REVIEW

YJBM

In vivo Detection of Alzheimer's Disease

Antoine Leuzy[a], Kerstin Heurling[b], Nicholas J. Ashton[b], Michael Schöll[b,c], and Eduardo R. Zimmer[d,e,f,*]

[a]Division of Clinical Geriatrics, Center for Alzheimer Research, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Huddinge, Sweden; [b]Wallenberg Centre for Molecular and Translational Medicine, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Sweden; [c]Clinical Memory Research Unit, Lund University, Sweden; [d]Department of Pharmacology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; [e]Graduate Program in Biological Sciences: Biochemistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil; [f]Brain Institute of Rio Grande do Sul (BraIns), Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Recent revisions to the diagnostic criteria for Alzheimer's disease (AD†) incorporated conceptual advances in the field. Specifically, AD is now recognized to encompass a continuum, spanning from preclinical (accruing brain pathology in the absence of symptoms) through symptomatic predemenia (prodromal AD, mild cognitive impairment) and dementia phases. The role of biological markers (biomarkers) of both the underlying molecular pathologies and related neurodegenerative changes has also been acknowledged. In this abridged review, we provide an overview of fluid (cerebrospinal fluid and blood) and molecular imaging-based biomarkers used within the field and discuss the potential role of computer driven artificial intelligence approaches for both the early and accurate identification of AD and as a tool for population enrichment in clinical trials testing candidate disease modifying therapies.

INTRODUCTION

Alzheimer's disease (AD) is the leading cause of dementia, accounting for between 50 and 70 percent of cases [1]. Clinically, AD is characterized by progressive global cognitive decline, affecting memory, language, visuospatial abilities, and executive function, leading ultimately, to dementia [2]. Neuropathologically, the two major hallmarks of AD are the deposition of insoluble amyloid-β (Aβ) plaques and the formation of neurofibrillary tangles (NFTs), composed of hyperphosphorylated tau. It is currently believed that the dysmetabolism of Aβ triggers a cascade of secondary abnormalities that involved tau-associated neurodegeneration as well as unresolved neuroinflammation [3] (reviewed in [4]).

During the past two decades, advances in the abil-

*To whom all correspondence should be addressed: Eduardo R. Zimmer, PhD, Department of Pharmacology, Federal University of Rio Grande do Sul, 500 Sarmento Leite Street, 90050-170, Porto Alegre, RS, Brazil; Tel: +55 51 33085558, Email: eduardo.zimmer@ufrgs.br.

Table 1. Performance of AD biomarkers.

Biomarker		Sensitivity / Specificity	References
CSF	$A\beta_{1-42}$	0.86/ 0.90	[7]
	$A\beta_{1-42}/A\beta_{1-40}$	0.93/ 1.0	[8]
	p-tau	0.80/ 0.92	[7]
	t-tau	0.81/ 0.90	[7]
Plasma	$A\beta_{1-42}$	0.93/ 0.96	[9]
	$A\beta_{1-42/}A\beta_{1-40}$	0.90/ 0.90	[10]
	$APP_{669-711}/A_{1-42}$	0.85/ 0.95	[10]
	Composite	0.95/ 0.95	[10]
PET	$A\beta$	0.85/ 0.88	[11]
	Tau	0.93-0.97/ 1.0	[12]
	FDG	0.84/ 0.86	[11]
MRI		0.81/ 0.75	[11]

Sensitivity and specificity figures for AD biomarkers, for the comparison AD dementia *versus* controls. Though these are in line with recommendations by the 1998 Biomarker Working Group, stating that an ideal AD biomarker should have sensitivity and specificity > 80% [13], performance is imperfect; this relates to the use of patients possibly misdiagnosed or harbouring mixed pathologies, the use of amyloid-positive "controls" and the high overlap in pathology between AD and other dementia disorders. Composite = $APP_{669-711}/A\beta_{1-42}$ to $A\beta_{1-40}/A\beta_{1-42}$.
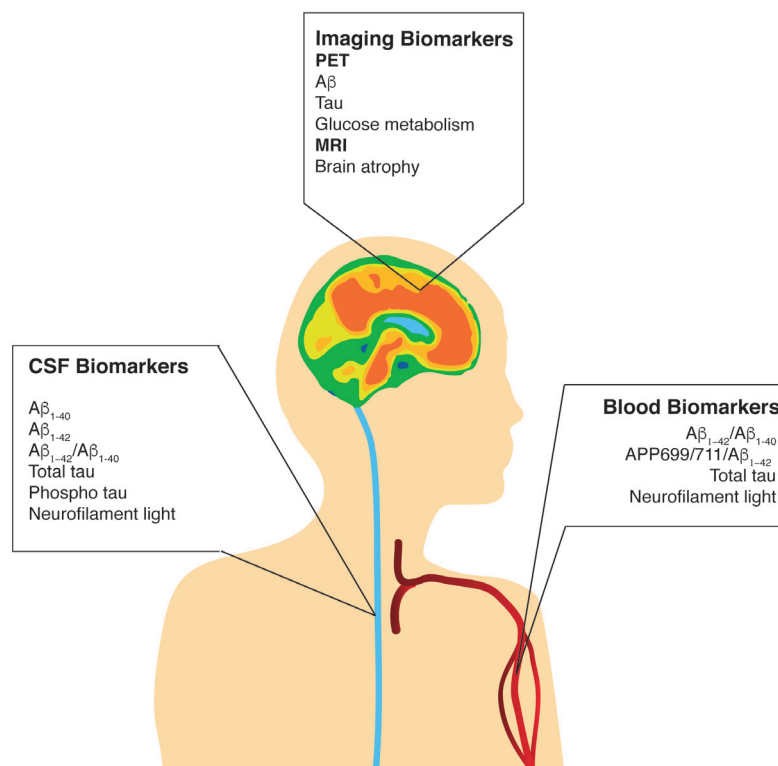
Figure 1. **Fluid and imaging biomarkers in Alzheimer's disease**. A schematic overview of established and candidate imaging and fluid-based AD biomarkers.
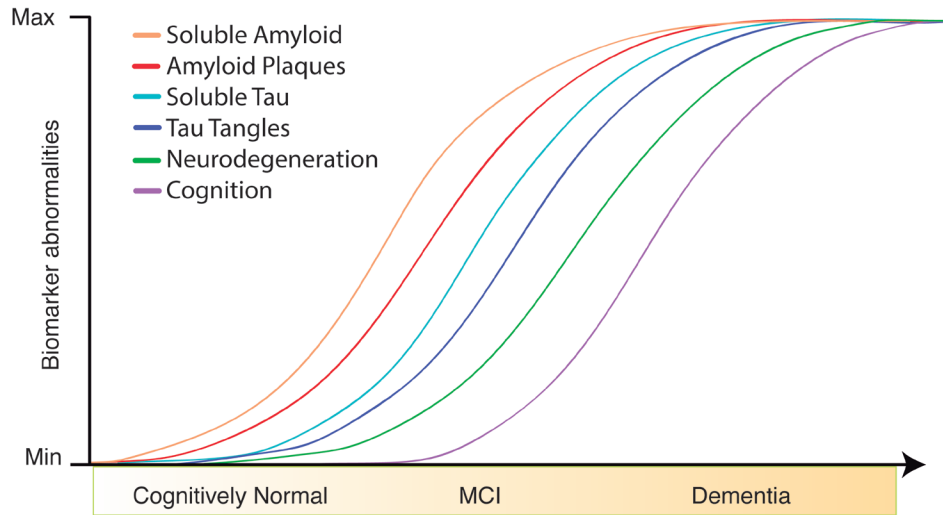
Figure 2. Hypothetical model of biomarker changes in Alzheimer's disease. Biomarkers for Aβ are believed to become abnormal first (CSF Aβ$_{1-42}$ preceding PET), followed by tau (CSF p-tau preceding PET), and neurodegeneration (CSF t-tau, MRI, [^{18}F]FDG PET) and cognitive decline. Adapted from [6].

ity to identify AD pathology using biological markers (biomarkers) have changed the way we understand AD entirely. A biomarker, by definition, is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [5]. Currently, AD hallmarks can be assessed *in vivo* by analyzing biomarkers in two main categories: i) fluid biomarkers, including cerebrospinal fluid (CSF) and promising developments in blood; and ii) imaging biomarkers, such as positron emission tomography (PET) and magnetic resonance imaging (MRI) [6] (Figure 1; Table 1). These biomarkers are now providing reliable quantitative measures that can support the early and accurate diagnosis of AD and carry the potential to surpass precision levels using clinical measures [14].

In this short review, we discuss the recent revisions to the conceptualization of AD and provide an overview of fluid and imaging-based biomarkers commonly used in the assessment of AD. Further, we address the use of artificial intelligence (AI) as a strategy to help identify individuals at risk for developing AD.

DEFINING AD: THREE DECADES OF ADVANCES

When the first diagnostic criteria for AD were published in 1984 by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [15], the typical amnestic syndrome that defines AD clinically and AD neuropathological

changes were considered interchangeable. AD has since become a clinical-biomarker construct with the introduction of the International Working Group (IWG) [16-18] and National Institute on Aging and the Alzheimer's Association (NIA-AA) guidelines [2,19,20]. Incorporating biomarkers for Aβ, tau, and neurodegeneration, these guidelines highlighted how AD pathology and resulting symptoms are not related in a one-to-one fashion, and delineated a continuum spanning preclinical (AD neuropathology begins to accumulate in the brain, yet in the absence of overt symptoms) and clinical stages (encompassing both a prodromal or mild cognitive impairment (MCI) phase, characterized by objective cognitive impairment, no dementia, and a dementia phase, each supported by biomarker evidence of AD pathology) (Figure 2). This separation of AD neuropathology from the signs/symptoms of the disease was further refined in recent revisions to the NIA-AA guidelines, with AD now an entity defined entirely on the basis of biomarkers for amyloid, tau, and neurodegeneration [21]. Though the IWG and 2011 NIA-AA guidelines incorporated criteria intended, in part, to assist in clinical decision-making, these guidelines overall are largely intended to provide a common framework for defining and staging AD.

FLUID BIOMARKERS

Cerebrospinal Fluid Biomarkers

The core CSF biomarkers used in the diagnostic work-up of and research in AD are residues from the enzymatic cleavage of the amyloid precursor protein (APP) at different lengths (Aβ$_{1-38}$, Aβ$_{1-40}$, and Aβ$_{1-42}$), total tau

(t-tau), and phosphorylated tau (p-tau, mainly phosphorylated at threonine 181 or serine 199) [22].

Of the different cleavage residues of APP, $A\beta_{1-42}$ is the main constituent of $A\beta$ plaques. Multiple studies have demonstrated a marked decrease in CSF $A\beta_{1-42}$ levels in AD, possibly due to the deposition of the peptide in $A\beta$ plaques. Levels of $A\beta_{1-38}$ and $A\beta_{1-40}$, however, are not affected in AD [23] and may be useful for the normalization of interindividual differences in $A\beta$-production [24] in the form of ratios ($A\beta_{1-42}/A\beta_{1-38}$ and $A\beta_{1-42}/A\beta_{1-40}$), though some studies have failed to show increased diagnostic accuracy using these [23,25].

In contrast to $A\beta$, cognitive decline in AD better relates to tau and neurodegeneration, processes that are reflected in the CSF as elevated t-tau and p-tau. Measurement of t-tau is considered to reflect the intensity of neurodegeneration at a given point while p-tau is thought to reflect a pathologic state of hyperphosphorylation leading to the formation of NFTs [22]. Both have been shown to be of value in the separation of AD from cognitively unimpaired (CU) controls and non-AD disorders, as well as prognostic abilities in predicting the conversion from MCI to AD [26]; here, ratios with $A\beta_{1-42}$ have been shown to provide increased sensitivity and specificity [25,26]. Interestingly, the axonal neuron-specific protein neurofilament light (NFL), which has been shown to correlate with low $A\beta_{1-42}$ and elevated tau levels [27], may serve as a useful marker of progression in AD.

Blood Biomarkers

The invasiveness of lumbar puncture as a procedure for collecting CSF stands as a drawback when considering its widespread use in the clinical work up of patients presenting with cognitive complaints, and for participant selection in therapeutic trials. A blood-based measure for AD pathology would therefore have significant practical advantages.

Establishing robust blood biomarkers for $A\beta$ and tau species has proven troublesome. $A\beta$ peptides are readily measured in plasma but historically the correlation with brain $A\beta$ levels (measured by $A\beta$ PET or CSF) has been weak, with levels being confounded by platelet production and other extra-cerebral tissues [28]. However, this generalized opinion is beginning to change with recent mass spectrometric and ultrasensitive immunoassay (single-molecule array, Simoa) evidence suggesting that $A\beta$ peptide ratios ($A\beta_{1-42}/A\beta_{1-40}$, $APP_{669-711}/A\beta_{1-42}$ and a composite $APP_{669-711}/A\beta_{1-42}$ to $A\beta_{1-40}/A\beta_{1-42}$) identify $A\beta$-positive individuals with high sensitivity and specificity [10,29,30]. There are currently no robust immunoassays for p-tau in plasma whereas the Simoa platform allows for femtomolar measures of t-tau. Nominal group differences between AD and CU individuals are con-

sistently observed [31], however the lack of correlation of plasma t-tau with CSF suggests that these two body fluids are differentially regulated [32]. The half-life of tau appears to be much shorter in plasma (hours) than in CSF (weeks) [33,34]. In contrast, plasma and CSF NFL tightly correlate [35,36], with plasma levels shown to have a marked elevation in AD compared to MCI and healthy controls [37], comparable to differences seen with core CSF biomarkers [38]. However, it is likely that increased plasma NFL lacks disease specificity as it is found to be a feature in many conditions [36,39,40].

Research has also focused upon the detection of other circulating blood proteins which may serve as peripheral indicators for clinical AD (reviewed in [41]). Increasingly, promising "endophenotype" studies that utilize imaging surrogates of brain atrophy [42] and amyloid-β PET [43] have pointed to peripheral biomarker panels indicative of on-going disease pathophysiology. However, these pilot data should be interpreted with some caution as they are derived from multi-marker panels and as a mechanistic understanding of the associations is currently lacking.

MOLECULAR IMAGING

$A\beta$ and tau PET

Since the first application of the PET ligand ^{11}C-Pittsburgh Compound B (^{11}C-PIB) to the *in vivo* study of $A\beta$ plaques in AD, numerous studies using ^{11}C-PIB have shown that the global amount of $A\beta$ in AD dementia patients is typically 50 to 70 percent above levels seen in CU older individuals [44]. Similar studies in CU individuals and MCI patients have shown $A\beta$ positivity in a high proportion (30 percent and 60 percent, respectively) [45,46], consistent with autopsy data in both groups showing comparable percentages meeting neuropathological criteria for AD [47,48]. $A\beta$ positivity among patients diagnosed clinically as AD dementia lies around 90 percent, with $A\beta$-negative cases assumed to represent clinical misdiagnosis [49]. Driven by the success of ^{11}C-PIB and limitations tied to its short half-life (~20 min, making it impractical for clinical use), several $A\beta$ labelling ^{18}F compounds have been developed (half-life ~110 min), including ^{18}F-florbetapir, ^{18}F-flutemetamol, ^{18}F-florbetaben, and ^{18}F-NAV4694 (previously ^{18}F-AZD4694) [50]. Using these tracers, findings similar to those for ^{11}C-PIB have been reported [49] (Figure 3). Importantly, ^{18}F-florbetapir (Amyvid), ^{18}F-flutemetamol (Vizamyl), and ^{18}F-florbetaben (Neuraceq) are now approved for clinical use. Interestingly, preliminary findings suggest that retinal levels of $A\beta$ plaques, measured *in vivo*, may provide an index of overall $A\beta$ brain levels [51]. Other autofluorescence based techniques have also shown potential for future *in vivo* use [52,53].
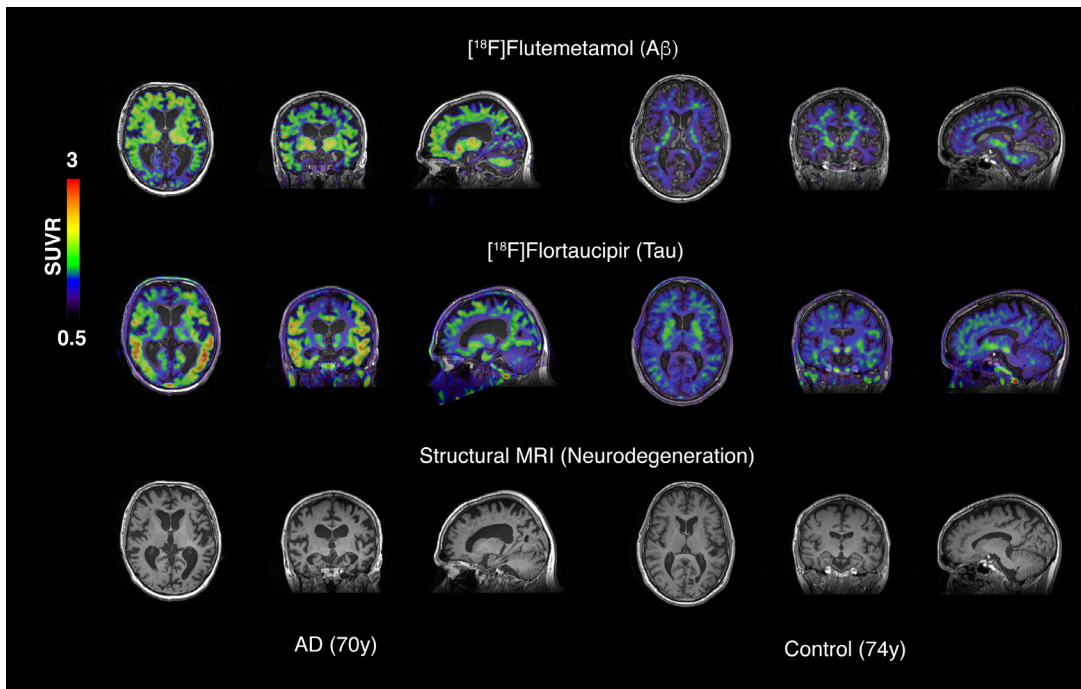
Figure 3. Molecular imaging in Alzheimer's disease. Representative images showing ^{18}F-flutemetamol (beta-amyloid) and ^{18}F-flortaucipir (tau) PET, along with T1-weighted MRI, in a patient with AD and a cognitively unimpaired older control. In rows one and two, warmer colors indicate areas of increased ligand retention, expressed as standard uptake value ratio (SUVR = ligand uptake in target region / reference region (cerebellar cortex), devoid of specific binding). Copyright Swedish BioFINDER, PI professor Oskar Hansson, MD, PhD, Clinical Memory Research Unit, Lund University, Malmö, Sweden.

Building on the success of Aβ imaging, PET ligands selective for AD-related paired-helical filament (PHF) tau have recently made their entry into the field. Using "first generation" tracers, including ^{18}F-THK5317, ^{18}F-THK5351, and ^{18}F-flortaucipir (also known as ^{18}F-AV-1451, ^{18}F-T807) (reviewed in [54]), robust differences in uptake have been reported between CU elderly controls and patients with AD have been observed, as well as elevated uptake in subjects with MCI [55,56] (Figure 3). Further, several tau imaging studies have shown good correspondence between ligand retention and Braak staging of *post mortem* tau pathology [56,57], and tight associations with neurodegenerative markers such as cortical atrophy measurements and ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET [58,59]. Comparative studies between tau ligands suggest varying sensitivity/specificity toward AD type tau [60,61], highlighting the complexity of tau pathology and the likely need for multiple tracers to properly address the full range of tauopathies. Importantly, first generation tracers have been shown to exhibit "off-target" binding, notably to monoamine oxidase B. Early findings from "second generation" tracers, including ^{18}F-MK-6240 and ^{18}F-PI-2620, appear promising; presented data, however, has so far mostly been limited to international conferences.

^{18}F-FDG PET

Since approved by the Food and Drug Administration (FDA) in 2004 for use in the diagnostic work up of dementia disorders, ^{18}F-FDG PET has come to play an important role in the evaluation of patients with suspected AD. A marker of neurodegeneration, decreased uptake of ^{18}F-FDG is interpreted as largely reflecting synaptic depletion [62]. In AD, a characteristic pattern of glucose hypometabolism is observed, involving the precuneus/posterior cingulate, inferior parietal lobule as well as posterolateral and medial aspects of the temporal lobe, including the hippocampus and entorhinal cortex [63,64]. In patients with MCI, the presence of this metabolic signature has been shown to predict progression to AD dementia [65,66]. Further, ^{18}F-FDG has shown high sensitivity and specificity in identifying AD and related neurodegenerative diseases using neuropathologic diagnosis as the standard of truth [67] and has been shown to be a helpful adjunct in clinical practice [68]. Moreover, ^{18}F-FDG hypometabolism has been shown to relate closely to cognitive decline, predicting progression from normal cognition through MCI and AD dementia [69,70]. ^{11}C-UCB-J, a marker of the synaptic vesicle glycoprotein 2A [71], also shows promise as a marker of synaptic loss;
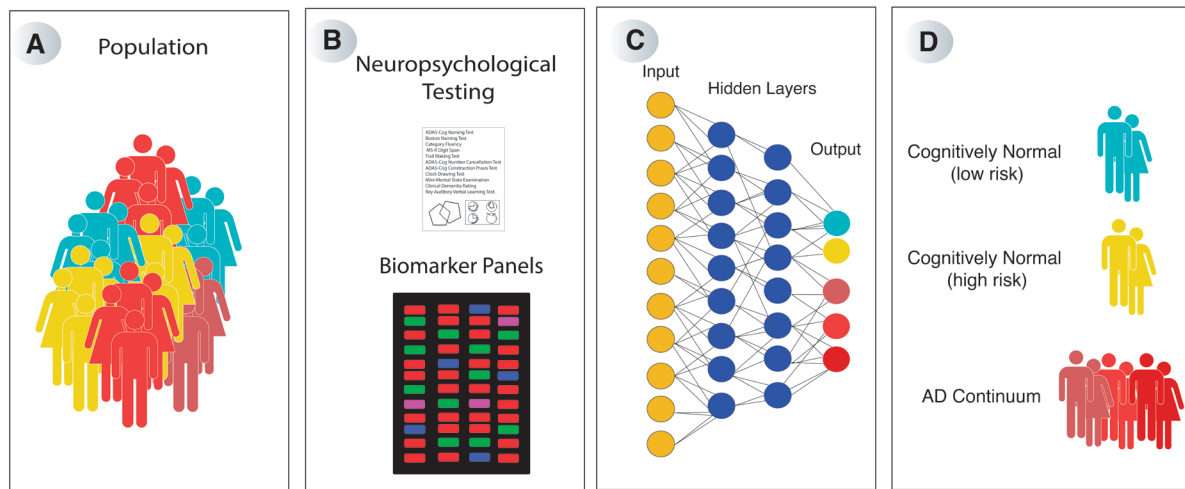
Figure 4. Representative artificial intelligence workflow for predicting patients at-risk for developing Alzheimer's disease. Large-scale multicentric studies in heterogeneous populations (**a**) collecting a wide range of biomarkers (imaging and fluid), genetics and neuropsychological data (**b**) can provide enormous quantity of valuable data, which can be promptly analyzed and shared. Using these big databases as input variables in highly refined machine learning and deep machine learning algorithms (**c**) it is expected that in the near future we will be able to identify and predict patients at-risk to develop Alzheimer's disease (**d**).

potentially more specific than ^{18}F-FDG, the field awaits findings from current cohort studies.

Magnetic Resonance Imaging

The use of structural MRI is an important component in the clinical work-up of patients with suspected AD. Atrophy within characteristically vulnerable medial temporal regions, including the hippocampus and entorhinal cortex, is the most established and validated measure, with visually based ratings showing high sensitivity and specify, both in the differentiation of AD from vascular and non-AD neurodegenerative pathologies [72] and in the prediction of conversion to AD dementia among MCI patients [73]. Further, given the high co-occurrence of cerebrovascular pathologies with AD [74], T2-weighted images can be used to gauge the proportion of cognitive impairment that can be ascribed to each; this, in turn, can help guide the magnitude of therapeutic emphasis placed on the control of vascular risk factors, for instance [75]. A related development has been the ability to image cerebral microbleeds (CMBs) using T2* and susceptibility weighted imaging [76]; CMBs have been shown to affect some 20 percent of AD patients [77] and to carry prognostic value [78].

BIOMARKER MODELLING OF AD

Though there exist differing schools of thought as to

the cause of AD [79], the predominant view is that dyshomeostasis of Aβ is the initiating event [80,81]. According to this hypothesis, aggregation of $Aβ_{1-42}$ leads to tau pathology, neurodegeneration, and clinical symptoms. Accordingly, the first biomarkers to become abnormal are CSF $Aβ_{1-42}$ followed by Aβ PET; Aβ then induces tau pathology in the medial temporal lobe (an alternative model stipulates that medial temporal tau in fact precedes Aβ [82]) and fosters its spread into neocortical areas; this process is reflected by abnormal CSF p-tau, and later, tau PET. This tauopathy would then lead to abnormalities in biomarkers of neurodegeneration, namely t-tau, structural MRI and ^{18}F-FDG PET, with clinical symptoms following these markers [83-85]. Though Aβ biomarkers, and, possibly, CSF tau [86,87] are thought to reach a plateau during the early symptomatic phase of the disease (*i.e.* prodromal AD) [88], tau PET and neurodegenerative biomarkers are believed to remain dynamic though the dementia phase [6,89]. Importantly, the relationship between a given individual's cognitive status and biomarker profile is thought to be influenced by genetic risk factors, comorbid pathologies, and cognitive reserve [90].

COMPUTER-ASSISTED BIOMARKER DRIVEN APPROACHES

One of the most pressing challenges facing the field currently is the failure of clinical trials testing candidate disease modifying treatments. Given the recognition that

therapies may prove ineffective in those already exhibiting dementia, focus has shifted toward the inclusion of minimally symptomatic and asymptomatic CU elderly individuals with biomarker evidence of AD pathology [91]. In order to facilitate reductions in required sample size, and to better assess treatment outcomes, identifying individuals who will show disease progression within a reasonable time frame is critical. Given the likely need to move beyond single variable approaches [92], instead accounting for high number of variables, as well as complex interactions between them, computer aided approaches, including AI, are emerging as a useful strategy for population enrichment (Figure 4). Findings derived using machine learning or deep learning, which involve computer algorithms extracting patterns from a dataset, and then learning to predict an outcome of interest [93], indeed suggest that the risk to develop AD can be detected prior to symptomatic onset [94].

CONCLUSIONS AND OUTLOOK

While further studies are required to more fully assess the utility of fluid and imaging biomarkers, particularly for tau PET given its recent introduction, findings are thus far promising and suggest that the informed and appropriate use of biomarkers can be of help in the early and accurate identification of AD. The complexity of many findings, aggravating the definition of thresholds for biomarker "positivity," and the high cost of these investigations, however, remain as obstacles to their routine use in clinical settings. Further, additional markers for targets such as α-synuclein and transactive response DNA binding protein 43 kDa (TDP-43) are likely needed to increase capabilities with respect to characterizing the full range of proteinopathies that can underlie dementia disorders. Given the recognition that potential disease modifying treatments are most likely to succeed if administered during the preclinical phase of AD, biomarker driven strategies for population enrichment are critical. In this respect, AI driven approaches may help optimize selection algorithms in order to increase study power and decrease observational periods.

REFERENCES

1. Winblad B, Amouyel P, Andrieu S, Ballard C, Brayne C, Brodaty H, et al. Defeating Alzheimer's disease and other dementias: a priority for European science and society. Lancet Neurol. 2016;15(5):455–532.
2. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263–9.
3. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med. 2016;8(6):595–608.
4. Polanco JC, Li C, Bodea LG, Martinez-Marmol R, Meunier FA, Gotz J. Amyloid-beta and tau complexity - towards improved biomarkers and targeted therapies. Nat Rev Neurol. 2018;14(1):22–39.
5. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89–95.
6. Jack CR Jr, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol. 2013;12(2):207–16.
7. Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurol. 2003;2(10):605–13.
8. Lewczuk P, Lelental N, Spitzer P, Maler JM, Kornhuber J. Amyloid-beta 42/40 cerebrospinal fluid concentration ratio in the diagnostics of Alzheimer's disease: validation of two novel assays. J Alzheimers Dis. 2015;43(1):183–91.
9. Kaneko N, Nakamura A, Washimi Y, Kato T, Sakurai T, Arahata Y, et al. Novel plasma biomarker surrogating cerebral amyloid deposition. Proc Jpn Acad, Ser B, Phys Biol Sci. 2014;90(9):353–64.
10. Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Dore V, et al. High performance plasma amyloid-beta biomarkers for Alzheimer's disease. Nature. 2018;554(7691):249–54.
11. Frisoni GB, Bocchetta M, Chetelat G, Rabinovici GD, de Leon MJ, Kaye J, et al. Imaging markers for Alzheimer disease: which vs how. Neurology. 2013;81(5):487–500.
12. Mattsson N, Smith R, Strandberg O, Palmqvist S, Scholl M, Insel PS, et al. Comparing (18)F-AV-1451 with CSF t-tau and p-tau for diagnosis of Alzheimer disease. Neurology. 2018;90(5):e388–95.
13. Consensus report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. Neurobiol Aging. 1998;19(2):109–16.
14. Henriques AD, Benedet AL, Camargos EF, Rosa-Neto P, Nobrega OT. Fluid and imaging biomarkers for Alzheimer's disease: where we stand and where to head to. Exp Gerontol. 2018.
15. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984;34(7):939–44.
16. Dubois B, Feldman HH, Jacova C, Cummings JL, Dekosky ST, Barberger-Gateau P, et al. Revising the definition

of Alzheimer's disease: a new lexicon. Lancet Neurol. 2010;9(11):1118–27.

17. Dubois B, Feldman HH, Jacova C, Dekosky ST, Barberger-Gateau P, Cummings J, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol. 2007;6(8):734–46.

18. Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol. 2014;13(6):614–29.

19. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):270–9.

20. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):280–92.

21. Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14(4):535–62.

22. Blennow K, Dubois B, Fagan AM, Lewczuk P, de Leon MJ, Hampel H. Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. Alzheimers Dement. 2015;11(1):58–69.

23. Schoonenboom NS, Mulder C, Van Kamp GJ, Mehta SP, Scheltens P, Blankenstein MA, et al. Amyloid beta 38, 40, and 42 species in cerebrospinal fluid: more of the same? Ann Neurol. 2005;58(1):139–42.

24. Leuzy A, Chiotis K, Hasselbalch SG, Rinne JO, de Mendonca A, Otto M, et al. Pittsburgh compound B imaging and cerebrospinal fluid amyloid-beta in a multicentre European memory clinic study. Brain. 2016;139(Pt 9):2540–53.

25. Palmqvist S, Zetterberg H, Mattsson N, Johansson P, Alzheimer's Disease Neuroimaging I, Minthon L, et al. Detailed comparison of amyloid PET and CSF biomarkers for identifying early Alzheimer disease. Neurology. 2015;85(14):1240–9.

26. Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. JAMA. 2009;302(4):385–93.

27. Zetterberg H, Skillback T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, et al. Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression. JAMA Neurol. 2016;73(1):60–7.

28. Zetterberg H. Plasma amyloid beta-quo vadis? Neurobiol Aging. 2015;36(10):2671–3.

29. Ovod V, Ramsey KN, Mawuenyega KG, Bollinger JG, Hicks T, Schneider T, et al. Amyloid beta concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. Alzheimers Dement. 2017;13(8):841–9.

30. Janelidze S, Stomrud E, Palmqvist S, Zetterberg H, van Westen D, Jeromin A, et al. Plasma beta-amyloid in Alzheimer's disease and vascular disease. Sci Rep. 2016;6:26801.

31. Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol. 2016;15(7):673–84.

32. Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, et al. Plasma tau levels in Alzheimer's disease. Alzheimers Res Ther. 2013;5(2):9.

33. Randall J, Mortberg E, Provuncher GK, Fournier DR, Duffy DC, Rubertsson S, et al. Tau proteins in serum predict neurological outcome after hypoxic brain injury from cardiac arrest: results of a pilot study. Resuscitation. 2013;84(3):351–6.

34. Hesse C, Rosengren L, Andreasen N, Davidsson P, Vanderstichele H, Vanmechelen E, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci Lett. 2001;297(3):187–90.

35. Gisslen M, Price RW, Andreasson U, Norgren N, Nilsson S, Hagberg L, et al. Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. EBioMedicine. 2016;3:135–40.

36. Hansson O, Janelidze S, Hall S, Magdalinou N, Lees AJ, Andreasson U, et al. Blood-based NfL: A biomarker for differential diagnosis of parkinsonian disorder. Neurology. 2017;88(10):930–7.

37. Zhou W, Zhang J, Ye F, Xu G, Su H, Su Y, et al. Plasma neurofilament light chain levels in Alzheimer's disease. Neurosci Lett. 2017;650:60–4.

38. Mattsson N, Andreasson U, Zetterberg H, Blennow K. Alzheimer's Disease Neuroimaging I. Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. JAMA Neurol. 2017;74(5):557–66.

39. Rohrer JD, Woollacott IO, Dick KM, Brotherhood E, Gordon E, Fellows A, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology. 2016;87(13):1329–36.

40. Rojas JC, Karydas A, Bang J, Tsai RM, Blennow K, Liman V, et al. Plasma neurofilament light chain predicts progression in progressive supranuclear palsy. Ann Clin Transl Neurol. 2016;3(3):216–25.

41. Baird AL, Westwood S, Lovestone S. Blood-Based Proteomic Biomarkers of Alzheimer's Disease Pathology. Front Neurol. 2015;6:236.

42. Thambisetty M, Simmons A, Hye A, Campbell J, Westman E, Zhang Y, et al. Plasma biomarkers of brain atrophy in Alzheimer's disease. PLoS One. 2011;6(12):e28527.

43. Ashton NJ, Kiddle SJ, Graf J, Ward M, Baird AL, Hye A, et al. Blood protein predictors of brain amyloid for enrichment in clinical trials? Alzheimers Dement (Amst). 2015;1(1):48–60.

44. Klunk WE. Amyloid imaging as a biomarker for cerebral beta-amyloidosis and risk prediction for Alzheimer dementia. Neurobiol Aging. 2011;32 Suppl 1:S20–36.

45. Aizenstein HJ, Nebes RD, Saxton JA, Price JC, Mathis CA, Tsopelas ND, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. Arch Neurol. 2008;65(11):1509–17.

46. Forsberg A, Engler H, Almkvist O, Blomquist G, Hagman

G, Wall A, et al. PET imaging of amyloid deposition in patients with mild cognitive impairment. Neurobiol Aging. 2008;29(10):1456–65.

47. Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ, et al. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. Arch Neurol. 2006;63(5):674–81.

48. Price JL, McKeel DW Jr, Buckles VD, Roe CM, Xiong C, Grundman M, et al. Neuropathology of nondemented aging: presumptive evidence for preclinical Alzheimer disease. Neurobiol Aging. 2009;30(7):1026–36.

49. Jack CR Jr, Barrio JR, Kepe V. Cerebral amyloid PET imaging in Alzheimer's disease. Acta Neuropathol. 2013;126(5):643–57.

50. Blennow K, Mattsson N, Scholl M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. Trends Pharmacol Sci. 2015;36(5):297–309.

51. Abbasi J. A Retinal Scan for Alzheimer Disease. JAMA. 2017;318(14):1314.

52. Condello C, Schain A, Grutzendler J. Multicolor time-stamp reveals the dynamics and toxicity of amyloid deposition. Sci Rep. 2011;1:19.

53. Kwan AC, Duff K, Gouras GK, Webb WW. Optical visualization of Alzheimer's pathology via multiphoton-excited intrinsic fluorescence and second harmonic generation. Opt Express. 2009;17(5):3679–89.

54. Saint-Aubert L, Lemoine L, Chiotis K, Leuzy A, Rodriguez-Vieitez E, Nordberg A. Tau PET imaging: present and future directions. Mol Neurodegener. 2017;12(1):19.

55. Chiotis K, Saint-Aubert L, Savitcheva I, Jelic V, Andersen P, Jonasson M, et al. Imaging in-vivo tau pathology in Alzheimer's disease with THK5317 PET in a multimodal paradigm. Eur J Nucl Med Mol Imaging. 2016;43(9):1686–99.

56. Scholl M, Lockhart SN, Schonhaut DR, O'Neil JP, Janabi M, Ossenkoppele R, et al. PET Imaging of Tau Deposition in the Aging Human Brain. Neuron. 2016;89(5):971–82.

57. Schwarz AJ, Yu P, Miller BB, Shcherbinin S, Dickson J, Navitsky M, et al. Regional profiles of the candidate tau PET ligand 18F-AV-1451 recapitulate key features of Braak histopathological stages. Brain. 2016;139(Pt 5):1539–50.

58. Chiotis K, Saint-Aubert L, Rodriguez-Vieitez E, Leuzy A, Almkvist O, Savitcheva I, et al. Longitudinal changes of tau PET imaging in relation to hypometabolism in prodromal and Alzheimer's disease dementia. Mol Psychiatry. 2017.

59. Xia C, Makaretz SJ, Caso C, McGinnis S, Gomperts SN, Sepulcre J, et al. Association of In Vivo [18F]AV-1451 Tau PET Imaging Results With Cortical Atrophy and Symptoms in Typical and Atypical Alzheimer Disease. JAMA Neurol. 2017;74(4):427–36.

60. Jang YK, Lyoo CH, Park S, Oh SJ, Cho H, Oh M, et al. Head to head comparison of [(18)F] AV-1451 and [(18)F] THK5351 for tau imaging in Alzheimer's disease and frontotemporal dementia. Eur J Nucl Med Mol Imaging. 2018;45(3):432–42.

61. Chiotis K, Stenkrona P, Almkvist O, Stepanov V, Ferreira D, Arakawa R, et al. Dual tracer tau PET imaging reveals different molecular targets for (11)C-THK5351 and (11) C-PBB3 in the Alzheimer brain. Eur J Nucl Med Mol

Imaging. 2018.

62. Millien I, Blaizot X, Giffard C, Mezenge F, Insausti R, Baron J, et al. Brain glucose hypometabolism after perirhinal lesions in baboons: implications for Alzheimer disease and aging. J Cereb Blood Flow Metab. 2002;22(10):1248–61.

63. De Santi S, de Leon MJ, Rusinek H, Convit A, Tarshish CY, Roche A, et al. Hippocampal formation glucose metabolism and volume losses in MCI and AD. Neurobiol Aging. 2001;22(4):529–39.

64. Minoshima S, Giordani B, Berent S, Frey KA, Foster NL, Kuhl DE. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. Ann Neurol. 1997;42(1):85–94.

65. Mosconi L. Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease. FDG-PET studies in MCI and AD. Eur J Nucl Med Mol Imaging. 2005;32(4):486–510.

66. Chételat G, Desgranges B, de la Sayette V, Viader F, Eustache F, Baron JC. Mild cognitive impairment: can FDG-PET predict who is to rapidly convert to Alzheimer's disease? Neurology. 2003;60(8):1374–7.

67. Silverman DH, Small GW, Chang CY, Lu CS, Kung De Aburto MA, Chen W, et al. Positron emission tomography in evaluation of dementia: regional brain metabolism and long-term outcome. JAMA. 2001;286(17):2120–7.

68. Jagust W, Reed B, Mungas D, Ellis W, Decarli C. What does fluorodeoxyglucose PET imaging add to a clinical diagnosis of dementia? Neurology. 2007;69(9):871–7.

69. Jagust W, Gitcho A, Sun F, Kuczynski B, Mungas D, Haan M. Brain imaging evidence of preclinical Alzheimer's disease in normal aging. Ann Neurol. 2006;59(4):673–81.

70. Herholz K, Nordberg A, Salmon E, Perani D, Kessler J, Mielke R, et al. Impairment of neocortical metabolism predicts progression in Alzheimer's disease. Dement Geriatr Cogn Disord. 1999;10(6):494–504.

71. Finnema SJ, Nabulsi NB, Eid T, Detyniecki K, Lin SF, Chen MK, et al. Imaging synaptic density in the living human brain. Sci Transl Med. 2016;8(348):348ra96.

72. Burton EJ, Barber R, Mukaetova-Ladinska EB, Robson J, Perry RH, Jaros E, et al. Medial temporal lobe atrophy on MRI differentiates Alzheimer's disease from dementia with Lewy bodies and vascular cognitive impairment: a prospective study with pathological verification of diagnosis. Brain. 2009;132(Pt 1):195–203.

73. Yuan Y, Gu ZX, Wei WS. Fluorodeoxyglucose-positron-emission tomography, single-photon emission tomography, and structural MR imaging for prediction of rapid conversion to Alzheimer disease in patients with mild cognitive impairment: a meta-analysis. AJNR Am J Neuroradiol. 2009;30(2):404–10.

74. Toledo JB, Arnold SE, Raible K, Brettschneider J, Xie SX, Grossman M, et al. Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. Brain. 2013;136(Pt 9):2697–706.

75. Frisoni GB, Fox NC, Jack CR Jr, Scheltens P, Thompson PM. The clinical use of structural MRI in Alzheimer disease. Nat Rev Neurol. 2010;6(2):67–77.

76. Shams S, Wahlund LO. Cerebral microbleeds as a

biomarker in Alzheimer's disease? A review in the field. Biomarkers Med. 2016;10(1):9–18.

77. Cordonnier C, van der Flier WM. Brain microbleeds and Alzheimer's disease: innocent observation or key player? Brain. 2011;134(Pt 2):335–44.

78. Henneman WJ, Sluimer JD, Cordonnier C, Baak MM, Scheltens P, Barkhof F, et al. MRI biomarkers of vascular damage and atrophy predicting mortality in a memory clinic population. Stroke. 2009;40(2):492–8.

79. Trojanowski JQ. Tauists, Baptists, Syners, Apostates, and new data. Ann Neurol. 2002;52(3):263–5.

80. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science. 2002;297(5580):353–6.

81. Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun. 1984;120(3):885–90.

82. Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol. 1999;45(3):358–68.

83. Fox NC, Scahill RI, Crum WR, Rossor MN. Correlation between rates of brain atrophy and cognitive decline in AD. Neurology. 1999;52(8):1687–9.

84. Hyman BT. Amyloid-dependent and amyloid-independent stages of Alzheimer disease. Arch Neurol. 2011;68(8):1062–4.

85. Jack CR Jr, Petersen RC, Xu Y, O'Brien PC, Smith GE, Ivnik RJ, et al. Rates of hippocampal atrophy correlate with change in clinical status in aging and AD. Neurology. 2000;55(4):484–9.

86. Andreasen N, Minthon L, Clarberg A, Davidsson P, Gottfries J, Vanmechelen E, et al. Sensitivity, specificity, and stability of CSF-tau in AD in a community-based patient sample. Neurology. 1999;53(7):1488–94.

87. Rosen C, Andreasson U, Mattsson N, Marcusson J, Minthon L, Andreasen N, et al. Cerebrospinal fluid profiles of amyloid beta-related biomarkers in Alzheimer's disease. Neuromolecular Med. 2012;14(1):65–73.

88. Jack CR Jr, Wiste HJ, Lesnick TG, Weigand SD, Knopman DS, Vemuri P, et al. Brain beta-amyloid load approaches a plateau. Neurology. 2013;80(10):890–6.

89. Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 2010;9(1):119–28.

90. Jack CR Jr, Holtzman DM. Biomarker modeling of Alzheimer's disease. Neuron. 2013;80(6):1347–58.

91. Holland D, McEvoy LK, Desikan RS, Dale AM. Alzheimer's Disease Neuroimaging I. Enrichment and stratification for predementia Alzheimer disease clinical trials. PLoS One. 2012;7(10):e47739.

92. Dubois B, Epelbaum S, Nyasse F, Bakardjian H, Gagliardi G, Uspenskaya O, et al. Cognitive and neuroimaging features and brain beta-amyloidosis in individuals at risk of Alzheimer's disease (INSIGHT-preAD): a longitudinal observational study. Lancet Neurol. 2018.

93. Lemm S, Blankertz B, Dickhaus T, Muller KR. Introduction to machine learning for brain imaging. Neuroimage. 2011;56(2):387–99.

94. Adaszewski S, Dukart J, Kherif F, Frackowiak R, Draganski B. Alzheimer's Disease Neuroimaging I. How early can we predict Alzheimer's disease using computational anatomy? Neurobiol Aging. 2013;34(12):2815–26.