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Update on the biomarker core of the Alzheimer's Disease Neuroimaging Initiative subjects

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Abstract

Here, we review progress by the Penn Biomarker Core in the Alzheimer's Disease Neuroimaging Initiative (ADNI) toward developing a pathological cerebrospinal fluid (CSF) and plasma biomarker signature for mild Alzheimer's disease (AD) as well as a biomarker profile that predicts conversion of mild cognitive impairment (MCI) and/or normal control subjects to AD. The Penn Biomarker Core also collaborated with other ADNI Cores to integrate data across ADNI to temporally order changes in clinical measures, imaging data, and chemical biomarkers that serve as mileposts and predictors of the conversion of normal control to MCI as well as MCI to AD, and the progression of AD. Initial CSF studies by the ADNI Biomarker Core revealed a pathological CSF biomarker signature of AD defined by the combination of Aβ1-42 and total tau (T-tau) that effectively delineates mild AD in the large multisite prospective clinical investigation conducted in ADNI. This signature appears to predict conversion from MCI to AD. Data fusion efforts across ADNI Cores generated a model for the temporal ordering of AD biomarkers which suggests that Aβ amyloid biomarkers become abnormal first, followed by changes in neurodegenerative biomarkers (CSF tau, F-18 fluorodeoxyglucose-positron emission tomography, magnetic resonance imaging) with the onset of clinical symptoms. The timing of these changes varies in individual patients due to genetic and environmental factors that increase or decrease an individual's resilience in response to progressive accumulations of AD pathologies.

[1]Since this is an update on the progress of the Penn Biomarker Core of ADNI-1, most citations listed here are from progress by this Core since 2004 and they are identified by *. Due to the explosion in publications on AD biomarkers, research from other centers are mainly cited in the reviews listed here.

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Further studies in ADNI will refine this model and render the biomarkers studied in ADNI more applicable to routine diagnosis and to clinical trials of disease modifying therapies.

Keywords: Alzheimer's disease; Cerebrospinal fluid; Plasma; Biomarkers; Mild cognitive impairment

1. Introduction[1]

1.1. Alzheimer's disease and the quest for informative biomarkers

Alzheimer's disease (AD) is the most common dementia [1,2], and the hallmark lesions of AD are Aβ plaques and neurofibrillary tangles (NFTs) formed by abnormal tau. Clinical symptoms closely relate to NFTs, neurodegeneration, and synapse loss [3–5]. AD can be divided into a presymptomatic phase in which subjects are cognitively normal but have AD pathology, a prodromal phase known as mild cognitive impairment (MCI), and a third phase when patients show dementia with impairments in multiple domains and loss of function in activities of daily living [4,6–8]. Although it has been suggested that diagnostic criteria for early AD should be redefined by the presence of memory impairments plus biomarker evidence of AD [9], this is still controversial despite numerous studies showing that AD biomarkers predict conversion from MCI to AD [10–19], and the diagnosis of AD still requires the presence of dementia [20]. However, it is timely to consider developing strategies to identify AD onset before symptom onset to optimize potential efficacy of disease modifying therapies, and to enable drug development aimed at AD prevention. Initial data emerging from Alzheimer's Disease Neuroimaging Initiative (ADNI) offer encouragement that success in accomplishing this may be close at hand. Thus, here we summarize progress by the Penn Biomarker Core of ADNI toward developing a pathological cerebrospinal fluid (CSF) and plasma biomarker signature for mild AD subjects as well as a biomarker profile that predicts conversion of MCI and normal control (NC) subjects to AD. Furthermore, the Penn Biomarker Core has collaborated with other ADNI Cores in data fusion efforts across ADNI to temporally order changes in clinical measures, imaging data, and chemical biomarkers that serve as mileposts and predictors of the conversion of NC to MCI as well as MCI to AD, and the progression of AD. Thus, we also briefly summarize this work and the hypothetical model of temporal changes in AD biomarkers which emerges now from the initial funding period of ADNI (ADNI-1) and informs future AD Biomarker Core studies in the ADNI renewal period (ADNI-2) and the ADNI Grand Opportunity (ADNI-GO) grant.

1.2. ADNI biomarker core progress: 2004–2009

The Penn Biomarker Core made considerable progress to implement the Aims of ADNI-1 including: (1) the establishment of biofluid collection, (2) the shipping and storage of standard operating procedures (SOPs), (3) establishing an archive of ADNI biofluids, and (4) the launch of our studies of these fluids (see also the ADNI website http://www.adni-info.org/index where all biomarker data are posted). Other progress includes establishing the Resource Allocation Review Committee (RARC), conducting meetings with ADNI Industrial Scientific Advisory Board (ISAB) members, meetings with international biomarker scientists, establishing an international CSF quality control program for continuing quality assessment of biomarker assays in World Wide ADNI (WW-ADNI), and support for ADNI ''add-on'' studies. In keeping with the ADNI mission, all ADNI biomarker data are posted on the ADNI website after they are obtained following data analyses and quality assessment.

1.2.1. Development of SOPs for biofluid collection, shipping, aliquoting, storage and curation

The Penn Biomarker Core established ADNI biomarker SOPs at the outset of ADNI-1. This was done in consultation with ISAB and other biomarker scientists to establish consensus for collection, handling, shipment, labeling, aliquoting, storage and tracking 24 hours/day, 7 days/week for 365 days/year (24/7/365) of DNA, CSF, plasma, serum and urine samples. We also worked closely with the ADNI Clinical Core to develop biofluid tracking forms that provide a detailed history for each sample. These SOPs are essential to assure: (a) sample integrity, (b) accurate identification of samples received and aliquots prepared from them, and (c) sample stability.

An example of the value of detailed characterization of each collected biofluid is documenting the time to freezing on dry ice at study sites. Thus, for the RARC approved proteomic studies of ADNI plasma and CSF samples, data on the time at room temp before freezing each sample could be informative for interpreting results on time/temp sensitive AD biomarkers, and detailed sample history permits selection of samples using a specific time stipulation, e.g., 1 to 2 hours at room temperature, but not longer for plasma samples. Another aspect of sample timing involves CSF collection where knowing the length of time from collection to time of transfer is important. For example, some early CSF samples were mistakenly collected into polystyrene collection tubes at the sites. This was rapidly corrected, but this information enabled the Biomarker Core to understand the effects of this collection error, and simulations of the effects of brief exposure to polystyrene alleviated concerns about this potential confound. Finally, to illustrate the fastidiousness with which ADNI sites obtained and processed biofluid samples, the average time CSF was in contact with any transfer tube was 25.7 minute for ADNI Baseline CSF

samples thereby limiting significant exposure time to any inappropriate CSF collection tube.

1.2.2. Current status of the ADNI-1 biofluid archive

Shortly after SOPs for the collection, processing, bar code labeling, packaging and shipment of biofluids for *APOE* genotyping, cell immortalization and biomarker studies were finalized, these SOPs were incorporated into the ADNI procedure manual, distributed to all ADNI sites and the first ADNI biofluids started to arrive at the Penn Biomarker Core in August 2005. Through April 30, 2007 a total of 1,108 blood samples were collected at the screening visit and all were received and rapidly processed for *APOE* genotyping at Penn so results could be entered into the ADNI database within a week of receipt to balance the ADNI cohort for *APOE* status. Residual blood samples were stored at −80 °C for DNA preparation and genetic studies. As of June 2009 a total of 12,053 ADNI biofluid samples have been received and processed. To respond to the request of the ADNI Clinical Core that clinic visits take place 5 d/wk, we arranged for receipt and freezer storage of biofluids 6 d/wk including Saturdays. Thus far, a total of 119,106 aliquots of serum, plasma, CSF, and urine have been prepared, bar code labeled and stored in dedicated ADNI freezers at −80 °C. Temperature monitoring of each freezer is done 24/7/365 with a telephone alarm system and one Penn Biomarker Core staff person is always ''on-call'' to respond to an alarm. For each primary biofluid sample collected, the following information is maintained in the ADNI Biomarker Core database at Penn: biofluid type (CSF, plasma, serum, urine), coded subject and visit ID, six digit license plate number, visit date and time, date and time of receipt, condition of samples as received, biofluid sample volume and number of aliquots. The database is backed up daily on an external ''brick'' hard drive and on a DVD disk. The latter are stored outside the Biomarker Core laboratory in a secure location in a different building to assure data security in the event of a catastrophic failure of the server on which the database resides.

1.2.3. Round Robin Study to validate and standardize methods to measure CSF Tau and Aβ

To reliably measure Aβ and tau in ADNI CSF samples, the Penn ADNI Biomarker Core identified several sources of variation in quantifying T-tau, tau phosphorylated a threonine 181 (P-tau$_{181p}$) and A$\beta_{1\text{-}42}$ that were shown by Luminex or enzyme-linked immunosorbent assay methods to have at least 85% sensitivity and 80% specificity for diagnosing AD, predicting MCI progression to AD, and identifying elderly Clinical Dementia Rating (CDR) scale 0 individuals likely to progress to CDR >0 [*16,*21]. We also validated the INNO-BIA AlzBio3 reagents (Innogenetics, Belgium[2] [These reagents are for research only and not for use in any diagnostic procedures listed here]) in a seven site study that included academic and ISAB sites.

A pre-qualification, as well as a qualification study was done using a standardized protocol for testing. The pre-qualification round provided the required experience for each participating site. Each run of the qualification study included the use of seven ready-to-use calibrator mixtures for assay calibration, two control samples which are included in the kit for run acceptance, three run validation samples (prepared by the addition of reference materials in diluent (a pre-defined combination of synthetic Aß$_{1\text{-}42}$ and P-tau$_{181p}$ andrecombinant tau protein), and five pools of CSF, including two CSF pools prepared from AD patient CSF samples (0.5 mL aliquots into polypropylene tubes, frozen at −80 °C). Shipping of the samples to participants included temperature monitoring. Three analytical runs were completed using the standardized test protocol. Briefly, the precision study demonstrated the following.

1.2.3.1. Repeatability and reproducibility

Within each of the seven centers, the within-center coefficient of variation (%CV) (combined within-run and between run precision) for measurement of the three CSF biomarkers was ∼10% over the three runs for each biomarker. For the CSF pools, the between-center variability was greater than that for the aqueous-based controls, and studies are underway to explain differences between laboratories. Furthermore, we collaborate with the WW-ADNI CSF biomarker quality control program to improve laboratory performance of these tests. Finally, results using the pre-made calibrators are highly reproducible between the seven centers (for more details see the report ''ADNI Interlaboratory Study'' posted on the ADNI website).

1.2.3.2. Sample stability

Stability of the three aqueous-based and three CSF pools was demonstrated for the 8-month interval between the pre-qualification and qualification studies. Ongoing studies in the Biomarker Core are evaluating longer term stability. Notably, the effect of brief exposure of 20 freshly obtained CSF samples to polystyrene for 1 hour at room temperature, as compared with polypropylene, decreased the concentration of A$\beta_{1\text{-}42}$, by 14.5%, and tau by 11%, but there was no change in P-Tau$_{181P}$ concentration (a collaborative study with Kaj Blennow).

1.2.3.3. ADNI CSF sample analyses

Tolerance intervals were generated from the CSF pool data for the Biomarker Core as a guide for the acceptability of analytical runs of ADNI CSF samples.

1.2.3.4. Publication of results

These data together with the pre-qualification study data now are being prepared for publication (Shaw et al., in preparation). This article reports on data from individual participating laboratories regarding other aspects of CSF biomarker measurements related to pre-analytical sample handling (freeze-thaw, long-term/short-term stability; diurnal variation), as well as instrumentation aspects (comparison of instruments and algorithms). All these data were posted on the ADNI website following completion and brief summaries for the ADNI sites were prepared and distributed at ADNI

meetings as well as placed on the ADNI website. Moreover, we published a detailed review [*21] of the steps and procedures required to bring potential AD biomarkers from concept to their implementation as useful biomarkers with particular emphasis on how this is done for the multiplexed analysis of CSF Aβ and tau.

1.2.4. Baseline Studies of CSF Tau and Aβ in >400 ADNI Subjects

This study of ADNI baseline CSF samples sought to develop a pathological CSF biomarker signature for AD [*16]. To do this, $A\beta_{1\text{-}42}$, T-tau and P-tau_{181} were measured in: (a) CSF samples obtained at baseline for 100 mild AD, 196 MCI and 114 elderly NC subjects in ADNI, and (b) an independent set of 56 autopsy-confirmed AD subjects and 52 age-matched elderly NC followed in the National Institute of Aging funded Penn AD Core Center with ADNI SOPs using the Luminex platform based multiplex immunoassay [*16,*21]. Detection of an AD CSF profile for T-tau, P-tau_{181}, and $A\beta_{1\text{-}42}$ in ADNI subjects was achieved using receiver operating characteristic cutpoints and logistic regression models derived from the autopsy-confirmed CSF biomarker data. Our data showed that CSF $A\beta_{1\text{-}42}$ was the most sensitive biomarker for AD detection in CSF from non-ADNI autopsy-confirmed subjects with a receiver operating characteristic area under the curve of 0.913 and sensitivity for AD detection of 96.4%. A unique bimodal characteristic of the distribution of CSF $A\beta_{1\text{-}42}$ was detected in each ADNI subgroup, and a logistic regression model for $A\beta_{1\text{-}42}$, T-tau, and *APOE* ε4 allele count provided the best delineation of mild AD. An AD-like pathological baseline CSF profile for T-tau/$A\beta_{1\text{-}42}$ was detected in 33/37 ADNI MCI subjects who converted to probable AD during the first year of the study. Based on these data, we conclude that the pathological CSF biomarker signature of AD defined by the combination of $A\beta_{1\text{-}42}$ and T-tau in the Penn autopsy-confirmed AD cohort and tested in the cohort followed in ADNI for 12 months effectively detects mild AD in a large multisite prospective clinical investigation, and this signature appears to predict conversion from MCI to AD.

The cutoff values established by Shaw et al. [*16] were validated in a follow-up study with EU-ADNI and ISAB collaborators [*22] wherein we sought to identify AD biomarker patterns in an independent, unsupervised manner, without clinical diagnoses using a mixture modeling approach to analyze the ADNI CSF Aβ42, T-tau, and P-tau_{181} data in Shaw et al. [*16]. This analysis was validated on two additional data sets, one of which was an autopsy-confirmed EU cohort. Using the ADNI data set, a CSF Aβ1-42/P-tau_{181} biomarker mixture model identified one feature linked to AD, while the other matched the NC status. The AD signature was found in 90%, 72%, and 36% of patients in the AD, MCI, and NC groups, respectively. The NC group with the AD signature was enriched in *APOE* ε4 allele carriers.

Furthermore, we collaborated with Hopkins investigators outside ADNI to investigate the effect of CSF abnormalities on rate of decline in everyday function in NC, MCI, and AD [*23]. Briefly, CSF T-tau, P-tau_{181}, and $A\beta_{42}$ data from 114 NC, 195 MCI patients, and 100 mild AD ADNI subjects and their Functional Activities Questionnaire and AD Assessment Scale (ADAS-cog) data were analyzed by random effects regressions. All CSF analytes were associated with functional decline in MCI, and all but T-tau/$A\beta_{42}$ were associated with functional decline in controls. Among controls, P-tau_{181} was the most sensitive to functional decline whereas in MCI it was $A\beta_{42}$. CSF biomarkers were uniformly more sensitive to functional decline than the ADAS-cog among controls and variably so in MCI, whereas the ADAS-cog was more sensitive than CSF biomarkers in AD. The impact of CSF biomarkers on functional decline in MCI was partially mediated by their impact on cognitive status. Across all diagnostic groups, persons with a combination of tau and $A\beta_{42}$ abnormalities exhibited the steepest rate of functional decline. These data indicate that CSF tau and Aβ42 abnormalities are associated with functional decline, and thus with future development of AD in controls and MCI patients. However, they are not predictive of further functional degradation in AD. Hence, persons with AD-like CSF tau and $A\beta_{42}$ abnormalities are at greatest risk of functional impairment.

1.2.5. Assessment of sulphatides, homocysteine and isoprostanes as AD biomarkers in ADNI-1

1.2.5.1. Sulphatides

Plans to validate CSF sulphatides as AD biomarkers in ADNI-1 were based on promising reports in 2004, but subsequent studies did not confirm these findings so we did not pursue sulphatides further. Therefore, we re-budgeted the savings from this change to support the 2.5-fold increase in the number of CSF samples we obtained, i.e. an increase from 20% of all ADNI subjects described in the original application to >50% after ADNI-1 was launched.

1.2.5.2. Homocysteine

ADNI-1 included plans to study homocysteine in plasma and CSF. Using a validated enzyme immunoassay methodology, we measured homocysteine in 813 baseline ADNI plasma samples. These revealed that there was no significant difference in mean plasma homocysteine concentration in AD vs. MCI, but there was between NC and AD ($P < .01$) and NC and MCI ($P < .01$). These data are consistent with previous studies showing an association between elevated baseline plasma homocysteine concentration and risk for development of AD [*24]. Inclusion of homocysteine in the logistic regression model described above for $A\beta_{1\text{-}42}$, T-tau, and *APOE* ε4 allele number showed it was nonsignificant as a variable in this model so the value of measuring plasma homocysteine levels is uncertain. However, additional analyses are underway for plasma homocysteine including correlations with other biomarkers and longitudinal changes.

Measurement of homocysteine concentrations in CSF was achieved in 410 CSF samples collected at baseline using a validated enzyme immunoassay (developed and performed by Merck) designed specifically to measure the much lower concentrations present in this biofluid as compared with plasma. However, these studies showed no significant difference between NC and either AD or MCI in mean homocysteine values.

1.2.5.3. Isoprostanes

Since earlier studies suggested that quantification of F [2]-Isoprostanes is a reliable index of oxidative stress in vivo and is valuable in the diagnosis and monitoring of AD [reviewed in *24], the most relevant isoprostanes were studied in CSF, plasma and urine as described in the ADNI-1 application. To do this, we developed and validated a semiautomated high-throughput high performance liquid chromatography tandem mass spectrometry assay for the quantification of 8-iso-PGF_{2a} in human urine and plasma [*25]. Briefly, after protein precipitation, samples were injected into the high performance liquid chromatography system and extracted online. The extracts were then back-flushed onto the analytical column and detected with an atmospheric pressure chemical ionization-triple quadrupole mass spectrometer monitoring the deprotonated molecular ions [M-H](−) of 8-iso-PGF_{2a} (m/z = 353–>193) and the internal standard 8-iso-PGF_{2a} ,8-iso-PGF_{2a} −d(4) (m/z = 357–>197). We then applied these methods to studies of human urine and plasma and showed that the assay was linear from 0.025 to 80 μg/L and in human plasma from 0.0025 to 80 μg/L (r [2] > .99). Inter-day accuracy and precision for concentrations above the lower limit of quantification were <10%. Concentrations of 8-iso-PGF_{2a} in urine of 16 NC individuals ranged from 55 to 348 ng/g creatinine. In 16 plasma samples from NC individuals, free 8-iso-PGF_{2a} was detectable in all samples and concentrations were 3 to 25 ng/L. These data show that our assay meets all method performance criteria, allows for analysis of >80 samples/d, and has the sensitivity to quantify 8-iso-PGF_{2a} concentrations in plasma and urine from NC individuals.

Next, we undertook validation studies of a liquid chromatography method with tandem mass spectrometry detection for simultaneous analysis of 8-iso-PGF_{2a} and 8,12-iso-iPF_{2a}-VI. Notably, these are the most frequently studied isoprostanes in human CSF and brain tissue [*24,*25]. An API 5000 triple quadrupole instrument with an atmospheric pressure chemical ionization ion source was used in this study. We used this method to quantify both isoprostanes in CSF from non-ADNI Penn AD patients and age-matched NC as well as in postmortem brains from AD and non-AD controls. Our results do not confirm some previous reports that the CSF isoprostanes studied here are useful AD biomarkers. However, we currently conduct similar isoprostane assays of ADNI plasma samples to determine if plasma isoprostanes are informative for risk for conversion from MCI to AD since the elderly population studied here will have increased risk for cardiovascular and cerebrovascular disease which could affect risk for AD.

1.2.6. Cross-sectional and longitudinal measures in NC, MCI and AD subjects in ADNI

In collaboration with Petersen et al. [*26], we characterized NC, MCI, and mild AD subjects to enable the assessment of the utility of neuroimaging and chemical biomarker measures in 819 subjects (229 NC, 398 with MCI and 192 AD) enrolled at baseline in ADNI and followed up for 12 months. The MCI subjects were more memory impaired than the NC subjects, but not as impaired as the AD subjects. Non-memory cognitive measures were minimally impaired in MCI subjects. The MCI subjects progressed to dementia in 12 months at a rate of 16.5% per year and ∼50% of the MCI subjects were on anti-dementia therapies. There was minimal movement on the ADAS-cog for the NC subjects, slight movement of the MCI subjects of 1.1 and a modest change for the AD subjects of 4.3. Baseline CSF measures of Aβ42 separated the three groups as expected [*16] and successfully predicted the 12-month change in cognitive measures. Thus, the 12-month progression rate of MCI was as predicted, and the CSF measures heralded progression of clinical measures over 12 months.

1.2.7. Ventricular expansion and CSF biomarkers in NC, MCI and AD subjects in ADNI

A collaborative study with the Thompson lab [*27] sought to improve on the single-atlas ventricular segmentation method using multi-atlas segmentation. We also calculated minimal numbers of subjects needed to detect correlations between clinical scores and ventricular maps. Correlations were analyzed between AD tau and Aβ42 CSF biomarkers [*16] and localizable deficits in the brain, in 80 AD, 80 MCI, and 80 NC from ADNI. Lower CSF Aβ42 protein levels were correlated with lateral ventricular expansion, and these studies show that ventricular expansion maps correlate with pathological CSF and cognitive measures in AD.

1.2.8. Combined analysis of Pittsburgh compound B (PIB), positron emission tomography, CSF biomarkers and cognition in ADNI subjects

Collaborations with the Jagust lab [*28] compared PIB-positron emission tomography (PET), F-18 fluorodeoxyglucose (FDG)-PET, and CSF measures of Aβ42, T-tau, and P-tau_{181} in 10 AD, 11 NC and 34 MCI ADNI subjects. There was substantial agreement between PIB-PET and CSF Aβ42 measures (91% agreement, kappa = 0.74), modest agreement between PIB-PET and P-tau (76% agreement, kappa = 0.50), and minimal agreement for other comparisons (kappa < 0.3). The Mini-Mental State Examination (MMSE) score was significantly correlated with FDG-PET but not with PIB-PET or CSF Aβ42. Regression models showed that PIB-PET was significantly correlated with Aβ42, T-tau, and P-tau_{181}, while FDG-PET only correlated with

Aβ42. Thus, PET and CSF biomarkers of Aβ agree with one another while FDG-PET is modestly related to other biomarkers, but is better related to cognition.

1.2.9. Tensor based imaging, CSF biomarkers and cognition in ADNI subjects

In a collaborative study with Leow et al. [*29], the anatomical distribution of longitudinal brain structural changes were compared over 12 months in 20 AD, 40 NCs, and 40 MCI ADNI subjects. We detected widespread cerebral atrophy in AD, and a more restricted atrophic pattern in MCI. In MCI, temporal lobe atrophy rates were correlated with changes in MMSE scores, CDR, and logical/verbal learning memory scores. In AD, temporal atrophy rates were correlated with higher CSF P-tau levels, and a greater CSF tau/Aβ42 ratio. Temporal lobe atrophy was significantly faster in MCI subjects who converted to AD than in nonconverters. Thus, serial magnetic resonance imaging (MRI) scans relate ongoing neurodegeneration to CSF biomarkers, cognitive changes, and conversion from MCI to AD.

1.2.10. Hippocampal volume loss, APOE genotype and CSF biomarkers in early AD

A collaborative study with the Weiner lab [*30] included 112 NC, 226 MCI, and 96 AD subjects who had at least three successive MRI scans at 47 ADNI sites. The MCI and AD groups showed hippocampal volume loss over 6 months and accelerated loss over 1 year while increased rates of hippocampal loss were associated with the *APOE* ε4 allele in AD and lower CSF Aβ42 in MCI irrespective of *APOE* genotype. These data supports the concept that increased hippocampal volume loss is an indicator of AD pathology and a potential marker for efficacy of therapeutic interventions in AD.

1.2.11. Comparing MRI and CSF Biomarkers in NC, MCI and AD subjects in ADNI

Collaborative studies with Cliff Jack's lab [*31,*32] correlated MRI and CSF biomarkers with clinical diagnosis and cognitive performance in NC, MCI, and AD. Baseline CSF (T-tau, Aβ1-42 and P-tau$_{181}$) and MRI scans were obtained in 399 subjects (109 NC, 192 MCI, 98 AD). STAND-scores (STructural Abnormality iNDexscore) were computed, and correlated with CDR-SB and MMSE in MCI and AD ($P \leq .01$). STAND and all CSF biomarkers were predictors of clinical group (NC, MCI or AD) univariately ($P < .001$). These studies show that CSF and MRI biomarkers independently contribute to intergroup diagnostic discrimination and the combination of CSF and MRI provides better prediction than either source of data alone. Studies of these subjects were extended, and single-predictor Cox proportional hazard models for time to conversion from MCI to AD showed that STAND and log T-tau/Aβ42 were predictive of future conversion. Thus, these studies show that MRI and CSF tau/Aβ42 provide complimentary predictive information about time to conversion from MCI to AD and combination of the two provides better prediction than either source alone.

1.2.12. Rules Based Medicine (RBM) studies of CSF and plasma

ADNI RBM ''add-on'' studies are in progress now using plasma and CSF samples from the entire ADNI cohort, but data are not yet available. Briefly, the plan is to interrogate these ADNI samples with an RBM panel of ~190 analytes using the Luminex® bead-based immunoassay system to identify novel sets of CSF and plasma AD biomarkers associated with definite AD versus MCI or NC status. Preliminary data from RBM studies of non-ADNI Penn samples identified several potential CSF and plasma AD biomarkers for further investigation in ADNI-1 (Hu et al., unpublished observation) and completion of the RBM interrogation of the ADNI-1 CSF and plasma samples in the coming months will enable independent confirmation or rejection of these analytes for further study in the ADNI competing renewal period (ADNI-2).

1.2.13. Round Robin Studies to determine the role of plasma Aβ40/42 as AD biomarkers

We recently completed our Aβ plasma round robin study that includes 12 academic and industrial laboratories, including ISAB sites. Each site used INO-BIA plasma Aβ kits (Innogenetics, Belgium). Notably, these studies now establish that we can reliably assess the utility of measuring plasma Aβ to predict conversion from MCI to AD and/or to monitor AD progression in studies of plasma Aβ proposed for ADNI-2. Detailed statistical analyses of the full data set are underway, but the reproducibility data achieved by the Penn ADNI Biomarker Core using the Innogenetics multiplex assay for plasma Aβ42/40 in this interlab round robin study look very promising. For example, we were able to achieve within and between day %CVs ranging between 0.9% and 4.9% for plasma Aβ42, 1.2% to 6.6% for Aβ40 and up to 7.6% for the ratio of Aβ42/Aβ40.

1.2.14. A model for the temporal ordering of biomarkers of AD pathology

The model proposed here and illustrated in Fig. 1 is based on the view that AD begins with abnormal processing of amyloid precursor protein thereby increasing brain Aβ which leads to neuron dysfunction and death [*33–*37]. The model also assumes a lag phase between Aβ deposition and neuron loss, and differences in brain resiliency, plasticity, cognitive reserve, or other factors likely account for the variable duration of this lag phase. The presence of additional brain pathologies (e.g., alpha-synuclein, TDP-43 lesions) also may contribute to clinical variations in AD patients. Briefly, the hypothetical model proposed by ADNI investigators in Fig. 1 relates AD onset and progression to AD biomarkers based on the following assumptions: (1) these biomarkers become abnormal before clinical symptoms

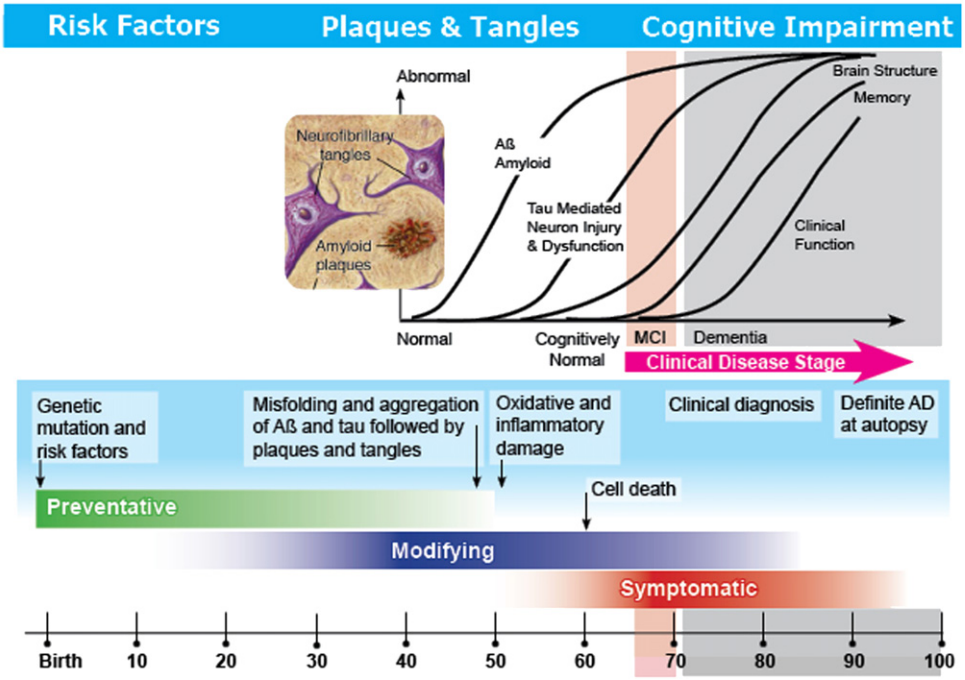

Fig. 1. This figure shows a hypothetical time line for the onset and progression of Alzheimer's disease (AD) neurodegeneration and cognitive impairments progressing from normal control to mild cognitive impairment (MCI) and on to AD. The only highly predictive biomarkers for AD years before disease onset are genetic mutations that are pathogenic for familial AD, and these can be detected from birth onwards to identify those individuals in familial AD kindreds who will go on to develop AD later in life. However, the emphasis in this review is on promising AD biomarkers studied in Alzheimer's Disease Neuroimaging Initiative for the diagnosis of AD and predicting conversion from normal control and/or MCI status to AD. Age from birth onwards is indicated in the timeline at the bottom of the figure and the green, blue and red bars indicate the time points at which preventive, disease modifying, and symptomatic interventions, respectively, are likely to be most effective, and the aqua bar identifies milestones in the pathobiology of AD that culminate in death and autopsy confirmation of AD. However, AD biomarkers are needed to accelerate efforts to test the efficacy of preventive and disease modifying therapies for AD. To do this, it is important to determine the temporal ordering of AD biomarkers, and the proposed Alzheimer's Disease Neuroimaging Initiative model illustrating the ordering of biomarkers of AD pathology relative to stages in the clinical onset and progression of AD is shown in the insert at the upper right of the figure adjacent to a depiction to the left of the defining pathologies of AD, i.e., plaques and tangles. In the insert on the right, clinical disease is on the horizontal axis and it is divided into three stages; cognitively normal, MCI and dementia. The vertical axis indicates the range from normal to abnormal for each of the biomarkers as well as for measures of memory and functional impairments. Amyloid imaging and cerebrospinal fluid Aβ are biomarkers of brain Aβ amyloidosis. Cerebrospinal fluid tau and F-18 fluorodeoxyglucose positron emission tomography are biomarkers of neuron injury and degeneration while structural magnetic resonance imaging is a biomarker of abnormal brain morphology.

appear, (2) Aβ biomarkers become abnormal before tau and neurodegenerative biomarkers, (3) tau and neurodegenerative biomarkers correlate with clinical disease severity, and (4) these biomarkers are temporally ordered. Growing evidence supports these assumptions as reviewed recently in Jack et al. [*36].

1.3. ADNI-2: the future

Consistent with the goals of ADNI-1 and building on our progress in the first ADNI funding period, the Specific Aims of the Biomarker Core in the ADNI renewal (ADNI-2) are to test our new AD biomarker hypotheses. To do this, we: (1) bank and curate biofluids from ADNI-1, ADNI GO, and ADNI-2 subjects, (2) distribute ADNI samples to investigators qualified by the ADNI RARC, and (3) study promising AD biomarkers. Selection of biomarkers for study in ADNI-2 is based on: (1) ADNI-1 Biomarker Core studies, (2) our studies of AD biomarkers in non-ADNI cohorts, and (3) other AD biomarker research. For example, our ADNI-1 data on CSF Aβ and tau established the importance of longitudinal studies of these analytes, while our Aβ plasma round robin data enable us to determine the utility of measuring plasma Aβ to predict conversion from MCI, including early MCI to AD or to monitor AD progression. Thus, the Specific Aims of the ADNI-2 Biomarker Core are designed to test the following hypotheses:

a. A panel of CSF/plasma biomarkers (rather than any single analyte) will predict conversion from normal to MCI (including early MCI) or to AD and conversion from MCI to AD as well as identify MCI subjects who have stable MCI and do not convert to AD.
b. A panel of CSF/plasma biomarkers will reflect the progression of AD from its prodromal phase through to moderate or severe stages of AD.
c. A panel of CSF/plasma biomarkers will predict the likelihood of healthy brain aging or resistance to AD in the NC population.

To accomplish this, the Specific Aims of the Penn Biomarker Core in ADNI-2 are to:

1. Continue to collect, aliquot, store, curate, track all samples collected from subjects in ADNI-1, ADNI GO, and ADNI-2.
2. Continue biomarker studies of CSF Aβ42, T-tau, and P-tau181p as well as plasma Aβ42 and Aβ40.
3. Validate promising new biomarkers including BACE, and analytes identified by the RBM panel of ~100 analytes.
4. Partner with the ADNI ISAB members and other investigators outside ADNI in RARC approved ''add-on'' biomarker studies that may include proteomic, metabalomic, and lipidomic methodologies.
5. Collaborate with all ADNI Cores/Investigators in analyses of biomarker, clinical, imaging and autopsy data.
6. Collaborate with WW-ADNI Sites in Europe, Japan, Korea, China, and Australia in joint studies and comparative analyses of previously collected and new biomarker data.

By implementing these Aims to test our new ADNI-2 hypotheses, we will advance understanding of the applications of validated and new AD biomarkers as predictive, diagnostic, and progression markers from NC to early MCI/MCI and thence to AD thereby contributing to the mission of ADNI-2.

2. Conclusions

The ADNI Biomarker Core at Penn has made substantial progress since ADNI-1 was launched in 2004, as reviewed here. Significantly, as a result of the intense collaborations across the ADNI Cores, we have generated a compelling model of the temporal ordering of AD biomarkers (Fig. 1) that will guide our studies of specific AD biomarkers for diagnosis and disease staging in ADNI-2 [*36]. The staging biomarkers and their temporal relationships with the phases of AD discussed here present opportunities to test AD biomarker hypotheses by ADNI investigators in ADNI-2 and by other investigators who mine ADNI data or develop their own biomarker data sets in WW-ADNI or other biomarker initiatives. In addition to their use in diagnostic tests, CSF biomarkers may be valuable in clinical trials, for enrichment of the patient sample with pure AD cases, for patient stratification, as safety markers, and to detect and monitor the biochemical effects of drugs [*37,38]. Thus, in the near future, we expect that data emerging from North American ADNI, WW-ADNI, and other biomarker initiatives will deliver on the promise to provide validated AD biomarkers for a wide variety of applications including predictive testing for AD, diagnosis of AD and for use in clinical trials to assess the response of disease modifying therapies in AD patients.

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