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

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Key points

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

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Abstract

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

Introduction

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

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

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

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

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

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

Neuropathological, clinical and imaging overview of non-Alzheimer NDDs

Parkinson's disease, Parkinson's disease dementia and dementia with Lewy Bodies

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

In addition to the Lewy body and α-synuclein pathology, DLB and PDD often show varying degrees of AD co-pathology[22]. The clinicopathologic correlation with the extent and severity of α-synuclein pathology is often blurred by the co-existing AD pathology, which has to be considered when the degree of probability is established regarding α-synuclein being the cause of clinical symptoms[9]. Less frequently, concomitant TDP-43 pathology is detectable[25].

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

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

Frontotemporal Dementia (FTD)

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

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

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

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

The core CSF biomarkers for AD (Aβ42, T-tau and P-tau), reflecting the defining Aβ and tau pathologies, consistently demonstrate diagnostically significant changes across studies[74] and now have prominent positions in the research diagnostic criteria for AD[5,6]. One way of refining Aβ pathology biomarkers is to combine Aβ42 and Aβ40 in a ratio. This ratio has repeatedly been shown to be a more reliable biomarker for cerebral Aβ pathology than CSF Aβ42 alone, most likely by normalizing for inter-individual differences in amyloidogenic APP-processing [75]. The concentrations of these core AD biomarkers are largely normal in the majority of dementias outside of AD[76,77]. This can be of great utility in the differential diagnosis of patients with cognitive symptoms. However, there are isolated exceptions to this rule; Aβ42 is abnormally decreased in half of DLB cases and many PDD patients[78,79] which highlights the overlapping pathologies with AD. Furthermore, marked increases of T-tau in Creutzfeldt-Jakob disease (CJD)[80] is a common observation whereas the concentrations P-tau remain normal or only marginally changed in CJD[81]. An unpredicted finding is that levels of CSF tau are largely normal in FTD. This includes concentrations of total tau and specific phosphorylated epitopes (P-tau$_{181}$, P-tau$_{231}$ and P-tau$_{199}$)[77,82,83] and N-terminal tau fragments truncated at 224 (tau6-224 or x-224)[84]. The same holds true for other primary tauopathies (*e.g.* PSP)[85,86]. The reason for this remains unclear but may suggest lower secretion of tau proteins to the extracellular space and the CSF, or alternative processing of full-length tau that are not captured by the commonly used mid-domain immunological assays.

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

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

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

Introduction to blood biomarkers in neurodegenerative research: challenges and technologies

Although easily accessible, the complexity of analysing blood content (plasma or serum) must not be underestimated. Firstly, due to its continuous and uninhibited exchange with the brain, truly brain-derived molecules will be considerably higher in concentration in the CSF for the same analyte in blood. Blood communicates with the brain across the blood brain barrier (BBB), via lymph vessels and through the glymphatic system[105] and on entering the bloodstream, a brain-derived analyte will be diluted in a complex matrix of highly abundant plasma proteins (*e.g.* albumin, IgG, transferrin, haptoglobin, and fibrinogen) that span >10 orders of magnitude. These "*matrix effects*" can have a large and inconsistent impact on the ability of an immunoassay to accurately quantify a specific target and can result in misleading conclusions. Moreover, protein biomarkers may

undergo protease degradation, have substantial peripheral expression including in blood cells such as platelets and erythrocytes, exist as multiple isoforms or contain stable and/or dynamic post-translational modifications. Lastly, analytical factors such as interference from heterophilic antibodies or variations in blood collection methods, processing and storage, can affect analytes in a different manner. All these factors will introduce a high degree of variability that is unrelated to the disease itself which can be difficult to account for. The choice of analyzing plasma or serum is also an important aspect to consider. An analyte of potential interest cannot merely be assumed to correlate well between these blood fractions[106]. While serum and plasma measures of NFL correlate well, with serum NFL exhibiting consistently higher concentrations[107], it is considered that plasma is the preferred matrix for Aβ42, Aβ40 and T-tau.

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

A final and important consideration in the development of a blood-based assay for neurodegenerative dissease is the intended context of use (COU) and translation from laboratory validation to clinical use. The Alzheimer Precision Medicine Initiative (APMI) recently published guidelines of a multi-tiered approach to biomarker evaluation as well as sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) suggestion depending on the intended COU[18].

The current state of blood biomarkers for AD

The search for robust blood biomarkers of AD pathology has now entered a second decade. Until recently, research on candidate blood biomarkers for AD had predominantly focused on proteins that are expressed at relatively high concentrations in the blood[109-111]. During recent years, technological advances in combination with better characterised clinical cohorts (including neuroimaging and CSF biomarker information on AD pathology) have led to a number of breakthroughs. The "endophenotype" approach has highlighted promising blood markers indicative of brain atrophy[111,112] and cerebral amyloid pathology[113-117]. Despite the commonality of these markers reaching nominal statistical significance across several studies, with supportive genomic and *in vitro* evidence (*e.g.* clusterin), they have not demonstrated the sensitivity and specificity required for clinical notoriety. Therefore, at this current time, the most promising blood biomarker candidates for AD are markers initially derived and converted from CSF assays (Table 3).

Aβ peptides can be readily measured in plasma using standard ELISA or ECL assays, but a large number of studies have historically shown no clear change between clinically diagnosed AD cases and cognitively unimpaired elderly[74]. However, this opinion is now being challenged as recent mass spectrometric[118-120], Simoa[121] and fully automated immunoassays[122] have provided evidence to suggest that Aβ peptide ratios can identify brain Aβ-

positive individuals with high sensitivity and specificity. The assessment of plasma T-tau in AD has been conducted in large research cohorts, with significant increases observed in AD[123,124]. However, the substantial overlaps between control groups, and poor correlations with CSF levels certainly limits plasma T-tau as being diagnostically useful[123]. Nonetheless, plasma T-tau may improve the prediction of future dementia. A prospective study performed in the Framingham Heart Study demonstrated that higher concentrations of plasma T-tau resulted in a 35% higher risk for AD dementia when adjusted for age and sex[124]. In regards to P-tau, a semi-sensitive assay for P-tau$_{181}$ (similar to the most employed CSF test) with ECL detection has been developed[125]. Using this assay, plasma P-tau concentrations are higher in AD dementia patients than controls. Using the same platform, data from two independent studies were recently presented at the Alzheimer's Association International Conference® (2019) In both studies, P-tau correlated tightly with [^{18}F]flortaucipir in Aβ-positive cases, CSF P-tau$_{181}$ and a step-wise increase of P-tau was observed with Braak Staging signifying that blood P-tau could be a very early indicator of AD pathology. Further, a study using an immunomagnetic reduction (IMR) assay for plasma P-tau$_{181}$ found a very clear increase in MCI-AD and AD dementia with area under curve (AUC) values of 0.79 and 0.84, respectively[126].

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

Blood biomarkers in non-Alzheimer's NDDs

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

Targeted protein biomarkers for non-AD NDD's

Amyloid-beta—The lowest plasma Aβ42 concentrations across non-AD NDDs have been reported in patients with DLB, but the difference did not reach statistical significance compared to other NDDs and no data on the Aβ42/Aβ40 ratio was provided[138]. In the same study, it was reported the patients with FTD exhibited Aβ42 concentrations significantly higher than all other groups[138], which is a potentially interesting finding given the low prevalence of Aβ binding in PET studies and subsequent higher concentrations of CSF Aβ42 in FTD studies. Clearly, more studies are needed on plasma Aβ in non-AD NDDs and whether reduced ratio of Aβ42/Aβ40 in plasma could be useful to detect Aβ pathology in DLB or exclude AD in non-Aβ-associated NDDs such as FTD and PSP remains to be examined. However, the peripheral expression of Aβ may confound an ultra-specific association of plasma Aβ concentrations with cerebral Aβ pathology[139].

T-tau—The expression of tau is brain-enriched and is detectable in multiple forms in plasma. However, as with Aβ, tau has peripheral expression and is detectable at both the mRNA and protein level in salivary glands [140] and kidney (http://www.proteinatlas.org/ENSG00000186868-MAPT/tissue). This is an important potential confounder that may explain the poor correlation between plasma tau with CSF tau, as previously seen in studies in AD[141]. The half-life of tau also appears to be much shorter (hours) in plasma[142] than in CSF (weeks)[143], which could also make it less reliable as a biomarker for neurodegeneration when measured in blood. Nonetheless, sensitive assays for T-tau have recently been developed on the Simoa and IMR platforms for its sub-femtomolar detection in plasma. Plasma concentrations of T-tau have diagnostic importance in specific NDDs tauopathies. Consistent with observations in CSF, patients with CJD, for example, have high levels of T-tau relative to other rapidly progressive dementias, AD and healthy controls[144,145] which positively associates with disease progression[145]. But, contrary to findings in CSF, IMR data demonstrates significantly increased plasma T-tau in patients with a clinical diagnosis of PD, DLB, and APD compared to controls[146], with a two-fold further increase in FTD with parkinsonism (FTD-P) or without parkinsonism[138]. Moreover, plasma T-tau is increased in bvFTD, PPA (irrespective of subgroup) and genetic FTD subtypes (*C9orf72*, *MAPT* and *GRN*) compared with controls when measured with Simoa[147]. However, the group overlaps are large, which negates diagnostic usefulness on a case-by-case basis, and there was no significant correlation with cross-sectional or longitudinal brain volume changes or disease duration[147].

Preliminary evidence suggests that the plasma N-terminus tau fragment (amino acids 6-198) is increased in MCI and AD[148]. Given that this tau fragment partly overlaps with the species measured by some commercial T-tau assays, it will be worth studying if $tau_{6\text{-}198}$ has diagnostic or prognostic importance in primary tauopathies as well as AD.

P-tau—There have been very few reports measuring plasma P-tau_{181} concentrations in AD[125,126,149] and in non-AD NDDs. By using IMR, P-tau_{181} was shown to be significantly increased compared to healthy controls in PD, DLB, CBS, MSA and PSP[138] but in combination with plasma Aβ42, P-tau_{181} concentrations were particularly prominent in separating FTD patients from PD, DLB and atypical parkinsonian disorders with 88.9% specificity and 92.9% sensitivity, a promising result in need of replication. In contrast to this, the promising P-tau data presented at Alzheimer's Association International Conference® (2019) demonstrated no increases in CBS, PSP and bvFTD as compared to control participants, suggesting that increases of P-tau in blood is AD specific and potentially amyloid related. The plasma concentrations of P-tau were able to distinguish AD cases from FTLD cases with an AUC >0.90 in two independent studies from BioFINDER and the University of California, San Francisco.

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

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

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

GFAp—GFAp is a marker of astrogliosis and is increased in the brains of NDDs[183]. Rapidly elevated blood GFAp levels are observed in acute structural disintegration of astroglial cells such as intracerebral hemorrhage and

traumatic brain injury[184]. Subtle changes in serum GFAp can now be observed using the Simoa platform. Serum GFAp has been shown to be increased in AD, though the levels in PD and bvFTD are normal[185]. Interestingly, serum GFAp levels are also increased in LBD and correlate with cognitive decline[185]. However, there is a disagreement between serum and CSF, as CSF GFAp levels are increased in most NDDs and only a weak correlation exists between serum and CSF in the same patients.

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

Non-targeted proteomic studies for non-AD NDD's

In PD or DLB, most biomarker discovery studies have relied on the proteome analysis of CSF with very little in plasma or serum. This proteomic profiling has identified changes in proteins such as ApoE, APP, cystatin C[191], Chitinase-3-like protein 1[191], Neuronal Pentraxin 1[192], Transthyretin[191] and Ubiquitin[76,191,193]. However, there exists only one study where all three disorders (PD, DLB and AD) were compared together using an isobaric labelling approach, 72 proteins – including ceruloplasmin and apolipoproteins, were uniquely associated to PD compared to AD and DLB[194]. Based on these findings, Zhang et al. validated a panel of eight proteins (tau, Aβ42, β2-microglobulin, interleukin-8, vitamin D-binding protein, apolipoproteins A and E and BDNF) that were highly effective at differentiating PD from other conditions[195]. The LC-MS proteomic analysis of blood samples has proved challenging although, recent studies successfully highlighted potential PD biomarkers in blood[196-198], of which the most promising and consistent being plasma apolipoprotein A1 (ApoA1). O'Bryant and colleagues, who used the mesoscale (MSD) panel approach and were able to determine two distinct plasma proteomic profiles. Firstly, with a diagnostic accuracy of 91%, they were able to distinguish LBD disorders from aged-matched controls. In contrast, a second protein panel could distinguish between DLB and PD with an accuracy of 92%. Overall, the proteomic profile of these panels reflected inflammation (*i.e*, IL5, IL6, Eotaxin), metabolic (*i.e*, Adiponectin) and vascular dysfunction (*i.e*, sVCAM1) in the periphery however; they had little overlap in their specific composition[199]. Using a similar approach King and co-workers also demonstrated a strongly increased inflammatory component in DLB patients, interestingly this was confined to the mild cognitive impairment (MCI) stage of disease and did not differ from MCI-AD[200].

Exosomes

Exosomes are a discrete population of cell-derived extracellular vesicles of between 30-100nm in size that are released into the extracellular space upon fusion of multivesicular bodies (MVBs) with the plasma membrane. Although exosome studies in the context of neurodegeneration are still developing, there has been an enormous growth over the past decade[201]. Once primarily thought to be the transporter of unwanted cellular debris it is now accepted that exosomes transfer biomolecules and pathogenic entities across biological barriers[202,203]. In recent times, Goetzl et al. have pioneered the isolation of 'neuronal specific' exosomes with the use of ExoQuick (System Biosciences) isolation coupled with IP with L1CAM (CD171). Their methodology has been used in numerous studies in AD and PD identifying proteins such as Aβ42[204-206], P-tau[204,205,207,208], Cathepsin D[205,206], REST[204-206], neurogranin[204,205,209], DJ-1[210] and α-synuclein[211]. Interestingly, exosomal GAP-43 and synapsin-1 are only altered in AD and not FTD compared to controls[212]. The potential of using exosome-based biomarkers as objective measures of target engagement has been recently demonstrated in neuronally derived exosomes with increased activity in the AKT pathway after GLP-1 receptor agonist treatment in PD patients[213]. There has been an exceptional increase in the number of studies focusing on extracellular vesicles, and growing interest in their potential as biomarkers[214] but challenges remain such as difficulties in reliably and efficiently enriching vesicles from biofluids and their differentiation. Methods of isolating exosomes rely on physical characteristics, such as size, flotation density, cell surface markers, and morphology[215]. These properties are at times used in combination, and a lack of standards and consensus within the field has led to variations in protocols used by researchers across laboratories worldwide[214].

RNA biomarkers

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

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

Metabolomics

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

The kynurenine pathway is modified with reduced levels of kynurenine[238] and increased levels of 3-hydroxykynurenine[239] (3-HK) and quinolinic acid[238], with 3-hydroxykynurenine also increased in the CSF of PD patients[244]. However, these shifts are not unique to PD, having also been reported in AD[245,246]. The kynurenine pathway is the main route of tryptophan breakdown in mammals, with 3-hydroxykynurenine produced by the breakdown of kynurenine by kynurenine-3-monoxygenase. Numerous studies have shown that 3-HK is neurotoxic[247,248], through its ability to produce highly reactive free radicals, the increased abundance of 3-HK will lead to greater production of free radicals and increased neurotoxicity. Quinolinic acid is a downstream product of 3-HK in the kynurenine pathway produced via anthranilate and 3-hydroxyanthranilate, and has been shown to be an endogenous excitotoxin[249,250] acting specifically via N-methyl-D-aspartate receptors. The methionine cycle describes metabolic pathways involved in the cytosolic recycling of homocysteine to methionine by means of remethylation. The maintenance of this cycle, which is dependent on the presence of vitamins B9 and B12, is often disturbed in PD and other dementias. In both cross-sectional and longitudinal PD cohorts[251], higher homocysteine levels in plasma is associated with cognitive decline. The overlap between the biomarkers reported

for PD and AD combined with the shared pathological features (e.g. via increased 3-HK) suggest the strategies for future biomarker studies need to identify individuals with specific pathologies rather than specific clinical phenotypes.

Future directions

The rapid advancement in highly sensitive quantitative technologies has led to promising developments in blood biomarker studies in AD. In the same manner, blood-based biomarkers have the potential to improve detection and diagnosis for non-AD NDD's by increasing accessibility, acceptability and ease of testing, as well as reducing costs. However, far fewer blood-based biomarker studies exist for non-AD NDDs. Nonetheless, even at this early stage, clear examples are emerging of how current blood-based biomarkers can have a potential role in the differential diagnosis of NDDs. Firstly, despite NFL being a global marker for neurodegeneration, a clear reduction of NFL in PD compared to AD, FTD and APD has been documented. Furthermore, NFL has the potential to act as non-specific outcome measure in Phase II clinical trials[252,253] and this would also be of huge benefit in exploring therapeutic interventions for non-AD NDD. Secondly, while plasma Aβ species are being rigorously investigated by the AD community, plasma Aβ42 could play a role in predicting cognitive decline in other NDDs with reported amyloid pathology. At this time the plasma Aβ ratio has not been explored in any capacity outside of AD with very few considerations of Aβ42 in NDD's. Lastly, early indications demonstrate that P-tau$_{181}$ may have huge potential role in classifying AD from NDDs and more specifically the extent of amyloid and tau pathology. More studies are needed to test the validity of NFL, Aβ and tau species blood biomarkers in non-AD NDD and like the AD community, these need to be evaluated overtime. This includes larger sample sizes, including test and validation cohorts that satisfy outlined NPV and PPV[18]. Finally, establishing concentration cut-offs for the individual diagnostic accuracy against AD and healthy controls with age-dependent cut-offs

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

Contributions

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

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Competing interests

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

Figure Legends

FIG.1. PET scans from illustrative patients with Alzheimer's disease (AD), behavioral variant frontotemporal dementia (bvFTD), corticobasal syndrome (CBS) and progressive supranuclear palsy (PSP). In the top row, axial slices of [^{11}C]Pittburgh Compound B (PiB) scans reflecting amyloid-β (Aβ) neuritic plaque density are displayed for each patient. The scan of the AD patient is "Aβ-positive" (considerable tracer retention throughout the cortex, especially in contrast to unspecific retention in the white matter [WM]) and "Aβ-negative" in the three non-AD patients (non-specific tracer retention in the WM only). In the middle row, tau PET imaging using the tracer [^{18}F]flortaucipir (FTP) is shown, which reflects intracellular aggregates of abnormally phosphorylated tau. Tracer binding in the AD patient is highly elevated in temporo-parietal areas, including the posterior cingulate and precuneus, as well as dorsal prefrontal cortex. Arrowheads highlight areas of mild to moderate tracer binding in patients with bvFTD (frontal gray and white matter), CBS (peri-rolandic area, including white matter), and PSP (frontal regions, globus pallidus/putamen, dentate nucleus). Asterisks indicate brain regions of unspecific tracer retention ("off-target" binding), including the choroid plexus and extra-axial areas. Binding patterns found in PSP and CBS in particular overlap with known patterns of off-target binding in the basal ganglia, midbrain regions, and cerebellum observed in healthy individuals. In the bottom row, glucose metabolic PET imaging using [^{18}F]FDG (FDG) is shown. Decreased FDG retention overlapped largely in regions of increased FTP across all patients. All scans courtesy of Dr Rabinovici / Dr La Joie, University of California, San Francisco, Memory and Aging Center. PiB PET scans were acquired 50-70 min post tracer injection, and standardized uptake values ratio (SUVR) were created using a cerebellar reference region; FTP scans were acquired at 80-100 min post tracer injection and SUVR created using an inferior cerebellar reference region; FDG scans were acquired at 30-60 min post tracer injection and SUVR created using the pons as reference region. MMSE, Mini Mental State Examination.

FIG. 2. Current strategies for blood biomarker discovery in neurodegenerative disorder research.
Abbreviations. ELISA, enzyme-linked immunosorbent assay; ECL, electrochemiluminescence; MSD, Meso Scale Discovery; IMR, immunomagnetic reduction; TMT, tandem mass tagging

Tables

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

NDD	Proteinopathy	Main regional involvement	Clinical characteristic	Biomarkers
AD	Aβ, tau	medial temporal lobe, parietal lobe	Memory, language, apraxia, agnosia	sMRI, FDG-PET, Aβ PET, tau-PET, CSF Aβ, CSF T-tau, CSF P-tau
FTD	TDP-43, tau, FET	frontal and anterior temporal lobes	Behavior, language/speech	sMRI, FDG-PET
LBD	α-synuclein, Aβ	Substantia nigra, limbic, neocortex	executive, visuospatial, park, visual hallucinations, fluctuating cognition, autonomous dysfunction, REM-sleep behavior disorder	DATscan, MIBG, PSG, EEG, MRI

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

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

Biomarker	Fluid matrix	Observation in AD	Interpretation / application
Aβ42	CSF	Decreased Aβ42 in AD and prodromal AD (sensitivity >90%)	Reflects cerebral Aβ deposition. Diagnostic biomarker with two fully validated mass spectrometry Reference Measurement Procedures (RMP) approved
	Blood (plasma)	IP-MS show decreased plasma Aβ42 in AD. Plasma Aβ42 levels show a weak–moderate concordance with amyloid PET.	Reflects cerebral Aβ deposition but influenced by peripheral expression. Candidate screening tool
Aβ42/Aβ40	CSF	Low Aβ42/Aβ40 ratio is found in AD and prodromal AD. Increased sensitivity and specificity than Aβ42 alone.	The Aβ42/Aβ40 ratio is thought to compensate for between-individual variations in 'total' Aβ production. Diagnostic biomarker.
	Blood (plasma)	Simoa and IP-MS methods show reduced plasma Aβ42/40 ratio in AD dementia and prodromal AD. Plasma Aβ42/40 ratio shows moderate-high concordance with amyloid PET outcomes	Aβ42/Aβ40 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 Aβ 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

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

Biomarker	Proteomic platform	Sample matrix	Observations *versus* 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$_{181}$	IMR	plasma	↑		↑	↑	↑ CBD, ↑ PSP, ↑ MSA
	MSD (*unpublished*)	plasma				↔	↔ CBD, ↔ PSP, ↔ MSA
Aβ_{42}	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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