Specific protein biomarker patterns for Alzheimer’s disease: improved diagnostics in progress

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Abstract This short review looks at Alzheimer's disease (AD) diagnosis through my own point of view, going from imaging through cerebrospinal fluid to blood proteins. Over the last couple of years, we have published two papers targeted at Alzheimer’s diagnosis. In one paper, we took an approach of selecting a specific target, namely, activity-dependent neuroprotective protein (ADNP), and our results tightened the association of ADNP blood expression with intelligence. In another paper, we took an unbiased approach of analysis of all genes expressed in lymphoblastoid cells lines and discovered changes in expression of the regulator of G-protein signaling 2 (RGS2) as a potential AD predictor. This review will assess our data in comparison to selected independent studies focusing on blood protein biomarkers as well as assessing saliva and urine samples with potential predictive value for AD. Furthermore, the review will provide directions for a combination of innovative markers, stratifying the population toward disease prevention and personalized medicine.

Keywords Predictive · Preventive · Personalized medicine · Alzheimer’s disease (AD)

AD onset and progression, imaging of protein biomarkers and cerebrospinal fluid analysis

In an article cited > 2000 times, Jack and colleagues outlined the dynamic progression of AD, starting from the accumulation of amyloid beta plaques, continuing with tau pathology, followed by changes in brain structure, then memory loss, and finally accumulation of clinical deterioration [1]. This article is coupled with another article entitled multi-modal techniques for diagnosis and prognosis of Alzheimer’s disease [2] and the timing of appearance of tau versus amyloid toxicity/pathology is still under investigation.

While imaging of AD amyloid beta plaques is commercially available since August 2014 (https://www.itnonline.com/content/first-us-commercial-neuraceq-scan-beta-amyloid-plaque-imaging-performed-wvu-healthcare), future studies are required regarding beta-amyloid thresholds associated with the first signs of accelerating rates of cognitive decline [3, 4].

I have extensively reviewed tau pathology for predictive diagnostics, targeted preventive and personalized medicine and application of advanced research in medical practice [5]. As the field is progressing, we have further recently reviewed the status of tau imaging (Höglund, Höglinger, Quinn, Hooper, and Gozes, submitted for publication). In short, $^{[18F]}$AV1451 used to label tau tangles showed a significant correlation between the degree of regional MRI brain atrophy and the extent of binding [6]. However, $^{[18F]}$AV1451 interacts also with neuromelanin [7] and shows differences between AD and progressive supranuclear palsy (PSP) [8]. Thus, the interpretation of $^{[18F]}$THK5351 PET images, with respect to tau, is confounded by the high MAO-B availability across the entire brain [9, 10], paving the path to future studies with the recent development of Merck’s MK-6240 and Piramal’s PI-2620.

As brain imaging is expensive, cerebrospinal fluid (CSF) biomarkers are further explored including, but not limited to
amyloid beta and tau. In this respect, AD presents a decrease of CSF amyloid-β42 (Aβ42) burden and an increase in cerebrospinal fluid total tau (T-tau). Genome-wide association study (GWAS) and interaction study of T-tau/Aβ42 robustly replicated previously reported AD-related genes APOE4, APOC1, and TOMM40 and suggested other influencing factors, warranting future investigation [11]. Focusing on phosphorylated tau, GWAS identified a panel of five SNPs, rs6766238, rs1143960, rs1249963, rs11975968, and rs4836493, that are predictive for p-tau181/Aβ1-42 ratio (high/low), suggesting a panel of SNPs as a potential prognostic biomarker in ApoE4-negative MCI patients [12]. There is also much interest in the use of CSF fragments of the tau endopeptidom [13] as biomarkers, with the asparagine endopeptidase mediated-NTF, taurine activity-dependent neuroprotecting protein (ADNP) in early AD patients [18]. Interestingly, not the same proteins were picked up as major blood players in the two studies cited above, suggesting potential impact for the disease status, non-demented elderly exhibiting amyloid plaques vs. early AD.

Discovered in our laboratory [19, 20], ADNP is essential for brain formation [21], and ADNP deficits in mice results in tau pathology coupled to cognitive impairments [22]. In this respect, de novo, mostly truncating mutations in ADNP lead to cognitive impairments and autism in children [23–26]. Importantly, we have recently shown that serum ADNP content [27] is correlated with premorbid intelligence. Thus, age adjustment showed significant associations between higher premorbid intelligence and greater serum ADNP, in agreement with the results described above. Furthermore, greater cortical amyloid correlated with lower ADNP and its family member ADNP2 blood-borne mRNAs. Significant increases in lymphocyte ADNP mRNA levels were observed in patients ranging from mild cognitive impairment (MCI) to AD dementia. ADNP is suggested as a novel biomarker toward screening and tracking AD.

Taking a different approach, we applied genome-wide transcriptomic profiling of lymphoblastoid cells lines (LCLs) from healthy individuals and AD patients for identifying genes that predict sensitivity to Aβ. Lower expression of RGS2 (regulator of G-protein signaling 2), a key control element of G-protein-coupled receptor signaling, was discovered compared to controls as listed below: (1) LCLs from healthy individuals exhibiting high vs. low Aβ sensitivity. (2) AD LCLs correlated with cognitive function. (3) Postmortem AD brain tissues. (4) MCI and AD blood samples. RGS2 is also suggested as a novel AD biomarker (alongside other genes) [28].

The differences observed in the potential protein markers identified attests to differential protein synthesis and protein stability in plasma, serum and blood cells, requiring a specific routine and critical requirements as implemented by large consortia like ADNI [29]. Furthermore, the possibility of personalized stratification is also tantalizing. Regardless, looking at ADNP in two geographically/ethically different groups, in one paper from Taiwan [18] and in one paper including a collaborative work between the USA and Israel [27], correlated serum ADNP levels with cognition/AD.
Current and future studies are also looking at blood exosomes [30]. Targeted measurements of proteins revealed that the levels of autolysosomal proteins in neurally derived blood exosomes distinguish patients with AD from controls, reflecting the pathology of AD years before clinical onset. These proteins include cathepsin D, lysosome-associated membrane protein 1 (LAMP-1), and ubiquitinylated proteins. In this respect, it is interesting to add that ADNP regulates cathepsin expression [31]. ADNP was further associated with autophagy [32]. Developing an algorithm that will take into account variations and relationships among the potential biomarkers listed above and beyond will bring us closer to precision medicine.

**Saliva biomarkers**

While blood serves a minimally invasive source for surrogate biomarkers, other body fluids, which do not require any body invasiveness include, for example, saliva. Recent comprehensive reviews on AD biomarkers exist [33], and specifically about saliva, suggesting early diagnosis of MCI and AD based on salivary lactoferrin, an iron- and also Aβ-binding glycoprotein [34].

In contrast, it is worth mentioning that the activity of salivary acetylcholinesterase (AChE), which has been proposed as a potential biochemical marker of cholinergic function associated with AD, only trended toward reduction in the AD cohort compared to the control group and this apparent reduction was insignificant [35].

It should be noted that the literature on saliva biomarkers for AD is still very limited (42 articles are currently available on PUBMED for saliva and Alzheimer’s), however, for the purpose of this review and future improved diagnostics it is worth mentioning some, as follows.

As a starting point, saliva is believed to be essential for the preservation of oral health and function, thus, unstimulated and stimulated submandibular salivary gland secretions were collected from non-medicated and otherwise healthy patients suffering from dementia and of the AD type (early disease stages) and compared to age-matched healthy controls. Results showed significantly lower salivary flow rates in the dementia sufferers, suggestive of a selective impairment in submandibular gland function in essentially healthy patients with early-stage dementia [36]. In this respect, cortisol can also be measured in saliva linking to stress/anxiety and depression as opposed to well-being, risks and protectors, respectively in terms of AD [37]. Furthermore, Dehydroepiandrosterone (DHEA) can be measured as well and correlated with cortisol [38].

In general related to body fluids, future studies are warranted combining proteomics, metabolomics [39] and genomic evaluations with large cohorts.

**Urine biomarkers**

Like in the saliva, urinary free cortisol (UFC) and creatinine (Cr) were measured, and a UFC/Cr ratio was calculated at a personal level showing that UFC/Cr level and UFC/Cr variability were significant predictors of AD risk an average of 2.9 years before AD onset. It was thus suggested that cortisol dysregulation as above may modulate the downstream clinical expression of AD pathology or present a preclinical marker for AD [40].

However, urine samples are not used for AD diagnosis, although those might be attractive for a relatively non-invasive sample collection. Previous studies have indicated some other utility for such samples, e.g., assessing environmental hazards, such as cadmium [41] and measuring oxidative stress markers such as F4-neuroprostanes, F3-neuroprostanes-6 DPA, and F2-dihomo-isoprostanates, metabolites of non-enzymatic lipid peroxidation of polyunsaturated fatty acids [docosahexaenoic acid, n-6 docosapentanoic acid, and adrenic acid, respectively] [42]. Importantly, these studies await robust clinical validations [43] and protein biomarkers have yet to be evaluated in depth.

**Expert recommendations and outlook**

In summary, here, we contribute to further understanding of biomarkers that can facilitate early diagnosis, prediction, classification/patient stratification, prognosis, and potentially treatment response, which could be used in clinical practice in line with follow-up steps in AD-dedicated research to satisfy patients’ needs and to advance healthcare as elegantly outlined before for other diseases and for disease states in general [44].

We focus here on proteins, broadly speaking proteomics, the study of the entire population of proteins and peptides in an organism or a part of it, such as a cell, tissue, or fluids like CSF, plasma, serum, urine, or saliva. We also discuss some transcriptomics. The proteomics field has been reviewed for AD describing methodology that was applied in > 100 studies, extracting a list of 366 proteins and peptides as potential targets in AD [45]. The current short review adds complexity to the list with more recent publications, leading to an outlook for future examinations and direct recommendations.

The next steps to be performed in order to advance the healthcare in the area should include additional advanced proteomics as well as bioinformatics mining and modeling methods for the identification of disease mechanisms in AD [46]. Future studies are also looking at the microbiome, an emerging active field of research [47]. The identification of disease mechanisms allows the development of novel biomarkers, targeted at disease prevention, monitoring and halting disease progression and drug responsiveness toward disease modification. Large consortia like ADNI [11; 29] will
facilitate these important studies toward personalized, precision medicine.

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**Compliance with ethical standards**  

**Conflict of interest**  Professor Illana Gozes serves as the Chief Scientific Officer of Coronis Neurosciences.

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