

# Latest advances in cerebrospinal fluid and blood biomarkers of Alzheimer's disease

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*Ther Adv Neurol Disord*

2019, Vol. 12: 1–23

DOI: 10.1177/  
1756286419888819

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**Abstract:** Alzheimer's disease (AD) is the most common neurodegenerative disease and its diagnosis has classically been based on clinical symptoms. Recently, a biological rather than a syndromic definition of the disease has been proposed that is based on biomarkers that reflect neuropathological changes. In AD, there are two main biomarker categories, namely neuroimaging and fluid biomarkers [cerebrospinal fluid (CSF) and blood]. As a complex and multifactorial disease, AD biomarkers are important for an accurate diagnosis and to stage the disease, assess the prognosis, test target engagement, and measure the response to treatment. In addition, biomarkers provide us with information that, even if it does not have a current clinical use, helps us to understand the mechanisms of the disease. In addition to the pathological hallmarks of AD, which include amyloid- $\beta$  and tau deposition, there are multiple concomitant pathological events that play a key role in the disease. These include, but are not limited to, neurodegeneration, inflammation, vascular dysregulation or synaptic dysfunction. In addition, AD patients often have an accumulation of other proteins including  $\alpha$ -synuclein and TDP-43, which may have a pathogenic effect on AD. In combination, there is a need to have biomarkers that reflect different aspects of AD pathogenesis and this will be important in the future to establish what are the most suitable applications for each of these AD-related biomarkers. It is unclear whether sex, gender, or both have an effect on the causes of AD. There may be differences in fluid biomarkers due to sex but this issue has often been neglected and warrants further research. In this review, we summarize the current state of the principal AD fluid biomarkers and discuss the effect of sex on these biomarkers.

**Keywords:** Alzheimer's disease, biomarkers, blood, cerebrospinal fluid, gender, neurodegeneration, sex

Received: 8 October 2019; revised manuscript accepted: 21 October 2019.

## Introduction

Alzheimer's disease (AD) accounts for 60–80% of all cases of dementia worldwide. There are some rare familial AD cases that are caused by inherited mutations in the *APP*, *PSEN1*, and *PSEN2* genes, but most cases are 'sporadic' or late-onset. The main risk factors for late-onset AD are aging and carrying the allele  $\epsilon 4$  of Apolipoprotein E (*APOE- $\epsilon 4$* ).<sup>1</sup>

According to the latest guidelines of the National Institute of Aging and Alzheimer Association (NIA-AA),<sup>2</sup> the term 'Alzheimer's disease' is

applied whenever there is biomarker evidence of pathological deposits of the amyloid- $\beta$  peptide ( $A\beta$  plaques) and tau neurofibrillary tangles (NFTs). The AT(N) classification summarizes biomarkers into three groups, 'A' refers to aggregated amyloid- $\beta$  ( $A\beta$ ), 'T' refers to aggregated tau, and 'N' refers to neurodegeneration.<sup>3</sup> Currently, the most widely accepted biomarkers for each of the categories are amyloid positron emission tomography (PET), cerebrospinal fluid (CSF)  $A\beta 42$  and CSF  $A\beta 42/40$  (A), tau PET and CSF phosphorylated tau (p-tau) (T), and structural magnetic resonance imaging,

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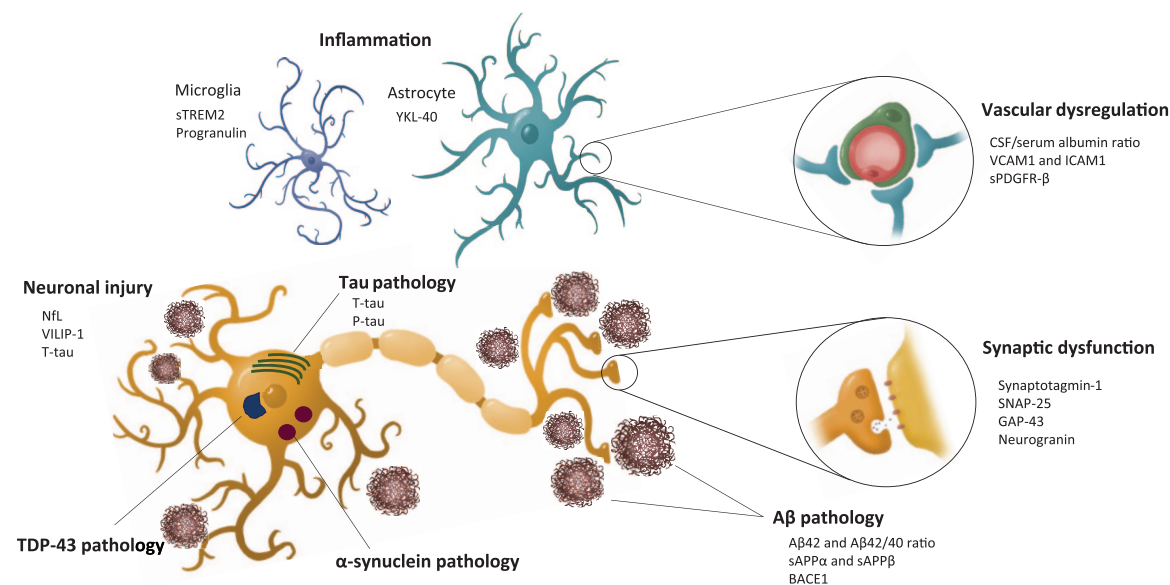
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**Figure 1.** Pathological events in Alzheimer's disease and their corresponding fluid biomarkers.

Aβ, β-amyloid; BACE1, β-site amyloid precursor protein cleavage enzyme 1; GAP-43, Growth-associated protein 43; ICAM-1, intercellular adhesion molecule-1; NfL, neurofilament light; p-tau, phosphorylated tau; sAPP, soluble N-terminal fragment of APP; SNAP-25, synaptosome-associated protein 25; sPDGFR-β, soluble platelet-derived growth factor receptor-β; sTREM2, soluble triggering receptor expressed on myeloid cells 2; TDP-43, transactive response DNA-binding protein 43; t-tau, total tau; VCAM-1, vascular cell adhesion molecule-1; VILIP-1, visinin-like protein 1; YKL-40, chitinase 3-like protein 1.

fluorodeoxyglucose (FDG) PET, CSF total tau (t-tau), and neurofilament light (NfL) (N).

Although Aβ and tau deposits define AD pathology, a significant degree of copathology is common in patients with AD. In addition, multiple pathological processes co-occur in AD, including inflammation and innate immune response, synaptic and vascular dysfunction, or blood-brain barrier (BBB) impairment (Figure 1). Successful treatment of AD may require the targeting of several pathological processes. Therefore, there is a clear need for suitable biomarkers to track pathological processes and detect concomitant pathologies, diagnose more accurately, assess prognosis, monitor treatment response, test target engagement, and to stratify participants in research studies and clinical trials. CSF biomarkers are widely used in research and clinical practice but lumbar puncture, despite being a well tolerated procedure with a very low rate of adverse effects, is still an invasive procedure.<sup>4</sup> Therefore, increasing efforts have been made in recent years to develop biomarkers in more accessible biological matrices including urine, saliva, and blood.<sup>5,6</sup> The development of blood biomarkers has become a priority in the

AD field and several new developments have occurred over the last few months.

In this review, we will examine the current status of AD biomarkers in CSF and blood (Table 1 and Figure 1). Because recent evidence suggests that sex, gender, or both can have an influence on different aspects of AD, we also briefly discuss their effects on fluid biomarkers.

### Aβ pathology

Aβ peptides are generated physiologically by the sequential cleavage of the amyloid-β precursor protein (APP), a type 1 transmembrane protein. APP can be cleaved by two pathways. The amyloidogenic pathway comprises the cleavage by the β-secretase enzyme (β-site APP-cleaving enzyme 1, BACE1) generating the β soluble N-terminal fragment of APP (sAPPβ) and the APP carboxy-terminal fragment (βCTF). βCTF is subsequently cleaved in the membrane by γ-secretase and results in Aβ peptides of variable lengths and the amyloid precursor intracellular domain (AICD). The 40-amino acid form (Aβ40) is the predominant type in the brain in physiological conditions, but the 42-amino acid

**Table 1.** Summary of the main AD fluid biomarkers.

Pathological mechanism	Biomarker	Change in AD <i>versus</i> controls	
		CSF	Serum/plasma
<b>A<math>\beta</math> pathology</b>	A $\beta$ 42 or A $\beta$ 42/40	↓	↓
	BACE1	≈	↑
	sAPP $\alpha$ and sAPP $\beta$	≈	○
<b>Tau pathology</b>	t-tau	↑	↑
	p-tau	↑	↑
<b>Neuronal injury</b>	NfL	↑	↑
	VILIP-1	↑	↑
<b>Synaptic dysfunction</b>	Neurogranin	↑	=
	GAP-43	↑	○
	Synaptotagmin-1	↑	↓
	SNAP-25	↑	○
<b>Vascular dysregulation</b>	CSF/serum albumin ratio	NA	↑
	VCAM1/ICAM1	↑	↑
	sPDGFR- $\beta$	○	○
<b>Inflammation</b>	sTREM2	↑	=
	Progranulin	≈	≈
	YKL-40	≈	=
	IP-10	≈	≈
	GFAP	≈	↑
	MCP-1	=	≈
<b>TDP-43 pathology</b>	TDP-43	≈	↑
<b><math>\alpha</math>-synuclein pathology</b>	$\alpha$ -synuclein	≈	=

Overview of the main AD-related fluid biomarkers. The table depicts the changes observed in AD (prodromal, dementia, or both) compared with controls as follows: ↑ increased or ↓ decreased levels in most or all studies; = no changes; ≈ inconsistent results; ○ unknown; NA not applicable. To complete these table, we reviewed all studies available in AlzBiomarker (<https://www.alzforum.org/alzbiomarker>) for GFAP, MCP-1, neurogranin, NfL, sAPP $\alpha$ , sAPP $\beta$ , sTREM2, VILIP-1, YKL-40. For A $\beta$ , t-tau, and p-tau we reviewed those studies in AlzBiomarker with a total number of patients >300. We also included other biomarkers not available in AlzBiomarker that we, however, found relevant for AD. A $\beta$ ,  $\beta$ -amyloid; BACE1,  $\beta$ -site amyloid precursor protein cleavage enzyme 1; CSF, cerebrospinal fluid; GAP-43, Growth-associated protein 43; GFAP, Glial fibrillary acidic protein; ICAM-1, intercellular adhesion molecule-1; IP-10, interferon-inducible protein-10; MCP-1, Monocyte chemoattractant protein-1; NA, not applicable; NfL, neurofilament light; p-tau, phosphorylated tau; sAPP, soluble N-terminal fragment of APP; SNAP-25, synaptosome-associated protein 25; sPDGFR- $\beta$ , soluble platelet-derived growth factor receptor- $\beta$ ; sTREM2, soluble triggering receptor expressed on myeloid cells 2; TDP-43, transactive response DNA-binding protein 43; t-tau, total tau; VCAM-1, vascular cell adhesion molecule-1; VILIP-1, visinin-like protein 1; YKL-40, chitinase 3-like protein 1.

form (A $\beta$ 42) is the predominant type in amyloid plaques.<sup>7</sup> The AICD may act as a transcriptional regulator of several target genes and is thought to contribute to AD pathology.<sup>8,9</sup> The

nonamyloidogenic pathway comprises the cleavage by  $\alpha$ -secretase resulting in the  $\alpha$  soluble N-terminal fragment of APP (sAPP $\alpha$ ) and the APP carboxy-terminal fragment ( $\alpha$ CTF).

$\alpha$  CTF is cleaved by  $\gamma$ -secretase that generates a p3 fragment and an AICD fragment.<sup>7,10,11</sup> Of note, A $\beta$  pathology is highly associated with the *APOE* genotype. Individuals carrying *APOE- $\epsilon$ 4* develop more amyloid plaques when they age.<sup>12,13</sup> The *APOE- $\epsilon$ 4* allele decreases the brain clearance of A $\beta$ , triggering its aggregation.<sup>14</sup> In addition, the APOE-protein can bind A $\beta$ , but its affinity differs between the three APOE isoforms. Thus, it is important to interpret amyloid-related biomarkers in the context of the *APOE* genotype.<sup>15,16</sup>

### A $\beta$ peptides

CSF A $\beta$ 42, in combination with t-tau and p-tau, constitutes the widely accepted CSF signature for AD diagnosis. CSF A $\beta$ 42 has a high diagnostic accuracy to discriminate between AD and controls and, in addition, it is helpful for distinguishing AD from other neurodegenerative diseases. More importantly, CSF A $\beta$ 42 allows us to identify AD in its preclinical stage. CSF A $\beta$ 42 accurately predicts disease progression in cognitively unimpaired individuals and in those with mild cognitive impairment (MCI).<sup>17</sup> In contrast with amyloid PET, CSF A $\beta$ 42 plateaus early in the disease progression.<sup>18,19</sup> Despite the high agreement between amyloid PET and CSF A $\beta$ 42, there are some conflicting results that may be due to the fact that CSF A $\beta$ 42 reflects a biological process preceding the aggregation of A $\beta$  detected with amyloid PET.<sup>20</sup> Recently, optimal centiloid cut-offs have been defined maximizing the agreement between the PET and CSF levels of A $\beta$ 42.<sup>21</sup>

However, the use of CSF A $\beta$ 42 can be limited by the impact of pre-analytical factors and by inter-individual differences in A $\beta$  production. These limitations can be partially overcome by normalizing A $\beta$ 42 values with A $\beta$ 40. A $\beta$ 40 does not change in AD and its concentration in CSF is 10-times higher than that of CSF A $\beta$ 42 and can, therefore, be used as a 'proxy' of total A $\beta$  levels. The CSF A $\beta$ 42/A $\beta$ 40 ratio appears to be a better predictor of amyloid PET positivity in prodromal AD and performs better at distinguishing AD from other dementias than CSF A $\beta$ 42 alone.<sup>22,23</sup> Similar to CSF A $\beta$ 40, CSF A $\beta$ 38 does not vary between AD and controls and the CSF A $\beta$ 42/A $\beta$ 38 ratio shows a comparable predictive potential to that of the CSF A $\beta$ 42/A $\beta$ 40 ratio.<sup>22</sup>

Increasing efforts are focusing on the measurement of blood A $\beta$ . Most previous studies demonstrated inconsistent results and a lack of correlation between CSF and blood A $\beta$ .<sup>24–26</sup> That was probably due to the fact that A $\beta$  concentration in blood is low<sup>27</sup> and A $\beta$  measurements were affected by matrix effects. However, recent studies using ultrasensitive assays are very promising. A $\beta$  has been measured in blood using the Simoa platform,<sup>28,29</sup> immunoprecipitation coupled with mass spectrometry (IP-MS)<sup>30–32</sup> and stable isotope labeling kinetics followed by IP-MS.<sup>33</sup> These studies have shown that blood A $\beta$ 42, A $\beta$ 42/A $\beta$ 40 ratio, or both, correlate with those in CSF and with amyloid PET.<sup>31–33</sup> They have also shown that A $\beta$ 42, A $\beta$ 42/A $\beta$ 40 ratio, or both differ between AD and controls or other diagnostic groups, although the magnitude of the difference is smaller than that found in CSF. The xMAP technology has been used to measure A $\beta$ 42/A $\beta$ 40. While a study demonstrated that A $\beta$ 42/A $\beta$ 40 could not predict AD,<sup>34</sup> other studies showed that pretreatment of plasma with a mixture of protease and phosphatase inhibitors may help to detect amyloid positive individuals.<sup>35,36</sup> Despite these promising results, data presented at the *Alzheimer's Association International Conference (2019)* showed that the different technologies used to measure blood A $\beta$  have a poor correlation between them. This warrants further investigation to clarify whether these methods are measuring different A $\beta$  pools or whether there are other technical issues involved.

A $\beta$  oligomers have been measured in CSF and blood using different techniques including ELISA, single-molecule fluorescence microscopy, and protein misfolding cyclic amplification assays. However, results have not been consistent due to multiple methodological difficulties.<sup>37</sup>

### Other A $\beta$ pathology biomarkers

Soluble fragment sAPP $\alpha$  or sAPP $\beta$  can be readily measured in CSF, but results are conflicting with regards to differences between AD and controls, or between progressive MCI and stable MCI.<sup>38–41</sup> However, sAPP $\beta$  may be used as a target engagement biomarker of BACE inhibitors. BACE1 activity, or its protein levels, can be measured in CSF but there are some inconsistencies in the results for AD patients. Most studies showed an increase on BACE1 (either activity

or protein levels) in AD compared with controls.<sup>41–43</sup> In addition, patients with MCI appear to have higher levels of BACE1 activity and protein levels compared with controls or AD patients, and BACE1 has been shown to be a good progression marker in MCI patients.<sup>44</sup> However, other studies have reported different results, including no differences in BACE1 activity between controls, MCI and AD, or even decreased CSF levels in AD compared with controls.<sup>45,46</sup> Probably, the main advantage of measuring BACE1 is as a biomarker of target engagement for BACE1 inhibitors. Available studies testing plasma BACE1 have shown inconsistent results regarding the potential of elevated BACE1 to differentiate AD patients from controls and to predict progression to AD among MCI patients.<sup>47</sup>

### Tau biomarkers

NFTs containing hyperphosphorylated tau are, in combination with A $\beta$  plaques, the pathological hallmarks of AD pathology.<sup>48,49</sup> Tau inclusions, however, are found in a wide range of neurodegenerative diseases including progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), some types of frontotemporal lobar degeneration (FTLD-tau), and chronic traumatic encephalopathy. All diseases with tau deposits are collectively known as ‘tauopathies’ and they differ in the isoform of tau and phosphorylations present in the aggregates.<sup>50</sup>

#### *t-tau and p-tau*

It has been very well established that CSF t-tau and p-tau levels are increased in AD compared with controls. CSF t-tau is considered to be a marker of the intensity of neuronal injury and neurodegeneration in AD. Therefore, t-tau has a clear diagnostic value for differentiating AD from normal aging but it can be elevated in a number of other neurological diseases. Of note, however, t-tau does not equally increase in all neurological diseases. It dramatically increases in Creutzfeldt–Jacob disease (CJD), and in acute stroke<sup>51</sup> but, in contrast, it only marginally increases (or it does not change or can decrease) in other neurodegenerative diseases including Parkinson’s disease (PD), FTLD, PSP, and CBD.<sup>52</sup> It has been previously suggested that the increased t-tau in CSF was a result of leakage from damaged neurons. However, this theory

has been recently challenged and it has been demonstrated that there is an active secretion of tau that may differ between physiological and pathological conditions and it is especially sensitive to underlying A $\beta$  pathology.<sup>53,54</sup> In addition, it is important to note that t-tau is measured using antibodies against the mid-domain, and there are other truncated tau forms in CSF that are being investigated.

In contrast, CSF p-tau is considered to be a biomarker of tau deposition in AD. In fact, CSF p-tau appears normal or only slightly increased in most of the other neurological diseases. Thus, CSF p-tau is a more specific biomarker for AD. In the recently published AT(N) system,<sup>2</sup> CSF t-tau and p-tau, account for neurodegeneration (N) and tau pathology (T), respectively. However, these two biomarkers are highly correlated and it is difficult to determine which component of each biomarker reflects neurodegeneration and tau pathology. Phosphorylation at threonine 181 (p-tau<sub>181</sub>) is the most widely used target as a p-tau biomarker in the clinical setting and increases very early in the AD *continuum*, even before tau PET becomes positive. Other tau species are increased in AD, including those phosphorylated in other mid-domain residues (threonine 231, serine 199, and 231) and C-terminal residues (Serine 396 and 404).<sup>55</sup> Of note, the abundance of several other phosphorylation sites in tau differs in the brain and CSF of individuals with and without AD.<sup>56</sup>

In addition to being validated diagnostic markers of AD, CSF t-tau and p-tau are useful biomarkers to stage the disease, predict its prognosis, and monitor drug response. Tau deposition follows disease progression more effectively and has a stronger correlation with cognitive status than A $\beta$ .<sup>57,58</sup> However, longitudinal studies have shown that CSF t-tau and p-tau, and other neurodegeneration markers, may decrease in advanced stages of the disease. CSF t-tau and p-tau have been shown to be good predictors of progression from cognitively unimpaired to MCI and progression to AD dementia.<sup>17</sup> CSF t-tau and p-tau are normally monitored in clinical trials, but the association between these biomarkers and the therapeutic response remains unclear.

Similarly to blood A $\beta$ , the introduction of ultra-sensitive immunoassays now allows the reliable

measurement of tau in blood. Before this, most studies showed considerable variability in results.<sup>26</sup> Using the ultrasensitive techniques, several groups have shown an increase in blood t-tau in AD compared with controls,<sup>59–62</sup> but the overlapping values between groups precludes their use as a diagnostic tool. In addition, plasma t-tau predicts cognitive decline and the risk of dementia.<sup>59,60,63</sup> The measurement of blood p-tau is technically challenging but promising results are being produced. Higher plasma or serum p-tau<sub>181</sub> have been described in AD *versus* controls using different platforms, including MSD,<sup>64</sup> Simoa,<sup>65</sup> label-free real time surface plasmon resonance technology,<sup>66</sup> and immunomagnetic reduction (IMR).<sup>67</sup>

## Neuronal injury

### NfL

Neurofilaments are intermediate filaments that are abundant in neuronal axons. Among the neurofilaments, NfL has the most promising results as a biomarker. NfL leaks into the CSF after neuronal and axonal damage. CSF NfL increases in multiple neurological diseases and is widely accepted as a nonspecific biomarker of axonal injury.<sup>68–70</sup> Of note, this increase is more prominent in the neurological diseases where there is axonal degeneration, white matter injury, or both, including amyotrophic lateral sclerosis (ALS), FTLN, and CJD.

The development of ultrasensitive techniques enables the accurate measurement of NfL in blood. Blood NfL levels closely correlate with those of CSF.<sup>71,72</sup> Therefore, blood NfL can be considered to be a noninvasive proxy of CSF NfL. Plasma or serum NfL levels are increased in AD and MCI compared with controls and in other neurological diseases.<sup>72–75</sup> More importantly, blood and CSF NfL levels increase many years before the symptom onset in autosomal-dominant AD,<sup>76–78</sup> and in Down syndrome.<sup>79</sup> In addition, CSF and blood NfL are associated with disease severity markers including brain atrophy, glucose hypometabolism, and cognitive impairment, which favors its use as a disease staging biomarker.<sup>71,80</sup> Finally, increasing agreement favors the use of NfL, instead of t-tau, as an independent marker of neurodegeneration ‘N’ in the AT(N) classification for AD.<sup>3</sup>

### Visinin-like protein 1

Visinin-like protein 1 (VILIP-1) is a calcium sensor protein that is highly expressed in neurons.<sup>81</sup> Intracellular VILIP-1 expression is decreased in AD, especially in the entorhinal cortex.<sup>82</sup> VILIP-1 in CSF is correlated with t-tau and p-tau, supporting the theory that VILIP-1 is a neuronal injury biomarker.<sup>83</sup> Most studies, except one<sup>84</sup> have shown that VILIP-1 is increased in AD compared with controls,<sup>26,83,84</sup> Further studies have suggested that it could help to differentiate AD from other dementias.<sup>85–87</sup> In addition, VILIP-1 has been shown to predict cognitive impairment and atrophy rates and to identify the MCI individuals that will progress to AD dementia.<sup>84,87–89</sup> Longitudinal measurements in late-onset AD and autosomal-dominant AD have shown that the initial increase in CSF VILIP-1 is ameliorated as dementia develops, similar to what happens with other neural injury markers including CSF t-tau.<sup>90,91</sup> Studies on blood VILIP-1 are limited, but a significant increase in plasma VILIP-1 has been reported in AD compared with controls.<sup>87</sup> In autosomal-dominant AD mutation carriers, VILIP-1 was also found to be increased but this finding did not reach statistical significance.<sup>90</sup>

## Synaptic dysfunction

Synapse dysfunction and eventual synapse loss is an early process in AD pathogenesis.<sup>92,93</sup> Pathological studies have demonstrated that synapse loss is closely associated with cognitive impairment.<sup>94,95</sup> Synaptic biomarkers can be classified as presynaptic (axonal) and postsynaptic (dendritic) biomarkers. The main presynaptic biomarkers are synaptotagmin-1, synaptosomal-associated protein 25 (SNAP-25), and growth-associated protein 43 (GAP-43), and the main postsynaptic biomarker is neurogranin.

### Synaptotagmin-1

Synaptotagmin-1 is a calcium sensor protein located in the presynaptic plasma membrane that is involved in the exocytosis of synaptic vesicles and the release of neurotransmitters.<sup>96</sup> Synaptotagmin-1 was first detected in the CSF in the late 1990s.<sup>97</sup> A later study, using mass spectrometry, showed that CSF synaptotagmin-1 was increased in both MCI and dementia due to AD. Of interest, higher levels were found in MCI due to AD.<sup>98</sup>

### SNAP-25

SNAP-25 is a component of the SNAP receptor (SNARE) complex located in the synaptic vesicles where it plays a key role in their exocytosis.<sup>99</sup> CSF SNAP-25 is significantly increased in prodromal and AD dementia compared with controls.<sup>100</sup> To date, to the best of our knowledge, no data is available on blood SNAP-25 in AD.

### GAP-43

GAP-43 is a presynaptic protein involved in neuronal development and synaptogenesis in the adult brain. It is mainly expressed in the hippocampus, entorhinal cortex, neocortex, cerebellum, and olfactory bulb.<sup>101,102</sup> CSF GAP-43 is increased in AD patients compared with controls and, importantly, compared with other neurodegenerative diseases. Therefore, it is a potential specific biomarker for AD-associated synaptic dysfunction.<sup>103,104</sup> There is a lack of studies on blood GAP-43 in AD.

### Neurogranin

Neurogranin is a postsynaptic protein that is highly expressed in the dendritic spines of the hippocampus, amygdala, caudate, and putamen. It binds the calcium-binding protein calmodulin (CaM) and regulates calcium signaling and synaptic plasticity.<sup>105</sup> CSF neurogranin is increased in AD and MCI. It is also increased in progressive compared with stable MCI, and predicts cognitive decline in cognitively unimpaired individuals.<sup>26,68,106–108</sup> More importantly, CSF neurogranin is specific for AD, reinforcing the view that synapses, in particular, are affected in AD. In addition, high levels of CSF neurogranin are found in CJD.<sup>109</sup> Unexpectedly, a study in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort found a longitudinal decrease of CSF neurogranin in AD patients.<sup>91</sup> Studies of blood neurogranin are limited and they have not reported significant differences between AD and controls.<sup>26</sup>

### Emerging synaptic biomarkers

Synaptic markers are a current area of intensive research in the AD field and some interesting results are being produced. Among the most promising synaptic markers are the neural pentraxin 2 (NPTX2) and the synaptic vesicle

glycoprotein 2A (SV2A). NPTX2 is specifically localized in excitatory synapses and binds the AMPA glutamate receptors. CSF NPTX2 decreases in AD patients and is correlated with cognitive status and hippocampal volume.<sup>110</sup> Individuals with lower levels of CSF NPTX2 have a faster cognitive decline. SV2A is the specific target for the antiepileptic drug levetiracetam and PET tracers that bind SV2A have been developed. CSF SV2A decreases in AD and FTLT, but not in Lewy body dementia or vascular dementia (VaD), and is inversely correlated with CSF p-tau and t-tau and cognitive function (Nicholas Ashton; *Alzheimer's Association International Conference 2019*). In addition, proteomic analyses of CSF have identified new synaptic proteins that are altered in the very early stages of the disease,<sup>111</sup> or that differ between individuals that have a fast cognitive decline and those that are cognitively stable.<sup>112</sup>

### Vascular dysregulation

Cerebrovascular disease and AD share multiple risk factors and concomitant cerebrovascular disease is more frequent in AD than in any other neurodegenerative disease.<sup>113</sup> In fact, vascular dysregulation has been proposed as a causative factor for AD<sup>114</sup> and, in addition, recent studies have proposed that vascular dysfunction is the earliest event in the sequence of pathogenic events that occur in AD.<sup>115,116</sup>

Alterations in the integrity of the blood-brain barrier (BBB) are related to the pathogenicity of AD and other dementias.<sup>117,118</sup> From the fluid biomarker perspective, the CSF/serum albumin ratio has been the standard measure of BBB function in clinical routine practice.<sup>119,120</sup> However, measurement of CSF/serum albumin ratio in AD has produced conflicting results and the meta-analysis by Olsson and colleagues found a minor but statistically significant increase in AD compared with controls.<sup>26</sup> A large population study from the Swedish Dementia Registry observed an increase in the CSF/serum albumin ratio in late-onset AD that was even higher in DLB, VaD, and mixed dementia. The CSF/serum albumin ratio was associated with CSF NfL but not with AD CSF core biomarkers, suggesting that BBB damage is not a specific feature of AD.<sup>121</sup> Other

biomarkers that have been proposed to reflect BBB integrity include tight junction proteins, the proinflammatory cytokine CypA, MMP-9, and blood-derived fibrinogen and plasminogen.<sup>122</sup> In addition, the CSF soluble platelet-derived growth factor receptor- $\beta$  (sPDGFR- $\beta$ ) has been proposed as a marker of pericyte breakdown.<sup>117,123</sup>

The endothelial markers, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) have been studied in AD. Previous investigations of CSF and blood VCAM-1, and ICAM-1 have produced conflicting results, but a recent study found that the CSF levels of these biomarkers were increased in preclinical, prodromal, and dementia stages of AD, and correlated with CSF tau, A $\beta$ , cortical thinning, and subsequent cognitive deterioration in nondemented patients.<sup>124</sup> In addition, the same study found that an increase in CSF IL-15 and Flt-1, which are proteins also related to vascular dysfunction and inflammation. Blood levels of VCAM-1 are increased in AD and vascular dementia patients compared with elderly controls.<sup>125,126</sup>

### Inflammation

Inflammation and innate immunity play a crucial role in AD pathogenesis from the very early stages.<sup>127–129</sup> Misfolded and aggregated A $\beta$  and tau proteins bind to pattern recognition receptors on microglia and astroglia and trigger an innate immune response.<sup>127</sup> This inflammatory response is heterogeneous and may have a beneficial effect (e.g. clearance of A $\beta$  and tau deposits, removal of cellular debris)<sup>130</sup> or, in contrast, a detrimental one (e.g. accelerated neurodegeneration).<sup>131</sup> In addition, the pattern of neuroinflammatory response may change throughout the AD *continuum*, exerting opposite effects at different stages.<sup>132</sup> Several inflammatory-related biomarkers are available but only help to determine partial aspects of the complex innate immune response. Thus, there is a need to develop and validate biomarkers, or combinations of them, that reflect different patterns of inflammatory response along the Alzheimer's *continuum*. Unfortunately, results on inflammatory-related biomarkers are inconsistent between studies.<sup>133,134</sup> In this study we review the most promising ones.

### Soluble triggering receptor expressed on myeloid cells 2

Triggering receptor expressed on myeloid cells 2 (TREM2) is an innate immune receptor of the immunoglobulin family that is expressed on the plasma membrane of myeloid lineage cells, including microglial cells in the central nervous system (CNS). TREM2 has multiple roles in microglia including migration, proliferation, cytokine release, phagocytosis, lipid sensing, APOE binding, and shielding of amyloid plaques.<sup>135–141</sup> Homozygous loss-of-function mutations in *TREM2* cause Nasu–Hakola disease and FTD-like syndrome, that are rare neurodegenerative diseases characterized by an early-onset frontal syndrome and, in Nasu–Hakola disease, by a concomitant bone involvement.<sup>142,143</sup> Low frequency coding variants in *TREM2* have also been associated with an increased risk for AD<sup>144,145</sup> and other neurodegenerative diseases. TREM2 is a type-1 transmembrane protein that is shed at the C-terminus of histidine 157 by ADAM10 and 17, and its soluble ectodomain (sTREM2) is released into the extracellular space<sup>138,146</sup> and can be measured in CSF and blood.<sup>138,147</sup> CSF sTREM2 may reflect the amount of TREM2 competent signaling on the surface of microglia and can, therefore, be used as a marker of the TREM2-mediated microglia response. However, to the best of our knowledge, it is still not known whether sTREM2 has a specific biological function other than acting as a decoy receptor opposing full-length TREM2 signaling.

Most studies have reported increased levels of CSF sTREM2 in AD *versus* controls,<sup>128,148–155</sup> although this was not found in all studies.<sup>138,156</sup> Further studies have demonstrated that CSF sTREM2 dynamically changes throughout the AD *continuum* and reaches a peak in the later asymptomatic stages and early symptomatic stages of late-onset AD and autosomal-dominant AD.<sup>128,150,151,155</sup> CSF sTREM2 is closely associated with tau-related neurodegeneration but not with A $\beta$  pathology.<sup>150</sup> In addition, CSF sTREM2 increases in MS and other neuroinflammatory diseases,<sup>147,157</sup> HIV1 infections,<sup>158</sup> in dementia patients with delirium,<sup>159</sup> and in those patients with a biomarker profile for suspected non-Alzheimer pathology (SNAP).<sup>150,151</sup> This suggests that a TREM2-mediated microglial response occurs whenever there is neural injury, and not only in AD. Of note, CSF sTREM2 normalizes in MS after treatment with natalizumab or



mitoxantrone,<sup>157</sup> which indicates that CSF sTREM2 may be used to test treatment response. In contrast, CSF sTREM2 has not been shown to be increased in HD,<sup>160</sup> FTLT,<sup>138,161</sup> or in repetitive head impacts.<sup>162</sup>

Despite the changes found in CSF sTREM2 in AD, there is a considerable overlap in the values between AD and controls that precludes its use as a diagnostic marker. However, there is one situation where sTREM2 may be useful for diagnostic purposes. Both CSF and blood sTREM2 are undetectable in patients with Nasu–Hakola disease and FTD-like syndrome.<sup>138,156</sup> Individuals bearing low frequency *TREM2* coding variants, in contrast, may have increased, decreased, or unchanged sTREM2 levels compared with non-carriers of these variants.<sup>148,150,153,163</sup>

A major question in this field is whether TREM2 has a beneficial or a detrimental effect. Human neuroimaging cross-sectional and longitudinal studies have shown that higher CSF sTREM2 is associated with increased gray matter volume and reduced diffusivity in early AD.<sup>164,165</sup> More importantly, a recent longitudinal study in the ADNI cohort has demonstrated that in individuals with biomarker evidence of amyloid and tau pathology, regardless of the clinical syndrome, higher CSF sTREM2 at baseline is associated with an attenuated decline in memory and global cognition at follow-up.<sup>130</sup> In addition, common variants of the *MS4A* gene are associated with increased CSF sTREM2 concentrations. Of interest, these variants are additionally associated with reduced AD risk and delayed age at onset of disease.<sup>163</sup> Altogether, this suggests a beneficial effect of TREM2 function.

In contrast with CSF sTREM2, blood sTREM2 has been considerably less studied. No changes have been found in plasma or serum sTREM2 in MS, neuroinflammatory diseases, AD, FTD, or PD.<sup>138,147,148,153</sup> Of interest, higher serum sTREM2 levels predict a higher risk of developing dementia in the Japanese population.<sup>166</sup>

### Progranulin

Progranulin is a secreted glycoprotein mainly expressed in activated microglia and, to a lesser extent, in neurons in the CNS. It contains 7.5

tandem repeats, that form the granulin domains. Proteolytic processing of full-length progranulin leads to the formation of individual granulins that are released into the extracellular space.<sup>167,168</sup> Among other biological functions, progranulin and the granulins are involved in the modulation of neuroinflammation. Progranulin is best known for its relation to FTLT because loss-of-function mutations of the progranulin gene (*GRN*) cause FTLT-TDP. *GRN* mutation carriers have decreased blood and CSF progranulin levels. However, progranulin is related to AD because some *GRN* variants increase the risk for AD<sup>169,170</sup> and studies in mice models have shown that progranulin has an impact in  $\beta$ -amyloid and tau pathology.<sup>171–173</sup> In addition, these *GRN* variants have an effect on blood and CSF progranulin levels but to a lesser extent than the FTLT-related mutations.<sup>170,174,175</sup>

Unlike FTLT, most studies had reported no differences in CSF progranulin levels between AD, MCI, and controls.<sup>174–177</sup> However, a larger cross-sectional study carried out in the ADNI cohort demonstrated that CSF progranulin increases over the course of autosomal-dominant AD and late-onset AD and is associated with cognitive decline and markers of neurodegeneration.<sup>178</sup> Of note, higher CSF progranulin was associated with higher sTREM2 in AD but not in healthy controls.<sup>178</sup> This suggests that, when there is underlying pathology, microglia secrete sTREM2 and PGRN which, interestingly, may have opposing effects.<sup>179</sup> Results for blood progranulin are not very promising because no changes in AD have been found,<sup>175,180</sup> and blood progranulin levels do not reflect progranulin levels in the brain.<sup>174</sup> Overall, CSF and blood progranulin are only useful diagnostic markers to screen *GRN* mutations in FTLT, but not in AD.

### YKL-40

YKL-40, which is also known as chitinase 3-like protein 1 (CHI3L1) or human cartilage glycoprotein 39 (hCGP-39) is upregulated in several inflammatory diseases and cancers and its biological function may be related to remodeling during inflammation.<sup>181</sup> In the CNS there is an increasing amount of evidence suggesting that YKL-40 is predominantly an astroglial protein.<sup>181,182</sup> In AD, CSF YKL-40 levels are

associated with tau pathology and neuroimaging variables including cortical thickness in amyloid positive patients and gray matter volume in *APOE-ε4* carriers.<sup>183–185</sup>

Several studies have found an increase in CSF YKL-40 in AD patients compared with controls and these results have been confirmed by a recent meta-analysis.<sup>26,183</sup> CSF YKL-40 increases with disease progression as demonstrated in longitudinal studies<sup>91,186</sup> and it is positively correlated with biomarkers of neurodegeneration. Some studies have even reported a significant increase of CSF YKL-40 in preclinical AD.<sup>39,185</sup> CSF YKL-40 can predict progression from cognitively unimpaired to MCI<sup>183</sup> and from MCI to AD dementia.<sup>84</sup> Increases in CSF YKL-40 are not specific for AD and can be found in other neurological diseases.<sup>187–189</sup> Of interest, CSF YKL-40 levels are unchanged or even decreased in PD without dementia.<sup>190–192</sup>

A few studies have assessed YKL-40 in blood. There may be a trend for elevated plasma YKL-40 in AD compared with controls.<sup>183,193</sup> Overall, CSF YKL-40 might have a limited diagnostic value, but it can be useful for tracking astroglial activation, assessing response to treatments targeting neuroinflammation, and the identification of subgroups of patients or disease staging based on inflammatory processes.

#### *Other inflammation markers*

Several other inflammatory biomarkers for AD have been studied but with heterogeneous and inconsistent results.<sup>133</sup> CSF human interferon-inducible protein-10 (IP-10) appears to increase in MCI and mild AD, although this is not consistent across studies.<sup>192,194</sup> In addition, plasma IP-10 has shown conflicting results.<sup>115,195</sup> Glial fibrillary acidic protein (GFAP) in CSF does not differ in AD compared with controls in most studies.<sup>26</sup> However, a recent study found increased CSF and serum GFAP in AD patients and a significant correlation between serum GFAP and cognitive decline.<sup>196</sup> Monocyte chemoattractant protein-1 (MCP-1) is slightly elevated in CSF of AD patients, and no differences are found in the blood.<sup>26</sup> Other inflammatory markers have been reported to be elevated in AD.<sup>133,197</sup> In contrast, serum sirtuin1 decreases with aging, making this reduction more

pronounced in AD patients.<sup>198</sup> Sirtuin1, a NAD-dependent protein deacetylase nuclear receptor, has been proposed as a regulator of aging, metabolic and inflammatory processes involved in AD.<sup>199–201</sup> Overall, most AD-related inflammatory biomarkers do not demonstrate a clear diagnosis potential, but they can be useful in tracking different patterns of inflammatory response to AD pathology.

#### **TDP-43 pathology**

Transactive response DNA-binding protein of 43 kDa (TDP-43) is a DNA and RNA-binding protein that is found phosphorylated in the ubiquitin inclusions in most cases of ALS and in FTLTDP.<sup>202,203</sup> Remarkably, TDP-43 deposits are found in elderly people and in 20–50% of patients with AD.<sup>204–209</sup> TDP-43 copathology in AD is probably not a mere bystander but it is associated with memory loss and brain hippocampal atrophy.<sup>207,210,211</sup> Recently, a new entity called ‘limbic-predominant age-related TDP-43 encephalopathy’ (LATE) has been described.<sup>212</sup> The neuropathological changes of LATE are characterized by deposits of TDP-43 in the amygdala, hippocampus, and middle frontal gyrus, and is sometimes accompanied by hippocampal sclerosis. LATE usually presents with an amnesic syndrome and can, therefore, mimic AD. A few studies have investigated TDP-43 in AD. Plasma TDP-43 levels may indicate the amount of TDP-43 pathology in AD.<sup>213</sup> Another study showed that plasma TDP-43 is increased in AD and progressive MCI.<sup>214</sup> The availability of TDP-43 biomarkers would not only be useful to diagnose ALS and FTLTDP but also to detect the TDP-43 pathology burden in AD. They would help to discriminate between LATE and other neurodegenerative diseases. However, the use of TDP-43 as a biomarker is restricted by the technical difficulties to specifically detect the protein.<sup>215</sup>

#### **$\alpha$ -synuclein pathology**

$\alpha$ -synuclein is the major component of Lewy bodies and Lewy neurites, which are intracellular inclusions that constitute the pathological hallmark of PD and DLB.<sup>216</sup> In addition,  $\alpha$ -synuclein aggregates can be found in multiple system atrophy (MSA), predominantly in oligodendrocytes

and neurons.<sup>217</sup> In combination, PD, DLB, and MSA are referred to as  $\alpha$ -synucleinopathies. Of interest, over half of AD patients (including late-onset AD, autosomal-dominant AD, and Down syndrome) have Lewy pathology, especially in the amygdala.<sup>218,219</sup> The pathological contribution of  $\alpha$ -synuclein in AD is unclear, but it may promote  $\beta$ -amyloid and tau aggregation.<sup>220,221</sup> CSF  $\alpha$ -synuclein has mainly been studied as a biomarker for  $\alpha$ -synucleinopathies and most studies have observed a decrease in CSF  $\alpha$ -synuclein levels in PD and DLB compared with controls or AD.<sup>222–225</sup> In AD, CSF  $\alpha$ -synuclein is slightly higher compared with controls.<sup>26,226</sup> CSF  $\alpha$ -synuclein is higher in those MCI patients that rapidly progress to AD.<sup>227</sup> Of interest, a set of AD patients have high CSF p-tau but low CSF  $\alpha$ -synuclein, and it has been speculated that this may reflect AD patients with Lewy bodies. In CJD, CSF  $\alpha$ -synuclein is significantly increased.<sup>222,228</sup> In blood, no differences have been found in plasma  $\alpha$ -synuclein between AD and controls,<sup>229,230</sup> but one study observed that plasma  $\alpha$ -synuclein increased in PD compared with controls.<sup>231</sup> Of note, most studies on  $\alpha$ -synuclein have measured the full-length protein and research is being carried out to detect  $\alpha$ -synuclein fragments and oligomers in biological fluids. It is also important to note that  $\alpha$ -synuclein biomarkers may simply reflect age-related copathologies rather than specific AD pathologies.

### Sex differences in fluid AD biomarkers

There is an increasing body of evidence that sex, gender, or both have an effect on the epidemiology, pathogenesis, clinical presentation, course, and treatment response in AD. This is, however, a complex topic because multiple social and biological factors are involved. We will focus on sex differences, although gender (i.e. social and cultural differences rather than biological) have an impact on AD.<sup>232,233</sup> The prevalence of AD dementia is higher in women than in men<sup>234</sup> and women appear to have a faster cognitive decline and brain atrophy rate once symptoms have started.<sup>235</sup> Some AD risk factors (e.g. depression and sleep disorders) are more prevalent in women<sup>232,236</sup> and *APOE- $\epsilon$ 4* confers a greater AD risk in women than men.<sup>237</sup> In addition, early menopause is associated with a higher risk of cognitive decline and AD neuropathology.<sup>238,239</sup> Thus, understanding the role of sex in AD is

crucial to develop a precision medicine approach in AD.<sup>232</sup>

Although sex is generally included as a covariable in biomarker studies, only a few studies specifically focus on the effect of sex in biomarkers. Table 2 reviews the main differences on AD-related biomarkers between women and men. In most studies, no sex differences have been found in AD core biomarkers.<sup>240–244</sup> However, a few studies found higher basal levels of t-tau in women compared with men in the whole cohort,<sup>243</sup> or solely in the AD group,<sup>245</sup> which is consistent with the fact that women have higher levels of tau pathology compared with men in pathological and PET studies.<sup>246,247</sup> In addition, the association between *APOE- $\epsilon$ 4* and CSF t-tau and p-tau is stronger in women than in men.<sup>240,243</sup> Although most studies did not find differences in A $\beta$ 42 between women and men, one study demonstrated a modifying effect of gender and *APOE* on the evolution of CSF A $\beta$ 42 linked to age. Specifically, women carrying an *APOE- $\epsilon$ 4* allele have stable CSF A $\beta$ 42 levels until the age of 50 years old but they then show a faster decline.<sup>242</sup>

With regard to other biomarkers, the data available on sex differences is limited. A recent study including patients along the AD *continuum* found higher CSF NFL levels in men than in women.<sup>186</sup> CSF sAPP $\alpha$  and sAPP $\beta$  have been reported to be higher in women with AD,<sup>248</sup> and CSF E-selectin and ICAM1 and VCAM1 are higher in men.<sup>124,242</sup> Inflammatory biomarkers are the markers that appear to be more influenced by sex. CSF progranulin is consistently higher in men than in women<sup>174,175,178</sup> but, surprisingly, plasma or serum progranulin are higher in women. In addition, CSF sTREM2 may be slightly higher in men, although the difference is not as pronounced as in CSF progranulin.<sup>148</sup>

### Conclusion

Overall, the effect of sex and gender on AD and, in particular, on AD fluid biomarkers, requires further research because it will help to improve the understanding of the disease and, especially, in the design of preventive and therapeutic strategies. The Women's Brain Project and the Alzheimer Precision Medicine Initiative have

**Table 2.** Sex differences in AD-related fluid biomarkers.

	CSF		Plasma/Serum	
<b>A<math>\beta</math>42 or A<math>\beta</math>42/40</b>	F = M	No differences found <sup>88,240-244</sup>	○	unknown
<b>sAPP<math>\alpha</math> and sAPP<math>\beta</math></b>	F $\geq$ M	Most studies have not assessed it. One study found higher levels in AD women. <sup>248</sup>	○	unknown
<b>t-tau</b>	F $\geq$ M	Most studies have not reported differences. A few showed higher levels in women (controls, MCI, and AD) <sup>243,245</sup>	F = M	Most studies have not assessed it. One study reported no differences <sup>59</sup>
<b>p-tau</b>	F = M	Most studies have not reported differences <sup>240-243,249</sup>	○	unknown
<b>NfL</b>	F $\leq$ M	Most studies have not assessed it. A few had reported no differences, <sup>69,250</sup> but a recent study found higher levels in men. <sup>186</sup>	F = M	Only a few studies available and no differences found <sup>72,78</sup>
<b>VILIP-1</b>	F = M	Most studies have not assessed it. A study reported no sex differences in controls and AD <sup>88</sup>	○	unknown
<b>VCAM1</b>	F $\leq$ M	Most studies have not assessed it. A few have found higher levels in men. <sup>124,242</sup>	F $\leq$ M	One study found higher levels in men. <sup>125</sup>
<b>ICAM1</b>	F $\leq$ M	Most studies have not assessed it. One study found higher levels in men <sup>171</sup>	○	unknown
<b>sTREM2</b>	F $\leq$ M	Most studies have not reported differences. <sup>150,151</sup> One study found higher levels in men (controls and AD) <sup>148</sup>	F = M	Only one study available and did not find differences <sup>148</sup>
<b>Progranulin</b>	F $\leq$ M	A number of studies have reported higher levels in men (controls, MCI, and AD) <sup>174,175,178</sup>	F $\geq$ M	Very few studies available. One study found higher levels in women while another did not find differences <sup>174,175</sup>
<b>YKL-40</b>	F = M	A number of studies have assessed it and no differences have been reported <sup>183,186,251-253</sup>	F = M	Few studies have assessed it and reported no differences <sup>183,193</sup>
<b>MCP-1</b>	F = M	Most studies have not assessed it. One study reported no differences <sup>251</sup>	F = M	Most studies have not assessed it. A few have reported no differences <sup>194,254</sup>
<b><math>\alpha</math>-synuclein</b>	F = M	Most studies have not assessed it. Few have reported no differences <sup>226,255,256</sup>	○	unknown

The table depicts the differences found in AD-related biomarkers between women and men in AD (prodromal, dementia, or both), the controls or both. ○ indicates unknown. We reviewed all studies available in AlzBiomarker for GFAP, MCP-1, neurogranin, NfL, sAPP $\alpha$ , sAPP $\beta$ , sTREM2, VILIP-1, YKL-40, and  $\alpha$ -synuclein. For A $\beta$ , t-tau, and p-tau we reviewed those studies in AlzBiomarker with a total number of individuals >300. CSF/Serum Albumin ratio, GFAP, and neurogranin are not included in the table because there is no data available on sex differences either in CSF or blood. In addition, we included biomarkers where there is evidence of sex differences.

A $\beta$ ,  $\beta$ -amyloid; CSF, cerebrospinal fluid; ICAM-1, intercellular adhesion molecule-1; MCP-1, Monocyte chemoattractant protein-1; NfL, neurofilament light; p-tau, phosphorylated tau; sAPP, soluble N-terminal fragment of APP; sTREM2, soluble triggering receptor expressed on myeloid cells 2; t-tau, total tau; VILIP-1, visinin-like protein 1; YKL-40, chitinase 3-like protein 1.

recently published some recommendations to improve the design, analysis, and reporting of sex and gender differences in future studies.<sup>232</sup>

### Acknowledgments

The authors would like to thank Elsa Velasco for the scientific illustrations included in this review. We also recognize and have heartfelt gratitude to

Mrs Blanca Brillas for her outstanding and continued support to the Pasqual Maragall Foundation that makes possible a future without Alzheimer's.

### Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

### Conflict of interest statement

JLM received advisory honoraria from Alergan, Roche diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector and Raman Health. He also received research support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking AMYPAD grant agreement n° 115952; the EU/EFPIA Innovative Medicines Initiative Joint Undertaking EPAD grant agreement n° 115736; and 'la Caixa' Foundation. MSC received funding from the European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie action grant agreement No 752310.

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