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1 **Title:** An update on blood-based biomarkers for non-Alzheimer neurodegenerative disorders

2  
3 Nicholas J. Ashton<sup>1, 2, 3, 4</sup>, Abdul Hye<sup>3, 4</sup>, Anto P. Rajkumar<sup>3, 4, 5</sup>, Antoine Leuzy<sup>2, 6</sup>, Stuart Snowden<sup>7</sup>, Marc  
4 Suárez-Calvet<sup>1, 8, 9</sup>, Thomas K. Karikari<sup>1</sup>, Michael Schöll<sup>1, 2, 6, 10</sup>, Renaud La Joie<sup>11</sup>, Gil D. Rabinovici<sup>11</sup>, Kina  
5 Höglund<sup>1, 12</sup>, Clive Ballard<sup>13</sup>, Tibor Hortobágyi<sup>3, 14</sup>, Per Svenningsson<sup>3, 15</sup>, Kaj Blennow<sup>1, 16</sup>, Henrik Zetterberg<sup>1,</sup>  
6 <sup>16, 17, 18</sup> & Dag Aarsland<sup>3, 4, 19</sup>

7  
8 <sup>1</sup>Department of Psychiatry and Neurochemistry, Institute of Neuroscience & Physiology, the Sahlgrenska  
9 Academy at the University of Gothenburg, Mölndal, Sweden; <sup>2</sup>Wallenberg Centre for Molecular and Translational  
10 Medicine, University of Gothenburg, Gothenburg, Sweden; <sup>3</sup>King's College London, Institute of Psychiatry,  
11 Psychology & Neuroscience, Maurice Wohl Clinical Neuroscience Institute, London, UK; <sup>4</sup>NIHR Biomedical  
12 Research Centre for Mental Health & Biomedical Research Unit for Dementia at South London & Maudsley NHS  
13 Foundation, London, UK; <sup>5</sup>Institute of Mental Health, University of Nottingham, Nottingham, UK; <sup>6</sup>Clinical  
14 Memory Research Unit, Lund University, Malmö, Sweden; <sup>7</sup>Core Metabolomics and Lipidomics Laboratory,  
15 Metabolic Research Laboratories, Institute of Metabolic Science, University of Cambridge, Cambridge  
16 Biomedical Campus, Cambridge, UK; <sup>8</sup>Barcelonaβeta Brain Research Center (BBRC), Pasqual Maragall  
17 Foundation, Barcelona, Catalonia, Spain; <sup>9</sup>Department of Neurology, Hospital del Mar, Barcelona, Catalonia,  
18 Spain; <sup>10</sup>Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK; <sup>11</sup>Memory and  
19 Aging Center, University of California, San Francisco, San Francisco, CA, United States; <sup>12</sup>Department of  
20 Neurobiology, Care Sciences and Society, Center for Alzheimer Disease Research, Neurogeriatrics Division,  
21 Karolinska Institutet, Novum, Huddinge, Stockholm, Sweden; <sup>13</sup>Medical School, University of Exeter, Exeter,  
22 UK; <sup>14</sup>MTA-DE Cerebrovascular and Neurodegenerative Research Group, Department of Neurology, University  
23 of Debrecen, Debrecen, Hungary; <sup>15</sup>Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; <sup>16</sup>Clinical  
24 Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden; <sup>17</sup>Department of  
25 Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK; <sup>18</sup>UK Dementia Research  
26 Institute at UCL, London, UK; <sup>19</sup>Centre for Age-Related Medicine, Stavanger University Hospital, Stavanger,  
27 Norway

## 28 29 **Key points**

- 30 • The neurodegenerative disorders (NDDs) are characterized by protein and other pathologies which can  
31 be reflected in biofluids.
- 32 • The use of cerebrospinal fluid (CSF) analysis and molecular imaging has been critical in stratifying  
33 populations based on diagnosis and underlying pathology, but are limited as population screening tools.
- 34 • Advances in ultra-sensitive immunoassay measurement of amyloid- $\beta$ , neurofilament light and tau, as  
35 well as mass spectrometry-based methods for amyloid, have demonstrated that a blood-based screening  
36 tool for Alzheimer's disease (AD) is a realistic and plausible possibility.
- 37 • This evidence is now indicating that such blood biomarkers could be important for other common NDDs  
38 (e.g, LBD & FTD).

39  
40 **Corresponding author:** Professor Dag Aarsland, [dag.aarsland@kcl.ac.uk](mailto:dag.aarsland@kcl.ac.uk)

51 **Abstract**

52 In recent years, there has been an increasing emphasis on the importance of blood-based biomarkers in the first-  
53 in-line evaluation of patients with suspected neurodegenerative disorders (NDDs). While neuroimaging (structural  
54 and molecular) and cerebrospinal fluid (CSF) analyses identify the underlying pathophysiology at the earliest  
55 stage, a biologically relevant marker derived from blood would have greater utility in the primary care setting and  
56 in the early eligibility screening for therapeutic trials. The rapid advancement of ultra-sensitive assays has enabled  
57 the investigation of pathological proteins to be measured in blood samples, but research has been predominately  
58 focused on Alzheimer's disease (AD). Nonetheless, proteins that are currently under scrutiny as blood biomarker  
59 candidates for AD (amyloid- $\beta$ , tau and neurofilament light chain) are likely to have fundamental importance for  
60 Lewy body dementia's (LBD), frontotemporal dementia (FTD) and other NDDs in terms of shared pathologies,  
61 similar degenerative processes or in the differential diagnosis of clinical symptoms. This review gives an overview  
62 and update on the current status of blood-based biomarkers for the non-AD NDDs, focusing on how putative AD  
63 and novel protein, metabolomic and RNA biomarkers perform in these populations. As background information,  
64 we also briefly outline the neuropathological, clinical, molecular imaging and CSF features of the most common  
65 NDDs outside of the AD continuum.

66  
67 **Introduction**

68 Age-related cognitive disorders represent a major and escalating societal challenge due to the growing number of  
69 elderly people. Many failed anti-dementia trials have been published, and one potential reason is a lack of synergy  
70 between drug and disease mechanisms. Precision medicine, *i.e.* characterization of the individual's phenotype and  
71 genotype for stratifying the right patient to the appropriate therapy, is therefore fundamentally important. To  
72 achieve this, accurate, minimally invasive, safe, and inexpensive biomarkers are needed that can be broadly  
73 administered to communities worldwide.

74 The foremost neurodegenerative disorders (NDDs) are characterized by aggregates of abnormal proteins found in  
75 the central nervous system (CNS), which allows for a mechanism-based proteomic biomarker search. Six hallmark  
76 proteins enable the classification of most NDDs: two of them are extracellular, amyloid- $\beta$  (A $\beta$ ) and the prion  
77 protein (PrPsc), four are intracellular: tau, alpha-synuclein ( $\alpha$ -synuclein), TAR DNA-binding protein 43 (TDP-  
78 43) and fused in sarcoma (FUS)<sup>1</sup>, leading to amyloidopathies, prionopathies, tauopathies,  $\alpha$ -synucleinopathies,  
79 TDP43-proteinopathies, respectively. The neurodegenerative pathologies often coexist and additional vascular  
80 changes are also prevalent causing clinical and neuropathological heterogeneity<sup>1</sup>. The numerous triplet disease  
81 disorders (spinocerebellar ataxias, Huntington's disease) are not included in this list, because they form, to some  
82 extent, a separate group of genetically defined movement disorders.

83 The presenting clinical manifestations and syndromes vary between NDDs but are related to the severity, type,  
84 and regional distribution of the proteinopathies (Table 1). Whereas AD is typically characterized by memory  
85 impairment, aphasia, apraxia, and agnosia, related to the involvement of medial temporal lobe and parietal cortex,  
86 the frontotemporal dementias (FTDs) are characterized by behavioral and language changes, and Lewy body  
87 dementias (Parkinson disease dementia (PDD) and dementia with Lewy Bodies (DLB)) by executive, attentional,  
88 and visuospatial impairment and non-cognitive symptoms such as parkinsonism, REM-sleep behaviour disorder,  
89 autonomic symptoms and visual hallucinations. The neuroanatomical distribution of proteinopathy pathology help  
90 to establish consensus protocols for neuropathological assessment and diagnosis<sup>1</sup>. The clinico-pathological  
91 correlation is however difficult to establish. In addition, most NDDs are heterogeneous diseases, *i.e.* combinations  
92 of proteinopathies, thus biomarkers, such as imaging and proteomic analysis, are crucial for accurate diagnosis  
93 which may allow detection in early prodromal or even pre-clinical stages for early interventions when available.  
94 With the exception of AD, where the most recent diagnostic criteria<sup>2-5</sup> and research framework<sup>6</sup> include  
95 biomarkers to establish the typical proteinopathy, non-AD NDDs are diagnosed by clinical features, although  
96 biomarkers can aid in the identification process.

97 Structural Magnetic Resonance Imaging (MRI) provides regional measures of brain atrophy, reflective of  
98 neurodegenerative processes, including dendritic pruning, synaptic loss and neuronal depletion. As dementia  
99 disorders are associated with spatially distinct patterns of regional volume loss<sup>7</sup>, MRI based markers of atrophy  
100 are included in certain diagnostic criteria for non-AD NDDs<sup>8-10</sup>. The introduction of *in vivo* positron emission  
101 tomography (PET) brain imaging has had a transformative impact in the context of NDDs, helping to both refine  
102 disease progression models and serve as a powerful diagnostic aid, complementing clinical and cognitive  
103 evaluations. Beginning with metabolic imaging using 2-deoxy-2-[<sup>18</sup>F]fluoro-D-glucose ([<sup>18</sup>F]FDG), supposedly  
104 reflective of neuronal or synaptic integrity<sup>11</sup>, the field next saw the introduction of, amongst many others, ligands  
105 capable of mapping and quantifying fibrillar A $\beta$ <sup>12</sup> and more recently, ligands specific for paired-helical filament  
106 (PHF) tau<sup>13,14</sup> and synaptic density<sup>15</sup>.

107 The clearance of abnormal proteins via the cerebrospinal fluid (CSF) is an endogenous neuroprotective  
108 mechanism of the brain. Not only for extracellular A $\beta$ , but also intracellular and synaptic proteins can leak into  
109 the CSF, and their reductions or accumulation can be used as a disease or disease progression biomarkers.  
110 However, a blood-based measure of such pathologies has substantial practical and economic advantages over  
111 imaging and CSF biomarkers currently utilized in clinical and research settings. Molecular imaging is costly, and  
112 access is limited to specialised centres. CSF analysis is more affordable and attainable but there remains a  
113 perceived invasiveness attached to a lumbar puncture, which may limit its use in clinical practice, depending on  
114 the healthcare system. Therefore, a blood-based marker would be of extreme value as a simplified initial triage  
115 step in a multi-stage assessment for cognitive complaints, secondary prevention trial selection or monitoring  
116 response to intervention.

117 In AD, there is already excellent imaging (FIG.1.)<sup>16</sup>, CSF<sup>17</sup> and promising blood biomarkers being developed  
118 (Table 2)<sup>18</sup>. In contrast, fluid biomarkers in non-AD NDDs remain in their infancy but will greatly benefit from  
119 the developments in the AD field. In this review, as background, we first briefly summarize clinical and  
120 neuropathological features of the most common non-AD NDDs and discuss the main findings from imaging and  
121 CSF studies. The focus is on the developing topic of blood-based biomarkers in key non-AD NDDs, such as LBD  
122 or FTD. After briefly reviewing the lessons from AD, we will discuss how this can inform our understanding of  
123 non-AD NDDs and consider disease specific biomarkers in these other neurodegenerative conditions.

124

## 125 Neuropathological, clinical and imaging overview of non-Alzheimer NDDs

### 126 *Parkinson's disease, Parkinson's disease dementia and dementia with Lewy Bodies*

127 Parkinson's disease (PD) is the second most common neurodegenerative disease (exceeded only by AD) and is  
128 characterized by the accumulation of  $\alpha$ -synuclein in inclusions known as Lewy bodies and Lewy neurites. The  
129 frequency of PD increases with aging (the mean age of onset of approximately 60 years) and the lifetime risk is  
130 slightly higher for men than for women. Although most cases are sporadic, some rare cases are familial. The  
131 pathological hallmark is the progressive loss of nigrostriatal dopaminergic neurons of the substantia nigra pars  
132 compacta. As a result of this dopaminergic pathology, PD typically manifests with a parkinsonian syndrome or  
133 parkinsonism, which is defined by the combination of the following motor clinical features: rest tremor, rigidity,  
134 bradykinesia and gait dysfunction with postural instability<sup>19</sup>. Of note, PD is the most common cause of  
135 parkinsonism but not the only one. Other neurodegenerative disease (*e.g.* progressive supranuclear palsy (PSP),  
136 cortical basal degeneration (CBD) or FTD) or secondary causes (*e.g.* metabolic, toxic, drug-induced, and vascular)  
137 can also lead to a parkinsonism. Besides the motor clinical features, PD also manifests with non-motor features,  
138 including hyposmia, sleep disorders, autonomic dysfunctions, pain, behavioural disturbances and cognitive  
139 impairments. Remarkably, a considerable number of patients with PD will eventually develop cognitive  
140 impairment and dementia over the course of their illness, a condition termed Parkinson's disease with dementia  
141 (PDD)<sup>20,21</sup>. Yet, the timing of the onset of dementia is highly variable and some patients rapidly develop dementia  
142 while others display no signs of cognitive impairment for many years and in some cases never develop  
143 dementia<sup>22,23</sup>. In patients where dementia precedes or arises concomitantly with the motor clinical features, the  
144 patient is diagnosed as dementia with Lewy bodies (DLB)<sup>24</sup>. Together, PD, PDD and DLB constitute the Lewy  
145 body diseases and a considerable clinical and pathological overlap exist between them. In particular, PDD and  
146 DLB are distinguished solely based on the relative timing of parkinsonism and dementia, *i.e.* if dementia occurs  
147 more than one year after the diagnosis of PD, the clinical diagnosis is PDD; whereas patients where dementia  
148 occurs before or simultaneously with parkinsonism are diagnosed as DLB. This distinction is arbitrary, and many  
149 patients are difficult to classify because the timing of cognitive decline and parkinsonism can be difficult to  
150 establish. The cognitive profile of Lewy body diseases varies but differs from that in AD in that it is characterized  
151 by relatively more executive, attentional and visuospatial impairment, although memory is usually impaired and  
152 often the first reported symptom. Interestingly, there are lesions outside the brain, with involvement of the  
153 autonomic nervous system leading to characteristic symptoms such as orthostatic hypotension and constipation.  
154 Among the neuropsychiatric symptoms, visual hallucinations and REM-sleep behavior disorder (RBD) are typical  
155 of Lewy body diseases.

156 In addition to the Lewy body and  $\alpha$ -synuclein pathology, DLB and PDD often show varying degrees of AD co-  
157 pathology<sup>22</sup>. The clinicopathologic correlation with the extent and severity of  $\alpha$ -synuclein pathology is often  
158 blurred by the co-existing AD pathology, which has to be considered when the degree of probability is established  
159 regarding  $\alpha$ -synuclein being the cause of clinical symptoms<sup>9</sup>. Less frequently, concomitant TDP-43 pathology is  
160 detectable<sup>25</sup>.

161 Beyond the exclusion of secondary causes of parkinsonism— such as vascular, demyelinating or space-occupying  
162 lesions within the brainstem or basal ganglia—conventional T1- and T2-weighted MRI sequences are considered  
163 of limited use in the diagnosis of PD as visual reads are often normal<sup>26,27</sup>. The degree and regional distribution of

164 volumetric loss is variable in DLB, but absent or minimal atrophy of the medial temporal lobe has been identified  
165 as a consistent feature<sup>9</sup>. Recent advances in MRI methodology, including iron-sensitive techniques such as  
166 susceptibility-weighted imaging and quantitative susceptibility mapping, show promise in capturing abnormalities  
167 within the substantia nigra and nigrostriatal system<sup>28</sup>. Using [<sup>18</sup>F]FDG PET, a pattern of temporoparietooccipital  
168 hypometabolism is typically observed in DLB and PD/PDD<sup>29-32</sup>, with the latter additionally showing relative  
169 hypermetabolism in the motor cortex, striatum, thalamus and cerebellum<sup>33</sup>. In keeping with the degeneration of  
170 nigrostriatal dopamine neurons as a defining feature of DLB and PD/PDD, dopaminergic function, whether  
171 measured by SPECT or PET, is markedly decreased in both<sup>34,35</sup>. Using amyloid- $\beta$  imaging, retention levels have  
172 been shown to be low in PD patients, somewhat increased in PDD and elevated in DLB<sup>36-39</sup> and to associate with  
173 cognitive decline<sup>37,38,40</sup>. In DLB and PD/PDD, early tau PET findings have varied, yielding rather inconsistent  
174 results between studies, with cortical ligand binding overlapping with controls<sup>41-43</sup>. [<sup>18</sup>F]Flortaucipir—and,  
175 possibly, related newer tau compounds<sup>44</sup>—has been shown to bind to neuromelanin in the substantia nigra<sup>45,46</sup>. As  
176 such, tau PET may be of use in PD/PDD due the characteristic loss neuromelanin rich neurons in this region.  
177 Overall, is not yet clear how tau pathology contributes to the development of these disorders<sup>47,48</sup>.

178

### 179 Frontotemporal Dementia (FTD)

180 FTD is a clinically and pathologically heterogeneous group of NDDs that predominantly exhibit frontal and/or  
181 temporal involvement. There are two main clinical presentations of FTD: the behavioural variant (bvFTD), which  
182 mainly leads to personality alterations and behavioral problems, and the less common primary progressive  
183 aphasia (PPA), which cause progressive deterioration of speech and/or language<sup>49</sup>, and which can be further  
184 subtyped into semantic (svPPA), non-fluent (nfvPPA). The third subtype of PPA, the logopenic variant (lvPPA),  
185 is usually associated with classical AD pathology. The international consensus criteria<sup>50</sup> defines possible bvFTD  
186 by the persistence or recurrence of at least three of the following symptoms, (i) early behavioural disinhibition,  
187 (ii) apathy, (iii) loss of empathy, (iv) perseverative, stereotyped, compulsive or ritualistic behaviours, (v)  
188 hyperorality and dietary changes, (vi) executive deficits<sup>9</sup> with relative preservation of memory and visuospatial  
189 functions, and by progressive deterioration of behaviour and/or cognition<sup>50</sup>. Finally, it is worth mentioning that  
190 familial FTD is observed in approximately a third of all FTD cases<sup>49</sup>. The most common genes involved in FTD  
191 are *MAPT*, *GRN* and *C9orf72*. A probable FTD diagnosis is made, when a suspected clinical FTD is accompanied  
192 by either a causative genetic mutation or neuroimaging evidence of disproportionate involvement of frontal and/or  
193 temporal lobes<sup>51</sup>. Because of their clinical heterogeneity, and the lack of reliable peripheral biomarkers, FTD  
194 continues to pose major diagnostic challenges in clinical settings<sup>52</sup>.

195 While the term FTD is usually applied to the clinical syndromes, the term 'frontotemporal lobar degeneration'  
196 (FTLD) is the neuropathological term. Three hallmark proteins define the FTLD pathological subtypes: (1) TDP-  
197 43, (2) Tau or (3) FET proteins (FUS, EWS and TAF-15). Consequently, FTLD are pathologically classified in  
198 FTLD-TDP, FTLD-tau and FTLD-FET<sup>53</sup>. FTD may overlap clinically and pathologically with motor neuron  
199 disease (MND) or some extrapyramidal syndromes (cortical basal syndrome, CBS or PSP). In fact, the most  
200 common underlying pathology in MND (and, in particular, in amyotrophic lateral sclerosis (ALS), the most  
201 prevalent MND) is also TDP-43 pathology. Some ALS cases are caused by mutations in *C9orf72*, *FUS* (*i.e.* ALS-  
202 *FUS*)<sup>54,55</sup> and have inclusions of demethylated FUS<sup>56,57</sup>. Likewise, the underlying pathology in corticobasal  
203 degeneration (CBD) and PSP is deposits of tau in astrocytes.

204 In bvFTD, MRI studies demonstrate prominent, usually symmetric, atrophy of the frontal lobes<sup>8</sup>. In contrast to  
205 the pattern typically seen in AD, involving the posterior temporal/parietal lobes and the posterior  
206 cingulate/precuneus<sup>58-62</sup>, three main patterns of glucose hypometabolism can classically be observed in FTD:  
207 precentral and inferior frontal in nfvPPA, anterior temporal lobes in svPPA (usually with marked leftward  
208 asymmetry)<sup>63,64</sup>, and frontal as well as temporo-limbic predominant patterns in bvFTD<sup>65</sup>. In case series that have  
209 examined A $\beta$  status among FTD patients, low rates of A $\beta$  positivity have been reported (0-15%), in line with A $\beta$   
210 plaques not being a feature characteristic of the FTLD pathology spectrum<sup>66-68</sup>. In patients across FTD syndromes,  
211 a recent study found low-level elevated tau-PET binding in disease-typical regions in individuals suffering from  
212 nfvPPA (inferior frontal areas), CBS (precentral gyrus and frontal white matter in a subset of cases), and bvFTD  
213 (fronto-temporal regions)<sup>69,70</sup>. Yet, autoradiography studies have suggested that existing tau-PET tracers do not  
214 bind to tau isoforms underlying non-AD tauopathies<sup>71-73</sup>, urging for a cautious interpretation of the *in vivo* PET  
215 findings. Molecular (A $\beta$ , tau) and functional (glucose metabolism) PET scans for an illustrative case of bvFTD—  
216 along with findings in AD, CBS and PSP, for comparative purposes—are shown in FIG. 1.

217

### 218 Cerebrospinal fluid (CSF) biomarkers of non-Alzheimer's NDDs

219 The core CSF biomarkers for AD (A $\beta$ 42, T-tau and P-tau), reflecting the defining A $\beta$  and tau pathologies,  
220 consistently demonstrate diagnostically significant changes across studies<sup>74</sup> and now have prominent positions in  
221 the research diagnostic criteria for AD<sup>5,6</sup>. One way of refining A $\beta$  pathology biomarkers is to combine A $\beta$ 42 and  
222 A $\beta$ 40 in a ratio. This ratio has repeatedly been shown to be a more reliable biomarker for cerebral A $\beta$  pathology  
223 than CSF A $\beta$ 42 alone, most likely by normalizing for inter-individual differences in amyloidogenic APP-  
224 processing<sup>75</sup>. The concentrations of these core AD biomarkers are largely normal in the majority of dementias  
225 outside of AD<sup>76,77</sup>. This can be of great utility in the differential diagnosis of patients with cognitive symptoms.  
226 However, there are isolated exceptions to this rule; A $\beta$ 42 is abnormally decreased in half of DLB cases and many  
227 PDD patients<sup>78,79</sup> which highlights the overlapping pathologies with AD. Furthermore, marked increases of T-tau  
228 in Creutzfeldt-Jakob disease (CJD)<sup>80</sup> is a common observation whereas the concentrations P-tau remain normal  
229 or only marginally changed in CJD<sup>81</sup>. An unpredicted finding is that levels of CSF tau are largely normal in FTD.  
230 This includes concentrations of total tau and specific phosphorylated epitopes (P-tau<sub>181</sub>, P-tau<sub>231</sub> and P-  
231 tau<sub>199</sub>)<sup>77,82,83</sup> and N-terminal tau fragments truncated at 224 (tau6-224 or x-224)<sup>84</sup>. The same holds true for other  
232 primary tauopathies (e.g. PSP)<sup>85,86</sup>. The reason for this remains unclear but may suggest lower secretion of tau  
233 proteins to the extracellular space and the CSF, or alternative processing of full-length tau that are not captured  
234 by the commonly used mid-domain immunological assays.

235 Neurofilament light chain (NFL) is the smallest of the neurofilament triplet proteins that are the structural  
236 components of the axons. NFL is released from the axons in normal ageing, however, in response to axonal  
237 damage (via neurodegeneration, inflammation, vascular or traumatic), NFL release is accelerated into the  
238 extracellular space where its concentration increases in the CSF. Several studies have shown that CSF NFL levels  
239 are highest in brain disorders with subcortical pathology, such as vascular dementia (VaD) and normal pressure  
240 hydrocephalus<sup>87,88</sup>. Notably, CSF NFL concentrations are clearly higher in FTD than in AD with onset of a similar  
241 age<sup>89</sup>, which supports that NFL aids in this differential diagnostic specific situation. In addition, CSF NFL also  
242 shows a very marked increase in CJD (correlating with CSF T-tau)<sup>90</sup>, due to the very extreme level of  
243 neurodegeneration. Importantly, while CSF NFL is relatively normal in pure PD, several studies have shown a  
244 very marked increase in CSF NFL in atypical parkinsonian disorders (APD), specifically in CBS, multiple  
245 systemic atrophy (MSA), and PSP<sup>85,91,92</sup>.

246 Measurements of total monomeric  $\alpha$ -synuclein in CSF has been proposed as a biomarker for PD and DLB, but  
247 most studies only show minor reductions in PD, with considerable overlap between controls and other patient  
248 groups<sup>93</sup>. A meta-analysis that included >3000 subjects across 17 studies also reported significantly lower levels  
249 of CSF  $\alpha$ -synuclein in PD but concluded that  $\alpha$ -synuclein is not yet helpful in the diagnosis of PD or DLB<sup>94</sup>. This  
250 observation might be explained by two reasons: (1)  $\alpha$ -synuclein is present in 10,000-fold higher in blood,  
251 suggesting that CSF contamination may introduce peripheral  $\alpha$ -synuclein not related to neurodegeneration, and  
252 (2) that  $\alpha$ -synuclein levels might be linked to two “pathologies” e.g.  $\alpha$ -synuclein inclusion pathology but  
253 simultaneous leakage to the CSF as a consequence of neurodegeneration. Hence, identification of a brain-specific  
254 pathological forms of  $\alpha$ -synuclein is crucial to advancing disease-specific biomarkers. Recent developments allow  
255 for the assessment of the pathological forms of  $\alpha$ -synuclein in CSF using the real-time quaking-induced  
256 conversion (RT-QuIC) technology. This diagnostic platform explores the self-replicating property of  
257 proteinopathic proteins, with sensitivity and specificity figures for PD and DLB exceeding 90%<sup>95,96</sup>. Importantly,  
258 new variants of  $\alpha$ -synuclein RT-QuIC assays can be performed more rapidly, within 1-2 days<sup>95</sup>, supporting their  
259 use as a diagnostic tool for synucleinopathies and also prionopathies<sup>97</sup>.

260 High CSF levels of the postsynaptic protein neurogranin have repeatedly been found in AD<sup>98-101</sup>. A recent study  
261 confirmed that this marked increase is seemingly specific to AD, while normal concentrations were found in a  
262 wide range of other NDDs, including FTD and DLB<sup>102</sup> but contradictory reports for PD<sup>102,103</sup>. Similar finding has  
263 been reported for presynaptic protein growth-associated protein 43 (GAP-43)<sup>104</sup>. Thus, CSF neurogranin and  
264 GAP-43 may be the latest addition in the toolbox to differentiate AD from non-AD NDDs.

265

## 266 Introduction to blood biomarkers in neurodegenerative research: challenges and technologies

267 Although easily accessible, the complexity of analysing blood content (plasma or serum) must not be  
268 underestimated. Firstly, due to its continuous and uninhibited exchange with the brain, truly brain-derived  
269 molecules will be considerably higher in concentration in the CSF for the same analyte in blood. Blood  
270 communicates with the brain across the blood brain barrier (BBB), via lymph vessels and through the glymphatic  
271 system<sup>105</sup> and on entering the bloodstream, a brain-derived analyte will be diluted in a complex matrix of highly  
272 abundant plasma proteins (e.g. albumin, IgG, transferrin, haptoglobin, and fibrinogen) that span >10 orders of  
273 magnitude. These “matrix effects” can have a large and inconsistent impact on the ability of an immunoassay to  
274 accurately quantify a specific target and can result in misleading conclusions. Moreover, protein biomarkers may  
275 undergo protease degradation, have substantial peripheral expression including in blood cells such as platelets and

276 erythrocytes, exist as multiple isoforms or contain stable and/or dynamic post-translational modifications. Lastly,  
277 analytical factors such as interference from heterophilic antibodies or variations in blood collection methods,  
278 processing and storage, can affect analytes in a different manner. All these factors will introduce a high degree of  
279 variability that is unrelated to the disease itself which can be difficult to account for. The choice of analyzing  
280 plasma or serum is also an important aspect to consider. An analyte of potential interest cannot merely be assumed  
281 to correlate well between these blood fractions<sup>106</sup>. While serum and plasma measures of NFL correlate well, **with**  
282 **serum NFL exhibiting consistently higher concentrations**<sup>107</sup>, it is considered that plasma is the preferred matrix  
283 for A $\beta$ 42, A $\beta$ 40 and T-tau.

284 Proteomic approaches for blood biomarker studies can be simplified into two main strategies; targeted and non-  
285 targeted, where the latter tends to feed into a more analyte-specific platform (FIG. 2). A common non-targeted  
286 “*hypothesis generating*” methodology employed in neurodegenerative research is label-free or isobaric liquid  
287 chromatography tandem mass spectrometry (LC-MS/MS), where a protease-digested peptide mixture is typically  
288 ionised and fragmented for identification and quantification simultaneously. For blood analysis, LC-MS/MS  
289 methods are typically complemented with upfront peptide/protein fractionation or the immunodepletion of highly  
290 abundant plasma proteins which vastly improve the level of identification<sup>108</sup>. LC-MS/MS can also be employed  
291 in a targeted manner if an analyte of interest has been acquired. Selection reaction monitoring (SRM) methods  
292 allows for better precision, more accurate quantification and higher throughput than unbiased LC-MS/MS  
293 methods. Capture-based techniques, that typically involve paired antibodies in the sandwich immunoassay format,  
294 remain the most popular technique for all biomarker analyses.. The combination of antibody capture followed by  
295 mass spectrometry (IP-MS) has been a popular tool for detailed characterization of a target of interest, particularly  
296 in AD biomarker research (*e.g.* A $\beta$  peptides). The fundamental basis for most new generation immunocapture  
297 assay follows the same workflow as a colorimetric enzyme-linked immunoassay (ELISA) format. The emergence  
298 of electrochemiluminescent (ECL) assays has theoretically allowed for the multiplexing of 10 (MesoScale  
299 Discovery, MSD) to 100 (Luminex, xMAP) analytes. However, these assays still experience the typical issues of  
300 antibody-based capture methods (dynamic range variability, specificity and cross-reactivity) that restrict  
301 multiplexing to a much more modest number than initially stated. Therefore, an initial biomarker discovery screen  
302 may not be suited to these strategies but are of tremendous value for the high throughput validation of a specific  
303 target(s) or pathway(s). The next wave in variations of the capture-based methodology includes Proximity  
304 Extension Assay (PEA, Olink), SOMAscan (Somalogic), Single Molecule Counting (SMCxPro), Single  
305 molecular array (Simoa, Quanterix), as well as fully automated immunoassays with electrochemiluminescence  
306 detection, *e.g.*, Elecsys. In the case of blood biomarkers reflecting neurodegeneration, analytical precision of a  
307 single target has far more value than the simultaneous measurements of multiple analytes at the cost sensitivity.  
308 The SMCxPro and Simoa platforms utilise traditional antibody sandwich immunocomplex technology with a sub-  
309 femtomolar level of measurement in blood. In both occasions, individual immunocomplexes are isolated utilising  
310 novel microfluidics and the fluorophores are excited allowing for detection of single molecules of the target of  
311 interest. The Simoa or “*digital ELISA*” is now the preferred tool to measure A $\beta$ , Tau, NFL and Glial fibrillary  
312 acidic protein (GFAP) in blood for acute and chronic neurological injury.

313 **A final and important consideration in the development of a blood-based biomarker for neurodegenerative disease**  
314 **is the intended context of use (COU) and translation from laboratory validation to clinical use. The Alzheimer**  
315 **Precision Medicine Initiative (APMI) recently published guidelines of a multi-tiered approach to biomarker**  
316 **evaluation as well as sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV)**  
317 **suggestions depending on the intended COU**<sup>18</sup>.

318

### 319 **The current state of blood biomarkers for AD**

320 The search for robust blood biomarkers of AD pathology has now entered a second decade. Until recently, research  
321 on candidate blood biomarkers for AD had predominantly focused on proteins that are expressed at relatively high  
322 concentrations in the blood<sup>109-111</sup>. During recent years, technological advances in combination with better  
323 characterised clinical cohorts (including neuroimaging and CSF biomarker information on AD pathology) have  
324 led to a number of breakthroughs. The “endophenotype” approach has highlighted promising blood markers  
325 indicative of brain atrophy<sup>111,112</sup> and cerebral amyloid pathology<sup>113-117</sup>. Despite the commonality of these markers  
326 reaching nominal **statistical** significance across several studies, with supportive genomic and *in vitro* evidence  
327 (*e.g.* clusterin), they have not demonstrated the sensitivity and specificity required for clinical notoriety.  
328 Therefore, at this current time, the most promising blood biomarker candidates for AD are markers initially  
329 derived and converted from CSF assays (Table 2).

330 A $\beta$  peptides can be readily measured in plasma using standard ELISA or ECL assays, but a large number of studies  
331 **have historically shown** no clear change between clinically diagnosed AD cases and cognitively unimpaired  
332 elderly<sup>74</sup>. **However, this** opinion is now being challenged as recent mass spectrometric<sup>118-120</sup>, Simoa<sup>121</sup> **and fully**

333 automated immunoassays<sup>122</sup> have provided evidence to suggest that A $\beta$  peptide ratios can identify brain A $\beta$ -  
334 positive individuals with high sensitivity and specificity. The assessment of plasma T-tau in AD has been  
335 conducted in large research cohorts, with significant increases observed in AD<sup>123,124</sup>. However, the substantial  
336 overlaps between control groups, and poor correlations with CSF levels certainly limits plasma T-tau as being  
337 diagnostically useful<sup>123</sup>. Nonetheless, plasma T-tau may improve the prediction of future dementia. A prospective  
338 study performed in the Framingham Heart Study demonstrated that higher concentrations of plasma T-tau resulted  
339 in a 35% higher risk for AD dementia when adjusted for age and sex<sup>124</sup>. In regards to P-tau, a semi-sensitive assay  
340 for P-tau<sub>181</sub> (similar to the most employed CSF test) with ECL detection has been developed<sup>125</sup>. Using this assay,  
341 plasma P-tau concentrations are higher in AD dementia patients than controls. Using the same platform, data from  
342 two independent studies were recently presented at the Alzheimer's Association International Conference<sup>®</sup>  
343 (2019). In both studies, P-tau correlated tightly with [<sup>18</sup>F]florotau in A $\beta$ -positive cases, CSF P-tau<sub>181</sub> and a  
344 step-wise increase of P-tau was observed with Braak Staging signifying that blood P-tau could be a very early  
345 indicator of AD pathology. Further, a study using an immunomagnetic reduction (IMR) assay for plasma P-tau<sub>181</sub>  
346 found a very clear increase in MCI-AD and AD dementia with area under curve (AUC) values of 0.79 and 0.84,  
347 respectively<sup>126</sup>.

348 Although not disease specific, blood NFL has potential as a marker to identify or rule out neurodegeneration since  
349 NFL is consistently increased in AD<sup>127-129</sup>, prodromal AD<sup>127</sup> and familial AD<sup>130,131</sup>. Further observations within  
350 AD cohorts also show that blood NFL correlates with cognition<sup>127,128</sup>, CSF biomarkers, *post-mortem* pathology<sup>132</sup>  
351 and structural imaging modalities<sup>127</sup>. Interestingly, blood NFL can predict AD onset in patients with Down's  
352 syndrome<sup>133,134</sup>. The promising progress in CSF biomarkers for synaptic integrity in AD, *e.g.* neurogranin, has yet  
353 to translate to blood. Plasma concentrations of neurogranin are detectable by conventional ELISAs but are  
354 unchanged in AD with no correlation with CSF neurogranin, probably due to the contribution of peripherally  
355 expressed neurogranin peptides<sup>135,136</sup>. As new CSF assays for synaptic integrity emerge (*i.e.* GAP-43)<sup>137</sup> and as  
356 technology continues to advance, the hope for a synapse specific marker in blood still remains.

357

#### 358 **Blood biomarkers in non-Alzheimer's NDDs**

359 As we have previously declared, the vast majority of blood biomarker research in neurodegenerative disorders  
360 has focused on AD and this is principally owing to a larger population pool for biomedical research. As imaging  
361 and CSF biomarkers now guide the accurate classification of AD, blood biomarkers are becoming increasingly  
362 accurate. This enhanced *in vivo* characterization of pathologies and advances in ultra-sensitive technologies for  
363 blood biomarker detection has and will continue to benefit non-AD NDD's.

#### 364 **Targeted protein biomarkers for non-AD NDD's**

365 *Amyloid-beta*—The lowest plasma A $\beta$ 42 concentrations across non-AD NDDs have been reported in patients with  
366 DLB, but the difference did not reach statistical significance compared to other NDDs and no data on the  
367 A $\beta$ 42/A $\beta$ 40 ratio was provided<sup>138</sup>. In the same study, it was reported the patients with FTD exhibited A $\beta$ 42  
368 concentrations significantly higher than all other groups<sup>138</sup>, which is a potentially interesting finding given the  
369 low prevalence of A $\beta$  binding in PET studies and subsequent higher concentrations of CSF A $\beta$ 42 in FTD studies.  
370 Clearly, more studies are needed on plasma A $\beta$  in non-AD NDDs and whether reduced ratio of A $\beta$ 42/A $\beta$ 40 in  
371 plasma could be useful to detect A $\beta$  pathology in DLB or exclude AD in non-A $\beta$ -associated NDDs such as FTD  
372 and PSP remains to be examined. However, the peripheral expression of A $\beta$  may confound an ultra-specific  
373 association of plasma A $\beta$  concentrations with cerebral A $\beta$  pathology<sup>139</sup>.

374 *T-tau*—The expression of tau is brain-enriched and is detectable in multiple forms in plasma. However, as with  
375 A $\beta$ , tau has peripheral expression and is detectable at both the mRNA and protein level in salivary glands<sup>140</sup> and  
376 kidney (<http://www.proteinatlas.org/ENSG00000186868-MAPT/tissue>). This is an important potential  
377 confounder that may explain the poor correlation between plasma tau with CSF tau, as previously seen in studies  
378 in AD<sup>141</sup>. The half-life of tau also appears to be much shorter (hours) in plasma<sup>142</sup> than in CSF (weeks)<sup>143</sup>, which  
379 could also make it less reliable as a biomarker for neurodegeneration when measured in blood. Nonetheless,  
380 sensitive assays for T-tau have recently been developed on the Simoa and IMR platforms for its sub-femtomolar  
381 detection in plasma. Plasma concentrations of T-tau have diagnostic importance in specific NDDs tauopathies.  
382 Consistent with observations in CSF, patients with CJD, for example, have high levels of T-tau relative to other  
383 rapidly progressive dementias, AD and healthy controls<sup>144,145</sup> which positively associates with disease  
384 progression<sup>145</sup>. But, contrary to findings in CSF, IMR data demonstrates significantly increased plasma T-tau in  
385 patients with a clinical diagnosis of PD, DLB, and APD compared to controls<sup>146</sup>, with a two-fold further increase  
386 in FTD with parkinsonism (FTD-P) or without parkinsonism<sup>138</sup>. Moreover, plasma T-tau is increased in bvFTD,  
387 PPA (irrespective of subgroup) and genetic FTD subtypes (*C9orf72*, *MAPT* and *GRN*) compared with controls  
388 when measured with Simoa<sup>147</sup>. However, the group overlaps are large, which negates diagnostic usefulness on a



389 case-by-case basis, and there was no significant correlation with cross-sectional or longitudinal brain volume  
390 changes or disease duration<sup>147</sup>.

391 Preliminary evidence suggests that the plasma N-terminus tau fragment (amino acids 6-198) is increased in MCI  
392 and AD<sup>148</sup>. Given that this tau fragment partly overlaps with the species measured by some commercial T-tau  
393 assays, it will be worth studying if tau<sub>6-198</sub> has diagnostic or prognostic importance in primary tauopathies as well  
394 as AD.

395 *P-tau*—There have been very few reports measuring plasma P-tau<sub>181</sub> concentrations in AD<sup>125,126,149</sup> and in non-  
396 AD NDDs. By using IMR, P-tau<sub>181</sub> was shown to be significantly increased compared to healthy controls in PD,  
397 DLB, CBS, MSA and PSP<sup>138</sup> but in combination with plasma Aβ<sub>42</sub>, P-tau<sub>181</sub> concentrations were particularly  
398 prominent in separating FTD patients from PD, DLB and atypical parkinsonian disorders with 88.9% specificity  
399 and 92.9% sensitivity, a promising result in need of replication. In contrast to this, the promising P-tau data  
400 presented at Alzheimer's Association International Conference® (2019) demonstrated no increases in CBS, PSP  
401 and bvFTD as compared to control participants, suggesting that increases of P-tau in blood is AD specific and  
402 potentially amyloid related. The plasma concentrations of P-tau were able to distinguish AD cases from FTLN  
403 cases with an AUC >0.90 in two independent studies from BioFINDER and the University of California, San  
404 Francisco.

405 *Neurofilaments*—A close correlation between NFL concentrations in blood and CSF have been replicated in many  
406 studies spanning a broad range of conditions<sup>127,150-155</sup> and therefore many of the reported observations of CSF NFL  
407 have been replicated in blood. While studies on the AD spectrum report correlation coefficients of between 0.5-  
408 0.75, rapidly progressing conditions or NDDs that have a larger effect on the blood-brain barrier (e.g. ALS or  
409 HIV-dementia) have far stronger associations. Given these robust relationships, it has been postulated that blood  
410 NFL could replace CSF NFL for the assessment of on-going axonal injury for some NDDs. However, it remains  
411 unclear if blood NFL concentrations change concurrently with CSF without delay or if this correlation remains  
412 strong across a longitudinal trajectory, an important consideration for an early marker of neurodegeneration or  
413 monitoring therapeutic response. Another potential confounder is the degree of peripheral nerve disorders  
414 influencing blood NFL levels<sup>156,157</sup>. Elevations of NFL are observed in almost all NDD's but also inflammatory,  
415 traumatic and vascular conditions however blood NFL can be used to distinguish between patients with PD and  
416 APD with high diagnostic accuracy (AUCs 0.81–0.91) which is similar to the diagnostic accuracy of CSF NFL<sup>92</sup>.  
417 Patients with ALS demonstrate the most marked increases in blood NFL. However, within the spectrum of NDDs,  
418 patients with FTD<sup>158,159</sup> and CJD<sup>90</sup> approach concentrations levels similar to ALS. The serum concentrations of  
419 phosphorylated neurofilament heavy (pNFH) can also be accurately detected using the Simoa platform<sup>160</sup>, which  
420 correlate well with CSF pNFH in FTD and ALS patients<sup>160-162</sup>. This robust association suggests that pNFH  
421 concentrations in peripheral blood, in the same manner to NFL, is a potential peripheral biomarker for neuronal  
422 damage in non-NDD's. Indeed, pNFH concentrations in serum can separate ALS patients from controls with an  
423 AUC >0.90 and distinguish ALS from FTD with an AUC >0.85<sup>160</sup>. Sensitive measures of pNFH might be more  
424 robust than NFL<sup>163</sup> have a more favorable outcome against preanalytical variables<sup>164</sup> and exhibit different release  
425 and/or clearance dynamics<sup>160</sup>.

426 *Fatty acid-binding proteins (FABPs)*—these small intracellular proteins facilitate the transport of fatty acids  
427 between the cell membrane and different organelles. Enriched in neurons, increased CSF FABP has been linked  
428 to axonal neurodegeneration in AD<sup>165,166</sup>. Furthermore, reductions in heart-type FABP have been reported in brain  
429 tissue from patients with Down's syndrome and AD<sup>167</sup>. In serum, increases of FABP have been reported in AD<sup>168</sup>  
430 but also marked increases in CJD<sup>169</sup>, DLB<sup>168,170-172</sup> and PD<sup>172</sup>.

431 *α-synuclein*—Levels of total, oligomeric, and phosphorylated α-synuclein in peripheral tissues and body fluids of  
432 people with PD have been extensively evaluated<sup>173</sup>. Most studies investigating α-synuclein have used CSF, but  
433 findings have been disappointing. As mentioned previously, the contamination of blood during lumbar puncture,  
434 due to very high concentration of α-synuclein in red blood cells, is a major potential confounder affecting these  
435 studies. It is therefore unsurprising that measuring total α-synuclein<sup>174-176</sup> in the plasma of PD patients has yielded  
436 inconsistent results. However, increases in oligomeric α-synuclein in serum<sup>177</sup> and red blood cells<sup>178-180</sup> have been  
437 shown in PD patients with moderate diagnostic performance. Further, increases in phosphorylated forms of α-  
438 synuclein in plasma<sup>174</sup> and a panel of post-translational modifications on α-synuclein (e.g., Tyr125  
439 phosphorylation and glycosylation) have demonstrated modest discriminatory power, AUC 0.71 and 0.84  
440 respectively<sup>181</sup>. More recently, Lin et al<sup>182</sup> demonstrated that plasma total α-synuclein (with IMR) levels are  
441 significantly higher in people with PD compared with control subjects, and particularly in PD patients with more  
442 advanced disease stage and those with dementia. This was later supported by further evidence<sup>138</sup>, however α-  
443 synuclein levels in PD did not differ from atypical parkinsonian disorders but, among FTD patients, patients with  
444 parkinsonism had significantly higher α-synuclein levels than patients without combined parkinsonism.

445 *GfAp*—GfAp is a marker of astrogliosis and is increased in the brains of NDDs<sup>183</sup>. Rapidly elevated blood GfAp  
446 levels are observed in acute structural disintegration of astroglial cells such as intracerebral hemorrhage and  
447 traumatic brain injury<sup>184</sup>. Subtle changes in serum GfAp can now be observed using the Simoa platform. Serum  
448 GfAp has been shown to be increased in AD, though the levels in PD and bvFTD are normal<sup>185</sup>. Interestingly,  
449 serum GfAp levels are also increased in LBD and correlate with cognitive decline<sup>185</sup>. However, there is a  
450 disagreement between serum and CSF, as CSF GfAp levels are increased in most NDDs and only a weak  
451 correlation exists between serum and CSF in the same patients.

452 *TDP-43* - TDP-43 can be measured in CSF but the majority of its expression appears to be blood-derived and its  
453 CSF concentration does not reflect neuropathology in FTD<sup>186</sup>. Total<sup>187</sup> and phosphorylated<sup>188</sup> plasma TDP-43  
454 have been reported to be increased in FTD and correlate with more severe TDP-43 pathology in the brain<sup>188</sup>. In  
455 support of this, a more recent study found higher levels of phosphorylated TDP-43 in both the CSF and plasma of  
456 patients carrying the *C9orf72* or *GRN* mutations than in patients with other types of FTD and healthy controls<sup>189</sup>.  
457 Increased plasma TDP-43 has also been reported in ALS<sup>190</sup>. These findings need to be carefully interpreted given  
458 the ubiquitous peripheral expression of TDP-43 ([https://www.proteinatlas.org/ENSG00000120948-  
459 TARDBP/tissue](https://www.proteinatlas.org/ENSG00000120948-TARDBP/tissue)) and further efforts are needed to separate peripheral TDP-43 from CNS TDP-43. A limitation  
460 of biofluid studies investigating TDP-43 is the use of the commercially available antibodies for TDP-43, which  
461 are restricted to a peptide region or phosphorylation sites of TDP-43 that are not the reported disease-specific  
462 truncated form of TDP-43.

463

#### 464 *Non-targeted proteomic studies for non-AD NDD's*

465 In PD or DLB, most biomarker discovery studies have relied on the proteome analysis of CSF with very little in  
466 plasma or serum. This proteomic profiling has identified changes in proteins such as ApoE, APP, cystatin C<sup>191</sup>,  
467 Chitinase-3-like protein 1<sup>191</sup>, Neuronal Pentraxin 1<sup>192</sup>, Transthyretin<sup>191</sup> and Ubiquitin<sup>76,191,193</sup>. However, there  
468 exists only one study where all three disorders (PD, DLB and AD) were compared together using an isobaric  
469 labelling approach, 72 proteins – including ceruloplasmin and apolipoproteins, were uniquely associated to PD  
470 compared to AD and DLB<sup>194</sup>. Based on these findings, Zhang et al. validated a panel of eight proteins (tau, A $\beta$ 42,  
471  $\beta$ 2-microglobulin, interleukin-8, vitamin D-binding protein, apolipoproteins A and E and BDNF) that were highly  
472 effective at differentiating PD from other conditions<sup>195</sup>. The LC-MS proteomic analysis of blood samples has  
473 proved challenging although, recent studies successfully highlighted potential PD biomarkers in blood<sup>196-198</sup>, of  
474 which the most promising and consistent being plasma apolipoprotein A1 (ApoA1). O'Bryant and colleagues,  
475 who used the mesoscale (MSD) panel approach and were able to determine two distinct plasma proteomic profiles.  
476 Firstly, with a diagnostic accuracy of 91%, they were able to distinguish LBD disorders from aged-matched  
477 controls. In contrast, a second protein panel could distinguish between DLB and PD with an accuracy of 92%.  
478 Overall, the proteomic profile of these panels reflected inflammation (*i.e.* IL5, IL6, Eotaxin), metabolic (*i.e.*  
479 Adiponectin) and vascular dysfunction (*i.e.* sVCAM1) in the periphery however; they had little overlap in their  
480 specific composition<sup>199</sup>. Using a similar approach King and co-workers also demonstrated a strongly increased  
481 inflammatory component in DLB patients, interestingly this was confined to the mild cognitive impairment (MCI)  
482 stage of disease and did not differ from MCI-AD<sup>200</sup>.

483

#### 484 *Exosomes*

485 Exosomes are a discrete population of cell-derived extracellular vesicles of between 30-100nm in size that are  
486 released into the extracellular space upon fusion of multivesicular bodies (MVBs) with the plasma membrane.  
487 Although exosome studies in the context of neurodegeneration are still developing, there has been an enormous  
488 growth over the past decade<sup>201</sup>. Once primarily thought to be the transporter of unwanted cellular debris it is now  
489 accepted that exosomes transfer biomolecules and pathogenic entities across biological barriers<sup>202,203</sup>. In recent  
490 times, Goetzl et al. have pioneered the isolation of 'neuronal specific' exosomes with the use of ExoQuick (System  
491 Biosciences) isolation coupled with IP with LICAM (CD171). Their methodology has been used in numerous  
492 studies in AD and PD identifying proteins such as A $\beta$ 42<sup>204-206</sup>, P-tau<sup>204,205,207,208</sup>, Cathepsin D<sup>205,206</sup>, REST<sup>204-206</sup>,  
493 neurogranin<sup>204,205,209</sup>, DJ-1<sup>210</sup> and  $\alpha$ -synuclein<sup>211</sup>. Interestingly, exosomal GAP-43 and synapsin-1 are only altered  
494 in AD and not FTD compared to controls<sup>212</sup>. The potential of using exosome-based biomarkers as objective  
495 measures of target engagement has been recently demonstrated in neuronally derived exosomes with increased  
496 activity in the AKT pathway after GLP-1 receptor agonist treatment in PD patients<sup>213</sup>. There has been an  
497 exceptional increase in the number of studies focusing on extracellular vesicles, and growing interest in their  
498 potential as biomarkers<sup>214</sup> but challenges remain such as difficulties in reliably and efficiently enriching vesicles  
499 from biofluids and their differentiation. Methods of isolating exosomes rely on physical characteristics, such as  
500 size, flotation density, cell surface markers, and morphology<sup>215</sup>. These properties are at times used in combination,

501 and a lack of standards and consensus within the field has led to variations in protocols used by researchers across  
502 laboratories worldwide<sup>214</sup>.

503

#### 504 *RNA biomarkers*

505 Ribonucleic acids (RNA), especially microRNAs (miRNA), remain stable in blood by being protein bound or  
506 encapsulated within exosomes or microvesicles<sup>216</sup>. They can be detected and measured in all blood fractions using  
507 quantitative polymerase chain reaction (qPCR), northern blotting, oligonucleotide probe fluorescence assays, gene  
508 expression microarrays, or next-generation RNA-sequencing (RNA-Seq). It has been hypothesised that each NDD  
509 may have its own unique peripheral miRNA signature<sup>217,218</sup>. Circulating RNA biomarkers for AD have been  
510 investigated<sup>219-221</sup>, and expression levels of 12 miRNA in blood may reportedly distinguish AD from controls with  
511 93% accuracy<sup>222</sup>. Systematic studies investigating blood-based RNA biomarkers for non-AD NDD are few. An  
512 oligonucleotide microarray study has reported identifying 12 differentially expressed mRNA for Huntington's  
513 disease<sup>223</sup>, however, the panel showed substantial overlap among the gene expression changes in PD and acute  
514 ischaemic stroke<sup>224,225</sup>.

515 Total *SNCA* mRNA expression in leukocytes did not differ in DLB<sup>226</sup>, but significantly higher leukocyte  
516 expression levels of an alternatively spliced isoform encoding *SNCA*-126 in DLB has been reported<sup>226</sup>. Moreover,  
517 mitochondrial *MT-ATP8*, *MT-CO2*, *MT-CO3*, and *MT-ND2* are reportedly downregulated in DLB leukocytes<sup>227</sup>.  
518 Another study has indicated that people with idiopathic rapid eye movement sleep behaviour disorder and lower  
519 serum levels of miR-19b might have higher risk for developing DLB<sup>228</sup>. Notwithstanding the increasing interest  
520 on circulating RNA in PD<sup>229</sup>, systematic research on blood-based RNA biomarkers for PDD remains sparse.  
521 Another small study that investigated blood miRNA profiles of a heterogeneous non-AD NDD group (n=10)  
522 including DLB, vascular dementia, and FTD has found significant downregulation of miR-590-5p and miR-142-  
523 5p, and significant upregulation of miR-194-5p, compared with AD<sup>230</sup>. Furthermore, a study that investigated  
524 brain-enriched plasma miRNA reported that miR-7, miR-9\*, let-7e, miR-335-5p, and miR-451 expression levels  
525 could distinguish FTD from controls with 88% accuracy<sup>217</sup>. The need for further research investigating exosomal  
526 RNA profiles in non-AD dementias cannot be overemphasised.

527

#### 528 *Metabolomics*

529 Metabolomics can be defined as “the unbiased analysis of the composition of small molecule metabolites in a  
530 given biological tissue or fluid, under a specific set of environmental conditions”<sup>231</sup>. The sensitivity of the  
531 metabolome to environmental changes makes it an ideal molecular pool to look for biomarkers but does also make  
532 it susceptible to confounding factors making experimental design imperative. Several studies have used  
533 metabolomics in the search for peripheral biomarkers with some success, with metabolites including sphingolipids  
534 acyl-carnitines and amino acids shown to discriminate AD from controls<sup>232-235</sup>. However, of more interest, a small  
535 number of studies have looked to discriminate stable and converting MCI. Mapstone et al. reported a panel of  
536 biomarkers that discriminated converting from stable MCI patients with a sensitivity and specificity of up to  
537 90%<sup>236</sup>. Studies in PD describe 92 biomarker candidates, with three metabolites (5-acetyl-amino-6-amino-3-  
538 methyluracil, alanine and glutamate) validated between studies and three metabolites (indole acetate, theophylline,  
539 uric acid) that have contrary reports. Ten studies have investigated the classification of PD patients from healthy  
540 controls<sup>237-240</sup>, with the AUC's ranging from 0.83-0.95. Stoessel et al.<sup>241</sup> tested the predictive performance of their  
541 markers using a random forest model, achieving an accuracy of 66%. Interestingly, a cursory review of the  
542 literature showed that 14 candidate PD biomarkers have also been reported as potential biomarkers of AD, which  
543 is a greater overlap than between PD studies, suggesting that these may represent generic makers of NDDs rather  
544 than specific makers of PD or AD. In addition, studies report that plasma levels of uric acid, a product of purine  
545 breakdown, are indeed decreased early in PD patients<sup>242</sup>, and is associated with poorer attention, executive and  
546 visuospatial functions<sup>243</sup>.

547 The kynurenine pathway is modified with reduced levels of kynurenine<sup>238</sup> and increased levels of 3-  
548 hydroxykynurenine<sup>239</sup> (3-HK) and quinolinic acid<sup>238</sup>, with 3-hydroxykynurenine also increased in the CSF of PD  
549 patients<sup>244</sup>. However, these shifts are not unique to PD, having also been reported in AD<sup>245,246</sup>. The kynurenine  
550 pathway is the main route of tryptophan breakdown in mammals, with 3-hydroxykynurenine produced by the  
551 breakdown of kynurenine by kynurenine-3-monooxygenase. Numerous studies have shown that 3-HK is  
552 neurotoxic<sup>247,248</sup>, through its ability to produce highly reactive free radicals, the increased abundance of 3-HK will  
553 lead to greater production of free radicals and increased neurotoxicity. Quinolinic acid is a downstream product  
554 of 3-HK in the kynurenine pathway produced via anthranilate and 3-hydroxyanthranilate, and has been shown to  
555 be an endogenous excitotoxin<sup>249,250</sup> acting specifically via N-methyl-D-aspartate receptors. The methionine cycle  
556 describes metabolic pathways involved in the cytosolic recycling of homocysteine to methionine by means of

557 remethylation. The maintenance of this cycle, which is dependent on the presence of vitamins B9 and B12, is  
558 often disturbed in PD and other dementias. In both cross-sectional and longitudinal PD cohorts<sup>251</sup>, higher  
559 homocysteine levels in plasma is associated with cognitive decline. The overlap between the biomarkers reported  
560 for PD and AD combined with the shared pathological features (e.g. via increased 3-HK) suggest the strategies  
561 for future biomarker studies need to identify individuals with specific pathologies rather than specific clinical  
562 phenotypes.

563

#### 564 **Future directions**

565 The rapid advancement in highly sensitive quantitative technologies has led to promising developments in blood  
566 biomarker studies in AD. In the same manner, blood-based biomarkers have the potential to improve detection  
567 and diagnosis for non-AD NDD's by increasing accessibility, acceptability and ease of testing, as well as reducing  
568 costs. However, far fewer blood-based biomarker studies exist for non-AD NDDs. Nonetheless, even at this early  
569 stage, clear examples are emerging of how current blood-based biomarkers can have a potential role in the  
570 differential diagnosis of NDDs. Firstly, despite NFL being a global marker for neurodegeneration, a clear  
571 reduction of NFL in PD compared to AD, FTD and APD has been documented. Furthermore, NFL has the  
572 potential to act as non-specific outcome measure in Phase II clinical trials<sup>252,253</sup> and this would also be of huge  
573 benefit in exploring therapeutic interventions for non-AD NDD. Secondly, while plasma A $\beta$  species are being  
574 rigorously investigated by the AD community, plasma A $\beta$ 42 could play a role in predicting cognitive decline in  
575 other NDDs with reported amyloid pathology. At this time the plasma A $\beta$  ratio has not been explored in any  
576 capacity outside of AD with very few considerations of A $\beta$ 42 in NDD's. Lastly, early indications demonstrate  
577 that P-tau<sub>181</sub> may have huge potential role in classifying AD from NDDs and more specifically the extent of  
578 amyloid and tau pathology. More studies are needed to test the validity of NFL, A $\beta$  and tau species blood  
579 biomarkers in non-AD NDD and like the AD community, these need to be evaluated overtime. This includes  
580 larger sample sizes, including test and validation cohorts that satisfy outlined NPV and PPV<sup>18</sup>. Finally,  
581 establishing concentration cut-offs for the individual diagnostic accuracy against AD and healthy controls with  
582 age-dependent cut-offs

583 The AD biomarker field has taken advantage of available methods to detect tangle and plaque pathology to  
584 diagnose AD and preclinical AD pathology *in vivo*. This has ensured that research cohorts have been well stratified  
585 to maximise the likelihood of establishing a robust blood biomarker reflective of pathology. However, this has  
586 been far more prominent in proteomics studies whereas metabolomic or RNA studies remain largely dependent  
587 on cohorts with purely clinical outcomes. To establish biomarkers for non-AD NDDs, our ability to detect *in vivo*  
588 measures of other key proteonopathies (*i.e.* TDP-43 or  $\alpha$ -synuclein) has to be improved. This process is likely to  
589 follow a tiered approach where autopsy-confirmed pathologies guide CSF biomarker discovery. This would then  
590 provide candidates for targeted omics or aid the accurate stratification for non-targeted discovery studies to  
591 identify novel blood candidates for NDDs. If identified in the future, these markers can be utilised to track the  
592 development and interaction of co-pathologies over time as well as to characterise clinical syndromes according  
593 to a pathological signature, allowing for personalised treatment and clinical care.

594

#### 595 **Contributions**

596 N.J. and D.A. provided the initial idea and outline of content for the manuscript. G.D.R. and R.L.J. provided  
597 imaging data for creation of FIG. 1. All authors contributed to the content of the publication, critically reviewed  
598 and edited the manuscript.

599

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617

#### 618 **Competing interests**

619 D.A. has received research support and/or honoraria from Astra-Zeneca, H. Lundbeck, Novartis Pharmaceuticals  
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621 served as a consultant or at advisory boards for Alektor, Alzheon, CogRx, Biogen, Lilly, Novartis and Roche  
622 Diagnostics, all unrelated to the work presented in this paper. H.Z. has participated in scientific advisory boards  
623 for Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Biogen and  
624 Alzecure, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform  
625 company at the University of Gothenburg. M.S. has served on an advisory board for Servier. All additional authors  
626 have nothing to disclose.  
627

#### 628 **Figure Legends**

629  
630 **FIG.1. PET scans from illustrative patients with Alzheimer's disease (AD), behavioral variant**  
631 **frontotemporal dementia (bvFTD), corticobasal syndrome (CBS) and progressive supranuclear palsy**  
632 **(PSP). In the top row, axial slices of [<sup>11</sup>C]Pittsburgh Compound B (PiB) scans reflecting neuritic amyloid-β (Aβ)**  
633 **plaque density are displayed for each patient. The scan of the AD patient is "Aβ-positive" (considerable tracer**  
634 **retention throughout the cortex, especially in contrast to unspecific retention in the white matter [WM]) and "Aβ-**  
635 **negative" in the three non-AD patients (non-specific tracer retention in the WM only). In the middle row, tau PET**  
636 **imaging using the tracer [<sup>18</sup>F]flortaucipir (FTP) is shown, which reflects intracellular aggregates of abnormally**  
637 **phosphorylated tau. Tracer binding in the AD patient is highly elevated in temporo-parietal areas, including the**  
638 **posterior cingulate and precuneus, as well as dorsal prefrontal cortex. Arrowheads highlight areas of mild to**  
639 **moderate tracer binding in patients with bvFTD (frontal gray and WM), CBS (peri-rolandic area, including WM),**  
640 **and PSP (frontal regions, globus pallidus/putamen, dentate nucleus). Asterisks indicate brain regions of unspecific**  
641 **tracer retention ("off-target" binding), including the choroid plexus and extra-axial areas. Binding patterns found**  
642 **in PSP and CBS in particular overlap with known patterns of off-target binding in the basal ganglia, midbrain**  
643 **regions, and cerebellum observed in healthy individuals. In the third row, glucose metabolic PET imaging using**  
644 **[<sup>18</sup>F]FDG (FDG) is shown. Decreased FDG retention overlapped largely with regions of increased FTP across all**  
645 **patients. The bottom row shows structural T1-weighted MR images, with slices matched to those for FTP and**  
646 **FDG. All scans are shown in neurological convention and are courtesy of Dr Rabinovici / Dr La Joie, University**  
647 **of California, San Francisco, Memory and Aging Center. PiB PET scans were acquired 50-70 min post tracer**  
648 **injection, and standardized uptake values ratio (SUVR) were created using a cerebellar reference region; FTP**  
649 **scans were acquired at 80-100 min post tracer injection and SUVR created using an inferior cerebellar reference**  
650 **region; FDG scans were acquired at 30-60 min post tracer injection and SUVR created using the pons as reference**  
651 **region. MMSE, Mini Mental State Examination.**  
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653 **FIG. 2. Current strategies for blood biomarker discovery in neurodegenerative disorder research.**  
654 Abbreviations. ELISA, enzyme-linked immunosorbent assay; ECL, electrochemiluminescence; MSD, Meso  
655 Scale Discovery; IMR, immunomagnetic reduction; TMT, tandem mass tagging  
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#### 664 **Tables**

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**Table 1.** Clinical, pathologic and biomarker features of the most common neurodegenerative disorders

<b>NDD</b>	<b>Proteinopathy</b>	<b>Main regional involvement</b>	<b>Clinical characteristic</b>	<b>Biomarkers</b>
AD	A $\beta$ , tau	medial temporal lobe, parietal lobe	Memory, language, apraxia, agnosia	sMRI, FDG-PET, A $\beta$ PET, tau-PET, CSF A $\beta$ , CSF T-tau, CSF P-tau
FTD	TDP-43, tau, FET	frontal and anterior temporal lobes	Behavior, language/speech	sMRI, FDG-PET
LBD	$\alpha$ -synuclein, A $\beta$	Substantia nigra, limbic, neocortex	executive, visuospatial, park, visual hallucinations, fluctuating cognition, autonomous dysfunction, REM-sleep behavior disorder	DATscan, MIBG, PSG, EEG, MRI, <a href="#">RT-QuIC</a>

Abbreviations. sMRI, structural MRI; MIBG, 123- metaiodobenzylguanidine; SPECT; PSG, polysomnography;

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**Table 2.** A summary of fluid biomarkers for Alzheimer’s disease

Biomarker	Fluid matrix	Observation in AD	Interpretation / application
A $\beta$ 42	CSF	Decreased A $\beta$ 42 in AD and prodromal AD (sensitivity >90%)	Reflects cerebral A $\beta$ deposition. Diagnostic biomarker with two fully validated mass spectrometry Reference Measurement Procedures (RMP) approved
	Blood (plasma)	IP-MS show decreased plasma A $\beta$ 42 in AD. Plasma A $\beta$ 42 levels show a weak–moderate concordance with amyloid PET.	Reflects cerebral A $\beta$ deposition but influenced by peripheral expression. Candidate screening tool
A $\beta$ 42/A $\beta$ 40	CSF	Low A $\beta$ 42/A $\beta$ 40 ratio is found in AD and prodromal AD. Increased sensitivity and specificity than A $\beta$ 42 alone.	The A $\beta$ 42/A $\beta$ 40 ratio is thought to compensate for between-individual variations in ‘total’ A $\beta$ production. Diagnostic biomarker.
	Blood (plasma)	Simoa and IP-MS methods show reduced plasma A $\beta$ 42/40 ratio in AD dementia and prodromal AD. Plasma A $\beta$ 42/40 ratio shows moderate-high concordance with amyloid PET outcomes	A $\beta$ 42/A $\beta$ 40 ratio may reflect mechanisms associated with cerebral amyloidosis Candidate screening tool
T-tau	CSF	High T-tau is found in AD and prodromal AD (sensitivity >90%)	High T-tau reflects intensity of neurodegeneration Diagnostic biomarker
	Blood (plasma)	Weak-moderate increases in AD and prodromal AD	Influenced by peripheral expression Unlikely to have a biomarker role in AD
P-tau	CSF	High P-tau is found in AD and prodromal AD (sensitivity >90%).	High P-tau reflects phosphorylation state of tau and thus probably tau pathology in AD. P-tau is more specific for AD than for T-tau. Diagnostic biomarker
	Blood (plasma)	Increased P-tau is seemingly specific to A $\beta$ positive AD's. Concordance with amyloid PET and tau PET (MSD assay)	Candidate diagnostic and screening biomarker
Neurogranin	CSF	High neurogranin is found in AD and prodromal AD	Reflects synaptic dysfunction or degeneration Candidate diagnostic biomarker
NFL	Blood (plasma or serum)	Increased in AD, familial AD and prodromal AD	High plasma NFL is a general biomarker for neurodegeneration, and not specific for AD Candidate screening tool of global neurodegeneration

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**Table 3.** Findings from targeted blood biomarker proteomic studies in non-AD NDDs

Biomarker	Proteomic platform	Sample matrix	Observations <i>versus</i> healthy controls				
			Parkinson disease	Parkinson disease dementia	Dementia with Lewy bodies	Frontotemporal dementia	Other non-AD NDDs
T-tau	IMR	plasma	↑		↑	↑ (NB: highest in FTD without parkinsonism)	↑ CBD, ↑ PSP, ↑ MSA
	Simoa	plasma					↑ CJD
	ELISA	plasma					↑ CJD
P-tau <sub>181</sub>	IMR	plasma	↑		↑	↑	↑ CBD, ↑ PSP, ↑ MSA
	MSD (unpublished)	plasma				↔	↔ CBD, ↔ PSP, ↔ MSA
Aβ <sub>42</sub>	IMR	plasma	↔		↓ (non-significant)	↑	↔ CBD, ↔ PSP, ↔ MSA
NFL	Simoa	plasma or serum	↔	↑	↑	↑	↑ CJD, ↑ ALS
pNFH	Simoa	serum				↑	↑ ALS
α-syn	IMR	plasma	↑	↑	↑	↑ (NB: not FTD without parkinsonism)	↑ CBD, ↑ PSP, ↑ MSA
FABP	ELISA	serum	↑		↑		↑ CJD
GFAp	Simoa	serum	↔	↑	↑	↔ (bvFTD)	

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