Plasma multianalyte profiling in mild cognitive impairment and Alzheimer disease

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Supplemental data at www.neurology.org

Supplemental Data

ABSTRACT

Objectives: While plasma biomarkers have been proposed to aid in the clinical diagnosis of Alzheimer disease (AD), few biomarkers have been validated in independent patient cohorts. Here we aim to determine plasma biomarkers associated with AD in 2 independent cohorts and validate the findings in the multicenter Alzheimer's Disease Neuroimaging Initiative (ADNI).

Methods: Using a targeted proteomic approach, we measured levels of 190 plasma proteins and peptides in 600 participants from 2 independent centers (University of Pennsylvania, Philadelphia; Washington University, St. Louis, MO), and identified 17 analytes associated with the diagnosis of very mild dementia/mild cognitive impairment (MCI) or AD. Four analytes (apoE, B-type natriuretic peptide, C-reactive protein, pancreatic polypeptide) were also found to be altered in clinical MCI/AD in the ADNI cohort (n = 566). Regression analysis showed CSF Aβ42 levels and t-tau/Aβ42 ratios to correlate with the number of *APOE*4 alleles and plasma levels of B-type natriuretic peptide and pancreatic polypeptide.

Conclusion: Four plasma analytes were consistently associated with the diagnosis of very mild dementia/MCI/AD in 3 independent clinical cohorts. These plasma biomarkers may predict underlying AD through their association with CSF AD biomarkers, and the association between plasma and CSF amyloid biomarkers needs to be confirmed in a prospective study. *Neurology*® 2012;79:897-905

GLOSSARY

Aβ42 = β-amyloid 1–42; **AD** = Alzheimer disease; **ADNI** = Alzheimer's Disease Neuroimaging Initiative; **BNP** = B-type natriuretic peptide; **CDR** = Clinical Dementia Rating; **CRP** = C-reactive protein; **CV** = coefficient of variation; **IGF-BP2** = insulin growth factor binding protein 2; **IL** = interleukin; **IUT** = Intersection Union Test; **MCI** = mild cognitive impairment; **p-tau$_{181}$** = tau phosphorylated at threonine 181; **Penn** = University of Pennsylvania; **RBM** = Rules-Based Medicine; **SAM** = significance analysis of microarrays; **t-tau** = total tau; **TARC** = Texas Alzheimer's Research Consortium; **WU** = Washington University.

The clinical diagnosis of mild cognitive impairment (MCI) and probable Alzheimer disease (AD) is increasingly aided by biomarkers predictive of underlying pathology.[1,2] These include CSF biomarkers reflecting the plaque and tangle pathology underlying AD such as β-amyloid 1–42 (Aβ42), total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau$_{181}$)[3]; substrate-specific brain imaging such as ^{11}C and ^{18}F PET imaging[4,5]; and structural MRI findings such as hippocampal volume.[6] Each modality has a different sensitivity-specificity profile, and additional technical barriers and patient preferences may dictate the successful implementation of any biomarker into clinical practice, including aversion to having a lumbar puncture for CSF biomarkers and cost for advanced imaging. Thus, a blood-based test is an appealing alternative because of its simplicity and cost-effectiveness for widespread clinical use as well as in specialty centers.[7,8] A multi-analyte profiling approach to plasma proteins and peptides can also yield

Table 1 Demographic features of subjects included in plasma multianalyte profiling from the University of Pennsylvania, Washington University, and Alzheimer's Disease Neuroimaging Initiative

University of Pennsylvania	Normal cognition	MCI	AD	Other dementias
No. (% female)	126 (64)	16 (94)	88 (55)	37 (41)
Age, y (SD)	68.30 (10.87)	72.38 (8.60)	70.83 (11.69)	65.14 (9.80)
% (n) APOE4 positive[a]	22 (101)	80 (15)	58 (74)	43 (35)
MMSE (SD)	29.15 (1.15)	25.19 (2.26)	17.59 (6.70)	

Washington University	Normal cognition	CDR 0.5	CDR 1
No. (% female)	242 (65)	63 (52)	28 (50)
Age, y (SD)	71.6 (7.4)	74.6 (7.3)	76.8 (6.2)
% APOE4 positive	32	54	57
MMSE (SD)	28.9 (1.3)	26.3 (2.8)	22.5 (4.0)

ADNI	Normal cognition	MCI	AD
No. (% female)	58 (48.3)	396 (35.4)	112 (42)
Age, y (SD)	75.2 (5.8)	74.9 (7.5)	75.0 (8.0)
% APOE4 positive	9	53	68
MMSE	28.9 (1.2)	27.0 (1.8)	23.6 (1.9)
CDR 0	58	1	0
CDR 0.5	0	395	59
CDR 1	0	0	53
CSF (n = 352)			
Aβ42 (SD)	251.45 (20.47)	163.81 (54.18)	142.52 (39.75)
t-Tau (SD)	63.69 (23.56)	103.66 (61.00)	121.21 (57.12)

Abbreviations: AD = clinically probable Alzheimer disease; ADNI = Alzheimer's Disease Neuroimaging Initiative; CDR = Clinical Dementia Rating scale; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination score.
[a] APOE genotyping information missing in 42 subjects, with total number of subjects with genotyping information shown in parentheses. The WU cohort had a higher proportion of participants with normal cognition (73% vs 55%, $p < 0.0001$) and a lower proportion of subjects with clinically probable AD than the Penn cohort (8% vs 38%, $p < 0.001$).

biologically important signatures of disease and endophenotypes to allow for prognostication and novel therapeutic development.

A number of recent studies generated additional enthusiasm for a blood-based test predictive of underlying AD pathology.[7,9] Using clinically diagnosed patients, 1 study identified plasma analyte combinations that predicted AD diagnosis and subsequent conversion to AD among subjects with MCI.[7] However, results from biomarker studies such as these often suffer from failed replication due to a combination of causes including lack of methodologic standardization, insufficient sample size, and overtraining of imprecise data,[10] and the 2 published studies may overestimate the clinical utility of blood-based tests given the single-batch nature and reliance on internal cross-validation within each study. Recently, serum analytes predictive of AD were found to have only modest accuracy in plasma.[8] Here we attempt to focus on the overlap between plasma analytes associated with MCI/AD in 2 independently recruited and characterized cohorts,[3,11–14] and then validate these findings in an independent cohort of 566 participants from the multicenter Alzheimer's Disease Neuroimaging Initiative (ADNI)[15,16] while exploring whether plasma analytes associated with MCI/AD correlated with CSF AD biomarker levels.

METHODS Standard protocol approvals, registrations, and patient consents. The study protocol involving University of Pennsylvania (Penn) subjects and samples was approved by the Penn Institutional Review Board. The study protocol involving Washington University (WU) subjects and samples was approved by the Human Studies Committee at Washington University. Written and verbal informed consent was obtained at enrollment at each center.

Participants. Subjects in the 2 discovery sets were recruited and longitudinally followed at Penn and WU (table 1), while subjects in ADNI were previously described.[16,17] At Penn, participants (n = 267) were community-dwelling healthy volunteers and patients evaluated at subspecialty clinics dedicated to the evaluation of neurodegenerative disorders including MCI,[18] AD,[19,20] and related dementia.[21,22] Cognitively normal subjects were recruited through all subspecialty clinics to participate in biofluid studies. APOE genotyping was performed for 235 out of 267 Penn subjects.

At WU, participants (n = 333) were community-dwelling volunteers enrolled in longitudinal studies of healthy aging and dementia at the Knight AD Research Center at Washington University. Clinical diagnosis was evaluated based on criteria from the National Institute of Neurological and Communicative Diseases and Stroke–Alzheimer's Disease and Related Disorders Association.[19] Cognitive status was rated with the Clinical Dementia Rating scale (CDR): CDR of 0 indicates no dementia, CDR 0.5 indicates very mild dementia, and CDR 1 indicates mild dementia.[23] Some of the CDR 0.5 participants in the study met the criteria for MCI and some were less impaired and were considered "pre-MCI."[18,24] APOE genotyping was performed for all subjects enrolled at WU.

Procedures. Samples were collected from Penn and WU subjects according to strict protocols without protease inhibitors. At sample collection, participants were ≥50 years of age and in good general health (including no evidence of clinically significant liver disease or renal failure), having no other psychiatric or medical diagnoses that could contribute importantly to cognitive impairment or dementia other than the primary neurodegenerative disorder. At Penn, plasma was collected in 10 mL K_2EDTA tubes (BD Vacutainer®) without overnight fasting and refrigerated immediately (4°C) before transporting to a central site on ice for centrifuge (2,000 g × 15 minutes at 4°C) separation into plasma and cellular components within 4 hours of collection.
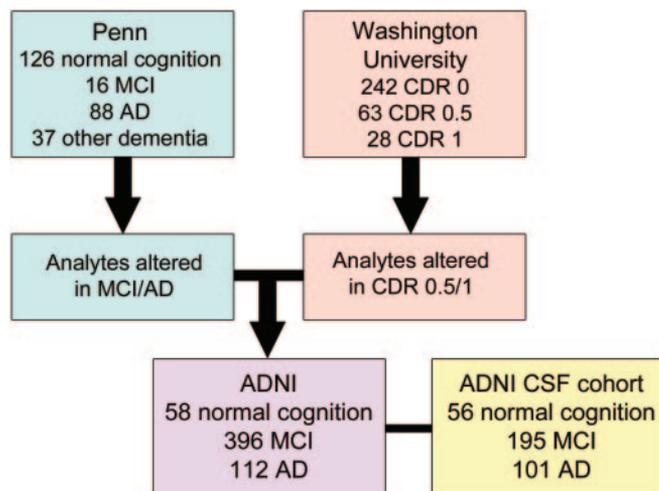
Subjects from University of Pennsylvania (Penn) and Alzheimer's Disease Neuroimaging Initiative (ADNI) were stratified into normal cognition, mild cognitive impairment (MCI), and clinically probable Alzheimer disease (AD) according to published criteria, along with additional patients from Penn with non-AD dementia. Subjects from Washington University were stratified according to Clinical Dementia Rating scale (CDR) according to published criteria (see Methods). In each cohort, mild impairment likely due to AD (mild cognitive and AD, or CDR of 0.5 and 1) were grouped and compared with subjects with normal cognition or CDR of 0.

Plasma aliquots (0.5 mL) were prepared, bar-coded, and then stored in polypropylene vials at −80°C until analysis. Quality control samples to determine coefficients of variation (CV) included duplicate plasma samples from 3 control subjects analyzed at the same time as the remaining Penn subjects, and an average intra-assay CV was obtained for each analyte of interest. At WU, plasma was collected in polypropylene tubes after overnight fasting between 7:30 and 8:00 AM and centrifuged (2,000 $g \times 15$ minutes at 4°C) for separation into plasma and cellular components. Plasma aliquots (0.5 mL) were stored at −80°C until analyzed.

Plasma aliquots from each center were interrogated consecutively in 2 batches (1 batch per center) in 2009 by Rules-Based Medicine (RBM, Austin, TX) for levels of 190 analytes using the multiplex Human DiscoveryMAP™ panel and a Luminex 100 platform (table e-1 on the *Neurology*® Web site at www.neurology.org).[12,25,26] The 190 analytes were assembled into preformatted assays designed for different diseases including cancer, autoimmune disorders, AD, Parkinson disease, and frontotemporal degeneration.[27] Plasma levels of 190 analytes in 566 subjects from the ADNI cohort (table 1) were also measured at RBM in 2010 using the same multiplexed immunoassays. Analytes below threshold of detection (11 for Penn and 21 for WU) were excluded. Dynamic range for each plasma analyte in the RBM panel is provided on the ADNI Web site (http://adni.loni.ucla.edu). A total of 352 subjects (56 normal cognition, 195 MCI, and 101 AD) also had CSF AD biomarker levels provided by the ADNI Biomarker Core.[3]

Statistical analysis. Statistical analysis in this study was performed in SPSS 17.0 (Chicago, IL) and significance analysis of microarrays (SAM).[28] In each cohort, cognitively impaired individuals (Penn: MCI and AD, WU: CDR 0.5 and 1) were grouped together as we sought to identify plasma analytes altered across various stages of the very mild dementia/MCI/AD spec-

trum, and because of the differential distribution of subjects within each impaired category. Power calculation was performed in PASS 11 (Kaysville, UT), which showed 89.0% power in the Penn cohort and 93.8% power in the WU cohort for each of 190 analytes to detect a true difference in expression of at least 0.5 with estimated group SD of 1.0 and a false discovery rate of 0.10 using a 2-sided 2-sample t test. All raw levels were log-transformed to achieve normal distribution. For initial identification of individual analytes different between normal cognition and very mild dementia/MCI/AD, logistic regression model was used adjusting for age and gender. We then used a model based on Intersection Union Test (IUT), which involves identification of overlapping results (analytes in the current study, genes in microarray studies) from distinct datasets.[29] As this method may be overly conservative and reduce the power in detecting true positives,[30] we used a more liberal threshold of significance at the univariate analysis stage of $p < 0.10$ (after adjusting for age and gender) to reduce type II errors. We further reduced type I errors by applying 2 additional filters by identifying 1) analytes from the modified IUT with common direction of change (vector direction) and 2) analytes from (1) that fulfill strict Bonferroni correction at the validation phase. Analytes with similar associations with very mild dementia/MCI/AD in each discovery cohort were then analyzed in the ADNI cohort (n = 566) for association with the diagnosis of MCI/AD with an α value of 0.0036 (0.05/14) for the 14 analytes that passed first level screening (figure). Univariate analysis was also repeated within each cohort using SAM,[28] and analytes found to be significant in more than 2 cohorts were identified.

In addition, we determined the relationship between novel plasma MAP biomarkers and CSF AD biomarker-drive diagnosis (CSF Aβ42 levels <193 pg/mL and t-tau/Aβ42 >0.39)[3] adjusting for age and gender ($p < 0.0036$), and the correlation between CSF AD biomarker and plasma marker levels using linear regression analysis. In these models, CSF AD biomarker (Aβ42, t-tau/Aβ42) levels were dependent variables, age and gender were entered in the first stage as independent variables, and number of *APOE*4 alleles and plasma biomarker levels were then entered in a stepwise fashion. Finally, as pancreatic peptide levels are influenced by ChEI therapy,[31] we analyzed the correlation between plasma and CSF AD biomarkers among subjects without ChEI (including donepezil, galantamine, and rivastigmine), including 58 subjects with normal cognition, 226 subjects with MCI, and 20 subjects with AD.

RESULTS Univariate analysis in Penn and WU cohorts. Among 190 analytes measured, 41 and 51 analytes were associated with mild dementia/MCI/AD in the Penn and WU cohorts ($p < 0.10$, table e-2). Among 23 analytes identified in both cohorts, 6 (apolipoprotein A1, apolipoprotein H, cystatin C, fibrinogen, myeloperoxidase, and neutrophil gelatinase-associated lipocalin; table 2) demonstrated opposite directions of association with diagnosis and were excluded from further analysis. In the remaining 17 analytes, 5 were reported by 1 previous serum study using the RBM panel to correlate similarly with clinical AD,[8] including C-reactive protein (CRP), interleukin (IL)-10, IL-15, pancreatic polypeptide, and resistin. Only 1 (IL-3) from a prior study using plasma and a different multiplex platform was in this list of 17, and showed an opposite

Table 2		Analytes associated with abnormal cognition in the Penn and WU datasets[a]				
Analyte		Penn	WU	Other dementia	Reference 7	Reference 8
Apolipoprotein A1		1.044	0.972			
Apolipoprotein E		0.945	0.965			
Apolipoprotein H		1.044	0.977			
Brain natriuretic peptide		1.083	1.074			
Cortisol		1.065	1.035			
C-reactive protein		0.824	0.921			<1
Cystatin C		1.035	0.972			
E-selectin		0.946	0.962			
FAS		1.031	1.017	1.005		
Fibrinogen		1.039	0.982			
Gamma-IFN-induced monokine		1.066	0.956			
IL-3		1.054	1.066		<1	
IL-10		1.039	1.023			>1
IL-12p40		1.017	1.065			
IL-13		1.050	1.045	0.992		
IL-15		1.031	1.046			>1
Myeloperoxidase		1.051	0.880			
NGAL		1.041	0.982			
Osteopontin		1.122	1.033	1.009		
Pancreatic polypeptide		1.078	1.093	1.013		>1
Resistin		1.059	1.022	1.009		>1
Serum amyloid protein		0.958	0.440			
Stem cell factor		1.039	1.054	1.013		<1

Abbreviations: AD = Alzheimer disease; IFN = interferon; IL = interleukin; MCI = mild cognitive impairment; Penn = University of Pennsylvania; WU = Washington University.
[a] Shown are odds ratios associated with diagnosis of very mild dementia/MCI/AD in the logistic regression models, in comparison to published studies of blood-based multianalyte profiling studies identifying analytes predictive of AD diagnosis. Positive values predict increases in log-transformed analyte levels associated with the diagnosis of AD, while negative values predict decreases in levels.

direction of association with AD.[7] SAM identified analytes associated with mild dementia/MCI/AD in each cohort, with 6 (α1-antitrypsin, apoE, CRP, N-terminal pro B-type natriuretic peptide, osteopontin, serum amyloid P) identified in both cohorts (table e-3).

Univariate analysis in ADNI cohort. We then sought to confirm the association between the 17 candidate AD plasma biomarkers and clinical diagnosis in ADNI. In the ADNI cohort, B-type natriuretic peptide (BNP) levels were measured instead of N-terminal pro B-type natriuretic peptide levels, and IL-10, IL-12p40, and IL-15 levels were not available. Using Bonferroni correction for the 14 analytes ($p < 0.0036$), we found 6 to be highly associated with the clinical MCI/AD diagnosis, including apoE, BNP, cortisol, CRP, IL-3, and pancreatic polypeptide (table 3). When ADNI participants with CSF were analyzed (n = 352), CSF findings predictive of

underlying AD pathologic changes (i.e., CSF Aβ42 levels <193 pg/mL and CSF t-tau/Aβ42 ratio >0.39) also were associated with levels of 4 plasma proteins, including apoE, BNP, CRP, and pancreatic polypeptide (table 3). All analytes had acceptable intra-assay variability (appendix e-1).

Applying SAM (false discovery rate of 10%) and then IUT to all 3 datasets yielded 14 analytes associated with mild dementia/MCI/AD (table e-4), including apoE, CRP, and insulin growth factor binding protein 2 (IGF-BP2), altered in all 3 cohorts, and BNP and pancreatic peptide altered in 2 cohorts. Given the larger type II error associated with applying IUT to all 3 datasets, we consider these results to be consistent with our discovery-validation approach using logistic regression.

Correlation between plasma and CSF AD biomarkers. As candidate plasma AD biomarker levels were associated with both the clinical diagnosis and CSF biomarker profiles consistent with AD, we analyzed whether CSF AD biomarker levels correlated with plasma AD biomarker levels. Multivariate linear regression modeling showed CSF Aβ42 levels to be strongly correlated with number of *APOE*4 alleles adjusting for age and gender ($p < 0.001$, $R = 0.559$, adjusted R^2 of 0.307), as well as levels of BNP and pancreatic polypeptide (table 4, $R = 0.596$, adjusted R^2 of 0.345). A similar association was found with CSF t-tau/Aβ42 ratios (table 4), although a weaker relationship was found between an increased t-Tau/Aβ42 ratio and candidate AD blood biomarkers (number of *APOE*4 alleles and plasma pancreatic polypeptide levels, $R = 0.404$, adjusted R^2 of 0.154). Plasma apoE levels correlated with *APOE*4 allele numbers and were not predictive of CSF biomarker levels independently of the latter. The addition of IGF-BP2 levels from SAM analysis or adjustment for ChEI use in either model did not affect the outcome (appendix e-1).

DISCUSSION The search for novel biochemical predictors of underlying neurodegenerative pathology has been greatly aided by high throughput multiplex platforms.[7,8,12] Using 2 large cohorts of well-characterized participants with normal cognition and cognitive impairment, we identified 17 plasma analytes associated with the clinical diagnosis of very mild dementia/MCI/AD in both cohorts. Among these, changes in apoE, BNP, CRP, and pancreatic polypeptide levels were also associated with MCI/AD diagnosis and CSF AD biomarker profiles in ADNI. Importantly, along with the known association between *APOE* genotyping and CSF AD biomarker levels, these AD plasma biomarkers also correlated with CSF Aβ42 levels and t-tau/Aβ42 ra-

Table 3	Effects of clinical or CSF-based diagnosis on analyte levels in the ADNI cohort (adjusted for age and gender)[a]	
	Odds ratio	p Value
Diagnosis of MCI or AD		
ApoE[b]	0.881	<0.001
BNP[b]	1.230	<0.001
CRP[b]	0.824	<0.001
IL-3[b]	1.141	<0.001
PP[b]	1.171	<0.001
Cortisol	1.030	0.015
E-selectin	0.636	0.134
FAS	1.017	0.19
IGF-BP2	0.984	0.419
IL-13	1.029	0.097
Osteopontin	0.947	0.006
Resistin	1.004	0.822
SAP	0.987	0.287
SCF	1.036	0.063
CSF Aβ42 <193 pg/mL		
ApoE	0.901	<0.001
BNP	1.175	<0.001
CRP	0.798	<0.001
PP	1.129	<0.001
Cortisol	1.031	0.004
E-selectin	0.673	0.098
FAS	1.001	0.929
IGF-BP2	1.003	0.873
IL-3	1.050	0.055
IL-13	1.005	0.735
Osteopontin	0.973	0.118
Resistin	1.006	0.647
SAP	0.993	0.511
SCF	0.997	0.858
CSF t-tau/Aβ42 >0.39		
ApoE	0.921	<0.001
BNP	1.113	<0.001
Cortisol	1.033	0.003
CRP	0.770	<0.001
PP	1.115	0.001
E-selectin	0.600	0.031
FAS	0.993	0.523
IGF-BP2	1.035	0.047
IL-3	1.035	0.171
IL-13	1.000	0.974
Osteopontin	0.970	0.076
Resistin	1.001	0.965
SAP	0.991	0.373
SCF	1.003	0.859

Table 4	Linear regression models showing associations between CSF AD biomarker levels (Aβ42 level, ratio of t-tau/Aβ42) and plasma AD biomarkers in all ADNI subjects with CSF and plasma analytes (n = 566)[a]	
	Regression coefficients	p
Aβ42		
Male	4.30	0.415
Age	0.68	0.090
No. of APOE4 alleles	−45.47	<0.001
BNP	−27.99	<0.001
Pancreatic polypeptide	−18.90	0.007
t-Tau/Aβ42		
Male	0.116	0.04
Age	0.001	0.711
No. of APOE4 alleles	0.297	<0.001
Pancreatic polypeptide	0.180	0.015

Abbreviations: Aβ42 = β-amyloid 1–42; AD = Alzheimer disease; ADNI = Alzheimer's Disease Neuroimaging Initiative; BNP = B-type natriuretic peptide; t-tau = total tau.
[a] Similar correlations were observed in ADNI subjects with CSF and plasma analytes not treated with cholinesterase inhibitors (n = 304; see appendix e-1).

tios. These plasma AD biomarkers may thus help predict underlying AD pathology through their relationships to established CSF biomarkers of AD, and could serve as the basis of a plasma-based screening battery for AD.

A major roadblock in the identification of candidate biomarkers through multi-analyte profiling has been the successful replication of 1 study's "hits" in other studies. There are many reasons for this, including preanalytical variables, different analytical platforms (such as 2D gel electrophoresis and Luminex multiplexing, among others), and lack of platform cross-validation, body fluid types (plasma, serum), subject selection, disease endophenotypes, and analytical algorithms. In the current study, all 3 cohorts (Penn, WU, ADNI) had the same platform,

Abbreviations: Aβ42 = β-amyloid 1–42; AD = Alzheimer disease; ADNI = Alzheimer's Disease Neuroimaging Initiative; BNP = brain natriuretic peptide; CRP = C-reactive protein; IGF-BP2 = insulin-like growth factor binding protein 2; IL = interleukin; MCI = mild cognitive impairment; Penn = University of Pennsylvania; PP = pancreatic polypeptide; SAP = serum amyloid protein; SCF = stem cell factor; t-tau = total tau; WU = Washington University.
[a] Levels of IL-10, IL-12p40, and IL-15 not available in the ADNI cohort at time of manuscript preparation.
[b] Analytes identified from both Penn and WU cohorts (table 3) significantly associated with a clinical diagnosis of MCI/AD or CSF biomarker pattern associated with pathologic AD with odds ratios shown.

body fluid type, and analytical approaches, with similar methods for subject selection and clinical characterization. Some analytes were previously identified in the multicenter Texas Alzheimer's Research Consortium (TARC) study using the same platform but a different biofluid (serum).[8] The difference in results between a plasma-based study and a serum-based study may be due to protein–protein interactions between analytes of interest and clotting factors, or differential interaction between analytes of interest, additives (e.g., EDTA, serum separation substrate), and potentially different plastic used in the construction of plasma ("purple top") and serum ("gold top") tubes. We collected plasma in tubes containing EDTA in part due to the indeterminate interaction between other additives (e.g., heparin, clot activator) and our analytes of interest. Beyond fluid type, the analytical platform likely contributes significantly to the lack of overlap between the current study and one prior study using the same biofluid (plasma) but a different platform.[7] This discrepancy can potentially be attributed to different antibody affinity, analyte- and platform-specific dynamic range, platform precision, and handling of noise and outliers by each platform and analysis. Finally, the use of IUT may generate conservative estimates of overlap,[29] but we focused on analytes with the highest likelihood of replication for further technical refinement. Future analytical strategies may use modified approaches such as Relaxed IUT to generate common lists, but any algorithm that biases discovery will increase the likelihood of type I error. However, the relatively higher degree of "biomarker concordance" between studies using the same platform would support that future discovery-type studies should be mindful of analytical platform selection as well as analyte identity, and analyte identity and levels should be additionally confirmed by independent means.

Among plasma AD biomarkers reported here, BNP, CRP, and pancreatic polypeptide levels were associated with CSF Aβ42 levels and t-tau/Aβ42 ratios regardless of ChEI use. BNP is a well-established marker of left ventricular dysfunction, and is elevated in acute strokes[32] and vascular dementia.[33] Elevated BNP levels are also associated with cognitive decline in vascular disease[34] and the development of AD and vascular dementia (independent of heart failure).[35] Elevated BNP may thus reflect shared risk factors between heart failure and AD or an unknown step in AD pathogenesis, and its effects on CSF Aβ42 levels with magnitudes similar to APOE4 genotype warrant further investigation in future prospective studies.

Pancreatic polypeptide levels were previously identified by TARC.[8] Pancreatic polypeptide is a small signaling peptide associated with postprandial appetite suppression[36] present in multiple brain regions, including those affected by AD such as the hippocampus[37] and locus ceruleus.[38] Increased plasma pancreatic polypeptide levels could reflect impaired transport across the blood–brain/CSF barrier through yet unclear mechanisms, but it is also elevated in the CSF of patients with AD.[12] Similar changes in patients with non-AD dementia further suggest that elevated pancreatic polypeptide levels may reflect neuronal loss irrespective of etiology, although such elevated levels can still serve as a potential plasma marker of neuronal injury. Similarly, CRP was not specifically associated with CSF Aβ42 levels or t-tau/Aβ42 ratios, although it complemented BNP and pancreatic polypeptides in predicting CSF AD biomarkers. The association between decreased CRP and AD diagnosis is perhaps perplexing, as CRP has been found to be decreased, increased, or unchanged in AD.[39] Alterations in CRP levels may again reflect neuronal injury, although its levels may be more susceptible to patient selection and endophenotypes than other plasma biomarkers.

This study included 3 large cohorts but there still exist a number of limitations. We sought to identify plasma biomarkers common to MCI and AD, and different proportion of patients with MCI in each cohort makes it challenging to identify MCI- or AD-specific changes. As few subjects recruited at the 2 centers or within ADNI have neuropathologic analysis, we did not examine which analytes provided the best pathologic prediction. This type of comparative analysis across centers can also be skewed by differential analyte stability, classification algorithm, and the choice of surrogate gold standard (clinical vs CSF-based diagnosis), although in-depth analyses are underway to address these. The differences in collection techniques between Penn and WU (fasting vs nonfasting, immediate centrifugation/freezing vs cold storage at 4°C before processing) and batch-to-batch variability may account for failed replication for some analytes, and the significance of analytes identified in only one cohort but validated in ADNI needs further interrogation. Finally, plasma biomarkers identified here accounted for some, but not all, changes in CSF AD biomarkers in the exploratory portion of the current study, although only analytes that passed our stringent selection criteria were tested in the ADNI cohort. These analytes, along with plasma amyloid peptides and nonamyloid analytes previously identified,[8,40] can be jointly analyzed for their correlation to CSF AD biomarkers. Nevertheless, we were successful in identifying plasma analytes whose levels were similarly altered in 3 independent cohorts using highly strict criteria. With 2 of these biomarkers (BNP, CRP) assayed routinely in clinical

laboratories, the utility of plasma AD biomarkers in screening patients at risk for AD should be readily assessed in prospectively recruited subjects.

AUTHOR CONTRIBUTIONS

Drafting/revising the manuscript for content, including medical writing for content: Drs. Hu, Holtzman, Fagan, Shaw, Arnold, Grossman, Xiong, Morris, Trojanowski, Soares. Study concept or design: Drs. Hu, Holtzman, Fagan, Shaw, Perrin, Arnold, Grossman, Xiong, Craig-Schapiro, Clark, McCluskey, Elman, Karlawish, Chen-Plotkin, Hurtig, Siderowf, Lee, Morris, Trojanowski, Soares. Analysis or interpretation of data: Drs. Hu, Holtzman, Shaw, Pickering, Kuhn, Chen, Van Deerlin, Swenson, Trojanowski, Soares. Contribution of vital reagents/tools/patents: Dr. Van Deerlin. Acquisition of data: Drs. Hu, Holtzman, Fagan, Shaw, Perrin, Arnold, Grossman, Craig-Schapiro, Clark, McCluskey, Elman, Karlawish, Chen-Plotkin, Hurtig, Siderowf, Lee, Morris, Trojanowski, Soares. Statistical analysis: Drs. Hu, Pickering. Study supervision or coordination: Drs. Holtzman, Shaw, Arnold, Xiong, Lee, Morris, Trojanowski, Soares. Obtain funding: Drs. Holtzman, Shaw, Arnold, Grossman, Swenson, Lee, Morris, Trojanowski, Soares.

DISCLOSURE

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Holtzman serves on scientific advisory boards for Satori Pharmaceuticals and EnVivo Pharmaceuticals; may accrue revenue on pending patents re: Methods for measuring the metabolism of neurally derived biomolecules in vivo; Use of anti-AB antibody to treat traumatic brain injury; Methods to treat Alzheimer's disease or other amyloid beta accumulation associated disorders; Humanized antibodies that sequester abeta peptide; Diagnostic for early stage Alzheimer's disease; and Predictive diagnostic for Alzheimer's disease; serves as a consultant to Merck Serono, Eli Lilly and Company, Takeda Pharmaceutical Company Limited, Abbott, Comentis, Inc., Eisai Inc., and AstraZeneca; is cofounder of and receives board of directors compensation from C2N Diagnostics LLC; receives research support from AstraZeneca, Pfizer Inc., Eli Lilly and Company, Elan Corporation, Forest Laboratories, Inc., the NIH, Cure Alzheimer's Fund, and Fidelity Foundation; has received compensation from Washington University from license revenue received for licensing of patent applications to C2N Diagnostics LLC; and may receive future royalty payments for Washington University licensing patents to C2N Diagnostics, LLC, and Eli Lilly and Company. 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