Trends in Molecular Medicine

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Review

Astrocyte Biomarkers in Alzheimer's Disease

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Astrocytic contributions to Alzheimer's disease (AD) progression were, until recently, largely overlooked. Astrocytes are integral to normal brain function and astrocyte reactivity is an early feature of AD, potentially providing a promising target for preclinical diagnosis and treatment. Several *in vivo* AD biomarkers already exist, but presently there is a paucity of specific and sensitive *in vivo* astrocyte biomarkers that can accurately measure preclinical AD. Measuring monoamine oxidase-B with neuroimaging and glial fibrillary acidic protein from bodily fluids are biomarkers that are currently available. Developing novel, more specific, and sensitive astrocyte biomarkers will make it possible to pharmaceutically target chemical pathways that preserve beneficial astrocytic functions in response to AD pathology. This review discusses astrocyte biomarkers in the context of AD.

Highlights

The neurocentric view of AD is evolving and the contributions astrocytes make to the disease's pathological processes are finally considered.

AD pathology triggers astrocyte reactivity, which imaging and fluid biomarkers can measure *in vivo*.

Astrocyte dysfunction in AD could contribute to [^{18}F]FDG-PET hypometabolism.

Astrocytes are promising targets for developing novel, specific fluid or imaging biomarkers for detecting preclinical AD.

Pharmacologically targeting astrocytes may lead to developing an effective treatment for AD.

From Neurons to Astrocytes: Evolution of Alzheimer's Disease Biomarkers

Our conceptualisation of Alzheimer's disease (AD) may have reached a point where a paradigm shift is required. The disease is the most common cause of dementia worldwide [1], accounting for 50%–70% of all cases [2], but the most common subtype, sporadic AD, remains incompletely understood. It is undisputed that the deposition of amyloid-beta (Aβ) into **Aβ plaques** (see Glossary) and the formation of **neurofibrillary tangles (NFTs)** composed of hyperphosphorylated **tau protein** are the main neuropathological features of AD [3]. Based on the **amyloid cascade hypothesis** [4], Aβ pathology triggers a cascade of events, leading to neurodegeneration, which drives AD towards the appearance of cognitive dysfunction [5]. Brain ageing and environmental and lifestyle factors in association with possession of genes like apolipoprotein E (*ApoE*) ε4 (Box 1) are instrumental in AD [6].

The predominant cascade hypothesis is open to criticism since many clinical trials that have failed were based on interventions targeting Aβ [7–9]. However, there are ongoing immunotherapy trials that have demonstrated significant promise (aducanumab & BAN2401) but the jury is still out [10]. The simple A → B → C causal chain that is currently accepted in AD research is insufficient and could be impeding therapeutic endeavours. More specifically, the pervading view is that removing Aβ (A) from the causal disease chain will prevent neuronal death (B) and stabilise cognitive function (C); ultimately this last step is where all trials have failed. It is far more probable that there are common, related, disease causes that interact, potentially at an individual patient level, which make any person more or less likely to develop AD. Discovering a common link between multiple causes could be the key to evolving our conceptualisation of AD and developing a successful treatment.

How AD is diagnosed has already evolved from the first criteria published in 1984 by McKhann *et al.* [11], to the 2011 McKhann *et al.* revisions [12], and to the 2018 research framework by Jack *et al.* [13]. The 1984 criteria were predominantly clinical with laboratory tests only used to exclude other causes of cognitive impairment, and diagnostic confirmation was only possible

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Box 1. *Apolipoprotein E*, a Major Genetic Risk Factor for AD, Is Produced by Astrocytes

ApoE exists as three alleles (*ApoE* ε2, *ApoE* ε3, and *ApoE* ε4), making six possible *ApoE* genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4). It is well accepted that the *ApoE* ε4/ε4 genotype is a major genetic risk for developing sporadic AD and *ApoE* ε2 is protective against AD pathology [133]. ApoE is predominantly produced by astrocytes having a role in degrading and clearing Aβ in the brain. More specifically, *ApoE* ε4–expressing mouse-derived astrocyte cultures present reduced autophagy with associated lower capacity for clearing Aβ plaques [134]. In addition, human induced pluripotent stem cell (iPSC)–derived astrocytes expressing ApoE ε4 allele provide less support to neurons, affecting survival and synaptogenesis [135]. Another study using astrocyte cultures derived from *ApoE* knockout mice demonstrated that ApoE seems necessary for the astrocytic ability of responding or internalising Aβ [136]. A recent article demonstrated that by changing *ApoE* ε3 for the *ApoE* ε4 gene in iPSCs from subjects without AD caused dramatic changes in neuronal, astrocyte, and microglial transcriptome [137].

with post-mortem evidence of Aβ plaques and NFTs in brain tissue. Using almost 30 years of scientific progress, the 2011 revisions incorporated clinical, neuropsychological, neuropathological, genetic, and biological characteristics of AD. The main step change was integrating *in vivo* fluid and imaging biomarkers to capture AD in its earliest stages. Jack *et al.*'s recently proposed biomarker-based diagnosis of AD primarily uses biological disease features and not clinical outcomes. This paradigm shift is dependent on reliable biomarkers for measuring Aβ (A), tau (T), and neurodegeneration (N), adopting an A, T, and N classification. This research framework still assumes a neurocentric perspective dominated by neuropathological features and neuronal dysfunction. Noteworthy, however, for the first time astrocytes have been suggested as a potential emerging biomarker target.

By its very nature, AD is a complicated, multifaceted disease and the new research framework attempts to accommodate that; however, the same simple causal chain, A → T → N, is conserved. Preventing synaptic loss and neuronal death caused by AD is obviously the primary objective of any treatment and no learned mind would argue that completely disregarding neurons is viable to prevent AD, but thinking about them in a different context may be. Neurons are very sophisticated biochemical electrical wires that can adapt their connectivity based on the signals they receive and the environment in which they exist. However, neurons cannot work properly without the support of specialised glial cells (Box 2) [14]. These cells have long been overlooked in AD, but evidence is growing that the neurocentric view of AD could be changing as evidenced by the inclusion of astrocytes as potential biomarkers. Among all glial

Box 2. Major Cellular Components of the Central Nervous System

Neurons (grey; Figure I), excitable cells in the CNS, communicate with each other through synapses and their signals are propagated by electrical impulses. The tripartite synapse has three components: a presynaptic neuron, a postsynaptic neuron, and an astrocytic process, which delineate a space called the synaptic cleft. For many years, glial cells ('glue' in Greek) were considered only the scaffold that keeps neurons together. However, it is now established that glial cells – astrocytes, microglia, and oligodendrocytes – are active components in the CNS. Astrocytes (green; Figure I) control synaptic neurotransmitters levels and maintain ionic homeostasis around the neurons, allowing for efficient synaptic signal transduction. They cover cerebral blood vessels with processes called endfeet and are part of the BBB. They provide metabolic and energetic support to neurons. In addition, astrocytes can release neuroactive molecules – gliotransmitters – in the synaptic cleft and reinforce the synaptic signal. Furthermore, they have neurotransmitter receptors and communicate with each other by calcium waves propagated via gap junctions from one astrocyte to another. Oligodendrocytes (blue; Figure I), CNS myelinating cells, wrap the cellular processes around axons providing insulation and protective layer. The myelin sheath, a lipid-rich membrane, interacts with surrounded axons, providing trophic support, promoting cell survival, and organising the distribution of ion channels along the axon. Periodic gaps in the myelin sheath allow for the passage of ions. Microglial cells (red; Figure I), CNS immune cells, are derived from the monocyte–macrophage lineage and migrate to the CNS during development and are carried by the blood. They are small and extremely plastic cells with numerous processes, which are widely distributed in the whole CNS. Similar to macrophages, microglial cells respond to the release of inflammatory molecules such as cytokines by becoming activated. When activated, microglia cells are recruited to areas of CNS infection or injury undergoing phenotypic changes and releasing several proinflammatory mediators that help to clear cellular debris and dead cells.

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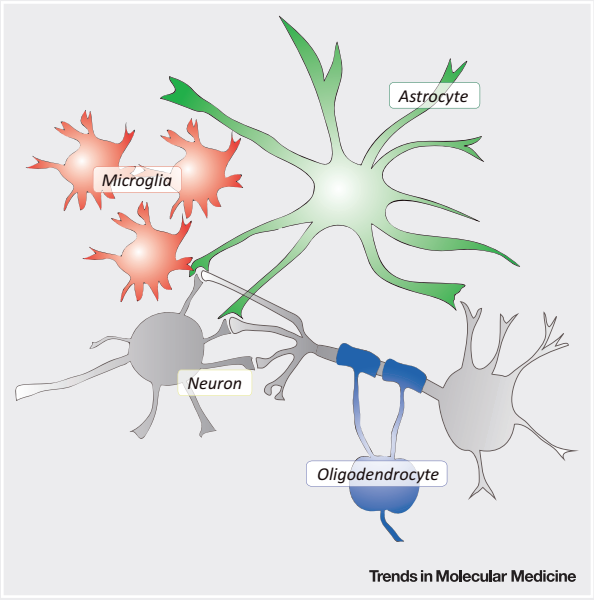

Figure I. The Fundamental Cells of the Human Brain Neurons (grey) and glial cells that include astrocytes (green), microglial cells (red) and oligodendrocytes (blue).

cells, astrocytes are probably the most versatile and incompletely understood. If an integrative, multifaceted evolution in the understanding of AD is needed, the cell type that may hold the key is the astrocyte (Figure 1).

The Unique Role of Astrocytes in the Central Nervous System

Although incompletely understood, it is established that astrocytes and microglia are important regulators of central nervous system (CNS) inflammatory response, while oligodendrocytes provide support and insulation to axons [15]. Indeed, oligodendrocytes wrap axons with myelin, a coating of compacted cell membrane, which promotes electrical insulation and facilitates the transit of action potentials along axons. Glial cells have been implicated as contributors to AD pathophysiology with most attention given to microglial activation as an AD biomarker (for review, see [16]).

Historically, astrocytes were thought of as relatively passive cells that glued neurons together, but this perspective has evolved substantially in recent years [14]. Astrocytes and neurons are closely embedded within brain tissue, sharing connections at synapses. It is now well recognised that one astrocyte can constantly exchange information with multiple neurons (the basis of the tripartite synapse) [17]. Astrocytes interact with blood vessels and are tightly linked to the basal lamina, which is a component of the vessel wall [18]. They are key components of the neurovascular unit (NVU) [19], which is a sophisticated multicellular matrix within the CNS; it comprises vascular cells (e.g., endothelial cells, pericytes) that together with astrocytic endfeet form the **blood–brain barrier (BBB)** [20]. The concerted action of the NVU controls BBB permeability and cerebral blood flow (CBF). Within the NVU, astrocytes are responsible for controlling neuronal and synaptic homeostasis by regulating ionic balance, removing or catabolising neurotransmitters, releasing bioactive molecules (such as growth factors or **gliotransmitters**), and playing a critical role in the maintenance of redox status [17]. They

Glossary

[^{11}C]BU99008: carbon-11-labelled positron emission tomography radiotracer that binds to the imidazoline2-binding site, highly expressed in astrocytes.
[^{11}C]-Deuterium-L-deprenyl ([^{11}C]DED): carbon-11-labelled positron emission tomography radiotracer that binds to monoamine oxidase-B, mainly expressed in reactive astrocytes.
Amyloid-β monomers: amyloid-β peptide usually constituted of 40 or 42 amino acids.
Amyloid-β oligomers: soluble toxic amyloid-β species composed by amyloid monomers (ranging from 12 to 150 kDa).
Amyloid-β plaques: β-sheet-insoluble conformation of amyloid-β, a histopathological feature of AD.
Amyloid cascade hypothesis: a hypothetical model put forward in 1992 by John Hardy and Gerald Higgins describing amyloid-β deposition as the causative agent in AD, which results in downstream NFTs formation, cell loss, vascular damage, and cognitive decline.
Amyloid precursor protein (APP): a transmembrane protein expressed in many tissues and concentrated in the CNS, which is cleaved into **amyloid-β monomers**.
Astrocyte–neuron lactate shuttle (ANLS): a hypothetical framework postulating that astrocytes take up glucose, metabolise it through glycolysis, and then fuel neurons with lactate in response to neuronal activity.
Blood–brain barrier (BBB): a highly selective 'physiological sieve' that divides the circulating blood from the brain, controlling the movement of ions and molecules between these two compartments.
Glial fibrillary acidic protein (GFAP): intermediate filament protein expressed mainly in astrocytes and overexpressed in reactive astrocytes.
Gliotransmitters: chemical transmitters released by astrocytes, including glutamate, D-serine, and ATP.
Glymphatic system: a fluid-clearance pathway composed by periarterial and perivenous spaces, and the interposed brain parenchyma that allows for clearance

are also key for brain energy homeostasis as an integral part of the **astrocyte–neuron lactate shuttle (ANLS)** [21], whereby astrocytes take up glucose, metabolise it through glycolysis, and shuttle lactate, via monocarboxylate transporters (MCTs), to neurons for energy.

The consensus is that astrocytes are not only monitoring and responding to fluctuations of synaptic transmission but also modulating behavioural state in health and disease [22]. Special focus should be given to astrocytes' role in modulating glutamatergic neurotransmission, which is responsible for around 90% of the excitatory neurotransmission in the human brain. Astrocytes are instrumental in taking up glutamate from the synaptic cleft and ceasing neurotransmission using a family of specific astroglial transporters, called high-affinity excitatory amino acid transporters (EAATs) [23]. To date, five EAATs have been described in mammals, among which GLAST (EAAT1) and GLT-1 (EAAT2) are predominantly found in astrocytes [24].

It is important to emphasise that astrocytes are highly heterogeneous and adjust to CNS demands. This remarkable ability to quickly adapt in response to the CNS environment gives them a key role in CNS defence and also suggests that astrocytes are one of the very first cells (if not the first ones) to react to CNS injury.

Reactive Astrocytes in Alzheimer's Disease

In pathological situations, astrocytes undergo a series of morphological and functional alterations collectively referred as reactive astrocytes. Reactive astrocytes overexpress **glial fibrillary acidic protein (GFAP)** and **vimentin** and re-express **nestin** (usually expressed in immature astrocytes) [25]. These proteins are astrocytic cytoskeletal components called intermediate filaments (IFs). IFs are the third fibrous component of the cytoskeleton, in addition to microtubules and microfilaments, having primary structural function but also playing important roles in regulating neuronal physiology [26,27]. Reactive astrocytes also undergo notable morphological alterations such as enlarged cell bodies and processes [25]. Furthermore, **monoamine oxidase-B (MAO-B)**, which is mainly located in the astrocyte outer mitochondrial membrane, is upregulated in reactive astrocytes [28] and correlates with different astrocyte markers in several neurodegenerative diseases [29]. The MAO-B enzyme catalyses the oxidative deamination of biogenic monoamines, primarily dopamine, having a key role in regulating monoaminergic neurotransmission [30]. The notion that reactive astrocytes are highly proliferative is now less accepted, with the current view suggesting that increased number of GFAP-positive astrocytes led to misinterpretation in the original definition of proliferating reactive astrocytes [31].

The first demonstration of abundant reactive glial cells surrounding Aβ plaques described by Alois Alzheimer was overlooked for many decades [32] and almost a century later, their role in AD remains poorly understood. Reactive astrocytes are typically found in post-mortem AD brain tissue in areas with high Aβ or tau pathology [33–35]. Other pathological components in AD such as microglial activation can also provoke astrocyte reactivity [36]. In fact, reactive astrocytes contribute to neuroinflammatory changes in AD by releasing cytokines, inflammatory factors, nitric oxide (NO), and reactive oxygen species (ROS) and promoting redox status imbalance [37] (please see Table 1 for an overview of astrocyte reactivity signature in AD).

Astrocytes and Microglial Cells: Partners in Neuroinflammatory Changes

Microglial activation has been extensively evaluated as an index of neuroinflammation in AD [16], but the neuroinflammatory contribution of astrocytes in AD is attracting more attention in

and redistribution of exogenous or endogenous molecules.

Magnetic resonance imaging (MRI): medical imaging technique that uses strong magnetic fields, electric gradients, or radio waves for acquiring anatomical and functional images.

Monoamine oxidase-B (MAO-B): enzyme that degrades biogenic and dietary amines predominantly located in the outer mitochondrial membrane of astrocytes but also in serotoninergic neurons.

Nestin: intermediate filament protein expressed in developing astrocytes (not adults) and re-expressed in adult reactive astrocytes.

Neurofibrillary tangles: insoluble twisted fibres primarily composed of hyperphosphorylated tau protein, a canonical histopathological feature of AD.

Positron emission tomography (PET): medical imaging technique that uses molecular agents labelled with positron-emitting radioisotopes allowing the visualisation of biological processes *in vivo*.

Tau protein: a microtubule-associated protein (MAP) responsible for maintaining and promoting cell microtubule stability.

Vimentin: intermediate filament protein expressed mainly in astrocytes and upregulated in reactive astrocytes.

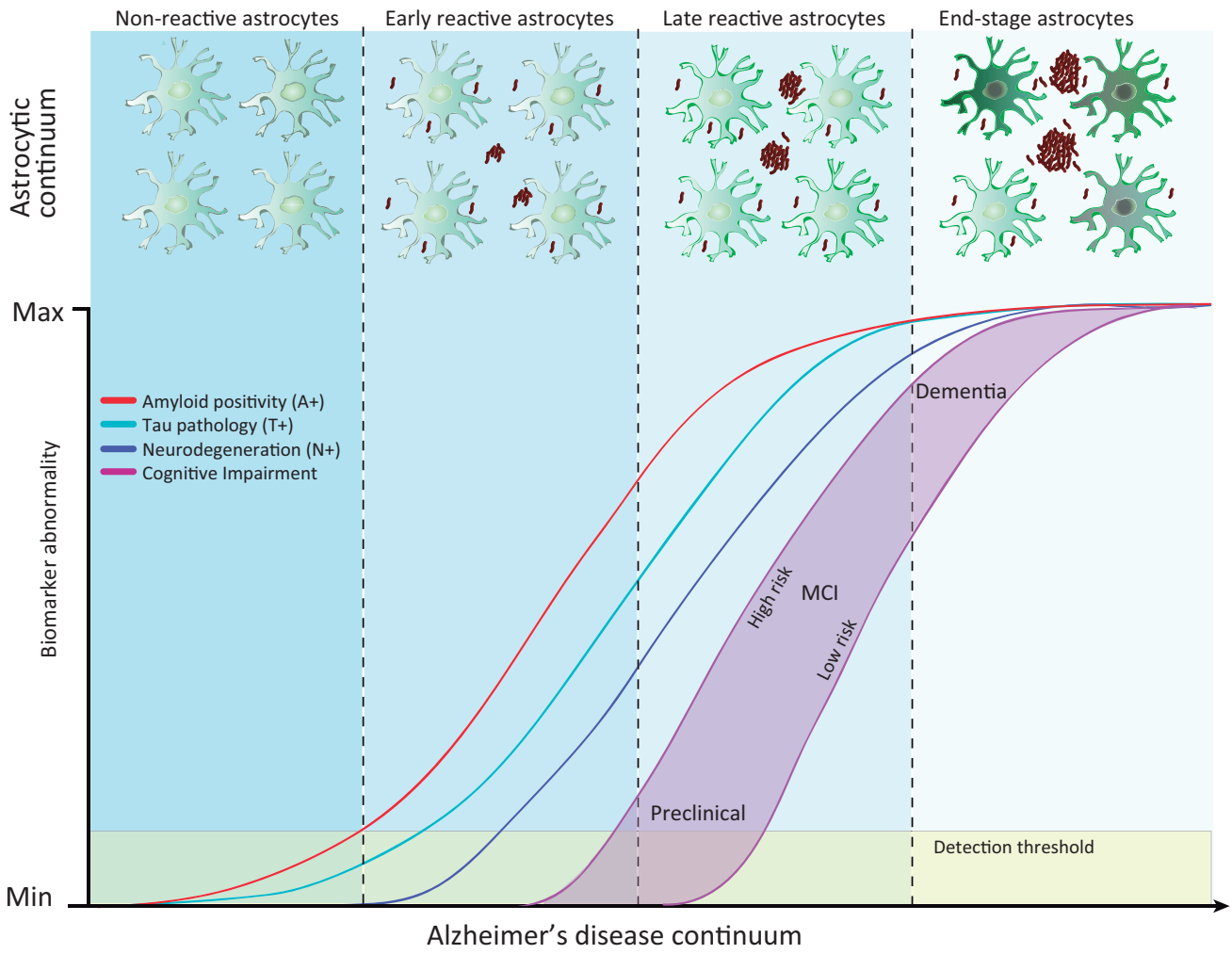

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Figure 1. Reactive Astrocytes in the Hypothetical Model of AD Biomarkers. The highly accepted hypothetical model of AD postulates that amyloid biomarkers become abnormal first (amyloid positivity, A+, red), followed by tau biomarkers (tau positivity, T+, light blue), which leads to neurodegeneration (N+, dark blue), cognitive impairment (violet), and finally dementia. Here, we propose a model including reactive astrocytes. In the absence of pathology, astrocytes exist in their nonreactive state supporting normal brain function. As amyloid-β (Aβ) pathology begins to develop, initially in the form of soluble Aβ oligomers (AβOs), astrocytes start to react to prevent toxicity to neurons. *In vivo* evidence suggests that MAO-B expression increases during the early stage of astrocyte reactivity. With further pathological progression – Aβ (red line) and tau (blue line) pathologies and neurodegeneration (purple line) – and the emergence of MCI and dementia, late- and end-stage reactive astrocytes can be measured with additional imaging and fluid biomarkers (e.g., GFAP, S100B, mI, and Glx). The decreasing opacity of the background colour for each astrocyte stage quadrant (nonreactive to end stage) reflects the loss of normal astrocytic function and therefore increasing vulnerability to symptomatic AD. Abbreviations: AD, Alzheimer's disease; GFAP, glial fibrillary acidic protein; Glx, glutamate + glutamine; MAO-B, monoamine oxidase-B; MCI, mild cognitive impairment; mI, myo-inositol.

the last years. A recent study demonstrates that reactive astrocytes are induced by activated microglia that release interleukin-1α (IL-1α), tumour necrosis factor-α (TNF-α), and complement component 1q (C1q), which induce phenotypic changes in astrocytes that assume a reactive neurotoxic form. The authors also proposed an A1/A2 nomenclature for astrocytes [38] (an analogue to M1 and M2 nomenclature, which has been used to characterise proinflammatory and anti-inflammatory macrophages, respectively [39]). Accordingly, toxic reactive astrocytes would be termed as 'A1 astrocytes' and protective reactive astrocytes as 'A2 astrocytes'; this nomenclature is gaining acceptance in the field [38].

Table 1. Reactive Astrocytes in Alzheimer's Disease: Key Evidence from Basic and Clinical Studies

Categories	Reactive astrocyte signature in Alzheimer's disease	Type of evidence	
		Basic study (animal models and astrocyte rodent culture)	Clinical study (human astrocyte culture, post-mortem brain tissue, and *in vivo* biomarkers)
Astroglial reactivity markers	↑ GFAP	[121]	[97,100,101,139]
	↑ Vimentin	[121]	[140]
	↑ S100B	[121,122]	[96–98,102–104,141]
	↑ Monoamine oxidase-B	[138]	[28,123,125]
Morphological changes	Atrophic	[142,143]	–
	Hypertrophy	[142,144]	[145–147]
Glutamatergic neurotransmission	↓ Glutamate transporters (GLT-1 and GLAST) levels	[148]	[152]
	↑ MGluR5 levels	[149,150]	[153]
	↑ Release of glutamate	[151]	–
GABAergic neurotransmission	↑ GABA production	[154]	–
	↑ GABA-transporter 3/4 (GAT3/4)	[155]	[155]
Cholinergic neurotransmission	↑ α7 Nicotinic acetylcholine receptor	–	[156–158]
Glucose metabolism	↓ Levels of GLUT-1	[159]	[73]
Amyloid-β metabolism proteins	↑ APP expression	[160]	[163]
	↑ BACE1 expression	[160,161]	[161,164]
	↑ Secretion of Aβ	[160]	–
	↑ Neprilysin levels near Aβ plaques	[48]	[165,166]
	↑ IDE levels near Aβ plaques	[162]	[165,166]
Cytokines/Chemokines	↑ Secretion of proinflammatory interleukins	[167]	[168–170]
	↑ Secretion of TNF-α and INF-γ	[167]	–
Growth factors	↓ Secretion of TGF-β1	[171]	[171]
Oxidative stress	↑ Glutathione (GSH) release	[172]	–
	↓ GSH levels	[173]	–
	↓ Superoxide dismutase	[174]	–
	↓ Catalase levels	[174]	–
	↑ ROS production	[175]	–
	↑ Inducible nitric oxide synthase levels	[176]	[177]
Calcium and potassium homeostasis	↑ Cytoplasmic calcium levels	[178]	–
	↑ Calcium waves amplitude, frequency, velocity, and travelling distance	[178]	–
	↓ Potassium channel Kir4.1 expression	[179]	[179]

Reactive astrocytes release several classes of molecules, which include cytokines [ILs, TNF-α, transforming growth factor-β (TGF-β) and others], chemokines (CXCL and CCL family), growth factors (brain-derived neurotrophic factor, nerve growth factor, and others), gliotransmitters (glutamate, D-serine, and ATP), and small molecules (NO and prostaglandins) [40,41]. Understanding the biological basis behind astrocyte reactivity is essential for understanding astrocyte–neuron communication and crucial for understanding the chemical pathways that lead to toxicity and cell death. Increased understanding of the relevant pathways will ultimately help to develop astrocyte-targeted biomarkers that have potential clinical usefulness in diagnosis and developing treatments.

Astrocytes Clearing, Degrading, Or Even Producing Amyloid-β

Studies have demonstrated that astrocytes have a major role in terms of clearing and degrading Aβ. In fact, Aβ enzymatic degrading proteases can be produced by reactive astrocytes. These proteases include neprilysin (NEP), endothelin-converting enzyme (ECE), insulin-degrading enzyme (IDE), and matrix metalloproteases. NEP, ECE, and IDE are metalloendopeptidases [42]. NEP exists mainly in cellular and intracellular plasma membrane of presynaptic neurons, but can be found in reactive astrocytes and microglia; ECE is present in the cytosol, cellular and intracellular membrane of neurons, endothelial cells, and astrocytes; and IDE is synthesised and secreted by neurons and glial cells acting extracellularly [43,44]. These enzymes cleave Aβ at a single site or at multiple sites (for review, see [45]). Complementary mechanisms for enhancing Aβ clearance involve ApoE, apoliprotein J, α2-macroglobulin (α2-M), and α1-21 antichymotrypsin (ACT) secretion or expression by astrocytes [46,47]. These extracellular protein chaperones, widely present in the plasma and cerebrospinal fluid (CSF), bind to Aβ species altering their ability to form insoluble aggregates and also facilitate Aβ clearance across the BBB [46].

Immunohistochemistry assays in aged wild-type and Tg2576 transgenic mice [that overexpresses the human **amyloid precursor protein (*APP*)** isoform 695 with the Swedish mutation KM670/671NL] and from post-mortem AD brain homogenates demonstrate that NEP is decreased in hippocampal and cortical regions [48–50]. Despite overall age-related decreases, NEP was upregulated in reactive astrocytes surrounding Aβ plaques in the Tg2576 mice, potentially reflecting astrocytic clearance of Aβ [48]. MMP-2 expression is also increased around Aβ plaques [51,52]. A large amount of extracellular Aβ deposits can be washed out from the brain by the astrocyte-mediated interstitial fluid bulk flow, namely, the **glymphatic system** [53]. Thus, accumulated evidence suggests that Aβ plaque-associated reactive astrocytes secrete proinflammatory factors and thereby break down or contribute to clearance of Aβ.

Beyond astrocytes' role in removing or degrading Aβ, there is evidence indicating that reactive astrocytes are capable of producing Aβ. Reactive astrocytes have increased levels of APP, β-secretase (BACE1), and γ-secretase. These form the essential machinery for releasing Aβ peptides prone to aggregate [54]. Remarkably, an immunocytochemical study in AD post-mortem entorhinal, hippocampal, and frontal regions demonstrated that BACE1 colocalises with neurons, but is barely present in nonreactive astrocytes, being substantially increased in reactive astrocytes, suggesting that astrocytes can contribute to Aβ production [55]. In summary, altered astrocyte systems responsible for degrading or clearing Aβ could be targeted as potential novel biomarkers in AD.

Glutamatergic Excitotoxicity

Around 30 years ago, evidence of reduced astrocyte glutamatergic transport in AD, mainly dysfunctional activity of the glutamate transporters GLAST and GLT-1, was found in post-mortem tissue and is now corroborated by an extensive literature [56].

In AD brains and mouse models, immunocontent and mRNA expression of GLT-1 and GLAST are reduced [57–60]. Cultured human astrocytes from post-mortem AD parietal cortices also present decreased glutamate uptake, measured by *ex vivo* radioactivity assays using [^{3}H] glutamate uptake, and reduced GLT-1 and GLAST immunocontent [61].

Greater attention has been given to GLT-1 since it is responsible for around 90% of astrocytic glutamate uptake in the brain [62]. A study in which mice lacking one allele of *GLT-1*$^{+/-}$ were crossed with mice harbouring *APP* Swedish and presenilin-1 (*PS1deltaE9*) mutations revealed that deficits in GLT-1 accelerate memory impairment [63]. A recent report demonstrated that astrocyte glutamatergic abnormalities – including reduced GLT-1 immunocontent – are found in the microenvironment surrounding Aβ plaques in the APP/PS1 mouse model {that over-express *APP* (KM670/671NL) and *PS1L166P* mutations} [64]. **Aβ oligomers (AβOs)** and preplaque Aβ species seem to decrease levels of GLT-1 and GLAST in hippocampal primary cultured astrocytes [65].

This bulk of evidence indicates that astrocyte glutamatergic homeostasis is disrupted in AD. Reduced glutamate transporters in association with increased levels of glutamate in the CSF are potential biomarkers to identify early changes. Of note, excitotoxicity has been implicated in several other brain disorders, but early astrocyte glutamatergic dysfunction in brain regions commonly associated with AD provides an interesting avenue with plenty of potential biomarker targets.

Abnormalities in Glucose Metabolism: The Contribution of Astrocytes

The dysfunction in glutamate homeostasis observed in AD is purported to contribute to glucose metabolism abnormalities. Glucose hypometabolism revealed by [^{18}F]fluorodeoxyglucose **positron emission tomography** ([^{18}F]FDG-PET) is classically associated with AD and this phenomenon is characteristically linked to neuronal dysfunction [66]. Interestingly, according to the ANLS hypothesis, astrocytic glutamate uptake acts as one of the triggers signalling for glucose uptake by astrocytes [67–70]. In fact, both human stem cell–derived neurons and astrocytes exposed to AβOs display significant glucose hypometabolism [71]. Post-mortem analysis in AD brains demonstrated that both glucose transporters, GLUT1 (predominant isoform on astrocytes and endothelial cells) and GLUT3 (neuronal specific) [72], are prominently reduced [73,74]. Thus, one could argue that hypometabolism in AD measured with [^{18}F]FDG-PET could therefore, in addition to indicating impaired synaptic function, be a nonspecific marker of astrocyte metabolic dysfunction.

In addition to glutamate, another key player in the metabolic response of astrocytes to neuronal activity is the potassium ion (K^+). K^+ is released by neurons during excitatory synaptic activity and produces rapid and transient glucose uptake in cultured astrocytes [75,76]. Elevated K^+ concentrations produce fast activation of glucose uptake, involving the sodium/bicarbonate cotransporter NBCe1, which is highly expressed in astrocytes [77–79]. Interestingly, recent data showed that high levels of extracellular K^+ can activate a novel lactate-permeable channel and by doing so, astrocytic lactate can be released independently of MCTs and against a concentration gradient [80,81]. Other emerging astrocytic modulators such as NO and ammonium (NH_4^+) have been shown to modulate astrocyte glucose metabolism [82,83]. Real-time monitoring of energy metabolites indicates that NO acutely stimulates glucose consumption and lactate accumulation in cultured astrocytes [84] while leading to long-term increase of glycolysis and lactate release [85]. Considering the alterations of neuronal nitric oxide synthase in AD [86,87] and its putative impact on glucose metabolism in astrocytes, it might be an additional factor involved in glucose hypometabolism. In addition to NO, NH_4^+, a major end

product of cellular amino acid metabolism, released by active neurons, was shown to induce an acute increase in astrocyte lactate production and its subsequent release to the extracellular space. Interestingly, NH_4^+ levels seem increased in the CSF of AD patients, but evidence of NH_4^+ affecting astrocyte glucose consumption is still lacking [88–90].

Apart from direct activity-dependent glucose utilisation for lactate production, astrocytes are able to constitute an energy reserve, synthesising and storing glucose-derived glycogen to eventually fuel neurons with lactate in specific, particularly energy-demanding, situations. In physiological conditions, glycogen is predominantly stored in astrocytes. Astrocyte glycogenolysis was shown to be necessary for learning, as well as for memory formation and storage in rodents [91]. Several studies have reported increases in glucose uptake, glycogenesis, and glycolysis during brain activation. A striking example is the rapid glycogen breakdown in the rat somatosensory cortex during whisker stimulation, paralleled by increased glucose uptake in astrocytes [92,93]. Considering the suggested role of glycogen in memory function and the well-described memory dysfunction in AD, any alteration in astrocyte glycogen metabolism could contribute to AD-associated difficulties to evoke/consolidate memories.

In summary, beyond synaptic dysfunction, it is very likely that complementary and synergistic mechanisms contribute to AD glucose hypometabolism. Glucose metabolism in astrocytes could be directly affected by (i) reduced glutamate uptake by astrocytes; (ii) reduced GLUT1 levels [73,94]; (iii) altered levels of energy metabolism modulators such as NH_4^+ and NO; and (iv) altered glycogen levels and/or metabolism. The cellular origin of AD glucose hypometabolism is unresolved, but it is becoming clear that more than one cell type is involved. It cannot be excluded that the other glial cells, such as oligodendrocytes and microglia, could also contribute to glucose hypometabolism and [^{18}F]FDG-PET signal in AD, but new studies are necessary to investigate these possibilities.

Astrocytic Biomarkers in Alzheimer's Disease

Sensitive and specific biomarkers already exist for the main pathological features of AD. In the A → T → N framework, Aβ and tau pathology can be assessed by measuring CSF concentrations of soluble Aβ and tau or fibrillar Aβ and tau with PET imaging; and neurodegeneration can be measured by [^{18}F]FDG-PET or structural **magnetic resonance imaging (MRI)**, as an index of brain atrophy. Typically, outcomes from these biomarkers are split into positive or negative results depending on a defined cutoff value. For example, if an Aβ-specific biomarker is acquired from a person and is abnormal (e.g., decreased Aβ in the CSF and/or increased Aβ in the brain), then the individual is classified as Aβ-positive (A+); a normal result would end with the individual being classified as Aβ-negative (A−), which is expected for healthy individuals.

Astrocytes are not integrated into this biomarker framework yet because there is a paucity of astrocyte biomarkers that can be incorporated into preclinical and human studies. Identifying novel candidates for use as fluid or molecular imaging biomarkers will go hand in glove with developing novel therapeutics for AD. Techniques are already available to measure astrocytes reactivity *in vivo* although their target specificity is questionable (Figure 2). The following subsections provide an overview of the so far used astrocyte biomarkers.

Fluid Biomarkers

Fluid biomarkers acquired from the body include saliva, urine, blood, or CSF. For the CNS, they are typically measured in the blood (serum/plasma) or CSF, provided they can cross, or leak out of, the BBB or are secreted/leak from cells and diffuse into CSF. This group of biomarkers should be specific and can be accurately measured with sensitive analytical techniques.

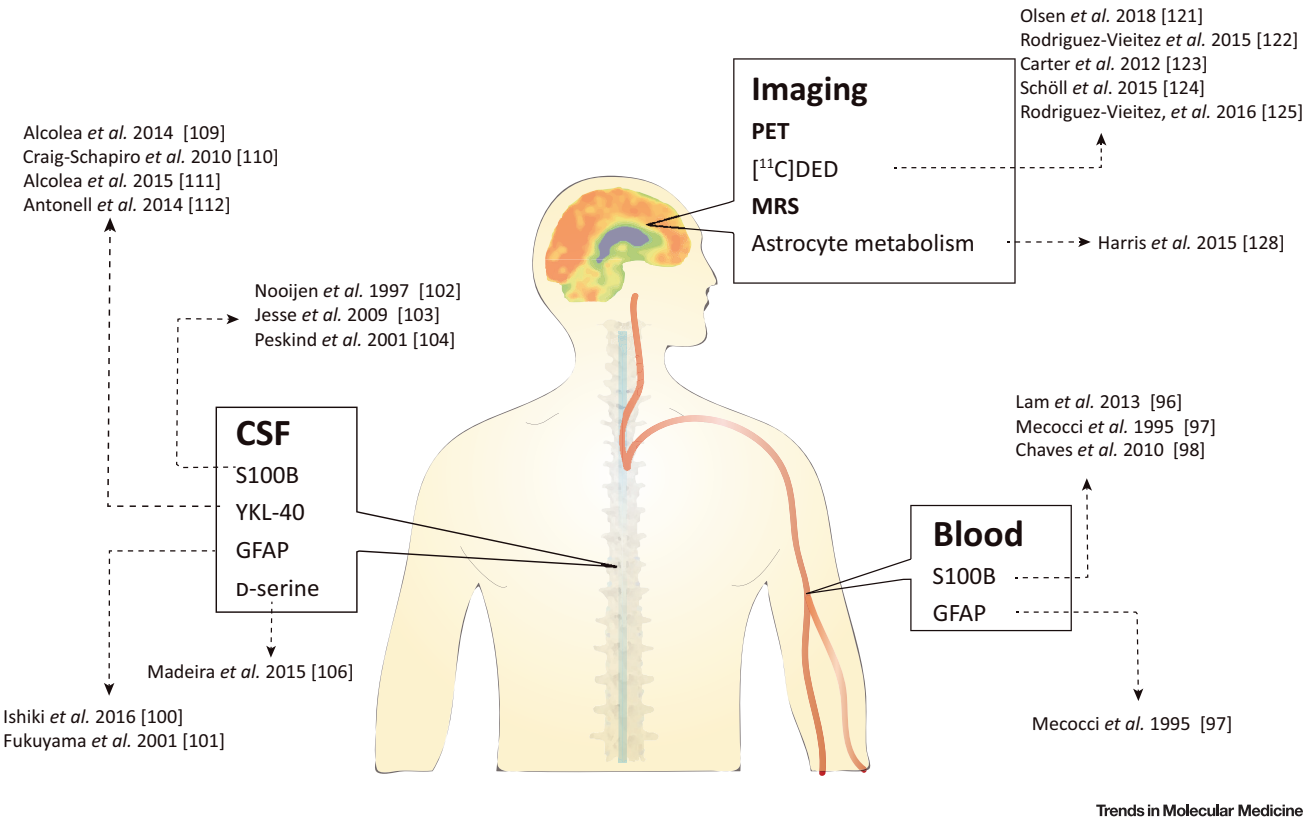

Figure 2. Fluid and Imaging Astroglial Biomarkers in Alzheimer's Disease. Astrocyte reactivity in Alzheimer's disease can be assessed by fluid biomarkers (blood and cerebrospinal fluid) and neuroimaging techniques – radiotracers for positron emission tomography (PET) and magnetic resonance spectroscopy (MRS). Abbreviations: [^{11}C]DED, [^{11}C]-deuterium-L-deprenyl; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein. See also [96–98,100–104,106,109–112,121–125,128].

Current biomarkers most assessed for measuring astrocyte's reactivity include S100B (a calcium-binding protein found mainly in the cytosol of astrocytes and Schwann cells) and GFAP (an astrocyte structural protein) [95].

Blood

Acquiring a blood-based biomarker is minimally invasive with minor side effects. Acquisition only requires venepuncture, which can be performed by any trained phlebotomist. In a sample of cognitively normal (CN) elderly individuals, sera levels of S100B were positively correlated with cognitive performance [96]. A report, that predates the current diagnostic frameworks, revealed that GFAP and S100B were increased in the serum of vascular dementia (VaD) and late-onset (>65 years old) sporadic AD patients. However, increases were not found in early onset (<65 years old) AD patients relative to CN controls. The VaD and late-onset AD patients were on average 10 years older, suggesting that the increases might be age related [97]. Another study demonstrated lower serum concentrations of S100B in late-onset AD patients compared with age-matched CN controls, although within the AD group, S100B concentrations significantly increased with dementia severity [measured using Mini-Mental State Examination (MMSE) and Clinical Dementia Rating (CDR)], but did not correlate with brain atrophy [98].

Cerebrospinal Fluid

Acquiring CSF is more invasive than blood as it requires lumbar puncture, which a trained medical doctor needs to perform and, although rare, side effects can be more severe. The most frequently used CSF AD biomarkers are $A\beta_{1-42}$, total tau, and p-tau concentrations; none of these are specific to astrocytes [99].

Information about CSF astrocyte AD biomarkers is sparse; GFAP is increased [100] and appears to correlate with dementia severity [101]. The data on CSF S100B levels are more confusing since they have been reported to be unchanged [102], slightly elevated but not significantly different to age-matched CN [103], or increased in the mild to moderate disease stages [104]. When compared to Creutzfeldt–Jakob disease (CJD) using an enzyme-linked immunosorbent assay, mean S100B levels were significantly lower in AD (CJD = 25 165 μg/L vs. AD = 4.69 μg/L) [102,103] and GFAP has been shown to be significantly elevated in AD (3.0 ng/mL) compared to CN controls (2.2 ng/mL), equivalent to dementia with Lewy bodies (3.4 ng/mL) but lower than in frontotemporal dementia (4.8 ng/mL) [100]. A large meta-analysis of fluid biomarkers found that there was no difference in GFAP concentrations between AD and CN controls [105]. Levels of CSF D-serine, a gliotransmitter released by astrocytes, are increased in probable AD patients compared to CN [106]. Another CSF biomarker is YKL-40 protein, which is overexpressed in a subset of astrocytes in AD [107]. The exact physiological role of YKL-40 in the CNS is unclear but it is thought to be involved in the activation of the innate immune system [108]. In AD patients, YKL-40 is elevated in the CSF even in early preclinical stages and it correlates with CSF p-tau and t-tau [109–112].

The fluid biomarkers from blood and CSF have not been fully validated to identify the earliest stages of chronic astrocyte dysfunction at asymptomatic or AD stages. The fluid biomarkers reviewed here are unlikely to be useful diagnostically as they are unable to differentiate health and disease due to the overlap between CN controls and other dementia types and confounding factors like ageing and disease severity. At this point GFAP, S100B, and YKL-40 will not provide any additional clinical insight compared to the CSF biomarkers already widely available ($A\beta_{1-42}$, total tau, and p-tau). This leaves the door wide open for the identification and development of more sensitive and specific astrocyte biomarkers that can be accurately measured in CSF or more preferably in peripheral fluids like blood.

Neuroimaging Biomarkers

Another avenue for probing astrocytic function *in vivo* is to use PET and MRI techniques. Both techniques are integral parts of the clinical examination of patients with suspected AD (Box 3).

Positron Emission Tomography

Investigating neuroinflammation with PET has long been possible by imaging the 18-kDa translocator protein (TSPO). TSPO is found within the outer mitochondrial membrane and TSPO expression, which is increased in activated microglia and can be imaged *in vivo* by using PET with [^{11}C]PK11195 or second-generation radiotracers like [^{11}C]PBR28, is increased in AD [113]. Interestingly, TSPO can be also expressed in astrocytes from post-mortem AD tissue and animal models, but it is not widely accepted as an astrocyte biomarker [114,115].

Presently, only two PET radiotracers are suggested to image astrocyte reactivity: **[^{11}C]-deuterium-L-deprenyl ([^{11}C]DED)** [116] and **[^{11}C]BU99008** [117]. Since both of these radiotracers incorporate the [^{11}C] isotope (half-life = 20.3 min), *in vivo* studies that utilise them can only be performed in specialised PET centres. These radiotracers possess different astrocyte molecular targets. The [^{11}C]DED radiotracer binds to the enzyme MAO-B and

Box 3. Positron Emission Tomography and Magnetic Resonance Imaging: Methodological Background, Pros and Cons

PET permits the noninvasive quantification of biological and pharmacological processes in the brain using molecules labelled with positron-emitting radioisotopes (e.g., [^{15}O], [^{11}C], and [^{18}F] that are produced by particle accelerators called cyclotrons). The labelled molecules called radiotracers (made in specialised radiochemistry facilities), which are intravenously administered to living humans (or animals) and have high biochemical sensitivity (nanomolar to picomolar resolution). In fact, PET's main versatility is its ability to quantitatively image different biochemical processes, owing to the wide range of radiotracers, at the molecular level. However, its versatility comes at a price since the technique involves ionising radiation and needs highly specialised research centres (especially for [^{11}C] radiotracers since [^{11}C] has a half-life of 20.33 min), housing a cyclotron and radiochemistry facilities. In addition, for true quantitative measures, PET requires online arterial blood sampling during scanning protocols that can last over 60 min.

MRI is primarily a noninvasive technique that does not require ionising radiation. MRI is dependent on the excitation and relaxation of protons in a strong magnetic field ($\geq$1.5 T) and uses radio-wave emissions to generate structural or functional images of the brain that can identify abnormalities like brain atrophy and vascular lesions. In addition, MRI can acquire information on the physicochemical state of tissues, blood flow, water diffusion, and motion. More recently, molecular targets have been tested in MRI, but are much less sensitive than PET (10^4–10^6 times less sensitive). In terms of clinical use on a large scale, MRI does not need the production of radioisotopes and radiotracers and individual MRI scans are much less expensive than PET.

Clinician's Corner

Astrocytes are important components of the neurovascular unit and are key to neuronal metabolic support, regulating cerebral blood flow, controlling the blood–brain barrier, regulating synapses and neurotransmission, and removing toxins from the brain. All of these processes are known to be dysfunctional in Alzheimer's disease (AD).

The main AD histopathological features are fibrillar amyloid-β (Aβ) plaques and intraneuronal neurofibrillary tangles. An AD biomarker model has been developed, in which abnormal Aβ biomarkers are the first to become detectable, possibly up to 30 years before the onset of symptoms. There is evidence that astrocytes are already responding to this first pathological insult and this response can be detected with imaging biomarkers.

Across the AD continuum, the type and magnitude of astrocytic response that can be detected with available biomarkers is variable. Increased expression of monoamine oxidase-B appears to be the earliest change followed by increased expression of glial fibrillary acidic protein (GFAP) and S100B at later symptomatic disease stages. However, doubts exist about the target specificity and the sensitivity of current biomarkers to accurately measure these changes *in vivo*.

Developing new, specific astrocyte biomarkers, either fluid or imaging based, will make it possible to pharmacologically target chemical pathways that either enhance the positive or suppress the negative aspects of astrocyte functions in the presence of AD pathology and measure treatment outcomes *in vivo* with the ultimate aim of preserving normal cognitive function into old age.

has been used in many different neurological conditions such as CJD, amyotrophic lateral sclerosis, and focal epilepsy [118–120]. In transgenic mice, early phases of Aβ deposition are associated with increased expression of MAO-B. By contrast, in late-stage Aβ deposition, MAO-B is not significantly elevated, whereas other astrocyte biomarkers such as GFAP are elevated [121,122]. Mouse data also show that MAO-B and GFAP do not colocalise, suggesting that they might not measure the same population of astrocytes. Human data demonstrate that [^{11}C]DED binding to MAO-B is significantly increased by approximately 17% in prodromal AD compared to CN controls in the frontal and parietal cortices [123] and that increased binding is present in autosomal dominant AD (ADAD) mutation carriers approximately 30 years before symptom onset [124]. In ADAD mutation carriers closer to the expected age of disease onset, [^{11}C]DED binding was decreased relative to CN. The largest cross-sectional mean cortical binding decreases relative to expected disease onset were observed in the parietal (0.0012 min^{-1}), anterior cingulate (0.0023 min^{-1}), and posterior cingulate (0.0036 min^{-1}) cortices. A longitudinal follow-up of the same ADAD patients revealed that regional [^{11}C]DED binding significantly decreased between 0.008 and 0.030 standardised uptake value ratio units (SUVR)/year as patients progressed to symptomatic phase [125].

The [^{11}C]BU99008 radiotracer quantifies imidazoline2-binding sites (I2BSs), which are located on astrocyte mitochondrial membranes and increase in post-mortem AD brains [126]. The first human [^{11}C]BU99008 PET study demonstrated that brain uptake was good and binding was consistent with the known distribution of I2BS [127]. Although studies are underway, at the time of writing, no published [^{11}C]BU99008 data are available for AD.

Magnetic Resonance Imaging

Developing an *in vivo* MRI astrocyte biomarker would be of great value since MRI is more widely available. At the moment, measuring specific aspects of astrocyte metabolism *in vivo* with [^{1}H]-magnetic resonance spectroscopy (MRS) has been performed with some success. It is possible to measure myo-inositol (mI), which is enriched in astrocytes, and neuron-to-astrocyte metabolic shuttling by calculating glutamate, glutamine, and lactate levels in rats [128]. The spectra generated from these MRS biomarkers could be useful to obtain an overall indication of astrocyte integrity. Several studies have revealed increased mI in the posterior cingulate and temporoparietal cortex in AD [129]. Since it is technically challenging to resolve glutamate and

glutamine levels in humans, a composite 'Glx' (glutamate + glutamine) peak is often measured and one study has revealed reduced Glx levels in AD [130], but another reported no difference from age-matched controls [131]. The discrepancy between studies could be caused by differences in patient selection with the study reporting no difference having older but less impaired patients, potentially reflecting individuals at an earlier disease stage. In a study that compared ante-mortem MRS with post-mortem immunohistochemistry in the posterior cingulate, elevated mI (scaled by creatine; mI/Cr) was associated with the occurrence of Aβ plaques in AD [132]. Across the whole AD continuum, higher Aβ was associated with elevated mI/Cr and lower N-acetyl aspartate (NAA)/mI ratios, whereas p-tau was associated with lower NAA/mI ratios. There is largely inconclusive, contradictory evidence for measured lactate levels *in vivo* [128].

Similar to the fluid biomarkers, PET and MRI astrocyte biomarkers have not been validated for clinical diagnosis at asymptomatic stages. Although imaging MAO-B with PET has potential to reveal astrocyte reactivity many years before symptom onset in ADAD, this has not been established in sporadic AD. More crucially, no imaging biomarkers offer true target specificity, since either measuring an enzyme (MAO-B), a binding site (I2BS), or a metabolite (mI) that is not solely found in astrocytes cannot fulfil the criteria. The development of imaging biomarkers for reactive astrocytes, to be used clinically in individuals at risk of developing AD, will rely on the identification of more specific astrocyte targets.

Concluding Remarks

Astrocytes have complex roles at different stages of AD pathophysiology and the conceptualisation of AD is shifting to an integrative perspective in which neurons and astrocytes work intricately with each other. Astrocytes are involved in metabolic support of neurons, neurotransmission, neurotransmitter recycling, regulating CBF, and clearing and degrading Aβ. All these processes are dysfunctional in AD. Current AD therapies are completely focused on neuronal dysfunction (cholinergic or glutamatergic) and clinical trials aimed at treating Aβ and tau pathology have so far failed to meet clinical endpoints (i.e., preventing cognitive deterioration). Since astrocytes are fundamentally involved in multiple processes, enhancing positive (e.g., A2 subtypes) and suppressing negative toxic functions pharmaceutically could help maintain normal brain function into advanced age.

Astrocyte biomarkers are therefore of high interest since diagnosis and therapies can be closely coupled. If the evolution of AD diagnostic criteria is to continue and fully integrate astrocytes into the scheme, then novel astrocyte biomarkers will need to be developed. This needs to coincide with a shift away from the linear causal logic (A → T → N) that permeates AD research today. From preclinical to end-stage AD the function of astrocytes, and therefore the appropriate biomarker to use, could vary significantly (Figure 3, Key Figure). Fluid (CSF and blood) and imaging astrocyte biomarkers (PET and MRI) can measure the earliest stages of astrocyte reactivity at asymptomatic stages, but they are unable to satisfactorily differentiate between health and disease due to the overlap between CN control, other dementia types (e.g., frontotemporal dementia and dementia with Lewy bodies), and confounding factors like patient age and disease severity. Although imaging MAO-B with PET has potential to reveal astrocyte reactivity years before symptoms in ADAD, it has not been established in sporadic AD. Another key point is related to the astrocyte specificity of these biomarkers. For example, S100B is exclusively derived from astrocytes in the CNS, but in the periphery S100B has other sources such as bone marrow and muscle. The imaging biomarkers similarly lack astrocyte specificity, since MAO-B is also expressed in serotonergic neurons.

Outstanding Questions

How early can we detect reactive astrocytes in Alzheimer's disease?

Which molecular targets could best serve as astrocyte biomarkers in AD?

Should astrocytes be included as potential therapeutic targets?

Is [^{18}F]FDG-PET, classically used for evaluating neuronal activity, also measuring astrocyte function?

Do populations of astrocytes respond to AD pathology differently (e.g., toxic A1 vs. protective A2)?

Could *in vivo* biomarkers differentiate between these 'good' and 'bad' astrocytes?

Would inhibition of 'bad' astrocytes and promotion of 'good' ones prevent the onset of symptomatic AD?

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Key Figure

Pathophysiological Alterations in Alzheimer's Disease Detected by Astrocyte Biomarkers

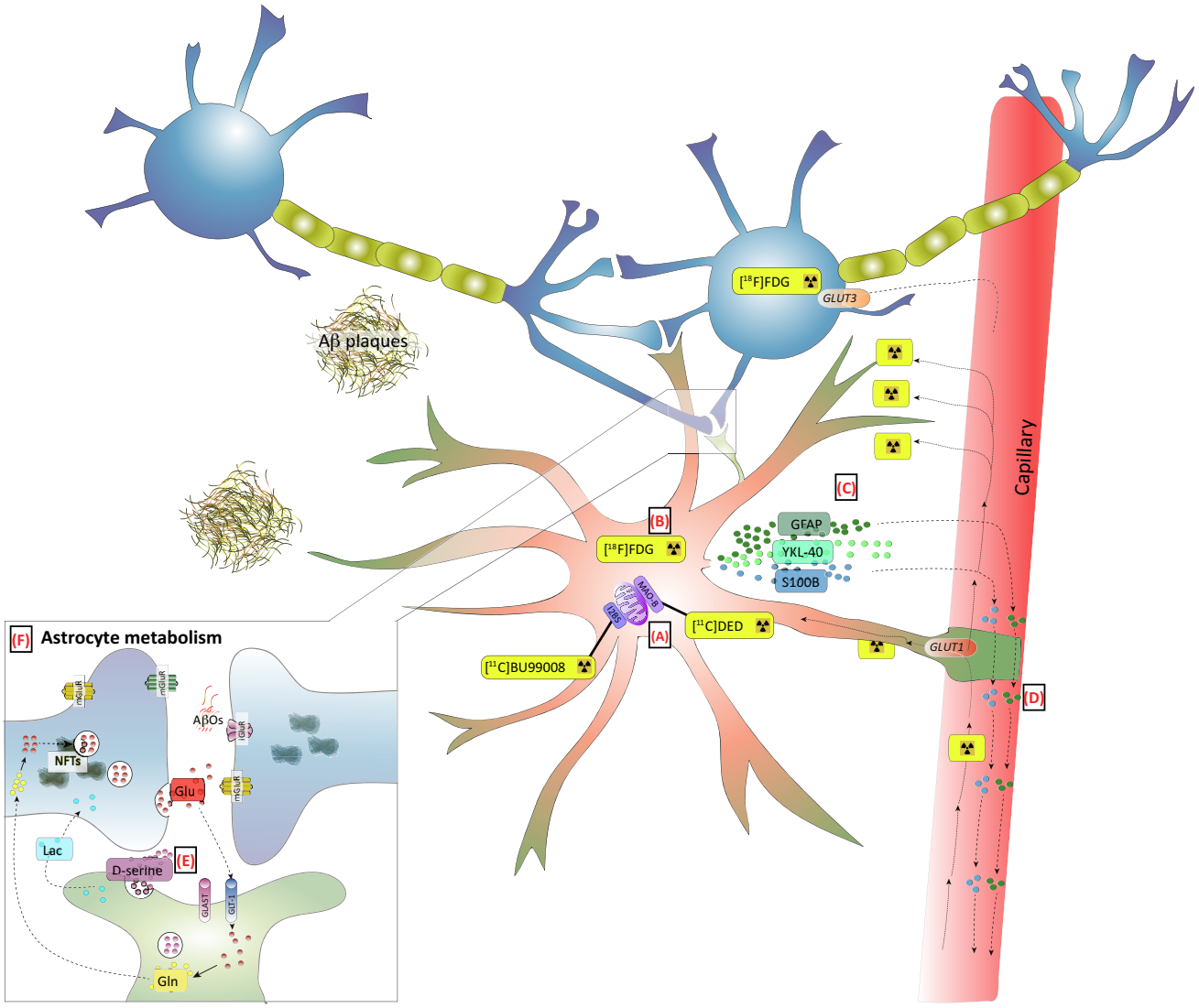

Figure 3. (A) [^{11}C]-l-deprenyl (DED) and [^{11}C]BU99008 positron emission tomography (PET) detect alterations in monoamine oxidase-B (MAO-B) and I2-imidazoline-binding sites (I2BS), both located in the outer mitochondrial membrane, respectively; (B) ^{18}F-fluorodeoxyglucose ([^{18}F]FDG-PET) detects astrocyte and neuron glucose hypometabolism, which is related to reduced levels of GLUT1 (located in part in astrocytes) and GLUT3 (predominantly located in neurons) in association with decreased levels of GLT-1 (the main transporter for taking up glutamate, acting as a trigger for signalling glucose uptake by astrocytes); (C and D) Glial fibrillary acidic protein (GFAP), S100B, and YKL-40 can be detected in the (C) cerebrospinal fluid and (D) blood. Insert: (E) D-serine, a gliotransmitter released by astrocytes, can be identified in the cerebrospinal fluid; (F) and astrocyte metabolism (metabolites such as glutamate, glutamine, and lactate) can be tracked by magnetic resonance spectroscopy. Abbreviations: Aβ, amyloid-β; AβO, amyloid-β oligomer; NFT, neurofibrillary tangle.

To at least complement the established AD biomarkers (amyloid and tau), more sensitive and specific astrocyte biomarkers that can be acquired with neuroimaging or from fluids, ideally blood, are needed. Novel *in vivo* biomarkers for GFAP or glutamatergic transporters (GLAST or GLT-1) that are more astrocyte specific are potential new targets. Developing new astrocyte biomarkers will enable measuring this vital cell population as it responds to pathology and although they may not generate a 'silver bullet' treatment, they will certainly evolve our conceptualisation of AD (see Outstanding Questions and Clinician's Corner).

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