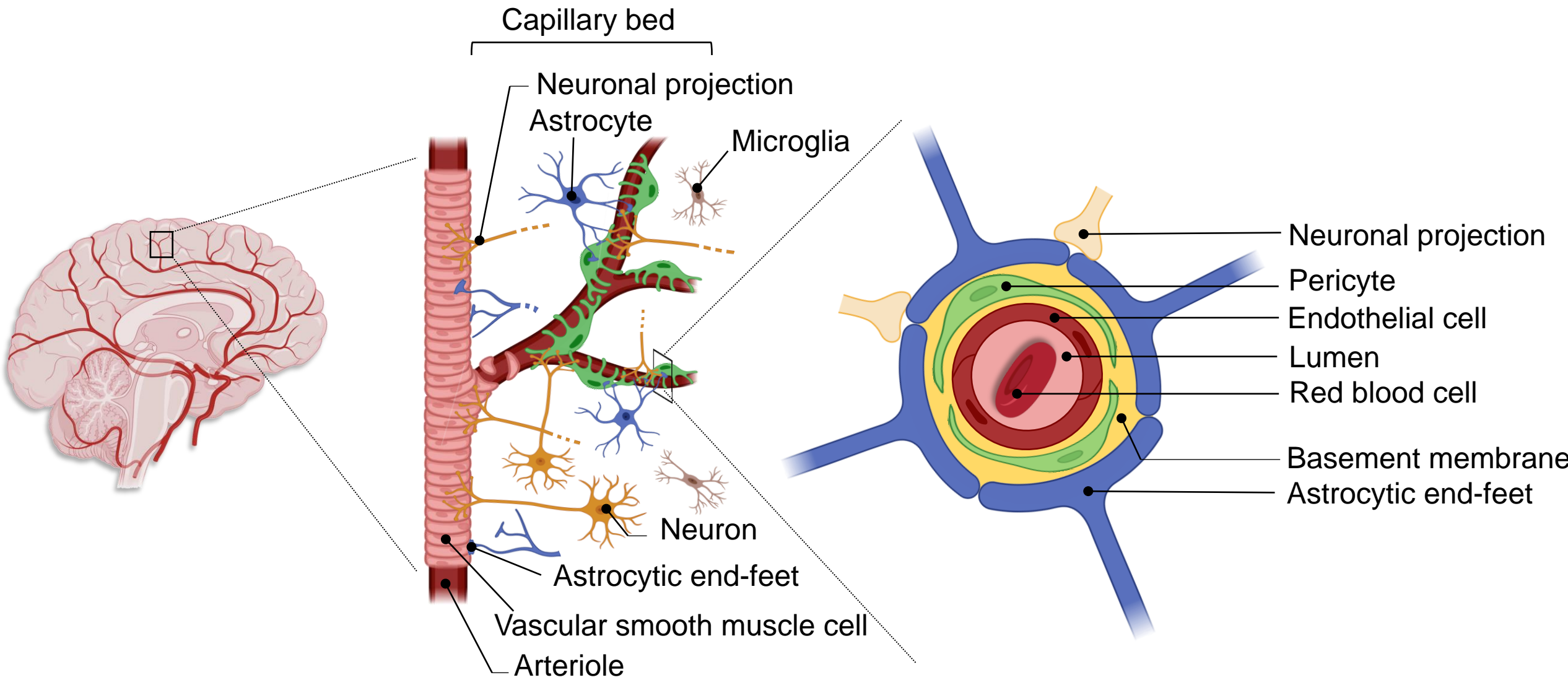
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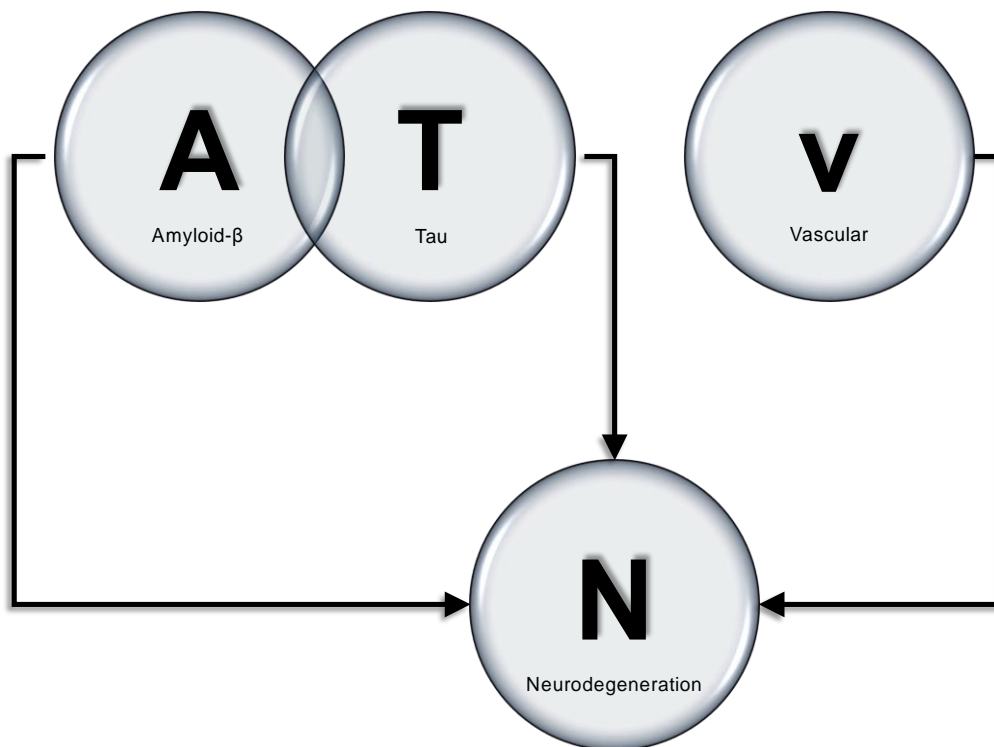
Figure 2. ATv(N) matrix. The AT(N) system classifies individuals in the Alzheimer's disease (AD) continuum by monitoring biomarkers for amyloid-β (A), tau (T), and neurodegeneration (N). Recent neuroimaging and fluid biomarker studies suggest that novel candidate biomarkers (X) for additional pathophysiological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

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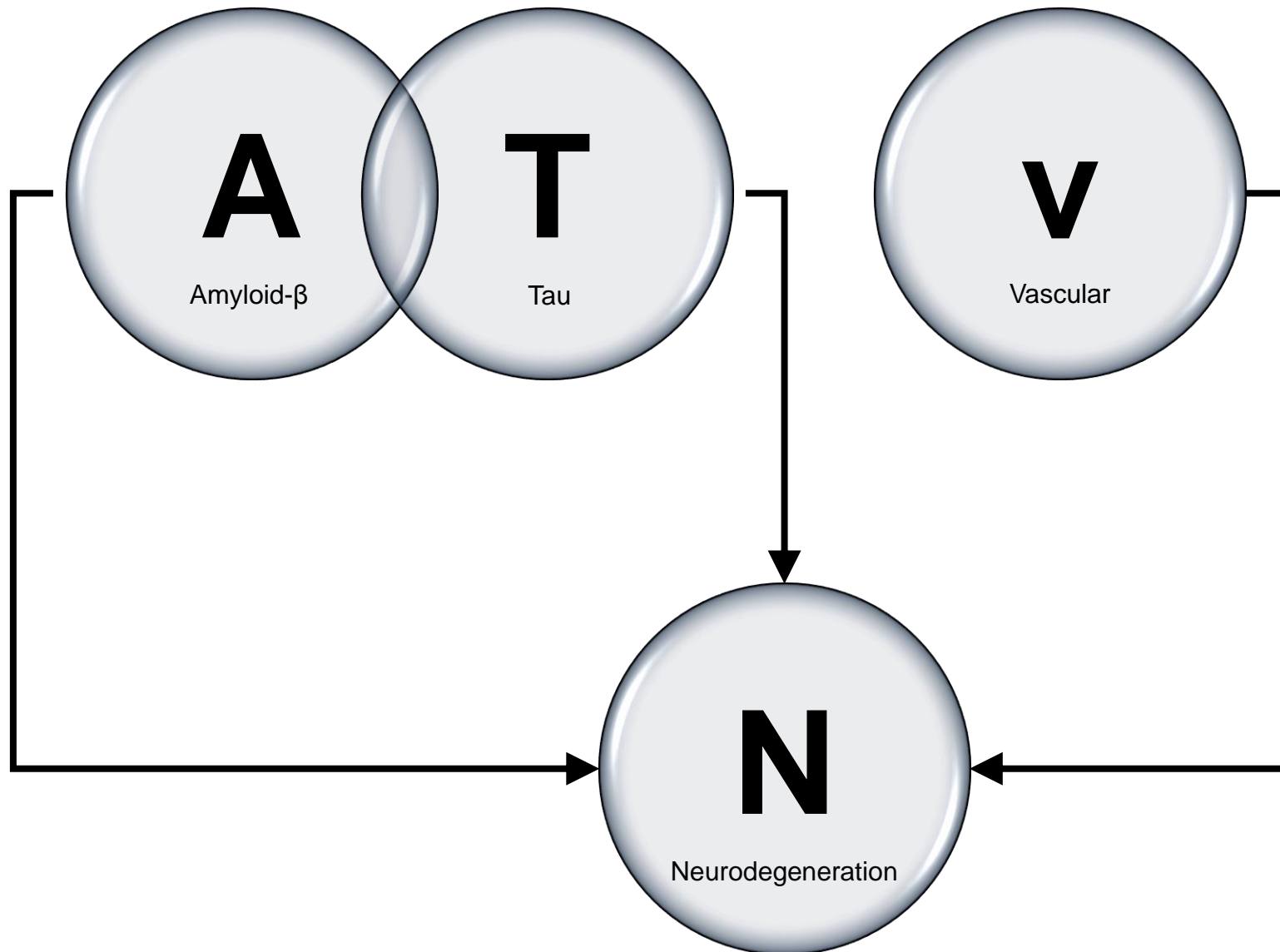
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Capillary bed

Neuronal projection

Astrocyte

Microglia

Neuronal projection

Pericyte

Endothelial cell

Lumen

Red blood cell

Basement membrane

Astrocytic end-feet

Neuron

Astrocytic end-feet

Vascular smooth muscle cell

Arteriole

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 Parahippocampus Caudate nucleus		73	✓	✓	Not studied	Yes* CSF, PET	Yes* CSF, PET	Yes*
	[35], C	Hippocampus Parahippocampus Caudate nucleus		245	✓	✓	✓	Yes* CSF, PET	Yes* CSF, PET	Yes*
	[41], C	GM, NAWM, Hippocampus		80	✓	✓	✓	Not studied		Yes*
	[64], C		Cortex GM (deep) Infratentorial	67	✓	Not studied		Not studied		Yes*
	[65], C		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		
	[66], C		Lobes GM (deep) Infratentorial	136	✓	Not studied	✓	Yes* CSF	Yes* CSF	Yes*
Early AD	[44], C	GM, NAWM		33	✓	✓	Not studied	Not studied		Yes
	[64], C		Lobes GM (deep) 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 ↑ Q_{alb}	64	✓	Not studied		Not studied		Yes*
	[24], C	↑ sPDGFRβ	141	✓	✓	Not studied	Yes* CSF, PET	Yes* CSF, PET	Yes*
	[35], C	↑ sPDGFRβ, ↑CypA, ↑MMP9 *APOE4* vs. *APOE3*	350	✓	✓	✓	Yes* CSF, PET	Yes* CSF, PET	Yes
AD	[74], M	↑ Q_{alb}	1295	Not studied			Not studied		
	[75], L	↑ sPDGFRβ, ↑ VEGF, ↑ VEGF/sVEGFR-1 ratio, ↑ Q_{alb}	1015	✓	Not studied	✓	Yes* CSF, PET	Not studied	
	[76], C	↑ sPDGFRβ (CSF and serum) ↑ albumin	78	✓	Not studied		Yes* CSF	Yes 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.