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Amyloid beta concentrations and stable isotope labeling kinetics of human plasma specific to CNS amyloidosis

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Abstract

INTRODUCTION—CSF analysis and other measurements of amyloidosis, such as amyloid-binding positron emission tomography (PET) studies, are limited by cost and availability. There is a need for a more practical Aβ biomarker for central nervous system (CNS) amyloid deposition.

METHODS—We adapted our previously reported Stable Isotope Labeling Kinetics (SILK) protocol to analyze the turnover kinetics and concentrations of Aβ38, Aβ40, and Aβ42 in human plasma.

RESULTS—Aβ isoforms have a half-life of approximately three hours in plasma. Aβ38 demonstrated faster turnover kinetics compared to Aβ40 and Aβ42. Faster fractional turnover of Aβ42 relative to Aβ40 and lower Aβ42 and Aβ42/Aβ40 concentrations in amyloid positive participants were observed.

DISCUSSION—Blood plasma Aβ42 shows similar amyloid-associated alterations as we have previously reported in CSF, suggesting a blood-brain transportation mechanism of Aβ. The stability and sensitivity of plasma Aβ measurements suggests this may be a useful screening test for CNS amyloidosis.

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BACKGROUND

In Alzheimer's Disease (AD) dementia, extensive neuronal loss occurs by the time symptoms begin, so simple screening tests for the pathology of AD are urgently needed. Aggregation and accumulation of amyloid-beta (Aβ), particularly Aβ42, is implicated in the pathogenesis of AD (1) with overproduction in autosomal dominant AD (2) and impaired clearance in the presence of amyloidosis contributing to the cause of AD (3). A pressing need exists for improved methods of detecting dysregulated Aβ metabolism for improved drug development, clinical trials, and pathologic diagnosis. Unfortunately, current diagnostic measures for AD have a number of limitations such as poor accuracy, with a recent study demonstrating sensitivity and specificity as low as 70.9% and 44.3%, respectively, when confirmed by post-mortem histopathology (4). Neuroimaging (i.e. PET-PIB) studies have emerged as tools for detection of cerebral amyloidosis; however, their use is limited by expense and availability (5). Furthermore, dysregulated Aβ kinetics may precede imaging-based amyloid detection by many years (3). Decreased cerebrospinal fluid (CSF) Aβ42 levels and increased CSF tau are associated with amyloidosis and risk of progression to dementia (6,7). However, CSF collection has perceived invasiveness, requires specialty training with relatively few practitioners for screening large numbers, and standardization of CSF biomarkers for clinical use is lacking. Plasma concentrations of Aβ40 and Aβ42 have been shown to increase with age and in early AD but may decrease with advancing AD. However, prior studies have not demonstrated highly significant differences in plasma Aβ concentrations in individuals with and without AD (8,9). Other blood tests have been in development; however, many do not measure the key pathological proteins of AD such as Aβ and have not been specific for AD pathology.

In order to understand the production, transport and clearance of Aβ, stable isotope labeling kinetics (SILK) studies of Aβ demonstrated the half-life of Aβ in the CNS is approximately 9 hours (10,11). A later study demonstrated that Aβ42 kinetics in CSF are specifically altered with amyloidosis, with faster Aβ42 turnover kinetics relative to Aβ38 and Aβ40 in amyloid positive individuals consistent with increased aggregation and deposition (3). Evidence supporting the transport of Aβ across the blood-brain-barrier and through CSF suggests that 30–50% of plasma Aβ originates from the CNS (12), and animal model BBB transporters of Aβ, RAGE and LRP, have previously been described (13,14). Understanding blood Aβ transportation, concentrations and kinetics is paramount to a more comprehensive understanding of whole body Aβ production, transport, and clearance between the brain, CSF and blood compartments. In this prospective study, we sought to determine blood Aβ kinetics and concentrations in late onset sporadic AD to determine if the pathophysiology previously found in the CNS could be detected in blood. We report for the first time the kinetics of Aβ turnover in the blood in both amyloid positive and amyloid negative individuals and also report our findings of Aβ isoform concentrations by amyloid status.

METHODS

Participants

Forty-one participants over the age of 60 were enrolled from the Knight Alzheimer's Disease Research Center at Washington University School of Medicine. Twenty-three

patients were determined to be amyloid negative by [^{11}C]PIB-PET imaging with mean cortical binding potential (MCBP) score of < 0.18 when available and otherwise by CSF Aβ42 concentration of 1 ng/ml or higher by immunoprecipitation mass spectrometry (IP/MS) as described elsewhere (11). Eighteen patients were amyloid positive by this criteria. Twenty-seven were rated as normal cognition defined by a Clinical Dementia Rating sum of boxes score of 0 (CDR 0) and had an average MMSE of 29. There were 14 with CDR>0 (range 0.5 for very mild dementia to 2 for moderate dementia) with an average MMSE of 25. The average age was 76.2 years (CDR 0 average age=75.2 and CDR>0 average age 78.1). The apolipoprotein E genotype was as expected for this general population (n=7 for 2/3 alleles, n=21 3/3, n=10 3/4, and n=3 4/4). This human study was approved by the Washington University Institutional Review Board and all participants completed informed written consent.

Sample collection

Participants were admitted to the Clinical Research Unit at Washington University at 7:00 AM following an overnight fast. An intravenous (IV) line was placed for serial blood draws. Hour zero (baseline) blood samples were obtained prior to tracer administration. For the IV bolus-labeled studies, the stable isotope tracer was prepared by the clinical pharmacy the morning of the study by dissolving 800 mg of L-[U-13_{C6}] leucine (Cambridge Isotope Laboratories, Inc.) into 150 ml sterile normal saline followed by transfer to an infusion bag through a 0.22 micron filter; it was stored at 4°C until use. For the oral-labeled studies, the tracer was administered orally by mixing 800 mg of L-[U-13_{C6}] leucine in 300 ml of grape Kool-aid mixed with sucralose sweetener. Participants had 10 minutes to consume the dose followed by a rinse of an additional 100 ml of grape Kool-aid mixed with sucralose sweetener without leucine. Following baseline blood samples, L-[U-13_{C6}] leucine was infused as an IV bolus over 10 minutes. Sixteen of the amyloid negative participants received IV bolus labeling and the remaining 7 received oral labeling. Fifteen of the amyloid positive participants received IV bolus labeling and the remaining three received oral labeling. 20 mL of blood were collected hourly for a total of 20 time points over 24 hours in EDTA tubes. Samples were centrifuged immediately upon collection, and the plasma, buffy coat and red blood cells were stored separately in polypropylene tubes (Axygen) at −80°C until time of sample processing.

Preparation of H4 APP695ΔNL Cell Media Standards, Determination of plasma $^{13}C_6$-leucine enrichment, and Immunoprecipitation of Aβ38, Aβ40, and Aβ42

All targeted Aβ isoforms (Aβ38, Aβ40, and Aβ42) were immunoprecipitated simultaneously from 2mL of plasma via a monoclonal anti-Aβ mid-domain antibody (HJ5.1, anti-Aβ13-28) conjugated to M-270 Epoxy Dynabeads (Life Technologies #14302D) according to manufacturer protocol. Each 1 mL aliquot of plasma was thawed on ice and pre-treated with 20μL of 100X protease inhibitor (Roche #11140920), 20μL of 2.5% (w/v) Tween-20 (Sigma #P9416), 50μL of 10X PBS (Sigma #P3813), and 100μL of 5M Guanidine (Sigma #G4505). After pre-treatment, 2 × 1mL aliquots from each corresponding collection time point were combined and spiked with 20μL of a solution containing 3.75 pg/μL $^{12}C^{15}N$-Aβ38, 25 pg/μL $^{12}C^{15}N$-Aβ40, and 2.5 pg/μL $^{12}C^{15}N$-Aβ42 in 4:1 0.1% NH_4OH:acetonitrile (ACN). A 50 μL aliquot of antibody-bead slurry containing 15 mg/mL

of epoxy-coupled dynabeads (10^9 beads/mL) was added and the mixtures were rotated at room temperature for 90 minutes. After incubation, the beads were washed twice with 1mL aliquots of 1X PBS and twice with 1mL aliquots of 100 mM Triethylammonium bicarbonate (TEABC, Sigma #17902). Washed beads were then aspirated to dryness and treated with 50 μL of neat formic acid (Fisher #A117-50) to elute Aβ species from the antibody-bead complex. The formic acid supernatant was transferred to a new 1.7 mL polypropylene tube and dried *in vacuo* without heat. The resulting dried precipitate was then treated with 50 μL acetonitrile and dried again *in vacuo* without heat to remove any residual formic acid. Each sample was then reconstituted in 50 L 100mM TEABC. Proteolytic digestion was initiated via the addition of a 50 μL aliquot of 2.5ng/μL LysN metalloprotease (Pierce # 90300) in 50mM TEABC. Digestion was performed overnight (~16 hrs) at 4°C and 1400 RPM. Digestion reaction were quenched via the addition of a 2μL aliquot of 50% Trifluoroacetic acid (TFA, Sigma # T6508) and 100μL of 2% ACN in 0.05% TFA. Quenched digests were loaded onto a C18 TopTip (Glygen #TT2C18.96) previously washed with 60% ACN in 0.05% TFA and equilibrated with 2% ACN in 0.05% TFA. After loading, digests were washed twice with 10% Acetonitrile/0.05% TFA and eluted with 150μL 60% ACN in 0.05% TFA. Solid phase extraction eluants were then dried *in vacuo* without heat and stored at −80°C until analysis.

Standard curves of $^{12}C^{15}N$-Aβ were prepared in a matrix of 50mg/ml of BSA in PBS in order to determine the percent coefficient of variation (%CV) and dilutional linearity. The standard curves demonstrated average %CV of 2.01% for Aβ38, 8.48% for Aβ40, 6.81% for Aβ42, and 5.73% for Aβ mid-domain. The linearity was demonstrated with r-squared values of >0.99 for each Aβ isoform (see supplemental figure 1).

LC-MS/MS analysis of Aβ peptides

Extracted digests were reconstituted with 25 μl of 20 nM BSA Digest (Pierce #1863078) in 10% Formic acid/10% acetonitrile. A 4.5 μL aliquot of each digest was then subjected to LC-MS/MS on a Thermo Orbitrap Fusion Tribrid mass spectrometer interfaced with a Waters nanoAcquity chromatography system. Reconstituted digests were loaded via direct injection from a 5 μL sample loop onto a Waters 100 × 0.075 mm Acquity M-class HSS T3 column at 10% ACN in 0.1% formic acid with a flow rate of 600 nL/min for twelve minutes. Peptides were then resolved using a 10 minute linear gradient at 300 nL/min from 10% ACN in 0.1% formic acid to 35% ACN in 0.1% formic acid. The initial gradient was followed by a steeper linear gradient to 90% ACN in 0.1% formic acid over 5 minutes also at 300 nL/min. The column was washed with 90% ACN in 0.1% formic acid for an additional 2 minutes at 600 nL/min prior to re-equilibration to initial conditions for 5 minutes also at 600 nL/min. A list of peptide precursor and product ions used for parallel reaction monitoring (PRM) as well as all pertinent data collection parameters is provided in the Supplementary Table.

RESULTS

Plasma SILK for Aβ38, Aβ40, and Aβ42

In order to determine whether Aβ kinetics in the blood differs between amyloid positive and amyloid negative individuals, SILK time courses were obtained for plasma Aβ38, Aβ40, and Aβ42. To determine plasma Aβ kinetic rates, isotopic enrichment ratios were calculated and plotted versus time to elucidate differences in the kinetics of Aβ isoforms in the blood. Notably, the half-life of the Aβ isoforms in plasma was found to be approximately three hours, considerably faster than previously reported in CSF SILK studies (approximately 9 hours, Figure 1A). For both amyloid negative and amyloid positive individuals, Aβ38 labeling kinetics peaked earlier and higher than Aβ40 and Aβ42, indicating a faster turnover rate. This pattern is unique to plasma Aβ kinetics and was not found in prior CSF Aβ SILK studies.

In prior CSF studies, Aβ42 peaked earlier than Aβ38 and Aβ40 in amyloid positive individuals (3) indicating a faster loss of soluble Aβ42 likely due to aggregation and deposition. In this plasma SILK study, the Aβ38/Aβ40 ratios were similar over time between amyloid groups (Figure 1B), indicating no difference in kinetic processing between Aβ38 and Aβ40. In contrast, the plasma SILK Aβ42/Aβ40 ratios demonstrated faster soluble Aβ42 turnover kinetics in amyloid positive individuals (Figure 1C), as seen in prior reports of Aβ CSF SILK (2,3). While the average SILK Aβ42/Aβ40 ratio remained close to unity in the amyloid negative group, a drop after hour 12 in the Aβ42/Aβ40 ratio of the amyloid positive group indicates faster Aβ42 turnover and aggregation in those with CNS amyloidosis (Figure 1C).

Absolute Aβ concentrations in human plasma

Human plasma samples were also analyzed for absolute concentrations of Aβ38, Aβ40, and Aβ42 at each time point to investigate the production rates and whether CNS amyloidosis is associated with plasma Aβ differences. Average Aβ concentrations for the amyloid positive and amyloid negative groups for all time points was 27.40 pg/ml and 23.81 pg/ml (Aβ38), 288.0 pg/ml and 272.4 pg/ml (Aβ40), and 37.13 pg/ml and 30.13 pg/ml (Aβ42), respectively. The Aβ42 concentrations and Aβ42/Aβ40 concentration ratios were significantly lower in the amyloid positive cohort compared to the amyloid negative cohort, and this finding was consistent in longitudinal samples over 24 hours ($p<0.001$, Figure 2). Average Aβ42/Aβ40 throughout the study demonstrated similar values within the amyloid negative group. While Aβ42/Aβ40 differences were small between amyloid groups, they were statistically significant at most time points measured throughout the study (Figure 3A). An average of Aβ concentrations over the 24 hour period demonstrates excellent precision for identifying CNS amyloidosis (Figure 3B). Regarding the diagnostic accuracy of detecting amyloidosis, CNS amyloidosis with plasma normal results were rare, while CNS non-amyloidosis with plasma positive was more common. A similar pattern of decreased Aβ42/Aβ40 ratios in the presence of amyloidosis is observed in CSF, which is hypothesized to be due to Aβ42 concentrations decreasing before detection of accumulation by amyloid PET.

The magnitude of the difference in plasma is less than detected in CSF. While Aβ42/Aβ40 ratios in the CSF are decreased by approximately 50% in the presence of amyloidosis, in plasma Aβ42/Aβ40 ratios are decreased by 14.3% on average in amyloid positive relative to amyloid negative individuals (Figure 3B). Despite these relatively small differences in Aβ42/Aβ40 concentration ratios between amyloid pathology groups, they were quantified by high resolution mass spectrometry with good stability over time suggesting reliability of this measurement as a biomarker for amyloidosis.

The degree of correlation between plasma and CSF Aβ42/Aβ40 concentration ratios is illustrated in Figure 4, where both plasma and CSF Aβ42/Aβ40 ratios are lower in amyloid positive individuals. This association has a correlation coefficient of approximately 0.7, indicating a strong relationship between these two measurements. Similarly, when plasma Aβ42/Aβ40 is plotted as a function of PIB-PET MCBP, all amyloid positive individuals by PIB-PET had plasma Aβ42/Aβ40 below the threshold of 0.1243. However, there were several amyloid negative participants by PIB-PET who also demonstrated plasma Aβ42/Aβ40 ratios below this limit. This subset of participants might represent “false positives” when using plasma Aβ42/Aβ40 to detect amyloidosis. Alternatively, this finding may reflect the early alterations in Aβ kinetics that occur prior to detection of amyloidosis by neuroimaging. Additionally, this subset may reflect participants who are in the process of converting from amyloid negative to amyloid positive, as the plasma sampling of some participants was performed up to several years after PIB-PET imaging.

To investigate the utility of measuring absolute plasma Aβ concentrations as biomarker for amyloidosis, a receiver operating characteristic (ROC) curve was generated from the averaged plasma Aβ42/Aβ40 concentration ratios. This ROC analysis demonstrates an area under the curve (AUC) of 0.8865. As a combined measure of sensitivity and specificity, the AUC describes the inherent validity of using this plasma biomarker as a metric for predicting amyloid status. An AUC of 0.8865 indicates the absolute Aβ42/Aβ40 concentration ratio from human plasma has good diagnostic accuracy for the detection of amyloidosis. In other words, there is an 89% probability that a randomly chosen individual with low plasma Aβ42/Aβ40 concentration ratio would have amyloidosis. Furthermore, using the Youden index to determine optimal cutoff values, a threshold of 0.1243 maximizes sensitivity and specificity of the plasma Aβ42/Aβ40 concentration ratio as a tool for amyloid status classification (Figure 5).

DISCUSSION

For the first time, we report the kinetics of Aβ turnover in human plasma by applying SILK methods previously utilized to study Aβ metabolism in human CSF. We found that the half-life of Aβ isoforms in human plasma is approximately three hours, indicating the turnover of these peptides in the blood is much more rapid than in the CSF, where the half-life was previously reported to be ~9 hours. Other differences in Aβ kinetics between the blood and CSF are evident with SILK analysis, including the faster turnover of Aβ38 in the blood of both amyloid positive and amyloid negative participants. The cause for the faster metabolism of Aβ38 may be due to faster clearance by peripheral clearance mechanisms in the liver or kidney or different transport rates.

The faster turnover of Aβ42 relative to Aβ40 is similar to alterations in CSF kinetics, but off a lesser magnitude. Consistent with a faster turnover rate, we found lower absolute concentrations of Aβ42 and Aβ42/Aβ40 in the blood of amyloid positive individuals similar to findings in CSF. Taken together, these findings suggest that plasma Aβ kinetics reflect the CNS pathology of amyloidosis in a similar fashion as CSF. Although the difference in concentrations and kinetics are of lesser magnitude compared to CSF, the ability to detect an aberrant amyloid state in the blood is possible with the high degree of specificity and precision by high resolution mass spectometry.

The physiology of Aβ production, transport and clearance are essential to understand the pathophysiology of AD and also to interpret diagnostic and therapeutic approaches. Our findings that labeled plasma Aβ can be detected within the first hour but is not detected in human lumbar CSF for nearly 5 hours suggest a direct brain-to-blood transport mechanism. Alternative explanations include a reverse blood-to-brain transport mechanism, or independent Aβ labeling and turnover of the blood and CNS compartments. However, our prior study on transport directly across the brain vasculature in humans indicates a brain-to-blood Aβ transport mechanism which accounts for 30% to 50% of Aβ in blood (12). Further, Aβ amyloidosis occurs exclusively in the CNS, making a peripheral amyloidosis-induced blood Aβ alteration unlikely without a CNS source. Thus, we conclude that a major clearance pathway of brain Aβ is into the plasma via blood-brain barrier transporters (e.g. LRP), which enable its rapid egress from the CNS. Therefore, a rapid and significant brain-to-blood transport mechanism likely exists which may be important in whole-body handling of Aβ, and consequently, an important mechanism in the pathogenesis of AD. We propose the hypothesis that active and rapid transport of brain-to-blood Aβ provides a direct and specific mechanism for detecting CNS amyloidosis. This hypothesis would need to be validated by a larger study of more individuals along with longitudinal follow-up to determine if the highly specific findings here represent some of the earliest detectable changes in AD amyloidosis.

Over two dozen studies have investigated blood Aβ concentrations as a biomarker for diagnosis of AD and results have been conflicting. Most analyses found no association between plasma Aβ40 and Aβ42 levels and risk of AD (15); others note elevated levels of Aβ42 in patients with MCI compared to cognitively normal controls and individuals with AD (16). Some prospective studies have shown that higher plasma Aβ42 levels are associated with increased risk of sporadic AD, but such elevations occur just prior to the onset of clinical symptoms as Aβ42 levels may subsequently decline as the disease progresses (17). However, these studies have not demonstrated a difference in plasma Aβ which would be useful for individual determination of amyloidosis due to very high overlap between groups. Low sensitivity and accuracy of measurement techniques may contribute to these frequently contradictory reports of plasma Aβ levels in AD and contribute to low specificity for amyloidosis. The greater sensitivity and specificity of the approach described here allows for the 10% to 15% differences in plasma Aβ concentrations to be quantified with a high degree of precision and therefore enables satisfactory determination of amyloid status. Furthermore, the longitudinal stability of these measurements over a 24 hour time period and within individual replicates increases the reliability of these findings, and consequently, the likely utility of plasma Aβ concentrations as a biomarker for AD.

Strengths of this study include its prospective registered design, blinded analyses, and careful selection of participants who were characterized by both CSF Aβ42/Aβ40 concentrations and PIB-PET imaging. Another strength is the consistency of two orthogonal approaches of kinetics and concentration measurements which indicate a similar specificity of plasma Aβ42 concentrations and kinetics for CNS amyloidosis. Additionally, the multiple longitudinal measures within the same individual demonstrate stability and consistency of Aβ concentrations associated with amyloidosis. Limitations of this study include its relatively smaller population size (n=41) and measurement of PIB-PET scan or CSF Aβ42/Aβ40 concentrations being obtained prior to plasma assessment.

To further validate the findings of this study cohort, external validation in a larger population of individuals with known amyloidosis status is needed. The stability and sensitivity of plasma Aβ measurements in this study suggest this Aβ blood test may be a useful screening test for CNS amyloidosis. Advantages of a simple blood test for amyloidosis could include greatly decreased cost, simplicity of the clinical collection procedure, high-throughput central lab processing of samples, avoidance of radiation, and controlled reproducibility of results. Development of a simplified blood test for amyloidosis with multi-center studies and central labs which can be implemented worldwide are needed to fully support rapid incorporation in the field. Measuring plasma Aβ as a biomarker for amyloidosis has the potential to provide a minimally invasive, simple screening test that reflects the underlying pathophysiology of AD for use in prevention trials as well as observational and longitudinal studies. Ultimately, with the advent of effective treatments for AD, this blood test may serve as a versatile diagnostic test for screening millions of individuals at risk for amyloidosis and AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med. 2016; 8(6):595–608. [PubMed: 27025652]
2. Potter R, Patterson BW, Elbert DL, Ovod V, Kasten T, Sigurdson W, Mawuenyega K, Blazey T, Goate A, Chott R, Yarasheski KE, Holtzman DM, Morris JC, Benzinger TL, Bateman RJ. Increased in vivo amyloid-β42 production, exchange, and loss in presenilin mutation carriers. Sci Transl Med. 2013; 5(189)
3. Patterson BW, Elbert DL, Mawuenyega KG, Kasten T, Ovod V, Ma S, Xiong C, Chott R, Yarasheski K, Sigurdson W, Zhang L, Goate A, Benzinger T, Morris JC, Holtzman D, Bateman RJ. Age and amyloid effects on human CNS amyloid-beta kinetics. Ann Neurol. 2015; 78(3):439–453. [PubMed: 26040676]

4. Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer's disease Centers, 2005–2010. J Neuropathol Exp Neurol. 2013; 71(4):266–273.

5. Cohen AD, Klunk WE. Early detection of Alzheimer's disease using PiB and FDG PET. Neurobiol Dis. 2014; 72(Pt A):117–22. [PubMed: 24825318]

6. Galasko D, Chang L, Motter R, Clark CM, Kaye J, Knopman D, Thomas R, Kholodenko D, Schenk D, Lieberburg I, Miller B, Green R, Basherad R, Kertiles L, Boss MA, Seubert P. High cerebrospinal fluid tau and low amyloid beta42 levels in the clinical diagnosis of Alzheimer disease and relation to apolipoprotein E genotype. Arch Neurol. 1998; 55(7):937–45. [PubMed: 9678311]

7. Clark CM, Xie S, Chittams J, Ewbank D, Peskind E, Galasko D, Morris JC, McKeel DW Jr, Farlow M, Weitlauf SL, Quinn J, Kaye J, Knopman D, Arai H, Doody RS, DeCarli C, Leight S, Lee VM, Trojanowski JQ. Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? Arch Neurol. 2003; 60(12):1696–702. [PubMed: 14676043]

8. Mayeux R, Honig LS, Tang MX, Manly J, Stern Y, Schupf N, Mehta PD. Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: relation to age, mortality, and risk. Neurology. 2003; 61(9): 1185–90. [PubMed: 14610118]

9. Yaffe K, Weston A, Graff-Radford NR, Satterfield S, Simonsick EM, Younkin SG, Younkin LH, Kuller L, Ayonayon HN, Ding J, Harris TB. Association of plasma beta-amyloid level and cognitive reserve with subsequent cognitive decline. JAMA. 2011; 305(3):261–6. [PubMed: 21245181]

10. Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM. Human amyloid-B synthesis and clearance rates as measured in cerebrospinal fluid in vivo. Nat Med. 2006; 12(7): 856–861. [PubMed: 16799555]

11. Mawuenyega KG, Kasten T, Sigurdson W, Bateman RJ. Amyloid-beta isoform metabolism quantitation by stable isotope-labeled kinetics. Anal Biochem. 2013; 440(1):56–62. [PubMed: 23714261]

12. Roberts KF, Elbert DL, Kasten TP, Patterson BW, Sigurdson WC, Connors RE, Ovod V, Munsell LY, Mawuenyega KG, Miller-Thomas MM, Moran CJ, Cross DT III, Derdeyn CP, Bateman RJ. Amyloid-B efflux from the CNS into the plasma. Ann Neurol. 2014; 76(6):837–844. [PubMed: 25205593]

13. Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, Welch D, Manness L, Lin C, Yu J, Zhu H, Ghiso J, Grangione B, Stern A, Schmidt AM, Armstrong DL, Arnold B, Liliensiek B, Nawroth P, Hofman F, Kindy M, Stern D, Zlokovic B. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. Nat Med. 2003; 9(7):907–913. [PubMed: 12808450]

14. Dean R, Wu Z, Sagare A, Davis J, Du Yan S, Hamm K, Xu F, Parisi M, LaRue B, Hu HW, Spijkers P, Guo H, Song X, Lenting PJ, Van Nostrand WE, Zlokovic BV. LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. Neuron. 2004; 5(3):333–44.

15. Fukumoto H, Tennis M, Locascio JJ, Hyman BT, Grwodon JH, Irizarry MC. Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels. Arch Neurol. 2003; 60:958–964. [PubMed: 12873852]

16. Sobów T, Flirski M, Kłoszewska I, Liberski PP. Plasma levels of alpha beta peptides are altered in amnestic mild cognitive impairment but not in sporadic Alzheimer's disease. Acta Neurobiol Exp (Wars). 2005; 65:117–124. [PubMed: 15960295]

17. Mayeux R, Schupf N. Blood-based biomarkers for Alzheimer's disease: plasma Ab40 and AB42, and genetic variants. Neurobio of Aging. 2011; 32(11):S10–S19.

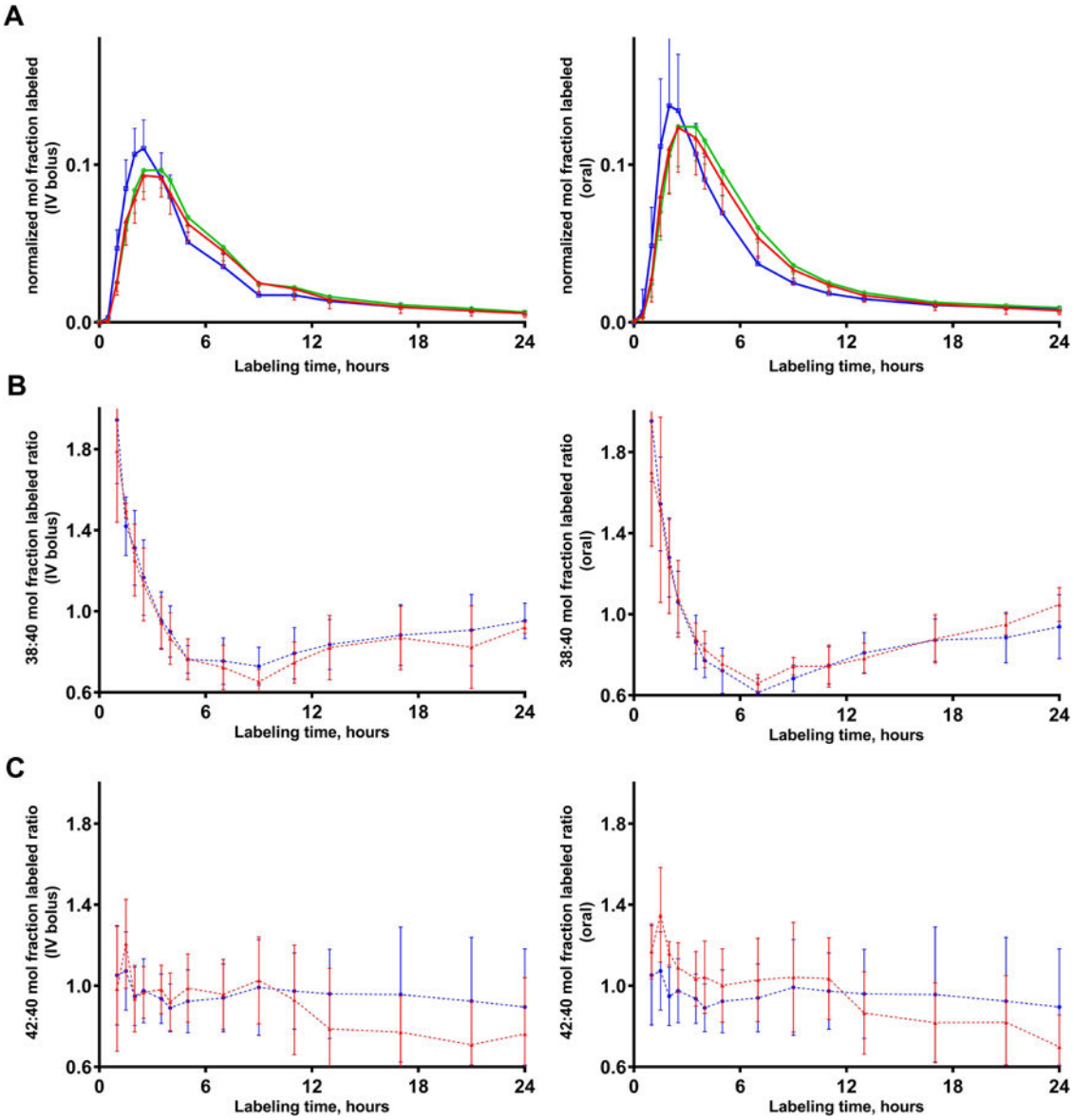

Figure 1. Plasma Aβ SILK for Aβ38, Aβ40, and Aβ42

(A): Average isotopic enrichment time course profiles normalized to plasma leucine for plasma Aβ38 (blue), Aβ40 (green), and Aβ42 (red) (mean +/− 95% CI) by labeling protocol (left: IV bolus; right: oral). Kinetic profiles of all three isoforms appear similar between labeling protocols, with Aβ38 reaching its labeling peak before Aβ40 and Aβ42.

(B): Average isotopic enrichment ratios for plasma Aβ38/Aβ40 displaying both amyloid groups on the same plot (blue, amyloid negative; red, amyloid positive) (mean +/− 95% CI) demonstrates similar rates of plasma Aβ38/Aβ40 turnover regardless of amyloid status or labeling protocol (left, IV bolus; right, oral).

(C): Average isotopic enrichment ratios for plasma Aβ42/Aβ40 displaying both amyloid groups on the same plot (blue, amyloid negative; red, amyloid positive) highlights the faster Aβ42 turnover kinetics in the amyloid positive group (mean +/− 95% CI) for both the IV-bolus (left) and oral-labeled groups (right).

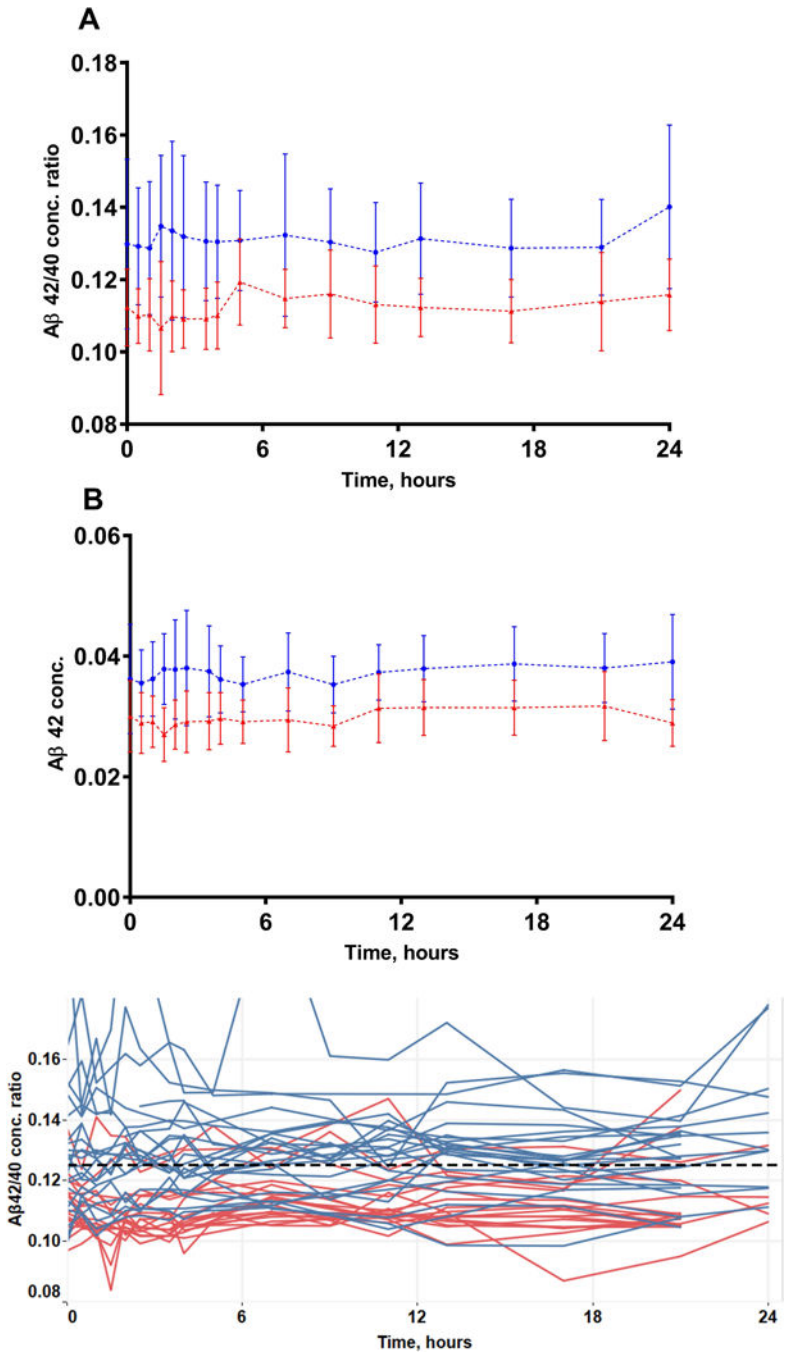

Figure 2. Absolute concentrations of Aβ by clinical group

(A): Absolute concentrations of Aβ42/Aβ40 over time averaged by clinical group (blue, amyloid negative; red, amyloid positive) (mean +/− 95% CI). Aβ42/Aβ40 concentrations were 10–15% lower in the amyloid positive group compared to the amyloid negative group at all time points measured.

(B): Absolute concentrations of Aβ42 over time averaged by clinical group (blue, amyloid negative; red, amyloid positive) (mean +/− 95% CI). Aβ42 concentrations were 10–15%

lower in the amyloid positive group compared to the amyloid negative group at all time points measured.

(C): Absolute concentrations of Aβ42/Aβ40 over time with individual participant time courses illustrates the consistency of concentration measurements (blue, amyloid negative; red, amyloid positive).

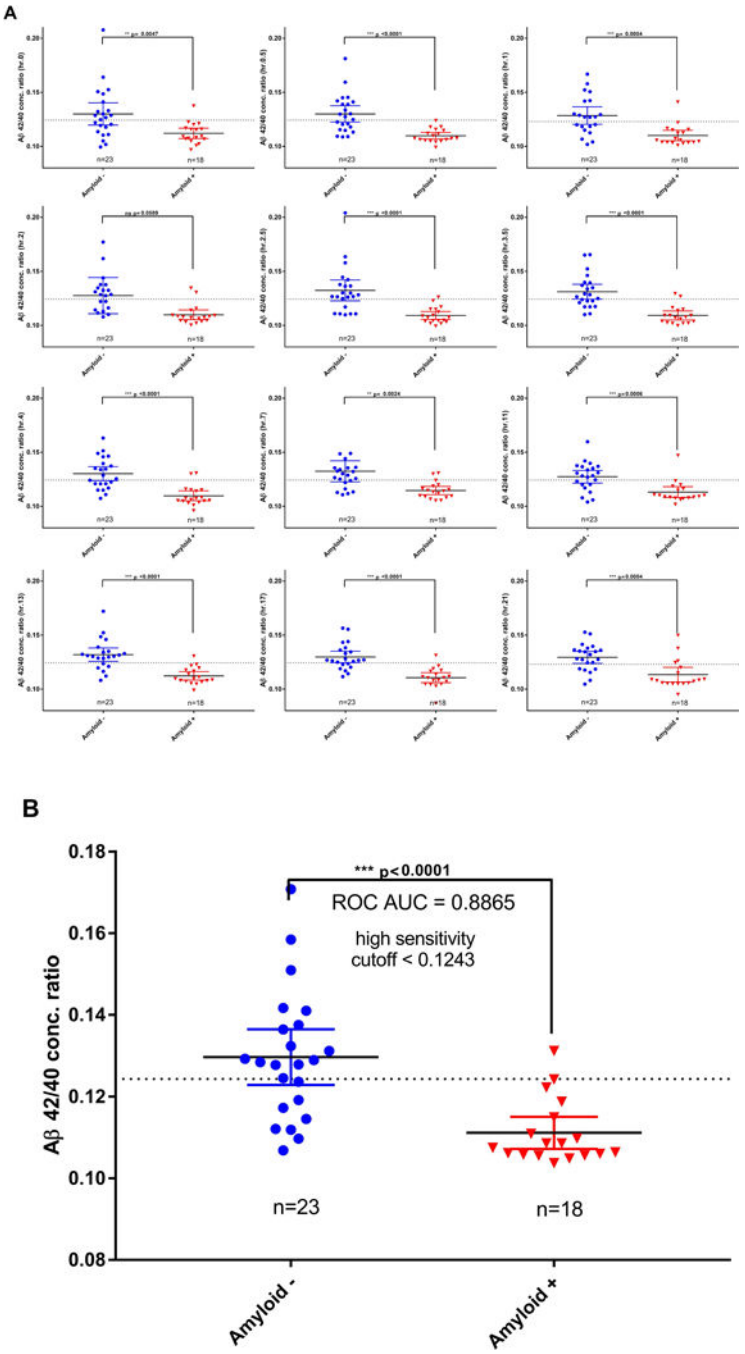

Figure 3. Absolute concentrations of Aβ42/Aβ40 remain relatively stable over time with measurable separation of clinical groups

(A): Average Aβ42/Aβ40 concentrations for each participant at all time points separated by amyloid status (blue, amyloid negative; red, amyloid positive) demonstrate the stability and reproducibility of this measurement over time.

(B): Aβ42/Aβ40 concentrations by amyloid status as an average of all time points (0 – 24 hours). On average, the Aβ42/Aβ40 concentration was 0.1297 +/− 0.0033 in the amyloid negative group (blue) and 0.1111 +/− 0.0019 in the amyloid positive group (red). This

reflects a 14.3% lower Aβ42/Aβ40 concentration in amyloid positive individuals compared to amyloid negative individuals overall (p value < 0.0001, mean +/− 95% CI shown).

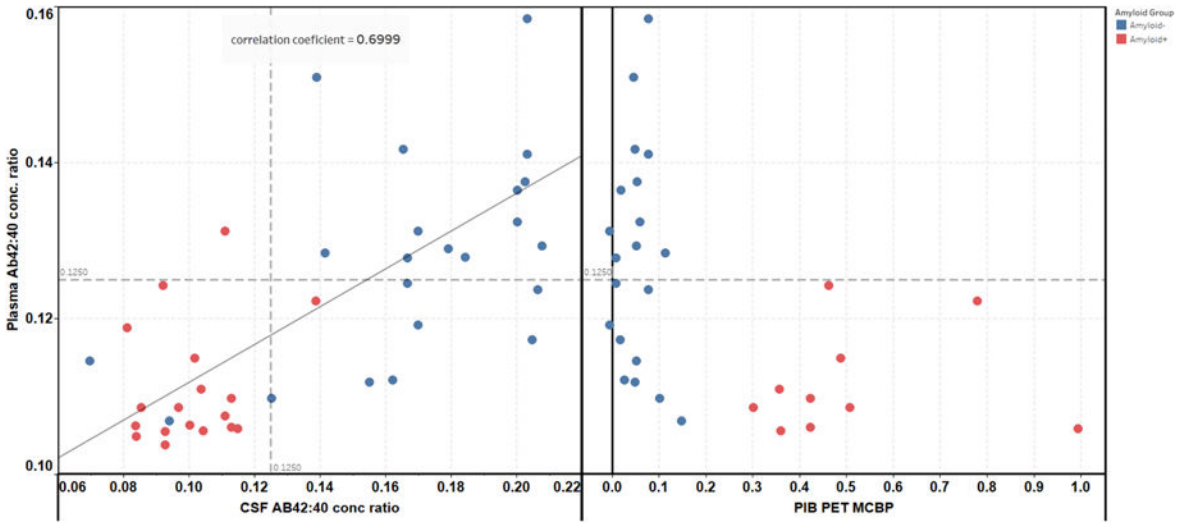

Figure 4. Plasma Aβ42/Aβ40 concentrations correlate with two different measures of amyloidosis

Plasma Aβ42/Aβ40 concentration ratios are lower in amyloid positive individuals (red) compared to amyloid negative individuals (blue) when amyloid status is determined by CSF Aβ42/Aβ40 concentrations. The relationship between decreased Aβ42/Aβ40 in both blood and CSF in the presence of amyloidosis has a correlation coefficient of 0.6999, indicating a strong positive correlation between these measurements ($p < 0.0001$). Similarly, plasma Aβ42/Aβ40 concentration ratios are lower in amyloid positive individuals (red) compared to amyloid negative individuals (blue) when amyloid status is determined by [^{11}C]PIB-PET imaging with amyloid positive individuals having a mean cortical binding potential (MCBP) > 0.18. While all participants with known amyloidosis by PIB-PET had correspondingly low plasma Aβ42/Aβ40 measurements, several participants classified as amyloid negative by PIB-PET were also found to have similarly low plasma Aβ42/Aβ40 values (below the threshold of 0.1243).

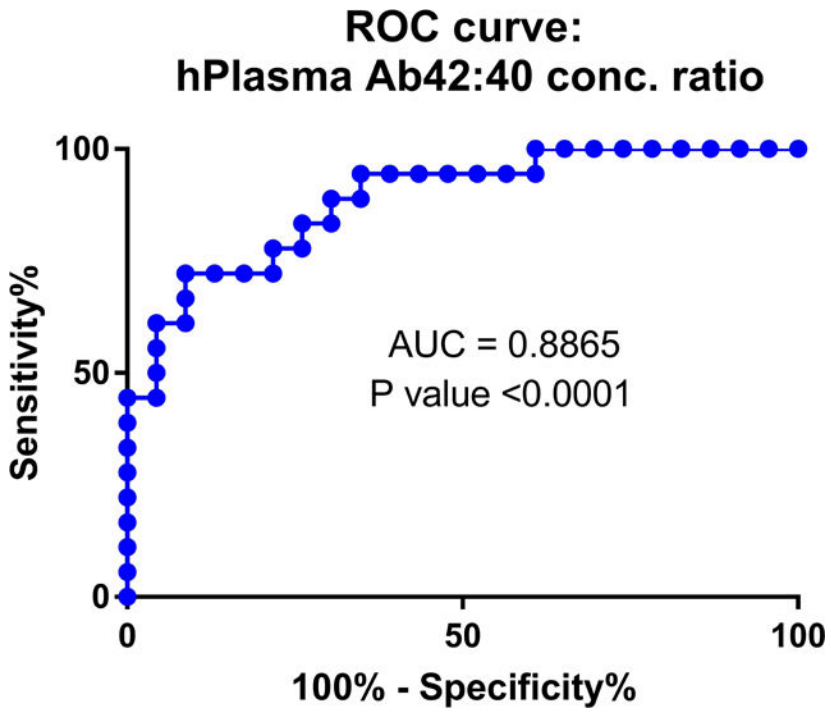

Figure 5. ROC curve

ROC curve analysis using average plasma Aβ42/Aβ40 concentration ratios over 24 hours demonstrates an AUC of 0.8865, indicating this assay has good accuracy as a diagnostic test to detect amyloidosis.