Blood-based Biomarkers of Alzheimer’s Disease: The Long and Winding Road

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Abstract: Background: Blood-based biomarkers can be very useful in formulating new diagnostic and treatment proposals in the field of dementia, especially in Alzheimer’s disease (AD). However, due to the influence of several factors on the reproducibility and reliability of these markers, their clinical use is still very uncertain. Thus, up-to-date knowledge about the main blood biomarkers that are currently being studied is extremely important in order to discover clinically useful and applicable tools, which could also be used as novel pharmacological strategies for the AD treatment.

Objective: The aim of this paper was to carry out a literature review on the major blood-based biomarkers for AD, connecting them with the pathophysiology of the disease.

Results: Recent advances in the search of blood-based AD biomarkers were summarized in this review. The biomarkers were classified according to the topics related to the main hallmarks of the disease such as inflammation, amyloid, and tau deposition, synaptic degeneration and oxidative stress. Moreover, molecules involved in the regulation of proteins related to these hallmarks were described, such as non-coding RNAs, neurotrophins, growth factors and metabolites. Cells or cellular components with the potential to be considered as blood-based AD biomarkers were described in a separate topic.

Conclusion: A series of limitations undermine new discoveries on blood-based AD biomarkers. The lack of reproducibility of findings due to the small size and heterogeneity of the study population, different analytical methods and other assay conditions make longitudinal studies necessary in this field to validate these structures, especially when considering a clinical evaluation that includes a broad panel of these potential and promising blood-based biomarkers.

Keywords: Alzheimer’s disease, biomarkers, blood, dementia, diagnosis, elderly, plasma.

1. INTRODUCTION

Similar to the human circulatory system, there is a long and winding road to travel down in order to find suitable blood-based biomarkers for the early diagnosis of Alzheimer’s disease (AD). Despite major efforts, researchers have not yet been able to identify a specific, sensitive and feasible biomarker for the screening of pre-clinical AD. It is well known that AD has a long pre-symptomatic phase, in which the symptoms are not yet manifesting, but the pathological markers, such as amyloid-β (Aβ) accumulation, are already present [1]. This is an interesting therapeutic window of opportunity to find targets or biomarkers that enable us to identify new strategies for diagnosis and against AD progression.

Cerebrospinal fluid (CSF) biomarkers, although being important protagonists in the understanding of AD pathology, are inappropriate for large scale screenings due to their invasive obtaining and necessity of trained staff characteristics. Positron emission tomography (PET) coupled to amyloid targeted ligands, such as 18F-labelled tracers and PIB, have been used in several studies and are currently considered as sensitive and specific tools for detecting amyloid accumulation. However, PET is also clinically unfeasible due to its requirements of specialized equipment and staff, and also to its use, which is limited to local production and regional distribution of the radioactive tracer [2].

Hereupon, blood-based specific AD biomarkers would be helpful, especially if they could be detected by a simple blood test in the pre-clinical phase of AD. However, the high complexity of blood, allied to confounding factors, such as the ageing process itself and some ageing-related comorbidities, the several pre-analytical and analytical variations in blood-based biomarker studies, especially those related to the collection and stocking of blood samples and the different platforms to analyze them, makes this a remarkable challenging task [3, 4]. The lack of reproducibility and the unclear route from basic discovery towards the clinical application of these biomarkers have hampered studies in the field. Nevertheless, despite all these challenges, knowing and understanding the mechanisms in which several blood-based AD biomarker candidates participate is of great importance in order to discover clinically usable and applicable biomarkers.

Based on these assumptions, here we performed a review on the current candidates of blood-based biomarkers for AD, showing the main results from different studies, focusing on their clinical applicability and association with AD pathogenesis. We have used a specific combination of words, which were inserted as a Boolean search that included, for each topic, the words “specific topic” AND “Alzheimer’s disease” AND “blood” AND “biomarker” NOT “cerebrospinal fluid” NOT “CSF”. The words inserted on the “spe-
cific topic” changed depending on the theme. For instance, for the inflammatory biomarkers heading we have structured searches using “inflammation” OR “inflammatory”, followed by the remaining of the Boolean structure. Any article not written in English has been excluded from the review. We also excluded any animal model from the searches, using the exceptions: NOT “mouse” NOT “mice” NOT “animal”. The searches were performed on Pubmed and Web of Science and covered articles published until September 2019. With this review, we hope to contribute to a better understanding of the state-of-the-art in the field and to encourage new research that can lead to opening new doors on the road to the clinical application of blood-based AD biomarkers and in formulating new pharmacological targets and/or strategies for the AD treatment.

2. INFLAMMATORY BIOMARKERS

AD pathology results in localized low-level inflammation early in the brain mainly due to Aβ deposition, which leads to glial activation within and surrounding senile plaques, triggering neuronal apoptosis [5]. The inflammatory process induced by activated glia and other cells as a response of Aβ deposition is a result of the release of pro-inflammatory cytokines, chemokines and other mediators of inflammation, which in turn intensifies amyloid production and toxicity, contributing to tau hyperphosphorylation, neuronal dysfunction and to the cognitive deterioration [6-8]. This creates a deleterious cyclical loop of exacerbated pathology in the central nervous system (CNS), in which the clearance of these hallmarks contributes to the neurodegenerative process due to the macrophages and other immune cell actions [9, 10].

Some studies using microglial markers and PET have confirmed that microglial activation increases in AD patients, which was related to the worsening of memory performance [11]. On the other hand, intracerebroventricular injection of interleukin 1 stimulates the release of high levels of peripheral interleukin-6 (IL-6), a well-studied pro-inflammatory cytokine [12]. Thus, the activation of inflammatory pathways in CNS is a response of the main signals of AD neuropathology and altered central levels of inflammatory cytokines are one of the AD hallmarks. Since they are quickly removed from CNS into the blood stream, the peripheral measure of these markers considers them as potential biological markers for AD, even in preclinical stages [13]. Moreover, epidemiological studies suggest that anti-inflammatory drugs are able to reduce the incidence of AD [14], although clinical trials with anti-inflammatory drugs have not yet been successful [15].

A complicating factor that has been already shown in the search for inflammatory-related AD markers is that the aging process per se is characterized by a chronic increase in the peripheral levels of inflammatory markers, which is called inflammaging [16]. Additionally, reduced activity in brain-blood flow and perfusion, as well as impairments in glucose metabolism during aging are well documented [17]. All these changes in brain metabolism may alter the ATP synthesis, which has a close relationship to the reactive oxygen species (ROS) production, and consequently to oxidative stress [18]. The aging process is also related with several metabolic pathologies such as diabetes, obesity and vascular diseases, which produce a state of chronic inflammation that may influence the uptake of some inflammation markers that can cross the blood-brain barrier (BBB), resulting in the onset or aggravation of AD [19]. Thus, results have extensively shown a correlation between peripheral inflammatory markers in AD from the onset of the degenerative process and even in the prodromal stage, which configures these cytokines as a potential treatment and/or diagnosis targets additionally to other recognized biological markers for AD [20]. However, due to the overlap between ROS production, inflammation and the aging process itself with neurodegeneration, a close relationship between metabolic disorders and AD has been demonstrated, making it difficult to establish suitable inflammatory biomarkers for the disease [21]. Together, these processes cause losses of neuronal cells, and consequently impairment of their function in the later stages of the disease (Fig. 1).

Interleukins and Tumor Necrosis Factors (TNFs) are the main molecules that mediate inflammatory responses. Despite the activation of the immune system by CNS as an intent to promote clearance and protect the brain, the imbalanced chronic release of cytokines plays a role in the neurodegenerative process [22]. As a consequence, cytokines were recognized as potential biomarkers of changes occurring in dementia, which could lead to the improvement of the accuracy of AD and cognitive decline diagnosis [23].

Higher levels of inflammatory markers can be detected in the elderly, mostly in the prodromal stages of AD and/or mild cognitive impairment (MCI) [24]. Activated microglia stimulates macrophage activity increasing the expression of the main pro-inflammatory cytokines, interleukin 1α, 1β, and 6 (IL-1α, IL-1β and IL-6) and tumor necrosis factor alpha (TNF-α) [7, 25]. It has been observed that the levels of these markers do not rise abruptly, but at low rates, which is recognized as a low-grade chronic inflammation. In this condition, elevated circulating levels of IL-6 and TNF-α have been found in MCI patients, compared to non-cognitively impaired controls [26, 27].

Some studies have been able to demonstrate the role of inflammation on the prodromal state of AD, confirmed by longitudinal evidence that described altered levels of inflammatory markers years before the AD onset [28, 29]. Moreover, a positive relationship between cytokine plasma concentrations throughout the progression of the disease from a mild to a moderate clinical stage was also described [30]. On the other hand, interestingly, severe AD patients did not present altered plasma cytokine levels, which may suggest a gradual decline of immunological activity in response to the progression of the degeneration.

An important meta-analysis that investigated 175 original reports performed after 2016 and containing measurements of peripheral inflammatory markers in AD patients and their healthy matched controls found that amyloidosis present in AD might induce an inflammatory response – triggered by glial cells – that cross the BBB and reach the circulation, starting a peripheral inflammatory reaction [31]. In this meta-analysis, 71 analytics were considered for inclusion and from these, 17 peripheral marker concentrations were found to be significantly higher in AD when compared to the levels of healthy controls. In AD patients, significantly higher peripheral blood cytokine concentrations were observed for IL-1β, IL-2, IL-6, IL-18, α1 anti-chymotrypsin, CXC (chemokine ligand)-10 (CXCL-10), epidermal growth factor (EGF), homocysteine, high sensitivity CRP (hsCRP), IFN-γ, soluble TNF-receptors 1 and 2, TNF-α converting enzyme (TACE) and vascular cell adhesion molecule-1 (VCAM-1) [31]. From these, IL-6 and TNF-α are the most frequently altered cytokines described in more than 40 studies that associate their role in AD. Longitudinal experimental design studies still have to be performed in order to ensure the participation of each cytokine in the degenerative AD process aiming to characterize them as AD biomarkers.

C-Reactive protein (CRP) has been described as a probable marker of the inflammatory process in the brain [32]. Hilal and co-workers [33] showed that CRP was related to the neurodegenerative process in dementia probable due to the effect of this inflammatory marker in blood vessels, but this association was independent of Aβ42 levels. In the same way, IL-6 is produced by cellular activation and transported from the periphery [34] to the CNS and vice versa, which stimulates microglia and astrocytes to release additional IL-6, also into the brain [35]. However, IL-6 plasma levels are also present as a vascular risk factor marker, and because of this lack of specificity, this cytokine may not be necessarily related only to the presence of dementia [36]. In this sense, despite Uslu et al. [35] have found a significant relationship between amyloid load and
MMSE, the data did not confirm the relationship between IL-6, TNF-α and cognitive performance.

TNF-α is a molecule present in most of the inflammatory processes, regardless of whether it is of chronic or acute occurrence. Taking into account that if patients evaluated do not have any acute activated inflammatory process as a result of some disease or other health condition, chronic elevated TNF-α levels may represent an important marker when AD patients are compared to healthy matched controls [37]. However, their short and variable half-life in serum may underestimate their role in the pathological process of AD [38]. Thus, it has been proposed that TNF transmembrane soluble receptor (s-TNFFR) isoforms TNFR1 and TNFR2, which have a longer half-life, are more easily detected especially in plasma and serum, thus reflecting the TNF-α level [39]. sTNF receptors stimulate Aβ production by activating transcription factors, such as nuclear factor-κB (NFκB) and accelerate cellular apoptosis by macrophage activity [40]. Consequently, in a chronic condition as AD, this marker provides better discriminatory power when AD patients were compared to healthy controls [39, 40]. In a research carried out with 137 MCI patients presenting 15% of conversion ratio for AD or vascular dementia followed for 46 years, it was found that s-TNF-R is one of the main markers involved in the early AD pathogenesis, which was associated with the amyloid metabolism [40]. Thus, a relationship can be seen between s-TNF receptors with amyloid production and consequently the activation of apoptotic signals that may result in neuronal death [41].

Taking this into account, these findings indicate that pathological AD hallmarks stimulate a chronic brain inflammatory cascade, activating microglia and consequently macrophages, which induces neuronal death. Accordingly, chronic exposure to inflammatory molecules has been associated with increased levels of Aβ, which creates an upward spiral that acts as a continued degenerative cascade. As the AD process may overlap the inflammaging and other related physiological and pathological characteristics cited above, the search for peripheral inflammatory markers for AD remains a challenge.

3. AMYLOID AND TAU PATHOPHYSIOLOGY BIOMARKERS

Over the past 20 years, different hypotheses have been formulated about the cause of AD. In the early 1990s, John Hardy proposed the amyloid cascade hypothesis, which posits the formation, aggregation, and deposition of insoluble amyloid-β peptides 1-42 (Aβ1-42) in extracellular spaces, as well as in the walls of blood vessels as one of the pathological hallmarks of AD. An excessive amount of Aβ1-42 peptides eventually results in the formation of amyloid plaques that in turn leads to neurotoxicity and neurodegeneration [42-46]. Aβ1-42 derives from the cleavage of β-Amyloid Precursor Protein (APP), a transmembrane protein found in many cell types including neurons, microglia and astrocytes. APP is processed either by two pathways. The first pathway is initiated by α-secretases and releases the soluble AβPPα (sAPPα) and carboxy-terminal fragment, α-CTF (or C85). The resulting C85 undergoes a further cleavage by γ-secretases to generate P3 peptide and the APP intracellular domain (AICD). However, APP can also be excised by cleaving β-secretase enzyme (BACE1) into the soluble sAPPβ (sAPPβ) and a 99-amino acid membrane bound fraction (C99).
Subsequently, the C99 fragment is cleaved by a similar enzyme, so-called γ-secretase. Additional cleaving of C99 results in the production of either Aβ40 or Aβ42 peptides, mainly responsible for generating senile plaque formation [47].

It is believed that 15-20 years before the onset of clinical symptoms of AD, the secondary structure of Aβ in α-helices becomes pathological (β-sheets), which aggregates and initiates the formation of toxic soluble oligomers, insoluble fibrils and finally the amyloid plaques [48-51]. Based on the amyloid cascade hypothesis, two main types of Aβ biomarkers for AD were established in clinical practice, amyloid PET and Aβ42 in CSF. Some evidence has focused on the cleavage products of the AβPP amyloidogenic pathway, which also includes the different Aβ species. However, these biomarker candidates still have relatively low reliability in the literature and are considered as a secondary class of markers [52]. Nevertheless, diagnostic criteria currently established for AD incorporate the use of CSF amyloidosis biomarkers, which, as already detailed in the introduction of this review, are invasive, expensive and have a high variability [53, 54]. Thus, the search for Aβ biomarkers is necessary due to the reduced costs and easier access.

Several studies have examined Aβ plasma and serum proteins to find new biomarkers for AD, however these findings have proven difficult to replicate in independent trials [55-58]. This is possibly due to multiple factors, such as the reduced plasma concentration of brain proteins, the action of blood proteases, elimination of these structures by the hepatic and renal systems, interference from the release of the same proteins by other peripheral tissues and excess of plasma proteins that may weaken the analyses [52]. Due to these factors, the literature presents controversial results regarding the Aβ plasma levels in AD compared to control participants. Some studies have shown that although the plasma level of Aβ42 alone is not enough to act as a biomarker, it is increased at the onset of AD and changes in its levels may indicate a transition from non-dementia to AD [59]. A cohort study showed that the plasma Aβ42/40 ratio is a useful biomarker to identify cognitively normal subjects at risk of developing AD [60]. However, other studies have shown that plasma levels of Aβ42 and Aβ40 present an overlap between preclinical and AD stages compared with cognitively healthy elderly controls [55]. These results reinforce the importance of using more sensitive analysis techniques to detect brain-specific proteins in blood samples [52].

In this proposal for more precise techniques, the study by Wang et al. [61] used a new type of enzyme-linked immunosorbent assay (ELISA) - the Multimeter Detection System (MDS) - to measure plasma Aβ oligomers. This assay has been developed for quantifying various oligomers using epitope-overlapping Aβ antibodies toward the N-terminus of the peptide and was originally designed to specifically detect prion oligomers in the blood. The MDS diagnosed AD with AUC of 0.84 and showed a strong correlation with other AD markers (MDS vs CSF Aβ42/40, r = -0.443, CSF phospho-Tau (pTau), r = 0.530, CSF total-Tau (tTau), r = 0.604). In this same subject, an immunoassay was able to monitor alterations of Aβ secondary structures in the blood plasma of patients with prodromal AD in a Swedish cohort (BioFINDER) and to correlate them with CSF biomarkers (Aβ42/40 ratio, tTau, p-Tau) and neuroimaging. The same Aβ biomarker in a 15-year longitudinal aging study comprising 10,000 participants (ESTHER, Germany) was able to predict AD eight years before the onset of symptoms, with a sensitivity of 71% and specificity of 91% [62]. Later, this same group of researchers reached even higher results of sensitivity and specificity (90% and 97%, respectively) when the values of the secondary structures of Aβ and tau in plasma and CSF were associated [63].

In the multicenter and prospective study of Palmqvist et al. [64], two cohorts were analyzed (Cohort 1: 2009-2015, n = 842, of which n = 513 Controls, n = 265 MCI and n = 64 AD; Cohort 2 for validation: 2000-2006; which n = 34 Controls, n = 109 MCI and n = 94 AD). Plasma Aβ42, Aβ40 and tau parameters were analyzed using fully automated assays and the best findings indicated that the association between Aβ42 and Aβ40 with Apolipoprotein E (APOE) genotype (AUC 0.85; 95% CI, 0.82 - 0.88) was able to predict the Aβ rate according to the cognitive level. It should be noted that slight or no improvement regarding the results was obtained with the plasma tau data addition.

Recently, SIMOA (single molecule array) was introduced as a new tool to identify earlier biomarkers to diagnose AD. Chatterjee and co-workers [65] investigated the plasma Aβ as a surrogate marker for brain Aβ deposition in 95 cognitively normal elderly individuals, ranked in low (Aβ-1) or high brain Aβ load (Aβ+) according to PET imaging, and detected plasma Aβ42/Aβ40 ratios higher in the Aβ group. The area under the receiver operating characteristic (ROC) curve shown to be 78% for distinguishing Aβ+ from Aβ- participants. In addition, in the large-scale longitudinal cohort, plasma Aβ42/Aβ40 ratio also assessed via SIMOA, achieved expressive accuracy values to predict cerebral amyloidosis in 276 older individuals with subjective memory complaints [66].

As AD is a multifactorial disease, it also involves tau hyperphosphorylation and aggregation leading to the intracellular paired helical filaments (PHF) and neurofibrillary tangles (NFTs) formation [67]. The molecular marker, called Alz-tau*, represents the high-molecular weight tau (HMW/tau) and the low-molecular weight tau (LMWtau). The ratio of these markers has been used as target of some studies [68, 69]. In a Chilean regional study, this HMW/LMW tau ratio showed significantly higher values in AD platelets compared to control subjects and was correlated with brain volume atrophy, measured by structural magnetic resonance imaging (MRI). This biomarker reached acceptable values for sensitivity (75.7%) and specificity (73.7%) for AD diagnosis [68]. Recently, this same platelet biomarker was analyzed in a Caucasian sample and was statistically higher in the AD group in relation to healthy subjects. Moreover, a high negative correlation was found between the MMSE scores and HMW/LMW tau ratio (r = -0.3454) [69].

In a study by Mukaetova-Ladinska and co-workers [70], two phosphorylation sites (Ser202/Thr205 and Thr181) were selected to discriminate AD presence, it was age-associated and a negative correlation with MMSE and the C-terminal end of tau protein was observed [70]. In another study, t-tau/Alz-tau ratio in plasma was highly predictive of brain tau deposition, also in typical sites for NFTs accumulation in AD. In addition, plasma t-tau/Alz-tau ratio was also highly associated with longitudinal AD brain changes and might be a biomarker for predicting brain tau pathology and neurodegeneration in AD [71]. Furthermore, through validated and comparative CSF biomarker criteria, Chen et al. [72] analyzed a novel tau protein fragment, known as tau N-terminal 1 (NT1), and verified that its levels were elevated in plasma of AD and AD-MCI groups compared with control subjects.

Therefore, the current literature demonstrates extensive works in the search for new peripheral biomarkers, preferably those that directly or indirectly involve the main neuropathological markers of AD. Although modest, these results are promising and meaningful towards an accurate, early and less invasive diagnosis of the disease.

4. NEUROTROPHINS AND GROWTH FACTORS

Neurotrophins are important regulators of neuronal survival, development, function, and plasticity, both in the central and peripheral nervous systems. Four neurotrophins were described in mammals: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). They derive from a common ancestral gene, are similar in sequence and structure, and were therefore collectively named neurotrophins.
BDNF is a growth factor related to the survival and functioning of neurons, and is a potent neuroprotective agent [74]. It has been described as a promising blood AD biomarker, since changes in its signaling pathways were reported in the disease [75]. However, the literature still presents many inconsistencies, so that some studies show increased [76, 77], decreased [78-80] or unchanged [81, 82] BDNF levels in AD compared to control participants. According to a recent review, these uncertain findings may involve multiple factors that influence the circulatory levels of BDNF, such as depressive symptoms, medication, lifestyle, experimental protocols, and others [74].

Recently, a meta-analysis compared the blood BDNF levels of more than 4,000 subjects from 33 studies. It was observed that BDNF levels, despite being significantly reduced in AD compared to controls (Hedges’ g = -0.492), showed expressive heterogeneity [83]. In the same study, other important blood factors and neurotransmitters such as vascular endothelial growth factor (VEGF); insulin-like growth factor-1 (IGF-1); insulin-like growth factor-2 (IGF-2); glial cell-derived neurotrophic factor (GDNF) and NGF were analyzed in 39 studies and showed no significant association with AD. On the other hand, the systematic review by Ng and collaborators [84] supports the fact that serum BDNF levels are reduced in AD compared to cognitively healthy subjects, but did not verify significant differences in serum BDNF between patients with AD and MCI and between MCI and controls. Another systematic review identified 98 articles and verified that blood BDNF levels were significantly decreased in AD patients compared with control subjects. However, blood IGF, NGF, and VEGF showed no differences among the groups [83].

On the other hand, the involvement of the beta-nerve growth factor (β-NGF) in the CNS is well known in critical hallmarks of AD and its peripheral changes (increased in MCI and mild AD or decreased in severe AD) are also involved in the progression of the disease [85]. The IGF-1 signaling pathway has emerged as a major regulator of the aging process. However, its role in cognitive function, as well as cerebrovascular and neurodegenerative disorders remain controversial [86] as IGF-1 serum activity levels reported earlier by Bruijn and co-workers [87] were associated with a higher prevalence and incidence of dementia. Moreover, in a recent clinical study, ApoE-e4 homozygote subjects presented lower serum levels of the IGF-1 receptor stimulating activity than heterozygotes and non-carriers [88].

5. BLOOD METABOLITES, HORMONES, TRANSPORTERS AND OTHER PROTEINS

As previously stated in this review, the metabolic basis of the AD onset and progression is still poorly understood; however, studies have suggested some alterations in blood metabolite profiles in people with the disease. Main findings demonstrate sphingolipids (particularly sphingomyelin - SM) [89, 90] and ceramides [91], glycerophospholipids (particularly glycerophosphatidylincholines – PCs – and LysoPCs) [92-94], acylceramines [89, 94], amino acids [89, 94] and asymmetric dimethylarginine (ADMA) [94] as classes of blood metabolites that are altered in AD.

SMs may be one of the most relevant biomarkers for the early detection of AD. Varma et al. [90] found that higher blood concentrations of four SMs [SM C16:0, SM C16:1, SM (OH) C14:1, SM C18:1] were associated with a significantly higher risk of future conversion to AD in cognitively healthy older adults. Accordingly, Toledo et al. [89] reported that increased blood levels of SMs (SM (OH) C14:1, and SM C16:0) were associated with increased Aβ in CSF and cognitive impairment in AD. SMs are enriched in the CNS as they are important constituents of membranes and play a critical role in neuronal cell signaling. In the brain, SMs mediate a diverse array of biological functions that are relevant to molecular mechanisms in AD, including amyloidogenic processing of the APP [90]. Among the simplest SMs are the ceramides, that were found to be elevated in brain tissue and CSF of early AD but appeared to be decreased in the blood of these patients [91]. Ceramides seem to be related to neuronal death due to their ability to activate caspase 3, the effector apoptotic molecule. Another possible factor is that oxidative stress in neurons, induced by amyloid accumulation (see item 6), can result in elevated ceramide levels, thereby contributing to the accelerated formation of pathogenic forms of amyloid by increasing β- and γ-cleavage of APP in the brain [91].

The second major class of metabolites possibly involved in AD pathogenesis are PCs and LysoPCs, essential components of cellular membranes, including those forming the cerebral tissue. Whiley et al. [92] demonstrated that AD patients presented lower plasma concentrations of distinct phosphatidylincholines (PC aa C36:5, PC aa C38:6, and PC aa C40:6), relative to controls. Accordingly, Fianiaca et al. [94] identified that all of the 13 PCs investigated were reduced during the preclinical stages of AD and Kim et al. [93] demonstrated that a decrease in PC38:6 and PC40:6 was linked to hippocampal atrophy in AD patients.

Acylcarnitines have important roles in the brain such as participation in mitochondrial function, energetics and neurotransmission. Some studies have shown that higher levels of acylcarnitines (C14:1, C16:1) in the blood were associated with cognitive impairment in AD [89, 94] and nine acylcarnitines that improved the predictive accuracy of AD diagnosis to 95% were found [94]. Specifically, the accumulation of acylcarnitine indicates a malfunction of fatty acid transport and/or β-oxidation in mitochondria, inefficient utilization of fatty acids as energy substrates, or alterations in tau metabolism, all changes that are characteristic of AD [89].

It is well reported that cholesterol transport in the brain is intimately linked to AD and several proteins involved in its transport may be potential biomarkers for AD. These include (ApoE) Apolipoprotein E and Apolipoprotein J (ApoJ). ApoE acts as a ligand for three cell surface receptors: the low-density lipoprotein (LDL) receptor; the LDL-receptor-related protein (LRP); and the very-low-density lipoprotein (VLDL) receptor [95]. There are six ApoE genotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, or E4/E4) and the presence of ApoE4 allele is correlated with increased AD risk [96]. Studies have shown that carriers of ApoE4 allele have lower serum and brain ApoE concentrations than carriers of the other isoforms [95], suggesting that lower ApoE4 levels might facilitate the accumulation of Aβ in the brain. This was supported by the findings that ApoE and Aβ levels are negatively correlated in multiple brain regions when analyzed in non-demented individuals [97]. On the other hand, ApoJ, also known as clusterin (CLU), acts as a ligand for high-density lipoprotein (HDL) and the brain has the ability to bind to Aβ, altering its aggregation and promoting its clearance, suggesting a neuroprotective role for this transporter [98]. Genetic variations within the ApoJ gene have been associated with AD and some evidence suggests that elevated plasma ApoJ levels are associated with increased white matter atrophy and AD risk [99]. These findings have led to the hypothesis that plasma ApoJ levels are elevated in response to brain pathology to exert its neuroprotective effects [98].

Studies that have investigated aminoacids showed that arginine, valine, asparagine, glycine, and others are possible biomarkers of AD [89, 94]. Metabolomic studies of plasma found that low levels of valine [89, 100] and asparagine [94] are associated with AD. Other studies found that glycine [100], arginine and polyamine [101] metabolism are altered in the plasma of AD patients, but the authors warned that plasma aminoacid levels show a large variability depending on the time of fasting that the subjects underwent, therefore requiring more standardized procedures to definitely prove their value as blood biomarkers.
Fiandaca et al. [94] found that ADMA is related to AD. ADMA is an endogenous inhibitor of nitric oxide synthase (NOS) and its elevated levels in the blood have been consistently associated with hypercholesterolemia, hypertension, chronic heart failure and atherosclerotic processes during pathological aging. ADMA is also considered as taking part in AD pathogenesis, most probably due to deteriorated cerebral circulation and dysfunction of vascular endothelium. Metabolomics studies suggest that specific metabolic changes described in this topic occur during the AD process and that blood-based metabolite markers could improve disease diagnosis [89, 90, 93, 94].

Sex hormones are one of the AD pathogenic factors and may be a way to understand the biology of sex-dependent variability in AD predisposition. One of the most common observations associated with AD onset is the decreased levels of estrogens, progesterone and androgens, pointing out the potential role of these hormones in AD pathogenesis [102]. Estrogens might have an important role in AD prevention through different mechanisms. The first is the ability of estrogens to reduce Aβ accumulation in several ways, including decreasing Aβ production by activation of the non-amyloidogenic APP pathway. Secondly, estrogens inhibit tau hyperphosphorylation and promote tau dephosphorylation through the inhibition of a large number of kinases and through the promotion of protein phosphatase 2A enzyme that is related to tau dephosphorylation [102]. Consistent with these data, it is well documented that women are at a greater risk of AD due to decreased sex hormones since they spend a large proportion of their life in the post-menopausal period as a result of increased longevity [103]. Similarly, the direct progesterone protecting actions against AD development and progression include the regulation of Aβ metabolism by reducing its production and increasing its clearance. Simultaneously, progesterone reduces tau hyperphosphorylation by modulating the activity of kinases involved in regulating tau phosphorylation, and some evidence suggests that serum levels of endogenous progesterone are inversely correlated with tau accumulation [102].

Androgens (such as testosterone) also have neuroprotective effects against AD. Many studies have detected lower testosterone levels in men with AD compared to normal age-matched controls, both in the blood and CSF [102]. As with estrogen and progesterone actions, the regulation mechanisms of the Aβ levels by testosterone are possibly performed by affecting both its production and clearance. Studies indicated that testosterone reduces Aβ production by altering APP processing towards the non-amyloidogenic pathway [104] and promotes Aβ clearance by stimulation of the Aβ-degrading enzyme action [105]. Male andropause occurs very slowly over a long period of time. During this phase, the total androgen levels start to decline in the thirties at a rate of 0.2–1% per year, while free testosterone decreases at a higher rate (2–3% yearly). This slow gradual andropause, relative to rapid menopause, may be one of the explanations of decreased male gender AD risk, delayed male MCI/AD conversion and slower AD cognitive deterioration [102].

Previous studies have demonstrated that other plasma protein profiles may be valuable diagnostic biomarkers for the early stage of AD. A group of eight plasma proteins was suggested as a predictor for AD diagnosis with high values of sensitivity and specificity. Among these proteins are BDNF (see item 4), angiogenin (AGT), osteopontin (OPN), cathepsin D, amyloid P component (SAP), complement C4, insulin-like growth factor binding protein 2 (IGFBP-2), and prealbumin (transthyretin, TTR) [106]. The AGT and SAP plasma protein levels were demonstrated to be reduced in AD, while IGFBP-2, OPN, cathepsin D, complement C4, and prealbumin seem to be elevated. The authors consider that the combination of these eight proteins has valuable potential as a diagnostic tool for AD [106]. However, some of these same proteins (IGFBP-2, complement C4, prealbumin) and others were found to be altered in AD using individual analyses. IGFBP-2 stands out as a well-studied protein and a possible biomarker in AD. IGFBP-2 is an IGF binding protein that regulates its activity in cells through a range of mechanisms. Research using animal models showed that overexpression of IGFBP-2 results in a volume reduction of the hippocampus, cerebellum, olfactory bulb, and prefrontal cortex [107]. In addition, IGFBP-2 levels were also reported to be significantly increased in the serum [108, 109] and in the plasma [106] of AD subjects. Likewise, complement C4 was observed to be elevated in the plasma of AD patients [106, 110]. Complement C4 is part of the complement system, which represents a series of proteins that are in place to destroy and remove foreign material within the human body. The system comprises 20 proteins that circulate in the blood and become activated in response to infection [110]. Prealbumin is a transport protein and has been found in CSF as an Aβ-binding molecule that suppresses the toxicity of oligomers [111]. Known as TTR, it seems to be altered in the plasma of AD subjects, but the literature is conflicting with some groups reporting elevated prealbumin in AD [106] and others showing lower levels [111].

Other possible blood-based protein biomarkers for AD are the neurofilaments (NF). NFs consists of three types of protein chains: NF medium (NFM); heavy (NFH) and light (NFL) [112], which are highly phosphorylated proteins, and the degree of this phosphorylation determines the axon diameter. When axonal damage occurs, NF molecules are released into the extracellular space, and consequently into body fluids, such as CSF or plasma. In this line, elevated CSF and blood NFL levels were found in AD [113, 114]. NFL might be useful as a screening test for neurodegeneration in the initial primary care evaluation of patients with cognitive impairments. A study including patients with familial AD (FAD) showed that blood NFL levels were increased not only in patients with the symptomatic disease, but also in pre-symptomatic mutation carriers [115].

A recent study of blood biomarker identification for AD used the combination of computational prediction and experimental validation to find potential blood protein biomarkers and found some proteins altered in the plasma of AD, including gelsolin (GSN), metalloproteinase inhibitor 1 (TIMP1), VLDL receptor (VLDLR), and amyloid-like protein 2 (APLP2) [116]. GSN can bind to Aβ, inhibit its action and promote its brain clearance, therefore having neuroprotective effects. TIMP1 plays an important role in the development of AD. It is a tissue inhibitor of matrix metalloproteinase 9 (MMP9), which is described to be associated with neurodegeneration processes by promoting extracellular Aβ degradation, neuronal degeneration and NFT formation [117], consequently functioning as an inflammatory mediator as a response to the elimination of amyloid deposition in AD [118]. VLDLR is an ApoE receptor involved in synaptic plasticity, while amyloid-like protein APLP2 helps presynaptic functions. Elevated levels of GSN and TIMP1, and lower levels of VLDLR and APLP2 were found in AD, compared to normal controls, indicating their potential as blood biomarkers for AD [116].

Another study identified candidate proteins to AD blood markers and investigated the relationship between AD and Aβ burden in the brain using the Slow Off-rate Modified Aptamer (SOMAmer®) technology. According to this study, two candidate proteins, pancreatic polypeptide (PPY) and M immunoglobulins (IgM) were associated with neocortical Aβ burden [119]. In addition, members of the neuropeptide Y (NPY) family, such as YY peptide (PPY) and PYY are described in the gut—brain axis, with bidirectional communication. The gut microbiota releases signaling molecules as well as peptides (plantins (PGN) and lipopolysaccharides (LPS), which also act promptly on CSN [120]. A study conducted by Boyle and colleagues [119] suggested that PYY would attend as a blood protein biomarker in AD, corroborating the claims of other studies [121]. Furthermore, a positive association between plasma amylin levels (a pancreatic peptide) and cognitive domains was
shown, pointing out a beneficial effect of this pancreatic peptide to brain function [122]. However, there are no indicators that PPY is produced in the brain [120]. Besides PPY, other proteins such as the macrophage inflammatory protein-1α and the vascular adhesion protein (VCAM-1) were also found to be increased in the group with high amyloid load, while immunoglobulin M-1 (IgM-1) and free thyroxine (FT4) levels were reduced [123]. The pancreatic hormone peptide islet amyloid polypeptide (IAPP) also plays a role in the clearance of Aβ, and its biological activity becomes modified in AD. Total plasma IAPP positively correlated with CSF t-tau and p-tau in control subjects and negatively with CSF Aβ42 in AD patients. These results encourage further research on the role of IAPP in AD [124].

The study by Marcello et al. [125] identified IgM autoantibodies directed against different Aβ epitopes in the plasma of AD, MCI and control subjects. Specifically, they investigated the levels of pGlu-Aβ-IgM, an auto-antibody with N-terminal truncated starting at position three with pyroglutamate levels and verified that its levels were significantly decreased in AD patients, compared to controls, suggesting that the immune system might be an initial sensor for a present pGlu-Aβ-IgM-induced pathological process in the brain of pre-symptomatic AD patients. The decreased pGlu-Aβ-IgM levels indicated that pGlu-Aβ-IgM peptides are observed early in the blood of patients with AD, possibly as a result of brain plaque aggregation [125].

Semicarbazide-sensitive amine oxidase, or vascular adhesion protein-1 (VAP-1) (SSAO/VAP-1), is another plasma protein associated with AD, specifically with dysfunctions of vascular system related to this type of dementia [126, 127]. Increased SSAO/VAP-1 activity and expression have been detected in the plasma of AD patients. As an enzyme, SSAO/VAP-1 metabolizes primary amines and destroys enzymes, inducing a pathological oxidative stress generation [127]. Furthermore, a recent study found that SSAO/VAP-1 expression is associated with endothelial activation by altering the release of proinflammatory, such as IL-6 and IL-8, and pro-angiogenic factors like VEGF [126]. Therefore, SSAO/VAP-1 may be further studied as a vascular dysfunction biomarker in AD.

Synaptic degeneration is one of the earliest pathological AD hallmarks and a plasma protein biomarker associated with low synaptic activity is the neuronal pentraxin 1 (NP1) [128]. NP1 is preferentially expressed in excitatory neurons, induced by Aβ oligomers, and involved in glutamate receptor internalization. Likewise, NP1 negatively regulates excitatory density and synaptic plasticity [129]. Studies have found elevated NP1 plasma levels and its fragments in MCI and AD patients compared to cognitively normal elderly. These elevations of NP1 at earlier stages of AD pathophysiology may be parallel to the process of synapse loss [128].

Other possible blood biomarkers for AD, such as IL-17, α2M, APOA1 and CXCL13 [119, 123, 125] did not reach significant results. Due to the complexity of the AD pathogenesis, combinations of plasma proteins associated with various biological pathways may be required as relatively reliable biomarkers for the diagnosis of this disease.

6. OXIDATIVE STRESS-RELATED BIOMARKERS

Free radicals are unstable molecules that have an electron that tends to be associated with positively charged molecules by reacting and oxidizing them [130]. They are constantly intrinsically produced by the organism, mostly as a result of the cellular respiration process, predisposing to a rapid association with other molecules, degrading them and generating the oxidation process denominated oxidative stress [131]. Oxidative stress is mainly an event caused by the imbalance between the generation of free radicals and the performance of the antioxidant defense system.

It was demonstrated that in AD occurs the overproduction of free radicals, which show high instability and reactivity. In addition to cell death, the oxidative stress phenomenon may also induce phosphorylation of the tau protein, thus, interfering in the formation and accumulation of NFTs, typical of AD [132]. Moreover, in the brain of individuals with AD, the NADPH oxidase enzyme located at the microglia membranes is activated, producing free radicals [130]. In addition, Aβ also represents an important source of free radicals in the pathological condition, due to their ability to react with active redox metals, such as Fe2+, which is more available in the brain of these individuals [133]. This is because, during neuroinflammation, glial cells are activated, which disrupts Fe2+ homeostasis [134]. Fe2+ facilitates lipoperoxidation, the reduction of hydrogen peroxide (H2O2) in a hydroxyl radical (HO·), and catalyzes the formation of OH· ions, a powerful reactive radical found in vivo, able to oxidize carbohydrates, lipids, proteins and DNA, leading to a reduction in neuronal connectivity, resulting in neuronal death [133, 135]. Thus, oxidative stress plays an important role in AD development and progression, preceding the main neuropathological marks of the disease [136].

It would be of great relevance to identify peripheral biomarkers of oxidative stress in order to contribute to the early identification of AD [137]. In general, increased free radical production in the brain during AD results in three main physiological events: lipid peroxidation and protein and DNA oxidation [137]. Lipid peroxidation can generally be described as a lipid-disruption process generated by free radicals. The plasma membrane of the cells can suffer from this event due to its lipid composition. In addition, an impairment in the enzyme’s activity attached to the plasma membrane is generated. Consequently, there is a cell death process, by apoptosis or necrosis, facilitating the development of several pathologies, including AD [138, 139].

Important biomarkers of the phenomena of lipid peroxidation and oxidative stress are F2-isoprostanes (F2-isopPs), which represent compounds formed by the non-enzymatic peroxidation of arachidonic acid induced by free radicals [140]. These lipid biomarkers are easy to detect due to their availability in many types of biological fluids, including blood [141]. A study by Irazarry et al. [142] quantified F2-IsopPs levels in plasma samples from MCI, AD and disease-free patients. The results indicated that although in CSF the levels of F2-IsopPs are high, the same was not found in the plasma of AD patients, limiting its potential as a peripheral biomarker. Additionally, lipid peroxidation causes the production of conjugated diene hydroperoxides and unstable substances that undergo disintegration in various aldehydes as 4-hydroxynonenal (4-HNE) and thiobarbituric-acid (TBARS) [143]. The 4-HNE is an unsaturated aldehyde formed by the peroxidative degradation of omega-6 fatty acid. Moreover, it is a neurotoxic product due to its property of reaction with aminoacids [144]. Some studies have confirmed that in the plasma of AD patients the quantity of 4-HNE was higher compared to the controls, indicating its role as a blood marker of lipid peroxidation [145-147]. Some studies have also shown that the serum or plasma levels of TBARS in AD subjects are significantly higher than in disease-free subjects [148-150], however, these results are still partially controversial considering that many other studies did not observe a significant difference in these levels between AD and age-matched control individuals [151, 152].

Beyond the free radical impacts, their accumulation has enough capacity to generate oxidation in proteins, causing a loss of function and increasing their degradation rate. This oxidative action results mainly in the formation of carbonylated proteins [137], which are direct products from the action of free radicals on proteins [153]. Carbonylated proteins were found to be significantly increased in the serum [154] and plasma of individuals with MCI and Dementia [155]. These studies emphasize that the specific oxidation analyses of blood proteins may be very useful in the investigation of AD biomarkers. In addition, the increased in vitro oxidative stress, in AD
patients plasma, has made the level of these proteins even more susceptible to the oxidation process [156].

The hydroxyl radical (\(\cdot\)OH) is an important oxidative stress derivate. It can be generated by the reaction of hydrogen peroxide with iron II (or copper I). This reaction occurs in vivo and contributes to brain tissue deterioration since the brain is vulnerable as it is largely composed of easily oxidizable lipids, has a high rate of oxygen consumption and lacks strong antioxidant defenses. This molecule is therefore able to damage nucleic acids, causing damage to nucleic acids, causing base substitution, addition, deletion, and other mutations both in the nucleus and mitochondrial DNA, leading to the production of various oxidized products [157].

The most common method used to determine oxidative damage to DNA is the measurement of modified bases, most often the nucleoside hydroxy-2′-deoxyguanosine (8-OHdG) [130]. Meocci and co-workers [158] evaluated 8-OHdG levels as an oxidative marker of DNA damage and demonstrated significantly higher levels in plasma of AD patients, compared to healthy participants, showing significantly higher levels in AD patients, compared to healthy subjects. In addition, in patients with AD, a significant inverse relationship was observed between the 8-OHdG molecule and plasma levels of antioxidants (lycopene, lutein, alpha-carotene and beta-carotene). Thus, it is understood that oxidative damage markers are increased in AD and correlate with decreased levels of antioxidants in plasma. Accordingly, the results of Bomba et al. [159] showed that increased 8-OH levels precede the formation of some of the typical AD pathological hallmarks, such as NFTs and Aβ plaques, representing important findings concerning the establishment of early biomarkers for AD.

Therefore, it can be observed that there is an increase in intra and extra-cerebral oxidative stress damage in AD and these products can diffuse into the blood and possibly be detected in the periphery, contributing to the early disease diagnosis. Nonetheless, these results are not fully consistent, since oxidative stress often accompanies other pathological conditions such as diabetes, cardiovascular and other neurodegenerative diseases, making the detection of oxidative reaction products not specific for AD [160].

7. NON-CODING RNAs

In the not too distant past, it was thought that the immense majority of genomic sequences would be widely transcribed into a diverse range of protein-coding RNAs and only some specific non-coding RNA (ncRNAs), such as transfer RNAs (tRNAs) or ribosomal RNAs (rRNAs), was discovered. Nowadays, the discovery of different types of ncRNAs has shown that the transcriptional landscape of all organisms is a far more complex process than initially imagined, making them fundamental components of the regulation of eukaryotic genomes.

ncRNAs are known as functional RNA molecules that do not encode a protein. They are represented by a myriad of types of RNA molecules and are divided according to their length, between small (<400 nucleotides) and long (>400 nucleotides) molecules [162]. The group of small ncRNAs comprises infrastructural and regulatory molecules, such as tRNAs, rRNAs, microRNAs (miRNAs), small interference RNAs (siRNAs), piwi-interacting RNA (piRNAs), small nuclear RNAs (snRNAs), small nuclear RNAs (snRNAs) and extracellular RNAs (exRNAs). The long ncRNAs (lncRNAs) are classified into four major classes, based on their biogenesis, genomic position and orientation related to protein-coding genes: (1) natural antisense transcript (NATs) or anti-sense RNAs, (2) bidirectional RNAs, (3) long intergenic RNAs (lincRNAs) and (4) sense-intronic RNAs [163, 164]. The list of lincRNAs continues to grow, motivating an increased interest in understanding their roles in biology and disease [165].

From these types of RNA molecules, miRNAs and lncRNAs have already been related to AD and can be potential biomarker candidates. miRNA was discovered in 1993 by Lee and colleagues [166]. They represent small ncRNAs with 20-22 base pairs (bp) that negatively regulate protein expression at the post-transcriptional level by binding complementary sequences at the 3′UTR of target mRNAs, either blocking translation or leading to mRNA degradation [167]. About 70% of known miRNAs are localized in the brain [168] and can also be found extracellular in fluids and in the circulation, such as in blood plasma [169], making them good candidates for blood-based biomarker development.

Several deregulated miRNAs in the brain, CSF, and blood have been associated with AD [170]. A review reported that 26 studies compared the measurement of miRNA in blood among AD patients and cognitively healthy controls [171]. In all the studies, at least one miRNA was found to be significantly differentially expressed between groups and, when making a comparison across the studies, six miRNAs had consistent patterns of differential expression in more than one study. Those were miRNA-107, 125b, 146a, 181c, 29b, and 342 and all had lower expression in AD compared to the controls [171]. From these, miRNA-107 has been the most extensively studied and shows direct involvement in AD pathology, since it is related to the dysregulation of BACE1 [172, 173], Hirano bodies, which represent rod-like structures within the brain, comprised actin and colillin [174], and the protein granulin (GRN) [175], all thought to be involved in AD pathogenesis. More recently, however, miRNA-181c-5p plasma levels, together with miRNA-92a-3p and miRNA-210-3p, were found to be upregulated in the plasma of MCI patients that progress to AD, but not in frontotemporal dementia (FTD) patients. Both miRNA181c-5p and 210-3p are involved in synaptic regulation and bind to NPI mRNA and its receptor, which negatively regulates synaptic activity [176] (see item 5).

Recently, it was demonstrated that miRNA-101a regulates autophagy phenomenon in an AD cell model, via the MAPK pathway. Autophagy – a process of self-degradation and recycling of macromolecules and cellular organelles – is described to be related to AD as a result of the accumulation of lysosomes and their hydrolases within neurons [177]. miRNA-101a was significantly reduced in the plasma of patients with AD and in the brain of transgenic mice bearing an APP mutation [178]. Nagaraj and co-authors [179] compared the miRNA profiles in the blood plasma of 15 MCI-AD patients, whose diagnoses were confirmed by CSF biomarkers, with 20 AD patients and 15 non-demented, age-matched individuals and found 6 miRNAs as the most promising biomarker candidates differentiating early AD. These are miRNA-483, 486, 30b, 200a, 502 and 142. According to the authors, miRNA-483 was the most deregulated miRNA and the best biomarker candidate for early AD detection. It has a binding site in tau and BACE mRNA, therefore able to damage neuronal activity.[180, 181]

MiRNA-501 and miRNA-137 regulate the expression of the α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptor subunit GluA1 in dentrites, suggesting its function in synaptic plasticity related to cognitive functions, including learning and memory [180, 181]. Serum miRNA-501 [182] and 137 [183] levels were downregulated in AD patients, which significantly correlates with lower MMSE scores [182]. MiRNA-34c belongs to the miRNA-34 family, comprising two additional members: miRNA-34a and -34b. These miRNAs negatively regulate Bcl2 – a protein related to cell survival/apoptosis and SIRT1 deacetylation involved in neuroprotection signaling. Accordingly, the levels of miRNA-34c are increased in plasma and PBMC AD patients, compared with age-matched normal elderly controls [185].

A systematic review found 20 studies evaluating miRNA deregulation in AD patients’ blood. From these studies, 56 deregulated miRNAs were found in serum, 10 in plasma, 11 in whole blood, 10 in BMC, and 15 in exosomes, totaling 102 miRNA differentially expressed in AD and age-matched controls [186]. Our own research group has studied the effects of some of some miRNA on a protein called a disintegrin and metalloproteinase 10...
(ADAM10), the α-secretase involved in the non-amyloidogenic cleavage of APP. We verified that miRNA-221 targets ADAM10 and it is downregulated in AD patients’ blood, compared to cognitively healthy subjects [187]. Even though this miRNA cannot be considered a direct biomarker, it is able to regulate the expression of ADAM10, which has been investigated as a possible blood-based biomarker for AD.

Only a few reports have suggested lncRNAs as candidates to blood-based AD biomarkers. BACE1-AS is a lncRNA that regulates both BACE1 mRNA expression and its protein levels. Different from other NATs that inhibit mRNA translation by forming a duplex with the sense mRNA, BACE1-AS modulates BACE1 mRNA expression increasing its stability, consequently generating additional Aβ production [188]. The lncRNA BACE1-AS was found to be elevated in the plasma of AD patients, despite the fact that no correlation was found between its levels and cognitive instruments such as MMSE [189]. 51A was also found to be upregulated in plasma from AD patients, curiously with a negative correlation with the disease progression evaluated by the MMSE score [190]. 51A is a lncRNA that drives a splicing shift of SORL1 from the synthesis of the canonical long protein variant A to an alternatively spliced protein form. Sortilin-related receptor 1 (SORL1) is a sorting receptor that acts during APP translocation, affecting its cleavage and protein form. Sortilin of the canonical long protein variant A to an alternative protein form. Sorting between normal aging.

The data described so far demonstrate an immense variability of miRNA and lncRNA types that can be potentially defined as AD biomarkers. Whether a specific miRNA or lncRNA or a set of different ncRNAs would be effectively considered as a blood-based AD biomarker will depend on the quality of the studies carried out, regarding the size and homogeneity of the sample, the inclusion of studies with subjects having different types of dementia, as well as large independent longitudinal studies with an appropriate selection of controls and normalization of analytical methods.

8. BLOOD CELLULAR COMPONENTS

So far, we have mentioned soluble AD biomarkers candidates. In this section, we will discuss some evidence pointing to cellular components as potential blood-based AD biomarkers. In platelets, in an opposite pathway to Aβ formation, the protein ADAM10 stands out in this field. Besides being abundant in CNS, ADAM10 was found to be reduced in platelets of AD patients, compared to cognitively healthy participants, with a significant positive correlation with neuropsychological tests, such as MMSE and the clock drawing test [193-197]. On the other hand, the ADAM10 gene expression in AD platelets and total blood did not show any differences between control groups or MCI patients [198] and, therefore, the lower protein levels are probably related to post-transcriptional mechanisms of gene regulation. Of note, an age-dependent increase in ADAM10 levels and activity in platelets was found among cognitively normal volunteers belonging to three groups of mean age of 25, 65, and 80 years [199], suggesting that ADAM10 has a great potential to be a biomarker for AD diagnosis, but also has effects on normal aging.

T lymphocytes and their receptors have been investigated to find potential AD biomarkers. Increased frequencies of T CD4+ [200] or T CD4+ expressing IL-10 cells were found in AD patients, compared to controls and patients with late onset depression [201], which can be a reflection of the deregulation and inflammation process present in the disease, as already discussed (see item 2).

Red blood cells (RBC) were studied to investigate the levels of α-synuclein in AD patients [202]. This protein is the main compo-

**Fig. (2).** Simplified diagram summarizing the main blood-based biomarkers of AD shown in this review.

†, increased levels in AD

쁠, decreased AD Levels

㎪, unvaried levels in AD or with diverging information (A higher resolution / colour version of this figure is available in the electronic copy of the article).
CONCLUSION

Here, we reported recent advances in the search for blood-based biomarkers for AD, which were summarized in Fig. (2). A series of limitations hamper the discovery of novel blood-based AD biomarkers. Some of them, but not restricted, are related to the lack of reproducibility of findings, which can be attributed to many variables in the studies, such as the small size and heterogeneity of the study population; selection of inadequate controls; different analytical methods or normalization of methods, statistical testing and data processing; few studies profiling biomarkers in more than one neurodegenerative disease; and a lack of broad validation in large independent longitudinal studies. Nevertheless, if such a marker exists, it is likely to correspond to a panel of biomarkers – rather than a single one – to establish thresholds that can be used to select individuals before more-invasive and specialized modalities, such as lumbar puncture or PET for diagnosis or prognosis, thereby enhancing clinical trial outcomes. In this regard, adhesion to international guidelines of standardization of blood assays, such as those from Joint Programming for Neurodegenerative Diseases (JPND) BIO-MARKAPD consortium can help investigators to produce more conclusive and congruent results, which could also be helpful in formulating novel pharmacological targets and/or strategies for the AD clinical treatment.

LIST OF ABBREVIATIONS

| SMs        | Sphingomyelins |
| ADMA       | Asymmetric Dimethylargine |
| PCs        | glycerophospholipidcholines |
| LysoPCs    | Lyso-glycerophospholipidcholines |
| APoE       | Apolipoprotein E |
| ApoJ       | Apolipoprotein J |
| miRNA      | MicroRNAs |
| IncRNA     | Long non-coding RNAs |
| BACE1      | β-amyloid precursor protein-cleaving enzyme 1 |
| BACE1-AS   | BACE1 antisense RNA |
| 4-HNE      | 4-hydroxynonenal |
| TBARS      | Thioarbituric-acid |
| F2-isopPS  | F2-isoprostanes |
| 8-OHdG     | Hydrox-2-deoxyguanosine |
| BDNF       | Brain-derived neurotrophic factor |
| OPN        | Osteopontin |
| IGFBP-2    | insulin-like growth factor binding protein 2 |
| NFL        | neurofilaments light |
| IgM        | M-immunoglobulins |
| PPy        | Pancreatic Polypeptide |
| GSN        | Gelsolin |
| TIMP1      | Metalloproteinase inhibitor 1 |
| VCAM-1     | Vascular cell adhesion molecule-1 |
| SSAO/VAP-1 | Semicarbazide-sensitive amine oxidase/vascular adhesion protein-1 |
| NP1        | Neuronal pentraxin 1 |
| RBC/α-Synuclein | Red blood cells/α-Synuclein |
| AGT        | Angiotensinogen |
| SAP        | Serum amyloid P component |
| pGluAβ-IgM | N-terminal truncated starting at position three with pyroglycolylated levels |
| VLDLR      | Very low-density lipoprotein receptor |
| APLP2      | Amyloid-like protein 2 |
| IgM-1      | Immunoglobulin M-1 |
| FT4        | Thyroxine |
| IL-1β      | Interleukin-1β |
| IL-6       | Interleukin-6 |
| TNF-α      | Tumor necrosis factor alpha |
| s-TNFR1    | Soluble tumor necrosis factor receptor 1 |
| s-TNFR2    | Soluble tumor necrosis factor 2 |
| T CD4+     | T lymphocytes |
| Aβ1-42     | Amyloid-β peptides 1-42 |
| pTau       | Phospho-Tau |
| Alz-tau    | High-molecular weight tau |
| tTau       | Total-Tau |
| NT1        | tau N-terminal |

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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