Journal of Alzheimer's Disease 46 (2015) 431–440
DOI 10.3233/JAD-142778
IOS Press

Soluble BACE-1 Activity and sAβPPβ Concentrations in Alzheimer's Disease and Age-Matched Healthy Control Cerebrospinal Fluid from the Alzheimer's Disease Neuroimaging Initiative-1 Baseline Cohort

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Handling Associate Editor: Stefan Lichtenthaler

Accepted 24 February 2015

Abstract. β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) plays an important role in the development of Alzheimer's disease (AD), freeing the amyloid-β (Aβ) N-terminus from the amyloid-β protein precursor (AβPP), the first step in Aβ formation. Increased BACE1 activity in AD brain or cerebrospinal fluid (CSF) has been reported. Other studies, however, found either no change or a decrease with AD diagnosis in either BACE1 activity or sAβPPβ, the N-terminal secreted product of BACE1 (sBACE1) activity on AβPP. Here, sBACE1 enzymatic activity and secreted AβPPβ (sAβPPβ) were measured in Alzheimer's Disease Neuroimaging Initiative-1 (ADNI-1) baseline CSF samples and no statistically significant changes were found in either measure comparing healthy control, mild cognitively impaired, or AD individual samples. While CSF sBACE1 activity and sAβPPβ demonstrated a moderate yet significant degree of correlation with each other, there was no correlation of either analyte to CSF Aβ peptide ending at residue 42. Surprisingly, a stronger correlation was demonstrated between CSF sBACE1 activity and tau, which was comparable to that between CSF $A\beta_{42}$ and tau. Unlike for these latter two analytes, receiver-operator characteristic curves demonstrate that neither CSF sBACE1 activity nor sAβPPβ concentrations can be used to differentiate between healthy elderly and AD individuals.

Keywords: ADNI, Alzheimer's disease, amyloid-β, amyloid-β protein precursor, BACE1, cerebrospinal fluid, ELISA, mild cognitive impairment, sAβPPβ, sBACE1, secretase

[1]Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu/). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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ISSN 1387-2877/15/$35.00

INTRODUCTION

Amyloid-β (Aβ) concentrations increase in the brains of subjects with Alzheimer's disease (AD) to form extracellular plaque which, together with neurofibrillary tangles containing the protein tau, associate with progressive neuron and synapse loss. Aβ is generated by sequential cleavage of the transmembrane amyloid-β protein precursor (AβPP) by the aspartic protease β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) and a second aspartic protease activity contained within the γ-secretase complex [1]. Aβ makes its way into the extracellular space and can be measured in cerebrospinal fluid (CSF). Following BACE1 cleavage of AβPP, an N-terminal secreted fragment, known as soluble AβPP beta (sAβPPβ) is also generated, providing the most proximal readout of BACE1 activity on AβPP. A fraction of the transmembrane-oriented, cellular BACE1 protein itself is cleaved between Ala429 and Val430 [2], losing its C-terminus [3] to form an extracellular, soluble, and catalytically-active BACE1 fragment (sBACE1). This cleavage event has been proposed as an alternate strategy to reduce or regulate AβPP processing, and sBACE1 may be a surrogate biomarker for brain BACE1 activity. Whether sBACE1 correlates in some way with ongoing brain BACE1 is unknown, but may be surmised if CSF sBACE1 activity and sAβPPβ (thought to be generated primarily in brain) correlate. Although sBACE1 is present in CSF (with pH around 7.3), both full-length, cell-associated BACE1 and sBACE1 have optimum activity around pH 4.5–5.5 [3–5]. A small study examining 31 AD patients [6] supported a correlation between CSF sBACE1 activity and amyloid positron emission tomography (PET) within brain regions abutting ventricles, but not with other regions typically containing amyloid deposition.

Several groups have reported increased BACE1 protein and enzymatic activity in AD brain [7–11] or CSF [12–15] compared with control subjects. However, others have observed either no change or reductions in BACE1 or sBACE1 concentrations or activity in brains or CSF from AD subjects [3, 16–19]. Similar to conflicting reports of sBACE1, CSF sAβPPβ has been measured by several groups, with varying results [20–27]. A few unique cohorts have been assessed for both CSF sBACE1 activity as well as sAβPPβ levels [18, 19], providing complementary means of assessing central nervous system BACE1 activity. Here, we applied robust assays for sBACE1 activity [3] and sAβPPβ measures [17] to baseline CSF samples from the well-characterized baseline ADNI-1 cohort.

METHODS

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 subjects but ADNI has been followed by ADNI-GO and ADNI-2. To date these three protocols have recruited over 1,500 adults, ages 55 to 90, to participate in the research, consisting of cognitively normal older individuals, people with early or late MCI, and people with early AD. The follow up duration of each group is specified in the protocols for ADNI-1, ADNI-2 and ADNI-GO. Subjects originally recruited for ADNI-1 and ADNI-GO had the option to be followed in ADNI-2. For up-to-date information, see http://www.adni-info.org/.

ADNI cohort

ADNI-1 was launched in 2004 by the National Institute on Aging, the Foundation for the National Institutes of Health and by a group of private-public partners as a 5-year precompetitive AD biomarker consortium. Written and verbal informed consents were obtained from participants at screening and enrollment. Further details regarding ADNI including participant

Table 1
ADNI-1 Patient demographics

	Control	MCI	AD
n	106	183	92
Age (range)	76 (62–90)	74 (55–89)	75 (57–89)
Gender (M/F)%	50/50	33/66	45/55
ApoE4 %	25%	54%	71%
MMSE (range)	29 (25–30)	27 (23–30)	24 (20–27)

MCI, mild cognitive impairment; AD, Alzheimer's disease; ApoE4, apolipoprotein E4; MMSE, Mini-Mental State Examination.

selection procedures and complete study protocols and standard operating procedures are available at the ADNI website http://adni.loni.usc.edu/. Participant demographics are shown in Table 1.

CSF samples

Patients fasted overnight, prior to morning CSF collection. Preanalytical handling followed ADNI standard operating procedures which required polypropylene tubes. Samples experienced one freeze-thaw cycle prior to analysis. Baseline samples [106 healthy control (HC), 92 AD, 183 MCI, and 20 technical replicates (401 total)] were analyzed for sBACE1 activity and sAβPPβ concentrations concurrently, using aliquots from the same vial at the same thaw on ice.

sBACE1 activity and sAβPPβ

Protocols for measuring sBACE1 activity and sAβPPβ were previously published [3, 17] and were followed precisely, including inhibitors of non-BACE1 aspartic proteases. CSF sBACE activity was defined as primarily sBACE1 versus sBACE2, including both genetic and biochemical studies [3, 28]. Briefly for sAβPPβ, a rabbit monoclonal antibody specific for the free carboxyl-terminal of wild type human sAβPPβ captured CSF sAβPPβ on an ELISA well. To detect sAβPPβ, mouse monoclonal antibody P2-1, conjugated to alkaline phosphatase was used, followed by a chemiluminesence-based substrate. To measure sBACE1 activity, a 2-step assay was employed. In the first step, cleavage of a biotinylated peptide substrate (biotin-KTEEISEVNFEVEFR) by the endogenous sBACE1 activity in CSF at the optimal pH of 4.5 generated biotin-KTEEISEVNF. This product was measured by one site ELISA using an NF-cleavage specific antibody after capture on a streptavidin-coated plate. Standard curves were generated with recombinant, soluble, baculovirus human BACE-1 or sAβPPβ (described in above publications) and were used to calculate absolute values within the ADNI CSF samples. Non-human primate, rhesus CSF collected from cisterna-magna ported monkeys [29] was used as quality control (QC) standards on each plate. Blinded data received a statistical QC review at Merck and Company and was forwarded, along with the raw data, to Dr. Leslie Shaw at the University of Pennsylvania for un-blinding; raw data was then posted to the ADNI website.

Statistical analysis

Details of data QC processing and the statistical analysis are provided in the ADNI data primer for sBACE1 activity and sAβPPβ, available at the ADNI website (http://adni.loni.usc.edu/). Receiver-operator characteristic (ROC) curves described the ability of each analyte to discriminate between diagnosis groups (AD versus HC, and sMCI versus pMCI). Linear models analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to compare analyte means among groups for diagnosis at baseline and with each group split into those for whom diagnosis was stable or progressed over the two year period. The same models were used to examine the effects of age, gender, and ApoE genotype. Analytes were modeled on the log scale, unless otherwise indicated. The Spearman (rank) correlation coefficient was used to measure the correlation between sBACE1 activity, sAβPPβ, and other CSF markers, namely $A\beta_{42}$, tau, and phospho-tau 181 (ptau181) which were measured previously [30] and data also posted to the ADNI website.

RESULTS

Intra-assay variability

Within the ADNI sample set were 20 technical CSF replicates that served to assess intra-assay variability, in addition to the rhesus QC samples that were present on each plate. While the rhesus CSF QCs for both analytes demonstrated coefficients of variability (CVs) of <20% across all plates, the human CSF duplicates ranged between 1-2 fold of each other for both analytes with sBACE1 activity more variable than sAβPPβ (CVs = 37% and 20%, respectively). Despite this degree of variability in the duplicates, the study was powered at 80% to detect >15–20% difference in the groups at $p < 0.05$.

sBACE1 activity and sAβPPβ concentrations across diagnosis groups

Mean CSF sBACE1 activity did not differ across groups (Table 2), whether stratified by baseline

Table 2
Summary statistics for CSF sBACE1 and sAβPPβ

		sBACE1 (pg/ml)					sAβPPβ (pg/ml)				
Group	n	mean	median	sd	se	95% CI	mean	median	sd	se	95% CI
sHC	97	44.9	42.0	17.8	1.8	(41.3, 48.5)	4062	3887	1348	137	(3790, 4333)
pHC	9	50.0	54.0	17.4	5.8	(36.6, 63.4)	4876	4486	1581	527	(3661, 6092)
Total HC	106	45.3	43.0	17.7	1.7	(41.9, 48.7)	4131	3983	1380	134	(3865, 4397)
rM	4	61.5	63.0	15.8	7.9	(36.4, 86.6)	5154	5237	1810	905	(2275, 8034)
sM	99	50.0	47.0	23.3	2.3	(45.3, 54.6)	3958	3773	1505	151	(3658, 4258)
pM	80	46.2	45.0	15.6	1.7	(42.7, 49.6)	4152	4216	1442	161	(3831, 4473)
Total MCI	183	48.5	45.5	20.2	1.5	(45.6, 51.5)	4069	3982	1487	110	(3852, 4286)
AD	92	44.4	42.0	16.8	1.7	(40.9, 47.8)	3934	3760	1307	136	(3664, 4205)

sHC, stable healthy control; pHC, healthy control progressing to either MCI or AD; rM, regressing MCI; sM, stable MCI; pM, progressing MCI; AD, Alzheimer's disease; MCI, mild cognitive impairment.

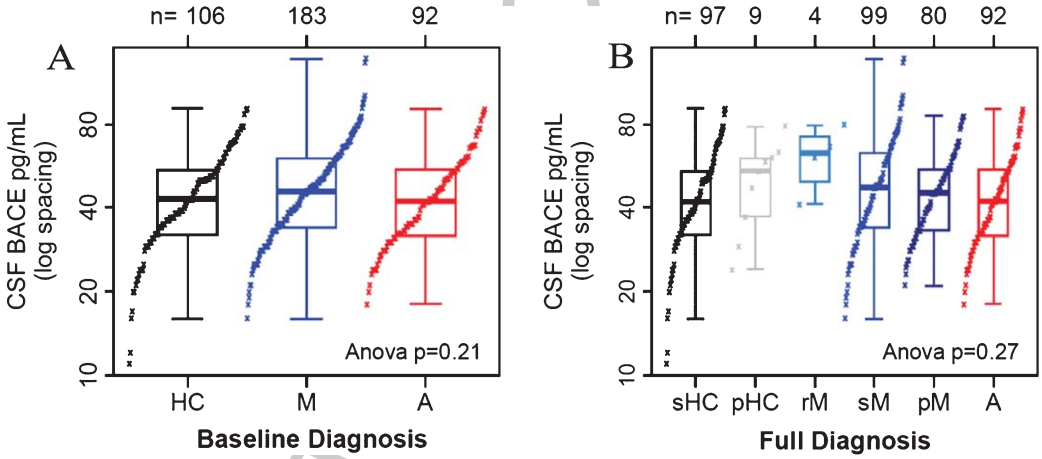

Fig. 1. CSF sBACE1 activity averages are not different across the three baseline diagnostic groups (A, $p = 0.21$), or in the subgroups based on diagnoses at baseline and 2 years after baseline (B, $p = 0.27$; sHC, stable healthy control; pHC, healthy control progressing to either mild cognitive impairment (MCI) or Alzheimer's disease (AD); rM, regressing MCI; sM, stable MCI; pM, progressing MCI; A, AD). Sample numbers for baseline and 2-year diagnosis are listed at the top of the respective figures.

diagnosis (Fig. 1A), or by a combination of the diagnosis at baseline and 2 years later (Fig. 1B). While some of the groups in Fig. 1B that were subdivided by clinical course trended higher (progressing healthy controls, pHC; regressing MCI, rMCI), there were few individuals in these groups ($n = 9$ and 4, respectively) and the differences were not statistically significant. Likewise, mean CSF sAβPPβ concentrations did not differ (Table 2) when grouped by either baseline diagnosis (Fig. 2A) or by the combination of diagnoses at baseline and 2 years later (Fig. 2B). ROC curves for sBACE1 activity and sAβPPβ failed to distinguish between AD and healthy control CSF (Fig. 3A, B) with an area under the curve for the healthy control and AD groups of 0.48 and 0.45, respectively. In addition, stable versus progressing MCI were also not distinguished by the assays, with AUC for sBACE1 activity of 0.477 and sAβPPβ of 0.547.

Effect of ApoE, age, and gender

The effects of ApoE4, age, and gender were explored by fitting nested ANCOVA models. There was no evidence that mean sBACE1 activity or sAβPPβ concentrations differed by the number of ApoE4 alleles either as a main effect among all samples (Fig. 4), or as an interaction with baseline diagnostic group (data not shown).

With age, sBACE1 activity increased approximately 1.8%/year (95% CI = 0.3%, 3.3%) in healthy controls ($p = 0.02$), but not in the MCI or AD groups (Fig. 5A). sBACE1 activity did not differ across gender (data not shown). CSF sAβPPβ demonstrated a negative 0.8% change/year with age, (95% CI = −1.3%, −0.2%) across all groups ($p = 0.0098$), with no evidence that the rate was different between groups. Mean CSF sAβPPβ concentration was 12% (3%, 22%) higher in males ($p = 0.002$) compared to females (Fig. 5B).

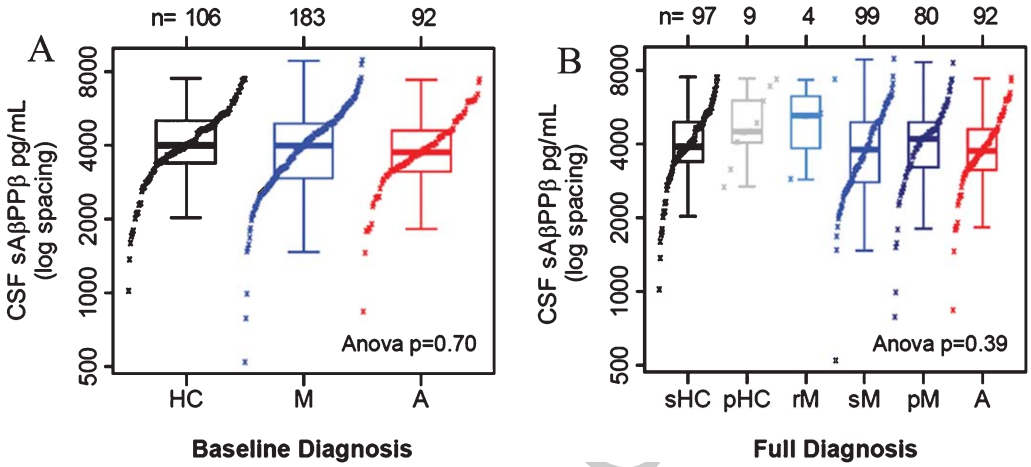

Fig. 2. CSF sAβPPβ concentration averages are not different across the three baseline diagnostic groups (A, $p = 0.70$), or in the subgroups based on diagnoses at baseline and 2 years after baseline (B, $p = 0.39$; sub group abbreviations and sample numbers as described in Fig. 2 legend).

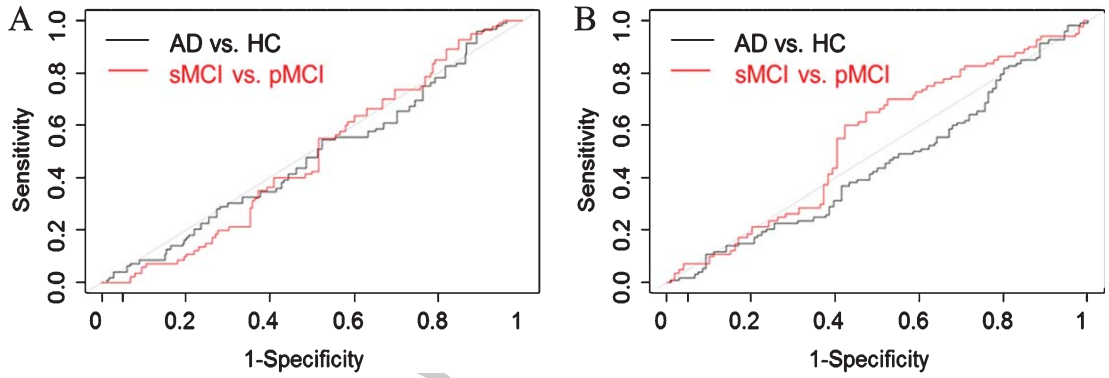

Fig. 3. Receiver-operator characteristic (ROC) curve analysis indicates neither CSF sBACE1 activity (A) nor CSF sAβPPβ (B) are useful to discriminate between either 1) healthy control versus AD (AUC = 0.478, standard error (SE) = 0.04 and AUC = 0.451, SE = 0.04, respectively) or 2) stable MCI versus progressing MCI populations (AUC = 0.477, SE = 0.04 and AUC = 0.547, SE = 0.04, respectively).

Correlation of sBACE1 activity and sAβPPβ with other CSF biomarker analytes

In addition to the sBACE1 activity and sAβPPβ measures performed here, additional analytes $A\beta_{42}$, tau, and ptau181 were previously measured from these individuals from parallel CSF aliquots [30]. Correlations were assessed between CSF sBACE1 activity and sAβPPβ, as well as between each of these analytes and $A\beta_{42}$, tau, and ptau181.

CSF sBACE1 activity and sAβPPβ demonstrated a modest, but significant correlation with each other ($r = 0.35$, $p = 3.2 \times 10^{-12}$), but neither analyte correlated with $A\beta_{42}$. Both CSF sBACE1 activity and sAβPPβ correlated with tau ($r = 0.43$, $p = 3.3 \times 10^{-18}$ and $r = 0.3$, $p = 2.1 \times 10^{-9}$, respectively), and somewhat weaker correlations to ptau181 ($r = 0.28$, $p = 1.6 \times 10^{8}$ and $r = 0.21$, p – 3.2×10^{-5}, respectively). The magnitude and significance of the sBACE1 activity and tau correlation is comparable to that for $A\beta_{42}$ versus tau ($r = -0.46$; $p = 3.4 \times 10^{-21}$).

DISCUSSION

Robust and well characterized assays were used to measure CSF sBACE1 activity and sAβPPβ in the ADNI-1 baseline cohort. These analytes were evaluated with respect to 1) disease status at baseline and after 2 years, 2) age, 3) gender, and 4) ApoE4 status. In addition, the correlation between CSF sBACE1 activity and sAβPPβ concentrations was assessed, as well as correlation of each of these measures to previously assayed tau, ptau181, and $A\beta_{42}$. Consistent with two recent reports [18, 19], no differences were found in either CSF sBACE1 activity or sAβPPβ between baseline AD, MCI patients, and HC; and also

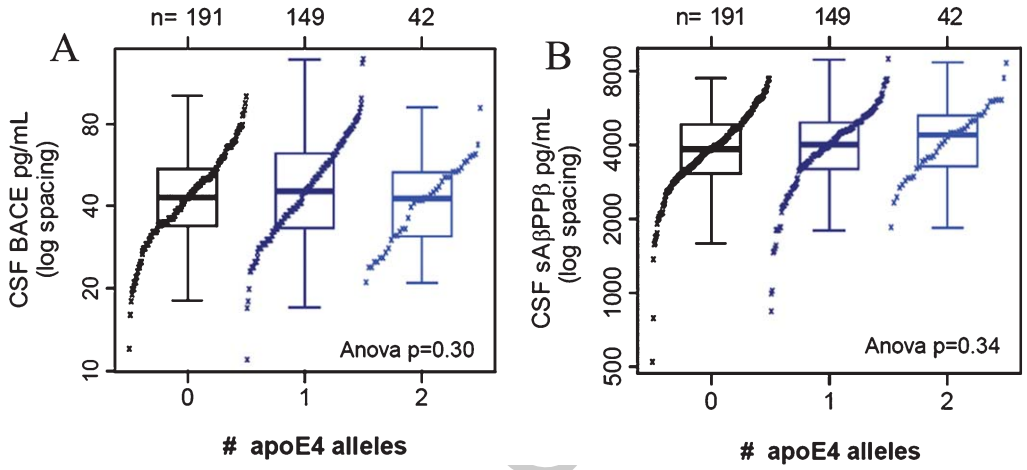

Fig. 4. CSF sBACE1 activity (A) and sAβPPβ concentration (B) are not different across individuals with different copy number of apolipoprotein E4 (apoE4) alleles ($p = 0.3$ and $p = 0.34$, respectively). Sample numbers per apoE4 allelle: zero E4 allele = 191, one E4 allele = 149, two E4 alleles = 42.

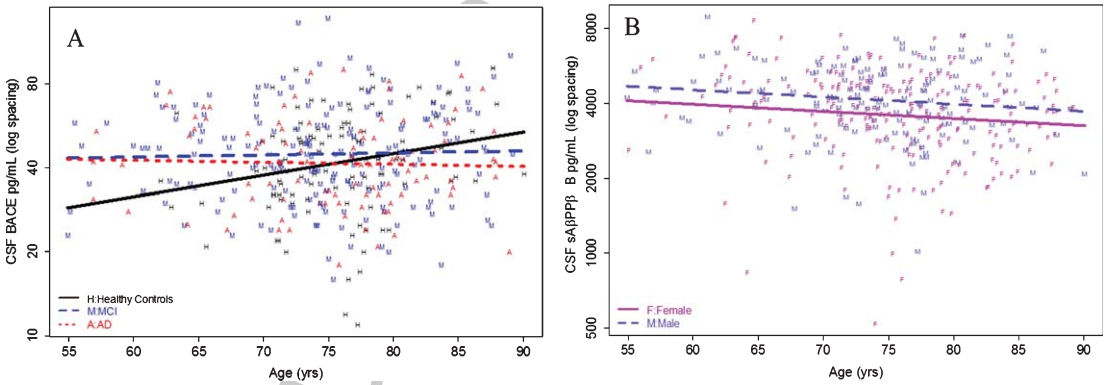

Fig. 5. Age dependence of CSF analytes: sBACE1 activity increased significantly with age in healthy control ($p = 0.02$), but not in either MCI or AD groups (A). CSF sAβPPβ concentrations decreased significantly with age across all groups ($p = 0.009$). Concentrations were significantly elevated in males versus females ($p = 0.002$).

relative to their subsequent diagnosis 2 years later. Importantly, the Rosén et al. study [18] employed the same assay format that was used in the ADNI-1 cohort study reported here. This finding of unaltered sBACE1 activity in HC versus AD patients is consistent with a report employing stable isotope labeling of newly-synthesized Aβ with $^{13}C_6$-leucine, which demonstrated no change in the Aβ synthetic rate but a reduced rate of Aβ clearance in AD CSF [31]. The relevance of CSF sBACE1 activity to brain membrane BACE1 activity is difficult to assess, however, using the same CSF sBACE1 assay reported here, Wu et al. [17] measured BACE1 activity and sAβPPβ in HC and AD brain and found unchanging BACE1 activity in the presence of reduced brain cortical sAβPPβ in AD versus HC; this result suggests reduced full-length AβPP substrate in late-stage AD subjects. While CSF sBACE1 activity has been compared to brain amyloid imaging within the same patient [6], and found a correlation only in brain regions close to ventricles, whether and how closely CSF sBACE1 activity and sAβPPβ correlate with brain AβPP processing is not entirely clear, although the correlation of these analytes to other CSF measures may give some clues.

The sAβPPβ sandwich ELISA employed used a rabbit monoclonal KM neo-epitope capture antibody and an N-terminal AβPP antibody for detection [17]. This assay demonstrated specificity, sensitivity, and quantitative assessment for sAβPPβ over sAβPPα, as confirmed using three methods: 1) recombinant protein standards, 2) immunodepletion of human brain homogenate and AβPP knockout mouse brain homogenate, and 3) pharmacological assessment with a BACE1 inhibitor in primary neuron cultures [17].

The solution-based BACE1 activity assay used an optimized, high affinity substrate and antibody detection of the 'NFEV' neo-epitope that was generated following sBACE1 cleavage of the biotin-KTEEISEVNFEVEFR substrate. This assay was specific for sBACE1 since all the non-BACE1 aspartyl protease activity in the CSF was completely inhibited with Pepstatin A and the remaining sBACE1 protease activity was inhibited dose-dependently with BACE1 specific inhibitors Merck-3 and Statin-Val- [3]. A previous report of elevated CSF sBACE1 activity [14] noted in the discussion that the result could have been confounded with non-BACE activity. Two studies that found elevated CSF sBACE1 activity [12, 14] did not provide assay validation parameters such as dynamic range, precision, reproducibility, sensitivity, recovery of sBACE1 spike into CSF samples, and dilution linearity. These parameters are important to assess a robust assay. Finally, two reports [12, 13] used very small sample sets of AD and HC patients ($n \leq 5$) so these studies should be viewed as preliminary.

The well-characterized BACE activity assay employed both in this and other studies [3, 15, 18] previously demonstrated acceptable variability parameters. Here, while the rhesus CSF QC samples demonstrated %CV within acceptable range, the human CSF technical replicate %CV were higher than expected. Wu et al. [3] found intra- and inter-day CV for neat and sBACE1-spiked rhesus CSF (not human CSF), to be between 4–10%, while intraday CV of rhesus CSF reported in our study was 20%, approximately 2–4 fold higher. Compared to the sAβPPβ assay, the sBACE1 activity assay is a multi-step procedure and this may have contributed to the increased variability. In addition, there were 401 total samples run concurrently within the same assay run, possibly also contributing to the somewhat higher variability. Despite this variability, the assay was sufficiently powered to detect relatively small differences.

While CSF sBACE1 activity increased with age approximately 1.8%/year in HC, activity was unchanged with age in either the MCI or AD groups, as found in other reports [18, 19]. At ages less than 75 years, sBACE1 activity in HC trended lower than either the MCI or AD groups, while at ages greater than 75 years, sBACE1 activity in the HC group appeared similar or slightly higher. Whether CSF sBACE1 activity was lower in the MCI or AD group at earlier ages would be interesting, but challenging to assess. One possibility is that there is a gradual, age-dependent increase in sBACE1 activity in all groups, but that a higher steady state was achieved at earlier ages in the MCI and AD group. Longitudinal samples from the same individual would be useful in this regard, including sporadic AD prodromal phases, or alternatively, from a familial AD cohort where each individual has a known mutation for autosomal dominant AD [32].

CSF sAβPPβ concentrations negatively correlated with age across all diagnostic groups and were on average higher in males versus females. In Rosén et al. [18], reduced concentrations of CSF sAβPPβ were found in AD patients with Mini-Mental State Examination (MMSE) scores between 0–20, compared with patients having MMSE above 20, or with healthy controls. This is also consistent with a recent study of 57 stable controls and 92 AD individuals with a high percentage of postmortem confirmation, which found a 20% reduction in levels of both sAβPPβ and sAβPPα in the AD group [33]. AD individuals in this study were more advanced, with a mean MMSE score of ∼13 compared with ∼23 in ADNI AD subjects. As with the age-dependent decrease found in our study, this association with cognitive decline could reflect the reduced brain volume and neuronal loss characteristic of disease progression. Consistent with an association between brain volume and sAβPPβ levels, two studies found reduced CSF sAβPPβ in frontotemperal dementia compared with AD [34, 35], or compared with control [34]. Reduced concentrations of brain sAβPPβ, sAβPPα (both pmol/g), and full length AβPP (% of control) have been reported in AD brain compared to aged control [17], also consistent with neuronal loss. In contrast, Pera et al. [19] reported increased CSF sAβPPβ with age.

sAβPPβ and sBACE1 activity positively correlated with each other, while levels of each also positively correlated with both tau and ptau181, but not with $A\beta_{42}$. CSF sBACE1 activity may indeed reflect brain activity as correlations are found with CSF sAβPPβ across all diagnostic groups, also found by others [18, 19]. The lack of a correlation between $A\beta_{42}$ and either CSF sBACE1 or sAβPPβ in any group [18 (sAβPPβ only), 19, 26], including healthy control, suggests that BACE1 activity may not be rate limiting for Aβ generation. This is consistent with findings in heterozygous BACE1 knockout mice with 50% reduced BACE1 activity which do not have reduced Aβ; complete gene knockout was required to change Aβ concentrations [36]. $A\beta_{42}$ sequestered into brain amyloid may also account for the lack of correlation with either CSF sBACE1 or sAβPPβ in the MCI or AD groups. In addition, formation of $A\beta_{42}$ oligomers, binding of $A\beta_{42}$ to chaperone-like proteins and sequestering of $A\beta_{42}$

in the plasma membrane or intracellularly could also lead to reduced apparent CSF $A\beta_{42}$ [37]. Alternatively, despite the significant correlation between CSF sBACE1 and sAβPPβ, CSF sBACE1 may not fully represent the membrane BACE1 activity in the brain, or may only represent brain BACE1 activity that is located near ventricles, A previous report found significant correlation between CSF sBACE1 activity and amyloid PET within brain regions abutting ventricles, but not with other regions containing amyloid deposition [6].

That the strongest correlation in the ADNI-1 baseline CSF samples was found between sBACE1 activity and tau, even in healthy normal individuals, suggests there may be related processes leading to the appearance of these normally cell-associated proteins in the CSF. This finding is consistent with others [15, 18], which found a correlation between sBACE1 and tau across all groups. Tau also correlated with sAβPPβ, albeit to a lesser degree. Correlation between these two analytes was also demonstrated in human brain [17]. As these proteins are neuron-enriched, their correlation may be linked with neuronal/synapse numbers in the brain.

In conclusion, our findings in the baseline ADNI-1 cohort are consistent with two recent publications employing unique human cohorts, one which used the same BACE1 activity assay as in this report; there is no apparent diagnostic utility of CSF sBACE1 or sAβPPβ beyond that of $A\beta_{42}$, tau, and ptau181 [30]. The further documentation of interesting correlations among the CSF biomarkers adds to the overall understanding of AD biology and disease progression.

ACKNOWLEDGMENTS

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through generous contributions from the following: Alzheimer's Association; Alzheimer's Drug Discovery Foundation; Araclon Biotech; BioClinica, Inc.; Biogen Idec Inc.; Bristol-Myers Squibb Company; Eisai Inc.; Elan Pharmaceuticals, Inc.; Eli Lilly and Company; EuroImmun; F. Hoffmann-La Roche Ltd and its affiliated company Genentech, Inc.; Fujirebio; GE Healthcare;; IXICO Ltd.; Janssen Alzheimer Immunotherapy Research & Development, LLC.; Johnson & Johnson Pharmaceutical Research & Development LLC.; Medpace, Inc.; Merck & Co., Inc.; Meso Scale Diagnostics, LLC.; NeuroRx Research; Neurotrack Technologies; Novartis Pharmaceuticals Corporation; Pfizer Inc.; Piramal Imaging; Servier; Synarc Inc.; and Takeda Pharmaceutical Company. The Canadian Institutes of Health Research is providing funds to support ADNI clinical sites in Canada. Private sector contributions are facilitated by the Foundation for the National Institutes of Health (http://www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of Southern California.

Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/14-2778r1).

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