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Laboratory diagnostics in dementia

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Abstract: Although recent evidence seems to suggest a steady or even declining prevalence and incidence of dementias, these disorders pose a tremendous threat to health care and caregivers. The most common, dominant cause of dementia is Alzheimer's disease (AD) followed by Levy body dementia (LBD) and vascular dementia (VD). Over the last 25 years, great progress has been made in understanding the pathogenesis of AD but not yet in its treatment. Advancements have been made by ever improving clinical and paraclinical definitions allowing for a continuously increasing differentiation of the various causes of dementias. Besides imaging, functional imaging using positron emission tomography (PET) is now being increasingly used to define the amyloid load *in vivo* in the brain. By the use of tau-specific tracers meaningful tau imaging may be achieved in the future. The discovery of the cleaving mechanisms of the amyloid precursor protein (APP) and the identification of its major products such as A β_{1-42} and A β_{1-40} as well the metabolism of tau and its phosphorylation have provided reasonably reliable markers to evaluate their usefulness for the diagnosis of AD, LBD, frontotemporal dementia (FTD), Parkinson's disease (PD), alcohol-related dementia (ARD), traumatic brain injury (TBI), mixed dementia (MD) and others first by cerebrospinal fluid (CSF) analysis and now, due to the introduction of a digital single molecule array (Simoa), by plasma testing. This promising new technique should open avenues for the laboratory validation of other markers such as neurofilament light chains (NfL), visinin-like protein-1 (VLP-1), heart fatty acid binding protein (HFABP) and YKL-40, facilitating further differentiation of the various forms of dementia thus leading to improved treatment.

Keywords: biomarkers; dementia; laboratory diagnosis; pre-analytical.

List of abbreviations: A β , amyloid beta protein; AD, Alzheimer's disease; ADAS-Cog, 13-item cognitive subscale of the Alzheimer's Disease Assessment Scale; ADNI, Alzheimer's Disease Neuroimaging Initiative; ALS, amyotrophic lateral sclerosis; APO, apolipoprotein; APP, amyloid precursor protein; ARD, alcohol-related dementia; BACE 1, β -secretase 1; CAA, cerebral amyloid angiopathy; CDR, clinical dementia rating; CJD, Creutzfeldt-Jakob disease or prion diseases; CSF, cerebrospinal fluid; CT, computerized tomography; eMCI, early mild cognitive impairment; FAD, familial Alzheimer's disease; FAQ, Functional Activities Questionnaire; FDG, fluorodeoxyglucose; FTD, frontotemporal dementia; HC, healthy controls; HD, Huntington's disease; HFABP, heart fatty acid binding protein; IP, immuno precipitation; LBA, ligand binding assay; LBD, Levy body dementia; MCI, mild cognitive impairment; MD, mixed dementia; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; MS, mass spectrometry; NPH, normal pressure hydrocephalus; OND(s), other neurological disease(s); Nf, neurofilament; NfL, neurofilament light chain; PACC, pre-clinical Alzheimer's cognitive composite; PD, Parkinson's disease; PET, positron emission tomography; PIP, Pittsburgh compound B; PrP^c, physiological (normal) isoform of prion protein; PrP^{Sc}, pathological isoform of prion protein; SMC, subjective memory complaints; TBI, traumatic brain injury; VD, vascular dementia; VLP-1, visinin-like protein-1.

Introduction

Dementias are an increasing worldwide problem with an estimated cost of \$1 trillion in 2018, affecting more than 46 million people worldwide [1]. Recent meta-analyses, however, appear to suggest a steady or even declining prevalence and incidence of dementias in countries such as France, Japan, the Netherlands, Nigeria, Sweden, Spain, the UK and the USA [2]. Epidemiological data for the relative frequency of dementia-causing diseases vary from less than 30% to over 65% for Alzheimer's disease (AD), followed by Levy body dementia (LBD) and vascular dementia (VD) both in about less than 15% to over 23% of cases [3], less than 5% to over 10% for Parkinson's disease (PD), alcohol-related dementia (ARD), traumatic brain injury (TBI), mixed dementia (MD) and less than

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2% each for Huntington's disease (HD) and normal pressure hydrocephalus (NPH); HIV-associated dementia and Prion diseases (collectively termed CJD) less than 1% in the general population [4–8]. All these numbers have to be viewed with great caution, as a definite diagnosis of dementias requires a combination of all available clinical, laboratory, imaging and, if possible, neuropathological findings. Even in cases in which this is achievable, a wide margin of uncertainty persists. Basically, the less stringent the criteria for defining any dementia are, the more likely are studies to seriously underreport incidence and prevalence of dementia [9]. Looking at clinical studies in any given population, the rate of undiagnosed cases of dementia varies between 43% and 93% [9]. Looking at the AD concept exemplifies the current difficulties in diagnosing the exact cause of dementia. A strategic roadmap has been proposed based on cancer biomarkers [10]. Any given marker has to have sufficient evidence of analytical validity (phase 1), clinical validity (phases 2 and 3) and clinical utility (phases 4 and 5) in order to identify AD [10]. Only phase 1 has been fulfilled so far.

Pathobiology

The discovery of the amyloid precursor protein (APP) by Müller-Hill and colleagues in 1987 gave the first insight into the origin of Alzheimer amyloid [11]. In 1999, three groups identified β -secretase 1 (BACE 1) as the enzyme generating insoluble forms of APP [12–14]. Proof of its pathogenic effects was shown 2 years later in mice deficient in BACE 1 [15] as was its major activity in neurons [16]. Despite a continuing debate on the role of the amyloid β -protein (the so-called $A\beta$ hypothesis) sufficient evidence has accumulated to support an altered homeostasis of $A\beta_{42}$ and related $A\beta$ peptides, i.e. an imbalance of their production and clearance as early and presumably often initiating factors in AD [17]. Tau, a microtubule-binding protein, can undergo hyperphosphorylation or conformational changes to a β sheet conformer. $A\beta$ amyloid sheet conformers and $A\beta$ oligomers can under certain circumstances cross-seed with intermediate tau species in β sheet conformation and with tau oligomers to exacerbate AD pathology [18]. Apolipoprotein 4 (ApoE4) has recently been shown to markedly exacerbate tau-mediated neurodegeneration in a mouse model, thus linking amyloid metabolism with tau and its phosphorylation [19]. Clearance of amyloid ($A\beta_{1-40}$, $A\beta_{1-42}$) takes place among other sites to a high degree in the choroid plexus, as shown by post-mortem and cell culture studies [20]. $A\beta$ -amyloid is cleared by APOE in an artificial model of human blood vessels [21]. In this model, the synergistic interaction of APOE and high-density lipoprotein facilitates $A\beta_{1-42}$

transport more efficiently than $A\beta_{1-40}$ consistent with $A\beta_{1-40}$ as the primary species accumulating in cerebral amyloid angiopathy (CAA) [21]. In addition, $A\beta$ increases proliferation and differentiation of neuronal progenitor cells in the choroid plexus but decreases survival of neurons [22]. Using the APP/PS1 murine model, Carro et al. could further show a significant reduction in brain $A\beta$ deposits, hyperphosphorylation of tau and astrocytic recovery by transplanting choroid plexus epithelial cells [23].

These findings provide compelling evidence for the clinical value of measuring $A\beta_{42}$ and related $A\beta$ peptides as well as tau and phospho-tau (p-tau) in cerebrospinal fluid (CSF) [24–32] and plasma [32–34].

Requirements for a biomarker

Potential biomarkers for diagnosing any of the multiple causes of dementia can be divided into several categories [35]:

1. A predictive one permits estimation of disease probability in the pre-clinical stage,
2. a diagnostic one allows for a sensitive and specific differentiation of one form of dementia from the other,
3. a prognostic one indicates the prognosis/chance of healing,
4. a treatment response biomarker facilitates estimating response to therapy,
5. a surrogate one aids in getting sufficient evidence on how an intervention influences an endpoint of interest,
6. trait markers give invariable characteristics of a disease, i.e. gene mutations, specific proteome patterns or specific mixtures of protein, DNA and RNA signatures and
7. state markers make it possible to follow disease progression [35].

Obviously, a diagnostic biomarker should be linked to the fundamental characteristics of a disease-specific neuropathology and detect the disease early in its course and distinguish it from other dementias. Furthermore, it should be simple to use, inexpensive and as less invasive as possible. Basic statistic parameters that are required are as follows:

- a sensitivity >85% (100% denotes all patients with the disease are correctly identified),
- a specificity >85% (100% of all disease-free individuals are identified),
- the prior probability is established (background prevalence of the disease in the population tested),
- a positive predictive value (PPV) >80% (100% means all people being positive for the biomarker have the disease at autopsy),

- a negative predictive value (NPV) (percentage of people with a negative test without the disease at autopsy).

Biomarkers used for AD (and other dementias) show an evident variability between batches and laboratories, due to a lack of a definite cut-off concentration [31]. International standards for CSF or serum/plasma biomarkers such as tau, p-tau, $A\beta_{42}$, $A\beta_{40}$, neuron-specific enolase (NSE), neurofilament light chain (NFL), visinin-like protein-1 (VLP-1), heart fatty acid binding protein (HFABP) and YKL-40 are still in a very early stage of development [30]. All available evidence points to a possible role of $A\beta$ plaques in AD pathogenesis. Severity of AD, however, correlates best with the burden of neocortical subfibrillary tangles (NFT), simplified by the vague term “amyloid burden” [36]. Labeling these with carbon-11-labeled Pittsburgh compound B (PIB) and newer tracers made positron emission tomography-computerized tomography (PET-CT) scanning the clinical reference tool to determine the severity of AD, also used as the diagnostic “gold standard” in the search for reliable CSF [27, 30, 36–40] and plasma biomarkers of AD [31, 35, 41–44]. Mass spectrometry (MS) for measuring $A\beta$ concentrations in CSF has been cross-validated to be a reliable marker of amyloid binding as measured by PET [30]. In this study, a certified reference material with exact levels of $A\beta_{1-42}$ using an MS-based reference measurement procedure allowed a first step toward international standardization. The cut-off for pathological $A\beta_{1-42}$ was distinctly higher than that with immunoassays. The authors suggest that this may be due to interferences in antibody-based assays such as epitope masking caused by the binding of $A\beta_{1-42}$ to other proteins or the use of denaturing agents in the sample preparation for MS increasing the level of accessible $A\beta_{1-42}$ [30]. The use of antibodies targeting the first and last aminoacids of $A\beta$ (i.e. $A\beta_{1-42}$) or a mid-domain epitope in combination with an end-specific antibody, termed $A\beta_{\xi-42}$, with different cut-offs, sensitivities and specificities may serve to explain at least some of the variability between assays [31]. Differing degrees of self-assembling properties of $A\beta_{1-40}$, $A\beta_{1-42}$, $APP_{669-711}$ and other as yet not identified components in CSF and plasma as well as other unknown mechanisms have led to the use of the $A\beta_{1-40}/A\beta_{1-42}$ ratio as a correcting factor for predicting brain amyloid burden and for improving the sensitivity, specificity and accuracy of CSF and plasma [31, 35, 37, 43]. Combining immunoprecipitation (IP) with MS (IP-MS) has recently been shown to yield a reasonably accurate determination of CSF and plasma $A\beta_{1-42}$ and $A\beta_{1-40}/A\beta_{1-42}$ ratios, and their composites [43]. Using PIB-PET and newer tracers as reference, the authors achieved an accuracy of about 90% in plasma samples from two carefully selected cohorts. As important as these data may be, the use of

different PET tracers of amyloid binding could introduce a new bias. Newer ^{18}F - $A\beta$ tracers such as florbetapir (FBP) or flutemetamol (FLUTE) performed slightly less well than PIB in differentiating $A\beta^+$ and $A\beta^-$ groups, which may only be due to their higher variance and lower performance [43].

Preanalytical diagnosis

Given the variety of different pathologies underlying dementia, an accurate clinical diagnosis needs to be accomplished by stringent criteria, neurocognitive testing and imaging techniques such as CT, magnetic resonance imaging (MRI) and PET, typically PET-CT. Looking at the most common form of dementia, AD, either as only or part of the cause(s) of dementia, it becomes evident that currently no universal clinical, biochemical and imaging criteria are available to definitely diagnose AD [42, 45]. A binary classification scheme has been proposed. It makes use of three parameters with “A” for amyloid biomarkers, “T” for p-tau biomarkers and “N” as a marker for neuronal injury or neurodegeneration [45]. “A” is either a positive amyloid marker (i.e. FBP, florbetaben and FLUTE) binding *in vivo* to fibrils by PET or PET-CT or decreased CSF $A\beta_{42}$. “T” is a tau pathobiology marker either a radionuclide (i.e. flortaucipir, [18F]-THK523, [11C]RO6924963) binding to intracellular tau or CSF p-tau (Table 1).

“N” yields a signature of neurodegeneration/neuronal injury by either structural MRI or FDG-PET or elevated CSF τ -tau [45, 46].

Reports on the prevalence of AD and non-AD brain pathologies are conflicting [40, 47]. A retrospective study described an increase of amyloid pathology with age in both cognitively healthy as well as impaired individuals [47]. Of the 179 patients with the clinical diagnosis of probable AD, 157 (87.7%) could be neuropathologically confirmed as having AD. In addition, out of a total of 179 patients, 82 patients (45.5%) had a mixed neuropathology, i.e. macroscopic infarcts ($n=54$), or neocortical LBD ($n=19$), or both, AD and LBD ($n=8$) [47]. In a cohort of 134 patients clinically diagnosed as having mild cognitive impairment (MCI), 73 (54.4%) had a neuropathological diagnosis of AD, but only 4.5% of brain infarcts without AD accounted for clinically probable AD [47]. To make matters more complicated, the Chicago group recently reported an extremely high individual variation of various pathologies in their follow-up on 1079 autopsy cases from two longitudinal clinico-pathological studies [48]. Although AD was most frequent in 65.3% of cases, it was frequently combined with other diseases. In those with at least one neuropathology (94.3%), 77.8% had two or more, 58.2% had three or more, 35% had four or more and 16.8% had

Table 1: Diagnostic criteria for clinically probable AD dementia.

A/T/N classification	NIA-AA ^a	2014 IWG ^b
A-/T-/N-	Dementia, unlikely due to AD	Not defined
A+/T-/N-	Intermediate likelihood; probable AD; based on clinical criteria	Typical AD (if A+ established by amyloid-PET)
A+/T+/N-	High likelihood probable AD; based on clinical criteria	Typical AD
A+/T-/N+	High likelihood probable AD; based on clinical criteria	Typical AD (if A+ established by amyloid-PET)
A+/T+/N+	High likelihood AD pathophysiology	Typical AD
A-/T+/N-	Probable AD; based on clinical criteria ^c	Not defined
A-/T-/N+	Intermediate likelihood; probable AD; based on clinical criteria ^c	Not defined
A-/T+/N+	Intermediate likelihood; probable AD; based on clinical criteria ^c	Not defined

From [45]. ^aNIA-AA, National Institute on Ageing-Alzheimer's Association; ^bIWG, International Working Group; ^cIn the event of conflicting results, biomarkers are regarded as "uninformative" and therefore do not alter the individual's diagnostic classification based on clinical assessment alone.

five or more. Interestingly, by examining the frequency of observed combinations of neuropathologies, if present AD accounted for ~60% of the total cognitive loss [48]. Surprisingly, the second most common finding was no pathology at a similar percentage, followed by AD and CAA in ~40%, AD, transactive response DNA binding protein 43 (TDP-43) and CAA in ~25%, macroscopic infarcts in about 23% and arteriosclerosis in about 21% of cases [44]. Most interestingly, the authors identified 236 unique combinations out of 256 possible combinations of pathology in their cohort. Of these 236 combinations, none were present in >6% of cases. These findings suggest a complex and multifactorial set of causes in brain aging and dementia. In a meta-analysis, the prevalence of amyloid positivity decreased from age 50 to 90 years for APOE ϵ 4 non-carriers to a higher degree than for APOE ϵ 4 carriers [40]. PET imaging findings correlated excellently with autopsy in APOE ϵ 4 carriers and slightly less well in APOE ϵ 4 non-carriers. In other dementias such as frontotemporal dementia (FTD), VD and LBD, amyloid burden increased with age and was higher overall for APOE ϵ 4 carriers. In corticobasal degeneration (CBD), amyloid burden, however, decreased with age and appeared to be twice as high in APOE ϵ 4 carriers as in APOE ϵ 4 non-carriers [40]. Interestingly, lower Mini-Mental State Examination (MMSE) scores were associated with amyloid positivity in both AD and non-AD dementia except for FTD as well as CBD showing no correlation with amyloid status [40]. In patients without dementia, the prevalence of amyloid burden increased from age 50 to 90 years from 10% to 44% [49]. In those with MCI, the prevalence rose from 27% at age 50 years to 71% at age 90 years. Again, APOE ϵ 4 carriers showed 2–3 times higher prevalence estimates than APOE ϵ 4 non-carriers [49]. The age at which 15% of participants were amyloid positive was 40 years for APOE ϵ 4 ϵ 4 carriers, 50 years for ϵ 2 ϵ 4 carriers, 55 years for those with ϵ 3 ϵ 4, 65 years for those with ϵ 3 ϵ 3 and 95 years for those with ϵ 2 ϵ 3. Taken together, these data suggest a cut-off of 15% for the onset of a cognitive decline.

Likewise this limit suggests a 20–30-year interval between the first development of amyloid positivity and the onset of dementia [49]. Further progress has been made in longitudinal studies such as the Alzheimer's Disease Neuroimaging Initiative (ADNI), which was started in 2004 with a cohort of 819 well-defined patients and controls including APOE ϵ 4 allele carriers [41, 50]. Based on clinical course, imaging, neuropsychological and CSF parameters, the authors identified several groups. These are healthy controls (HC), individuals with subjective memory complaints (SMC), early mild cognitive impairment (eMCI), MCI and AD. Besides clinical and neuropsychological assessments [MMSE, Clinical Dementia Rating (CDR) Sum of Boxes, 13-item cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog) Rey Auditory verbal learning test, preclinical Alzheimer's cognitive composite (PACC) and Functional Activities Questionnaire (FAQ)], MRI measurements of the hippocampal volume, entorhinal cortex, ventricular and total brain volume, PET analyses including amyloid burden measurements, CSF assays of A β , τ -tau and p-tau were serially analyzed [41, 50]. Although these groups were well characterized at entry, data analysis over time showed a considerable variation, likely due to the different degrees of underlying neuropathology [50]. Most interestingly, both HC and SMC groups seem to comprise at least three apparently dissimilar subgroups [41]. One subgroup appears akin to the earliest stage of AD, one has signs of brain atrophy without amyloid pathology and one appears normal in all regards.

CSF analysis

Until the turn of the millennium, analysis of CSF was the only available method to obtain laboratory support for a diagnosis of AD or other diseases such as CJD or FTD [38, 51–53]. The research into CJD and prion diseases can serve as an useful example, which can be achieved by studying the

“monocausal” process, apparently linked to the misfolding of the normal peptide PrP^c (the physiological (normal) isoform of prion protein) by its transformed counterpart PrP^{Sc} (the pathological isoform of prion protein [54]. Sensitivity, specificity, stability and PPV as well as NPV for a clinical diagnosis of CJD could be demonstrated at a satisfactory level by two-dimensional electrophoresis of p130/p131 later designated as 14-3-3 protein [53]. Diagnosing CJD in a national cohort by Western blot yielded a PPV of 94.7% and a NPV of 92.4% [52]. Further improvement in the development of new molecular methods for diagnosing CJD has been achieved by real-time quaking-induced conversion (RT-QuICA) yielding a sensitivity of 85% and a specificity of 99% [55].

For the diagnosis of AD, a recent review summarizes the clinical usefulness and accuracy of tau, p-tau, tau/Aβ ratio and p-tau/Aβ ratio [27]. In seven eligible studies, the authors found a median specificity of 72% with an estimated sensitivity of 75% [95% confidence interval (CI): 67–85] for the diagnosis of AD by measurement of tau. In six studies, p-tau yielded a median specificity of 47.5% with an estimated sensitivity of 81% [64–91]. In five studies of the p-tau/Aβ ratio, sensitivities between 80% and 96% with specificities between 33% and 95% were reported [27].

This comparison of a pathologically, biologically and molecularly well-defined and overall the fastest progressing type of dementia, CJD, with the most common form of dementia and a highly clinical variable course between a few years and more than a decade illustrates the limits of CSF analysis against a background of a still somewhat heterogeneous group of disorders grouped as AD.

Aβ₄₂ and tau (tau and p-tau)

Based on the findings by Motter and colleagues [56], Hulstaert and colleagues reported CSF analysis in a large multicenter cohort of 150 AD and 100 normal control (NC) patients in 1999 [38]. They demonstrated a significantly improved sensitivity of 85% and specificity of 86% each using the combination of lowered Aβ_{1–42} over an elevated level of p-tau, introducing the Aβ/tau ratio. Like others [51], they used polypropylene containers due to the binding of Aβ₄₂ to glass and polystyrene (Table 2).

Aβ₄₂, τ-tau and p-tau exhibited a good separation of AD patients from HC CSF Aβ₄₂ (average ratio: 0.56, 0.55–0.58, $p < 0.0001$), τ-tau (2.54, 95% CI: 2.44–2.64, $p < 0.0001$) and p-tau (1.88, 1.79–1.97, $p < 0.0001$). Patients in MCI cohorts could be well discriminated from AD patients and those with stable MCI (average ratio 0.67 for CSF Aβ₄₂, 1.72 for P-tau, and 1.76 for T-tau) [31].

Table 2: Pre-analytical handling.

Confounder	Recommendation
Sample withdrawal volume	12 mL
Type of needle	25G atraumatic needle
Location of LP	Intervertebral space L3–L5
Traumatic LP	Discard blood-contaminated CSF until sample is clear, followed by immediate centrifugation at 2000×g for 10 min at RT
Erythrocyte count	<500 erythrocytes/mL
Sample collection tube	PP tube, but preferably copolymer PP-PE tube
Sample storage tube	PP tube, but preferably copolymer PP-PE tube
Sample storage volume	1–2 mL, preferably filled to 75% of its capacity
Sample centrifugation	Not essential, only when CSF sample is blood-contaminated
Sample storage temperature	As soon as possible at –80 °C
Delayed freezing of samples	<4 h
Long-term stability	Up to 10 years at –80 °C
Freeze-thaw cycles	Maximum of two cycles

CSF, cerebrospinal fluid; LP, lumbar puncture; PP, polypropylene; PE, polyethylene; RT, room temperature. From [35].

NfL

Neurofilament (NF) proteins are type IV intermediate filament proteins and are specific for neurons of both the central nervous system (CNS) and the peripheral nervous system (PNS) [57]. In mature neurons, NFs exist as a triplet protein: the light chain (NfL with 68 kDa), a mid-sized subunit (NFM with 160 kDa) and a high molecular weight (NHM with 205 kDa) subunit [57]. In 1996, Rosengren and colleagues purified NfL and developed an enzyme-linked immunosorbent assay (ELISA) showing a significantly elevated level in CSF in a small group of AD patients as compared to NC [58], thus introducing NfL as a diagnostic tool. In diseases of both the PNS [57] as well as the CNS [59–66], the expression and metabolism of NFs are affected. NfL has been shown to be a biomarker of FTD and its variants behavioral variant FTD (bvFTD), the variant with motor neuron disease (FTD-MND), semantic variant primary progressive aphasia (PPA), nonfluent variant PPA, corticobasal syndrome (CBS), progressive nuclear palsy (PSP), active cerebral small vessel disease, TBI, disease severity in HD, multiple sclerosis, amyotrophic lateral sclerosis (ALS) and AD.

NfL in CSF has been shown to have a large effect size (2.35, 1.90–2.91, $p < 0.0001$) in separating HC from AD patients [31]. In the last 5 years, solid evidence of an association of elevated NfL levels in CSF with poor survival

in FTD and ALS has accumulated [8, 59, 67]. The same holds true for AD [67–71]. NFL cannot, however, be used to make a diagnosis of AD given the many causes of its elevation in CSF and blood. Its use as a marker for disease progression (state marker) has been shown to correlate with the speed of brain atrophy, decrease of hippocampal and ventricular volume as well as neurocognitive tests such as the MMSE and ADAS-cog [72]. As repeated CSF sampling in these patients is not used outside of carefully controlled clinical studies and is not superior to the non-invasive procedures described, the determination of NFL CSF levels is currently not recommended and pursued in clinical practice [35].

Other potential markers

VLP-1, a neuronal calcium-sensor protein, in CSF has been shown to differentiate HC from AD patients and suggested to be diagnostic for early AD and even predict future cognitive impairment [73]. HFABP was first shown in 2010 to be able to differentiate AD from other neurological disorders (ONDs) with a sensitivity of 87% and a specificity of 81%, while its sensitivity was reduced to 46% with a specificity of 94% in differentiating AD from MCI [74].

A marker of neuroinflammation, YKL-40, produced by astrocytes located close to amyloid plaques, has also been reported to be elevated in CSF of patients with very mild (CDR 0.5) and mild (CDR 1.0) AD [75].

NSE, a glycolytic enzyme localized in neurons and also in erythrocytes [76], has been shown to have a moderate effect size (average ratios 2.38–1.47) in the differentiation of AD from controls, as have been VLP-1, HFABP and YLK-40 [31]. Its determination is hampered by its presence in erythrocytes which artificially increase the NSE values in hemolytic samples (Table 2) [76].

The future: peripheral blood

In order to comprehend the role of blood biomarkers, it is wise to outline the so-called key contexts of use (COU) [77]. Drawing CSF is an invasive procedure and thus not suitable for large-scale prospective studies or randomized controlled clinical trials in the evaluation of new therapies for AD. Recent improvements in ligand binding assays (LBA) technologies such as the single molecule ELISA Simoa array have led a 1000-fold improvement

of the sensitivity of standard immunoassays either in a single or multiplex setting [78, 79].

A β

The introduction of a digital enzyme-linked immunosorbent assay or Single Molecule Array technology (Simoa) led to reliable determinations of A β_{1-42} [34, 80]. In three cohorts from the prospective and longitudinal Swedish BioFINDER, the lower limit of detection (LLoD), defined as a concentration corresponding to a signal level of 2.5 standard deviation (SD) above the assay background, was 0.019 and 0.16 pg/mL for Simoa A β_{42} and A β_{40} assays, respectively A β levels correlated with the CSF levels and the A β plaque burden as assessed by amyloid PET of the brain [80]. This approach also permits to assess the response to drug treatment. Similar results have been obtained for p-tau 181 in plasma [28, 81] and NfL [61].

In a recent publication, the amyloid- β precursor protein (APP)_{669–711}/A β_{1-42} and A β_{1-40} /A β_{1-42} ratios in plasma were correlated with individual brain amyloid- β -positive or -negative status as determined by amyloid- β -PET imaging [43]. Both the discovery cohort from Japan (n=121) and the validation cohort from Australia (n=252 including 111 individuals diagnosed using ¹¹C-labeled PIB-PET and 141 using other ligands) showed an excellent accuracy with 96.7% in the former and 94% in the latter group. The use of the above ratios is an advantage, helping to reduce the wide inter-individual ratios and overcome the obstacle of insufficient reliability in previous approaches [33]. Even using the Youden's index for correcting the cut-off in both groups, the composite biomarker [(APP)_{669–711}/A β_{1-42} and A β_{1-40} /A β_{1-42} ratios] showed 87.6% and 87.4% accuracy for the discovery and validation cohort, respectively [43]. Currently, owing to the complex technology, a lack of standardization and no correlation with at least one neuropathologically defined AD cohort so far, this approach is not yet feasible in routine laboratory praxis.

Neurofilament (Nf) light chain (NfL) and phosphorylated Nf heavy chain (pNfH)

A recent multicenter study using the Simoa platform showed a significant increase of NfL and pNfH in CSF and serum of patients with ALS in the early and later symptomatic phases [4]. The sensitivity of NfL measurement in

CSF of ALS patients vs. ONDs and ALS mimics was 89% (CI: 82%–93%) and 89% (CI: 71%–93%) with a specificity of 84% (CI: 73%–92%) and 89% (CI: 81%–91%), respectively. The corresponding sensitivity in serum was 79% (CI: 70%–86%) and 100% (CI: 84%–100%) with a specificity of 92% (CI: 80%–98%) and 84% (CI: 76%–90%), respectively. Similar sensitivity and specificity was found for CSF/serum using pNfH [4]. In another well-characterized cohort plasma, the NfL levels were shown to inversely correlate with cognitive performance but not with cerebral amyloid load as measured by PET [82].

In a smaller set of patients with familial AD (FAD) serum NfL correlated with years to/from the onset of FAD ($p=0.81$, $p<0.0001$), MMSE ($p=-0.62$, $p=0.0001$), CDR and sum of boxes ($p=0.79$, $p<0.0001$), baseline brain volume ($p=-0.62$, $p=0.0002$) and whole brain atrophy ($p=0.53$, $p=0.01$) indicating its usefulness as a biomarker of early AD [62].

In a larger cohort of sporadic cases of HC, MCI and AD, a considerable overlap of NfL plasma levels of 34.7 pg/mL, 43.0 pg/mL and 50.9 pg/mL showed the absence of evidence of NfL as a useful biomarker for the diagnosis of prodromal and dementia stages of AD [61]. A comparison of the median plasma levels of HC (12.7 pg/mL), presymptomatic carriers (16.7 pg/mL) and symptomatic carriers (46.0 pg/mL) [62] and sporadic AD cases [61] and the corresponding plots suggests that the discrepancies seen could be due to a bias by the smaller groups of patients in FAD [62] and AD [61] or a biological difference between FAD and AD.

These could be due to the serum sampled in the former and plasma in the latter or inherent to FAD. Findings in ONDs such as mild TBI [64], diffuse axonal injury [83], multiple sclerosis (MS) [66] and recent small vessel infarcts [65] suggest NfL in serum/plasma as a state marker useful for the general monitoring of the extent and/or severity of diffuse axonal damage common to neurodegeneration (ALS, AD, MCI, LBD, VD, HD, ARD, CJD, FTD, PD) inflammatory disorders (such as MS) and traumatic injuries (such as TBI).

Tau

In the validation of plasma or serum measurements of tau in HC, SMC, eMCI, MCI and AD, some studies yielded contradictory findings for tau plasma levels [28, 32, 84]. While a study in a small cohort suggested an association between plasma tau levels and the degree of dementia [84], two large studies could not identify a relevant and diagnostically significant correlation between plasma tau and cognitive decline [28, 32].

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